VACCINE GUIDE

NCVIA / VICP / VAERS

National Childhood Vaccine Injury Act

(VICP)

H.R.5546 - National Childhood Vaccine Injury Act of 1986

99th Congress (1985-1986)

Sponsor: Rep. Waxman, Henry A. [D-CA-24] (Introduced 09/18/1986)
Committees: House - Energy and Commerce; Ways and Means | Senate - Labor and Human Resources
Committee Reports: H.Rept 99-908 Part 1
Latest Action: Senate - 10/18/1986 Read twice and referred to the Committee on Labor and Human Resources. (All Actions)
Tracker: Introduced | **Passed House**

Summary(2) Text Actions(11) Titles(3) Amendments(0) Cosponsors(23) Committees(3) Related Bills(1)

There are 2 summaries for H.R.5546. Passed House amended (10/14/1986)

Bill summaries are authored by CRS.

Shown Here:
Passed House amended (10/14/1986)

(Measure passed House, amended)
National Childhood Vaccine Injury Act of 1986 - **Title I: Vaccines - Subtitle 1: National Vaccine Program** - Amends the Public Health Service Act to establish in the Department of Health and Human Services a National Vaccine Program to: (1) direct vaccine research and development within the Federal Government; (2) ensure the production and procurement of safe and effective vaccines; (3) direct the distribution and use of vaccines; and (4) coordinate governmental and nongovernmental activities. Requires the Director of the Program to report to specified congressional committees.

Establishes the National Vaccine Advisory Committee to recommend: (1) ways to encourage the availability of an adequate supply of vaccines; and (2) research priorities.

Authorizes appropriations for FY 1987 through 1991.

Subtitle 2: National Vaccine Injury Compensation Program - **Part A: Program Requirements** - Establishes the National Vaccine Injury Compensation Program as an alternative remedy to judicial action for specified vaccine-related injuries.

Prescribes the contents of any petition for compensation.

Grants U.S. district courts authority to determine eligibility and compensation. Requires the district court in which the petition is filed to designate a special master to serve as an adjunct to the court. Sets forth the responsibilities of the court.

Lists factors to be considered when determining the amount of a compensation award. Sets forth a table of injuries deemed vaccine-related for compensation purposes. Permits the Secretary of Health and Human Services to: (1) promulgate regulations to revise such table; and (2) recommend changes to the vaccines covered by the table.

Provides that compensation awarded under the Program shall be paid out of the National Vaccine Injury Compensation Trust Fund. Limits awards for actual and projected pain and suffering and emotional distress to $250,000. Prohibits awards for punitive damages.

Establishes the Advisory Commission on Childhood Vaccines to: (1) advise the Secretary on the implementation of the Program; (2) recommend changes to the Vaccine Injury Table; and (3) recommend research priorities.

Part B: Additional Remedies - Sets forth procedures under which the person who filed a petition for compensation under the program may elect to file a civil action for damages.

Provides that no vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death: (1) resulting from unavoidable side effects; or (2) solely due to the manufacturer's failure to provide direct warnings. Provides that a manufacturer may be held liable where: (1) such manufacturer engaged in the fraudulent or intentional withholding of information; or (2) such manufacturer failed to exercise due care. Permits punitive damages in such civil actions under certain circumstances.

Part C: Assuring a Safer Childhood Vaccination Program in the United States - Requires each health care provider who administers a vaccine listed in the Vaccine Injury Table to record certain information with respect to each such vaccine. Requires each health care provider and vaccine manufacturer to report certain information to the Secretary.

Requires the Secretary to develop certain vaccine information materials for distribution to the legal representatives of any child receiving a vaccine listed in the Vaccine Injury Table.

Directs the Secretary to promote the development of safer childhood vaccines.

Sets forth recordkeeping and reporting requirements for vaccine manufacturers. Imposes civil and criminal penalties for destroying, altering, or concealing any such report or record.

Part D: General Provisions - Allows any person to commence a civil action against the Secretary where the Secretary allegedly has failed to perform a duty under this Act. Provides for judicial review of the Secretary's regulatory actions in a court of appeals of the United States.

Allows the Secretary to provide licensing for unpatented vaccines for naturally occurring human infectious diseases under certain circumstances.

Requires the Secretary to conduct studies on pertussis, rubella, and radiculoneuritis vaccines and publish the results of such studies.

Directs the Secretary to study the risks to children associated with each vaccine listed in the Vaccine Injury Table and establish guidelines respecting the administration of such vaccines. Directs the Secretary to periodically review and revise such guidelines.

Directs the Secretary to review the warnings, use instructions, and precautionary information presently used by manufacturers of vaccines listed in the Vaccine Injury Table. Directs the Secretary to require manufacturers to revise and reissue any warning, instruction, or information found inadequate.

Grants the Secretary recall authority with respect to any licensed virus, serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or other licensed product which presents a danger to public health. Establishes civil penalties for recall violations.

Directs the Secretary to make annual reports to specified congressional committees on the impact this Act has on the supply of vaccines.

Title II: Miscellaneous - Provides that certain Federal provisions designed to reduce paperwork shall not apply to information required to carry out this Act.

Bruesewitz *v.* Wyeth Inc.

PETITIONER

Russell Bruesewitz, et al.

RESPONDENT

Wyeth, Inc., fka Wyeth Laboratories, et al.

LOCATION

United States District Court for the Eastern District of Pennsylvania

DOCKET NO.

09-152

DECIDED BY

Roberts Court (/courts?court=Roberts Court)

LOWER COURT

United States Court of Appeals for the Third Circuit

CITATION

562 US 223 (2011)
(https://supreme.justia.com/cases/federal/us/562/223)

GRANTED

Mar 8, 2010

ARGUED

Oct 12, 2010

DECIDED

Feb 22, 2011

ADVOCATES

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Facts of the case

Two hours after Hannah Bruesewitz received her six-month diphtheria, tetanus and pertussis vaccine in 1992, she started developing seizures and was hospitalized for weeks. Hannah has continued to suffer from residual seizure disorder that requires her to receive constant care, according to her parents. When their daughter was three-years-old, Russell and Robalee Bruesewitz filed a petition seeking compensation for her injuries. One month prior to the petition, new regulations eliminated Hannah's seizure disorder from the list of compensable injuries. The family's petition was denied. Three years later, in 1998, the drug company Wyeth withdrew the type of vaccine used in Hannah's inoculation from the market.

The Bruesewitzes filed a lawsuit against Wyeth in state court in Pennsylvania. They claimed the drug company failed to develop a safer vaccine and should be held accountable for preventable injuries caused by the vaccine's defective design. A federal judge dismissed the lawsuit, ruling that the National Childhood Vaccine Injury Act protected Wyeth from lawsuits over vaccine injury claims. The U.S. Court of Appeals for the 3rd Circuit affirmed.

Question

Can a federal law shield vaccine manufacturers from certain product liability lawsuits in state court that seek damages for serious health problems suffered by children?

Conclusion

6–2 DECISION FOR WYETH, INC.

MAJORITY OPINION BY ANTONIN SCALIA

The NCVIA's "no-fault" compensation program preempts design-defect claims against vaccine manufacturers brought by plaintiffs seeking damages for injury or death caused by vaccine side effects.

6–2 DECISION

MAJORITY OPINION BY ANTONIN SCALIA

Plaintiffs are entitled to seek compensation for such injury or death by filing a timely claim in the U.S. Court of Federal Claims, which holds special jurisdiction over such cases.

Yes. The Supreme Court affirmed the lower court decision in an opinion by Justice Antonin Scalia. The majority reasoned that Congress had set up a special vaccine court as a way to provide compensation to injured children without driving drug manufacturers from the vaccine market. Justice Stephen Breyer filed a concurring opinion. Justice Sonia Sotomayor filed a dissenting opinion, joined by Justice Ruth Bader Ginsburg. Justice Elena Kagan took no part in consideration of the case.

Cite this page

"Bruesewitz v. Wyeth Inc." *Oyez*, www.oyez.org/cases/2010/09-152. Accessed 5 Nov. 2022.

Syllabus

NOTE: Where it is feasible, a syllabus (headnote) will be released, as is being done in connection with this case, at the time the opinion is issued. The syllabus constitutes no part of the opinion of the Court but has been prepared by the Reporter of Decisions for the convenience of the reader. See *United States* v. *Detroit Timber & Lumber Co.,* 200 U. S. 321, 337.

SUPREME COURT OF THE UNITED STATES

Syllabus

BRUESEWITZ ET AL. *v.* WYETH LLC, FKA WYETH, INC., ET AL.

CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE THIRD CIRCUIT

No. 09–152. Argued October 12, 2010—Decided February 22, 2011

The National Childhood Vaccine Injury Act of 1986 (NCVIA or Act) created a no-fault compensation program to stabilize a vaccine market adversely affected by an increase in vaccine-related tort litigation and to facilitate compensation to claimants who found pursuing legitimate vaccine-inflicted injuries too costly and difficult. The Act provides that a party alleging a vaccine-related injury may file a petition for compensation in the Court of Federal Claims, naming the Health and Human Services Secretary as the respondent; that the court must resolve the case by a specified deadline; and that the claimant can then decide whether to accept the court's judgment or reject it and seek tort relief from the vaccine manufacturer. Awards are paid out of a fund created by an excise tax on each vaccine dose. As a *quid pro quo,* manufacturers enjoy significant tort-liability protections. Most importantly, the Act eliminates manufacturer liability for a vaccine's unavoidable, adverse side effects.

Hannah Bruesewitz's parents filed a vaccine-injury petition in the Court of Federal Claims, claiming that Hannah became disabled after receiving a diphtheria, tetanus, and pertussis (DTP) vaccine manufactured by Lederle Laboratories (now owned by respondent Wyeth). After that court denied their claim, they elected to reject the unfavorable judgment and filed suit in Pennsylvania state court, alleging, *inter alia,* that the defective design of Lederle's DTP vaccine caused Hannah's disabilities, and that Lederle was subject to strict liability and liability for negligent design under Pennsylvania common law. Wyeth removed the suit to the Federal District Court. It granted Wyeth summary judgment, holding that the relevant Pennsylvania law was preempted by 42 U. S. C. §300aa–22(b)(1), which

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provides that "[n]o vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after October 1, 1988, if the injury or death resulted from side-effects that were unavoidable even though the vaccine was properly prepared and was accompanied by proper directions and warnings." The Third Circuit affirmed.

Held: The NCVIA preempts all design-defect claims against vaccine manufacturers brought by plaintiffs seeking compensation for injury or death caused by a vaccine's side effects. Pp. 7–19.

(a) Section 300aa–22(b)(1)'s text suggests that a vaccine's design is not open to question in a tort action. If a manufacturer could be held liable for failure to use a different design, the "even though" clause would do no work. A vaccine side effect could always have been avoidable by use of a different vaccine not containing the harmful element. The language of the provision thus suggests the design is not subject to question in a tort action. What the statute establishes as a complete defense must be unavoidability (given safe manufacture and warning) with respect to the particular design. This conclusion is supported by the fact that, although products-liability law establishes three grounds for liability—defective manufacture, inadequate directions or warnings, and defective design—the Act mentions only manufacture and warnings. It thus seems that the Act's failure to mention design-defect liability is "by deliberate choice, not inadvertence." *Barnhart* v. *Peabody Coal Co.*, 537 U. S. 149, 168. Pp. 7–8.

(b) Contrary to petitioners' argument, there is no reason to believe that §300aa–22(b)(1)'s term "unavoidable" is a term of art incorporating Restatement (Second) of Torts §402A, Comment *k,* which exempts from strict liability rules "unavoidably unsafe products." "Unavoidable" is hardly a rarely used word, and cases interpreting comment *k* attach special significance only to the term "unavoidably unsafe products," not the word "unavoidable" standing alone. Moreover, reading the phrase "side effects that were unavoidable" to exempt injuries caused by flawed design would require treating "even though" as a coordinating conjunction linking independent ideas when it is a concessive, subordinating conjunction conveying that one clause weakens or qualifies the other. The canon against superfluity does not undermine this Court's interpretation because petitioners' competing interpretation has superfluity problems of its own. Pp. 8–12.

(c) The structure of the NCVIA and of vaccine regulation in general reinforces what §300aa–22(b)(1)'s text suggests. Design defects do not merit a single mention in the Act or in Food and Drug Administration regulations that pervasively regulate the drug manufacturing process. This lack of guidance for design defects, combined with

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the extensive guidance for the two liability grounds specifically mentioned in the Act, strongly suggests that design defects were not mentioned because they are not a basis for liability. The Act's mandates lead to the same conclusion. It provides for federal agency improvement of vaccine design and for federally prescribed compensation, which are other means for achieving the two beneficial effects of design-defect torts—prompting the development of improved designs, and providing compensation for inflicted injuries. The Act's structural *quid pro quo* also leads to the same conclusion. The vaccine manufacturers fund an informal, efficient compensation program for vaccine injuries in exchange for avoiding costly tort litigation and the occasional disproportionate jury verdict. Taxing their product to fund the compensation program, while leaving their liability for design defect virtually unaltered, would hardly coax them back into the market. Pp. 13–16.

561 F. 3d 233, affirmed.

SCALIA, J., delivered the opinion of the Court, in which ROBERTS, C. J., and KENNEDY, THOMAS, BREYER, and ALITO, JJ., joined. BREYER, J., filed a concurring opinion. SOTOMAYOR, J., filed a dissenting opinion, in which GINSBURG, J., joined. KAGAN, J., took no part in the consideration or decision of the case.

SUPREME COURT OF THE UNITED STATES

No. 09–152

RUSSELL BRUESEWITZ, ET AL., PETITIONERS *v.* WYETH LLC, FKA WYETH, INC., FKA WYETH LABORATORIES, ET AL.

ON WRIT OF CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE THIRD CIRCUIT

[February 22, 2011]

JUSTICE SCALIA delivered the opinion of the Court.

We consider whether a preemption provision enacted in the National Childhood Vaccine Injury Act of 1986 (NCVIA)[1] bars state-law design-defect claims against vaccine manufacturers.

I

A

For the last 66 years, vaccines have been subject to the same federal premarket approval process as prescription drugs, and compensation for vaccine-related injuries has been left largely to the States.[2] Under that regime, the elimination of communicable diseases through vaccination became "one of the greatest achievements" of public health in the 20th century.[3] But in the 1970's and 1980's vac-

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[1] 42 U. S. C. §300aa–22(b)(1).

[2] See P. Hutt, R. Merrill, & L. Grossman, Food and Drug Law 912–913, 1458 (3d ed. 2007).

[3] Centers for Disease Control, Achievements in Public Health, 1900–1999: Impact of Vaccines Universally Recommended for Children, 48 Morbidity and Mortality Weekly Report 243, 247 (Apr. 2, 1999).

cines became, one might say, victims of their own success. They had been so effective in preventing infectious diseases that the public became much less alarmed at the threat of those diseases,[4] and much more concerned with the risk of injury from the vaccines themselves.[5]

Much of the concern centered around vaccines against diphtheria, tetanus, and pertussis (DTP), which were blamed for children's disabilities and developmental delays. This led to a massive increase in vaccine-related tort litigation. Whereas between 1978 and 1981 only nine product-liability suits were filed against DTP manufacturers, by the mid-1980's the suits numbered more than 200 each year.[6] This destabilized the DTP vaccine market, causing two of the three domestic manufacturers to withdraw; and the remaining manufacturer, Lederle Laboratories, estimated that its potential tort liability exceeded its annual sales by a factor of 200.[7] Vaccine shortages arose when Lederle had production problems in 1984.[8]

Despite the large number of suits, there were many complaints that obtaining compensation for legitimate vaccine-inflicted injuries was too costly and difficult.[9] A

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[4] See Mortimer, Immunization Against Infectious Disease, 200 Science 902, 906 (1978).

[5] See National Vaccine Advisory Committee, A Comprehensive Review of Federal Vaccine Safety Programs and Public Health Activities 2–3 (Dec. 2008) (hereinafter NVAC), http://www.hhs.gov/nvpo/nvac/documents/vaccine-safety-review.pdf (as visited Feb. 18, 2011, and available in Clerk of Court's case file).

[6] See Sing & Willian, Supplying Vaccines: An Overview of the Market and Regulatory Context, in Supplying Vaccines: An Economic Analysis of Critical Issues 45, 51–52 (M. Pauly, C. Robinson, S. Sepe, M. Sing, & M. William eds. 1996).

[7] See *id.,* at 52.

[8] See Centers for Disease Control, Diptheria-Tetanus-Pertussis Vaccine Shortage, 33 Morbidity and Mortality Weekly Report 695–696 (Dec. 14, 1984).

[9] See Apolinsky & Van Detta, Rethinking Liability for Vaccine Injury, 19 Cornell J. L. & Pub. Pol'y 537, 550–551 (2010); T. Burke, Lawyers,

significant number of parents were already declining vaccination for their children,[10] and concerns about compensation threatened to depress vaccination rates even further.[11] This was a source of concern to public health officials, since vaccines are effective in preventing outbreaks of disease only if a large percentage of the population is vaccinated.[12]

To stabilize the vaccine market and facilitate compensation, Congress enacted the NCVIA in 1986. The Act establishes a no-fault compensation program "designed to work faster and with greater ease than the civil tort system." *Shalala* v. *Whitecotton*, 514 U. S. 268, 269 (1995). A person injured by a vaccine, or his legal guardian, may file a petition for compensation in the United States Court of Federal Claims, naming the Secretary of Health and Human Services as the respondent.[13] A special master then makes an informal adjudication of the petition within (except for two limited exceptions) 240 days.[14] The Court of Federal Claims must review objections to the special master's decision and enter final judgment under a similarly tight statutory deadline.[15] At that point, a claimant has two options: to accept the court's judgment and forgo a traditional tort suit for damages, or to reject the judgment and seek tort relief from the vaccine manufacturer.[16]

Fast, informal adjudication is made possible by the Act's Vaccine Injury Table, which lists the vaccines covered under the Act; describes each vaccine's compensable,

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Lawsuits, and Legal Rights: The Battle over Litigation in American Society 146 (2002).

[10] Mortimer, *supra*, at 906.

[11] See Hagan, 45 Food Drug Cosm. L. J. 477, 479 (1990).

[12] See R. Merrill, Introduction to Epidemiology 65–68 (2010).

[13] See 42 U. S. C. §300aa–11(a)(1).

[14] See §300aa–12(d)(3).

[15] See §300aa–12(e), (g).

[16] See §300aa–21(a).

adverse side effects; and indicates how soon after vaccination those side effects should first manifest themselves.[17] Claimants who show that a listed injury first manifested itself at the appropriate time are prima facie entitled to compensation.[18] No showing of causation is necessary; the Secretary bears the burden of disproving causation.[19] A claimant may also recover for unlisted side effects, and for listed side effects that occur at times other than those specified in the Table, but for those the claimant must prove causation.[20] Unlike in tort suits, claimants under the Act are not required to show that the administered vaccine was defectively manufactured, labeled, or designed.

Successful claimants receive compensation for medical, rehabilitation, counseling, special education, and vocational training expenses; diminished earning capacity; pain and suffering; and $250,000 for vaccine-related deaths.[21] Attorney's fees are provided, not only for successful cases, but even for unsuccessful claims that are not frivolous.[22] These awards are paid out of a fund created by an excise tax on each vaccine dose.[23]

The *quid pro quo* for this, designed to stabilize the vaccine market, was the provision of significant tort-liability protections for vaccine manufacturers. The Act requires claimants to seek relief through the compensation program before filing suit for more than $1,000.[24] Manufacturers are generally immunized from liability for fail-

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[17] See §300aa–14(a); 42 CFR §100.3 (2009) (current Vaccine Injury Table).

[18] See 42 U. S. C. §§300aa–11(c)(1), 300aa–13(a)(1)(A).

[19] See §300aa–13(a)(1)(B).

[20] See §300aa–11(c)(1)(C)(ii).

[21] See §300aa–15(a).

[22] See §300aa–15(e).

[23] See §300aa–15(i)(2); 26 U. S. C. §§4131, 9510.

[24] See 42 U. S. C. §300aa–11(a)(2).

ure to warn if they have complied with all regulatory requirements (including but not limited to warning requirements) and have given the warning either to the claimant or the claimant's physician.[25] They are immunized from liability for punitive damages absent failure to comply with regulatory requirements, "fraud," "intentional and wrongful withholding of information," or other "criminal or illegal activity."[26] And most relevant to the present case, the Act expressly eliminates liability for a vaccine's unavoidable, adverse side effects:

> "No vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after October 1, 1988, if the injury or death resulted from side effects that were unavoidable even though the vaccine was properly prepared and was accompanied by proper directions and warnings."[27]

B

The vaccine at issue here is a DTP vaccine manufactured by Lederle Laboratories. It first received federal approval in 1948 and received supplemental approvals in 1953 and 1970. Respondent Wyeth purchased Lederle in 1994 and stopped manufacturing the vaccine in 1998.

Hannah Bruesewitz was born on October 20, 1991. Her pediatrician administered doses of the DTP vaccine according to the Center for Disease Control's recommended childhood immunization schedule. Within 24 hours of her April 1992 vaccination, Hannah started to experience

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[25] See §300aa–22(b)(2), (c). The immunity does not apply if the plaintiff establishes by clear and convincing evidence that the manufacturer was negligent, or was guilty of fraud, intentional and wrongful withholding of information, or other unlawful activity. See §§300aa–22(b)(2), 300aa–23(d)(2).

[26] §300aa–23(d)(2).

[27] §300aa–22(b)(1).

seizures.[28] She suffered over 100 seizures during the next month, and her doctors eventually diagnosed her with "residual seizure disorder" and "developmental delay."[29] Hannah, now a teenager, is still diagnosed with both conditions.

In April 1995, Hannah's parents, Russell and Robalee Bruesewitz, filed a vaccine injury petition in the United States Court of Federal Claims, alleging that Hannah suffered from on-Table residual seizure disorder and encephalopathy injuries.[30] A Special Master denied their claims on various grounds, though they were awarded $126,800 in attorney's fees and costs. The Bruesewitzes elected to reject the unfavorable judgment, and in October 2005 filed this lawsuit in Pennsylvania state court. Their complaint alleged (as relevant here) that defective design of Lederle's DTP vaccine caused Hannah's disabilities, and that Lederle was subject to strict liability, and liability for negligent design, under Pennsylvania common law.[31]

Wyeth removed the suit to the United States District Court for the Eastern District of Pennsylvania, which granted Wyeth summary judgment on the strict-liability and negligence design-defect claims, holding that the Pennsylvania law providing those causes of action was preempted by 42 U. S. C. §300aa–22(b)(1).[32] The United States Court of Appeals for the Third Circuit affirmed.[33] We granted certiorari. 559 U. S. ___ (2010).

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[28] See *Bruesewitz* v. *Secretary of Health and Human Servs.*, No. 95–0266V, 2002 WL 31965744, *3 (Ct. Cl., Dec. 20, 2002).

[29] 561 F. 3d 233, 236 (CA3 2009).

[30] See *id.,* at *1.

[31] See 561 F. 3d at 237. The complaint also made claims based upon failure to warn and defective manufacture. These are no longer at issue.

[32] See *id.,* at 237–238.

[33] *Id.,* at 235.

II

A

We set forth again the statutory text at issue:

> "No vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after October 1, 1988, if the injury or death resulted from side effects that were unavoidable even though the vaccine was properly prepared and was accompanied by proper directions and warnings."[34]

The "even though" clause clarifies the word that precedes it. It delineates the preventative measures that a vaccine manufacturer *must* have taken for a side-effect to be considered "unavoidable" under the statute. Provided that there was proper manufacture and warning, any remaining side effects, including those resulting from design defects, are deemed to have been unavoidable. State-law design-defect claims are therefore preempted.

If a manufacturer could be held liable for failure to use a different design, the word "unavoidable" would do no work. A side effect of a vaccine could *always* have been avoidable by use of a differently designed vaccine not containing the harmful element. The language of the provision thus suggests that the *design* of the vaccine is a given, not subject to question in the tort action. What the statute establishes as a complete defense must be unavoidability (given safe manufacture and warning) *with respect to the particular design*. Which plainly implies that the design itself is not open to question.[35]

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[34] 42 U. S. C. §300aa–22(b)(1).

[35] The dissent advocates for another possibility: "[A] side effect is 'unavoidable' . . . where there is no feasible alternative design that would eliminate the side effect of the vaccine without compromising its cost and utility." *Post*, at 15 (opinion of SOTOMAYOR, J.). The dissent makes no effort to ground that position in the text of §300aa–22(b)(1).

A further textual indication leads to the same conclusion. Products-liability law establishes a classic and well known triumvirate of grounds for liability: defective manufacture, inadequate directions or warnings, and defective design.[36] If all three were intended to be preserved, it would be strange to mention specifically only two, and leave the third to implication. It would have been much easier (and much more natural) to provide that manufacturers would be liable for "defective manufacture, defective directions or warning, and defective design." It seems that the statute fails to mention design-defect liability "by deliberate choice, not inadvertence." *Barnhart* v. *Peabody Coal Co.*, 537 U. S. 149, 168 (2003). *Expressio unius, exclusio alterius.*

B

The dissent's principal textual argument is mistaken. We agree with its premise that "'side effects that were unavoidable' must refer to side effects caused by a vaccine's *design*."[37] We do not comprehend, however, the second step of its reasoning, which is that the use of the conditional term "if" in the introductory phrase "if the injury or death resulted from side effects that were unavoidable" "plainly implies that some side effects stemming from a vaccine's design are 'unavoidable,' while

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We doubt that Congress would introduce such an amorphous test by implication when it otherwise micromanages vaccine manufacturers. See *infra*, at 13–14. We have no idea how much more expensive an alternative design can be before it "compromis[es]" a vaccine's cost or how much efficacy an alternative design can sacrifice to improve safety. Neither does the dissent. And neither will the judges who must rule on motions to dismiss, motions for summary judgment, and motions for judgment as a matter of law. Which means that the test would probably have no real-world effect.

[36] W. Keeton, D. Dobbs, R. Keeton, & D. Owen, Prosser and Keeton on Law of Torts 695 (5th ed. 1984); Restatement (Third) of Torts §2 (1999).

[37] *Post*, at 3.

others are avoidable."[38] That is not so. The "if" clause makes total sense whether the design to which "unavoidable" refers is (as the dissent believes) any feasible design (making the side effects of the design used for the vaccine at issue avoidable), or (as we believe) the particular design used for the vaccine at issue (making its side effects unavoidable). Under the latter view, the condition established by the "if" clause is that the vaccine have been properly labeled and manufactured; and under the former, that it have been properly *designed*, labeled, and manufactured. Neither view renders the "if" clause a nullity. Which of the two variants must be preferred is addressed by our textual analysis, and is in no way determined by the "if" clause.

Petitioners' and the dissent's textual argument also rests upon the proposition that the word "unavoidable" in §300aa–22(b)(1) is a term of art that incorporates comment *k* to Restatement (Second) of Torts §402A (1963–1964).[39] The Restatement generally holds a manufacturer strictly liable for harm to person or property caused by "any product in a defective condition unreasonably dangerous to the user."[40] Comment *k* exempts from this strict-liability rule "unavoidably unsafe products." An unavoidably unsafe product is defined by a hodge-podge of criteria and a few examples, such as the Pasteur rabies vaccine and experimental pharmaceuticals. Despite this lack of clarity, petitioners seize upon one phrase in the comment *k* analysis, and assert that by 1986 a majority of courts had made this a *sine qua non* requirement for an "unavoidably unsafe product": a case-specific showing that the product was "quite incapable of being made safer for

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[38] *Ibid.*

[39] See Brief for Petitioners 29.

[40] Restatement §402A, p. 347.

[its] intended . . . use."[41]

We have no need to consider the finer points of comment *k*. Whatever consistent judicial gloss that comment may have been given in 1986, there is no reason to believe that §300aa–22(b)(1) was invoking it. The comment creates a special category of "unavoidably unsafe products," while the statute refers to "side effects that were unavoidable." That the latter uses the adjective "unavoidable" and the former the adverb "unavoidably" does not establish that Congress had comment *k* in mind. "Unavoidable" is hardly a rarely used word. Even the cases petitioners cite as putting a definitive gloss on comment *k* use the precise phrase "unavoidably unsafe product";[42] none attaches special significance to the term "unavoidable" standing alone.

The textual problems with petitioners' interpretation do

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[41] *Id.,* Comment *k*, p. 353; Petitioners cite, *inter alia*, *Kearl* v. *Lederle Labs.*, 172 Cal. App. 3d 812, 828–830, 218 Cal. Rptr. 453, 463–464 (1985); *Belle Bonfils Memorial Blood Bank* v. *Hansen*, 665 P. 2d 118, 122 (Colo. 1983).

Though it is not pertinent to our analysis, we point out that a large number of courts disagreed with that reading of comment *k*, and took it to say that manufacturers did not face strict liability for side effects of properly manufactured prescription drugs that were accompanied by adequate warnings. See, *e.g., Brown* v. *Superior Court*, 227 Cal. Rptr. 768, 772–775 (Cal. App. 1986), (officially depublished), aff'd 44 Cal. 3d 1049, 751 P. 2d 470 (1988); *McKee* v. *Moore*, 648 P. 2d 21, 23 (Okla. 1982); *Stone* v. *Smith, Kline & French Labs.*, 447 So. 2d 1301, 1303–1304 (Ala. 1984); *Lindsay* v. *Ortho Pharm. Corp.*, 637 F. 2d 87, 90–91 (CA2 1980) (applying N. Y. law); *Wolfgruber* v. *Upjohn Co.*, 72 App. Div. 2d 59, 61, 423 N. Y. S. 2d 95, 96 (1979); *Chambers* v. *G. D. Searle & Co.*, 441 F. Supp. 377, 380–381 (D Md. 1975); *Basko* v. *Sterling Drug, Inc.*, 416 F. 2d 417, 425 (CA2 1969) (applying Conn. law).

[42] See, *e.g., Johnson* v. *American Cyanamid Co.*, 239 Kan. 279, 285, 718 P. 2d 1318, 1323 (1986); *Feldman* v. *Lederle Labs.*, 97 N. J. 429, 440, 446–447, 479 A. 2d 374, 380, 383–384 (1984); *Belle Bonfils Memorial Blood Bank supra*, at 121–123; *Cassisi* v. *Maytag Co.*, 396 So. 2d 1140, 1144, n. 4, 1146 (Fla. App. 1981); *Racer* v. *Utterman*, 629 S. W. 2d 387, 393 (Mo. App. 1981).

not end there. The phrase "even though" in the clause "even though the vaccine was properly prepared and [labeled]" is meant to signal the unexpected: unavoidable side effects persist *despite* best manufacturing and labeling practices.[43] But petitioners' reading eliminates any opposition between the "even though" clause—called a concessive subordinate clause by grammarians—and the word "unavoidable."[44] Their reading makes preemption turn equally on unavoidability, proper preparation, and proper labeling. Thus, the dissent twice refers to the requirements of proper preparation and proper labeling as "two additional prerequisites" for preemption independent of unavoidability.[45] The primary textual justification for the dissent's position depends on that independence.[46] But linking independent ideas is the job of a coordinating junction like "and," not a subordinating junction like "even though."[47]

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[43] The dissent's assertion that we treat "even though" as a synonym for "because" misses the subtle distinction between "because" and "despite." See *post,* at 17, n. 14. "Even though" is a close cousin of the latter. See Webster's New International Dictionary 709, 2631 (2d ed. 1957). The statement "the car accident was unavoidable despite his quick reflexes" indicates that quick reflexes could not avoid the accident, and leaves open two unstated possibilities: (1) that other, unstated means of avoiding the accident besides quick reflexes existed, but came up short as well; or (2) that quick reflexes were the only possible way to avoid the accident. Our interpretation of §300aa–22(b)(1) explains why we think Congress meant the latter in this context. (Incidentally, the statement "the car accident was unavoidable because of his quick reflexes" makes no sense.)

[44] See W. Follett, Modern American Usage: A Guide 61 (1966).

[45] *Post*, at 9, 17.

[46] *Post*, at 3–5.

[47] The dissent responds that these "additional prerequisites" act "in a concessive, subordinating fashion," *post,* at 17, n. 14 (internal quotation marks and brackets omitted). But that is no more true of the dissent's conjunctive interpretation of the present text than it is of *all* provisions that set forth additional requirements—meaning that we could eliminate "even though" from our English lexicon, its function being entirely

Petitioners and the dissent contend that the interpretation we propose would render part of §300aa–22(b)(1) superfluous: Congress could have more tersely and more clearly preempted design-defect claims by barring liability "if . . . the vaccine was properly prepared and was accompanied by proper directions and warnings." The intervening passage ("the injury or death resulted from side effects that were unavoidable even though") is unnecessary. True enough. But the rule against giving a portion of text an interpretation which renders it superfluous does not prescribe that a passage which could have been more terse does not mean what it says. The rule applies only if verbosity and prolixity can be eliminated by giving the offending passage, or the remainder of the text, a competing interpretation. That is not the case here.[48] To be sure, petitioners' and the dissent's interpretation gives independent meaning to the intervening passage (the supposed meaning of comment *k*); but it does so only at the expense of rendering the remainder of the provision superfluous. Since a vaccine is not "quite incapable of being made safer for [its] intended use" if manufacturing defects could have been eliminated or better warnings provided, the entire "even though" clause is a useless appendage.[49] It would suffice to say "if the injury or death resulted from side effects that were unavoidable"—full stop.

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performed by "and." No, we think "even though" has a distinctive concessive, subordinating role to play.

[48] Because the dissent has a superfluity problem of its own, its reliance on *Bates* v. *Dow Agrosciences LLC*, 544 U. S. 431 (2005), is misplaced. See *id.,* at 449 (adopting an interpretation that was "the only one that makes sense of each phrase" in the relevant statute).

[49] That is true regardless of whether §300aa–22(b)(1) incorporates comment *k*. See Restatement §402A, Comment *k*, pp. 353, 354 (noting that "unavoidably unsafe products" are exempt from strict liability "with the qualification that they are properly prepared and marketed, and proper warning is given").

III

The structure of the NCVIA and of vaccine regulation in general reinforces what the text of §300aa–22(b)(1) suggests. A vaccine's license spells out the manufacturing method that must be followed and the directions and warnings that must accompany the product.[50] Manufacturers ordinarily must obtain the Food and Drug Administration's (FDA) approval before modifying either.[51] Deviations from the license thus provide objective evidence of manufacturing defects or inadequate warnings. Further objective evidence comes from the FDA's regulations—more than 90 of them[52]—that pervasively regulate the manufacturing process, down to the requirements for plumbing and ventilation systems at each manufacturing facility.[53] Material noncompliance with any one of them, or with any other FDA regulation, could cost the manufacturer its regulatory-compliance defense.[54]

Design defects, in contrast, do not merit a single mention in the NCVIA or the FDA's regulations. Indeed, the FDA has never even spelled out in regulations the criteria it uses to decide whether a vaccine is safe and effective for its intended use.[55] And the decision is surely not an easy one. Drug manufacturers often could trade a little less efficacy for a little more safety, but the safest design is not always the best one. Striking the right balance between safety and efficacy is especially difficult with respect to vaccines, which affect public as well as individual health. Yet the Act, which in every other respect micromanages manufacturers, is silent on how to evaluate competing designs. Are manufacturers liable only for failing to em-

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[50] See 42 U. S. C. §262(a), (j); 21 CFR §§601.2(a), 314.105(b) (2010).
[51] See §601.12.
[52] See §§211.1 *et seq.*, 600.10–600.15, 600.21–600.22, 820.1 *et seq.*
[53] See §§211.46, 211.48.
[54] See 42 U. S. C. §300aa–22(b)(2).
[55] Hutt, Merrill, & Grossman, Food and Drug Law, at 685, 891.

ploy an alternative design that the FDA has approved for distribution (an approval it takes years to obtain[56])? Or does it suffice that a vaccine design has been approved in other countries? Or could there be liability for failure to use a design that exists only in a lab? Neither the Act nor the FDA regulations provide an answer, leaving the universe of alternative designs to be limited only by an expert's imagination.

Jurors, of course, often decide similar questions with little guidance, and we do not suggest that the absence of guidance alone suggests preemption. But the lack of guidance for design defects combined with the extensive guidance for the two grounds of liability specifically mentioned in the Act strongly suggests that design defects were not mentioned because they are not a basis for liability.

The mandates contained in the Act lead to the same conclusion. Design-defect torts, broadly speaking, have two beneficial effects: (1) prompting the development of improved designs, and (2) providing compensation for inflicted injuries. The NCVIA provides other means for achieving both effects. We have already discussed the Act's generous compensation scheme. And the Act provides many means of improving vaccine design. It directs the Secretary of Health and Human Services to promote "the development of childhood vaccines that result in fewer and less serious adverse reactions."[57] It establishes a National Vaccine Program, whose Director is "to achieve optimal prevention of human infectious diseases . . . and to achieve optimal prevention against adverse reactions."[58] The Program is to set priorities for federal vaccine research, and to coordinate federal vaccine safety and effi-

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[56] See Sing & William, Supplying Vaccines, at 66–67.

[57] 42 U. S. C. §300aa–27(a)(1).

[58] §300aa–1.

cacy testing.[59] The Act requires vaccine manufacturers and health-care providers to report adverse side effects,[60] and provides for monitoring of vaccine safety through a collaboration with eight managed-care organizations.[61] And of course whenever the FDA concludes that a vaccine is unsafe, it may revoke the license.[62]

These provisions for federal agency improvement of vaccine design, and for federally prescribed compensation, once again suggest that §300aa–22(b)(1)'s silence regarding design-defect liability was not inadvertent. It instead reflects a sensible choice to leave complex epidemiological judgments about vaccine design to the FDA and the National Vaccine Program rather than juries.[63]

And finally, the Act's structural *quid pro quo* leads to the same conclusion: The vaccine manufacturers fund from their sales an informal, efficient compensation program for vaccine injuries;[64] in exchange they avoid costly tort litigation and the occasional disproportionate jury verdict.[65] But design-defect allegations are the most speculative and difficult type of products liability claim to

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[59] See §§300aa–2(a)(1)–(3), 300aa–3.

[60] See §300aa–25(b).

[61] See NVAC 18–19.

[62] See 21 CFR §601.5(b)(1)(vi) (2010).

[63] The dissent quotes just part of this sentence, to make it appear that we believe complex epidemiological judgments ought to be assigned in that fashion. See *post*, at 26. We do not state our preference, but merely note that it is Congress's expressed preference—and in order to preclude the argument that it is absurd to think Congress enacted such a thing, we assert that the choice is reasonable and express some of the reasons why. Leaving it to the jury may (or may not) be reasonable as well; we express no view.

[64] See 42 U. S. C. §300aa–15(i)(2); Pub. L. 99–660, §323(a), 100 Stat. 3784. The dissent's unsupported speculation that demand in the vaccine market is inelastic, see *post*, at 24, n. 22, sheds no light on whether Congress regarded the tax as a *quid pro quo*, most Members of Congress being neither professional economists nor law-and-economics scholars.

[65] See 42 U. S. C. §§300aa–11(a)(2), 300aa–22.

litigate. Taxing vaccine manufacturers' product to fund the compensation program, while leaving their liability for design defect virtually unaltered, would hardly coax manufacturers back into the market.

The dissent believes the Act's mandates are irrelevant because they do not spur innovation in precisely the same way as state-law tort systems.[66] That is a novel suggestion. Although we previously have expressed doubt that Congress would quietly preempt product-liability claims without providing a federal substitute, see *Medtronic, Inc.* v. *Lohr*, 518 U. S. 470, 486–488 (1996) (plurality opinion), we have never suggested we would be skeptical of preemption unless the congressional substitute operated like the tort system. We decline to adopt that stance today. The dissent's belief that the FDA and the National Vaccine Program cannot alone spur adequate vaccine innovation is probably questionable, but surely beside the point.

IV

Since our interpretation of §300aa–22(b)(1) is the only interpretation supported by the text and structure of the NCVIA, even those of us who believe legislative history is a legitimate tool of statutory interpretation have no need to resort to it. In any case, the dissent's contention that it would contradict our conclusion is mistaken.

The dissent's legislative history relies on the following syllogism: A 1986 House Committee Report states that §300aa–22(b)(1) "sets forth the principle contained in Comment k of Section 402A of the Restatement of Torts (Second);"[67] in 1986 comment *k* was "commonly understood" to require a case-specific showing that "no feasible alternative design" existed; Congress therefore must have intended §300aa–22(b)(1) to require that showing.[68] The

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[66] See *post*, at 21–24.

[67] H. R. Rep. No. 99–908, pt. 1, p. 25 (1986) (hereinafter 1986 Report).

[68] *Post*, at 7–8.

syllogism ignores unhelpful statements in the Report and relies upon a term of art that did not exist in 1986.

Immediately after the language quoted by the dissent, the 1986 Report notes the difficulty a jury would have in faithfully assessing whether a feasible alternative design exists when an innocent "young child, often badly injured or killed" is the plaintiff.[69] Eliminating that concern is why the Report's authors "strongly believ[e] that Comment k is appropriate and necessary as the policy for civil actions seeking damages in tort."[70] The dissent's interpretation of §300aa–22(b)(1) and its version of "the principle in Comment K" adopted by the 1986 Report leave that concern unaddressed.

The dissent buries another unfavorable piece of legislative history. Because the Report believes that §300aa–22(b)(1) should incorporate "the principle in Comment K" and because the Act provides a generous no-fault compensation scheme, the Report counsels injured parties who cannot prove a manufacturing or labeling defect to "pursue recompense in the compensation system, not the tort system."[71] That counsel echoes our interpretation of §300aa–22(b)(1).

Not to worry, the dissent retorts, a Committee Report by a later Congress "authoritative[ly]" vindicates its interpretation.[72] Post-enactment legislative history (a contradiction in terms) is not a legitimate tool of statutory interpretation. See *Jones* v. *United States*, 526 U. S. 227, 238

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[69] 1986 Report, at 26; see *ibid.* ("[E]ven if the defendant manufacturer may have made as safe a vaccine as anyone reasonably could expect, a court or jury undoubtedly will find it difficult to rule in favor of the 'innocent' manufacturer if the equally 'innocent' child has to bear the risk of loss with no other possibility of recompense").

[70] *Ibid.*

[71] *Ibid.*

[72] *Post*, at 12. This is a courageous adverb since we have previously held that the only authoritative source of statutory meaning is the text that has passed through the Article I process. See *Exxon Mobil Corp.* v. *Allapattah Services, Inc.*, 545 U. S. 546, 568 (2005).

(1999); *United States* v. *Mine Workers*, 330 U. S. 258, 281–282 (1947). Real (pre-enactment) legislative history is persuasive to some because it is thought to shed light on what legislators understood an ambiguous statutory text to mean when they voted to enact it into law. See *Exxon Mobil Corp.* v. *Allapattah Services, Inc.*, 545 U. S. 546, 568 (2005). But post-enactment legislative history by definition "could have had no effect on the congressional vote," *District of Columbia* v. *Heller*, 554 U. S. 570, 605 (2008).

It does not matter that §300aa–22(b)(1) did not take effect until the later Congress passed the excise tax that funds the compensation scheme,[73] and that the supposedly dispositive Committee Report is attached to that funding legislation.[74] Those who voted on the relevant statutory language were not necessarily the same persons who crafted the statements in the later Committee Report; or if they were did not necessarily have the same views at that earlier time; and no one voting at that earlier time could possibly have been informed by those later statements. Permitting the legislative history of subsequent funding legislation to alter the meaning of a statute would set a dangerous precedent. Many provisions of federal law depend on appropriations or include sunset provisions;[75] they cannot be made the device for unenacted statutory revision.

That brings us to the second flaw in the dissent's syllogism: Comment *k* did not have a "commonly understood meaning"[76] in the mid-1980's. Some courts thought it required a case-specific showing that a product was "unavoidably unsafe"; many others thought it categorically exempted certain types of products from strict liability.[77]

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[73] Pub. L. 99–960, §323(a), 100 Stat. 3784.

[74] H. R. Rep. No. 100–391, pt. 1, p. 701 (1987).

[75] See, *e.g.,* Pub. L. 104–208, §§401, 403(a), 110 Stat. 3009–655 to 3009–656, 3009–659 to 3009–662, as amended, note following 8 U. S. C. §1324a (2006 ed., Supp. III) (E-Verify program expires Sept. 30, 2012).

[76] *Post*, at 8.

[77] See n. 39, *supra; post*, at 7–8, n. 5.

When "all (or nearly all) of the" relevant judicial decisions have given a term or concept a consistent judicial gloss, we presume Congress intended the term or concept to have that meaning when it incorporated it into a later-enacted statute. *Merck & Co.* v. *Reynolds*, 559 U. S. ___, ___ (2010) (SCALIA, J., concurring in part and concurring in judgment) (slip op., at 5). The consistent gloss represents the public understanding of the term. We cannot make the same assumption when widespread disagreement exists among the lower courts. We must make do with giving the term its most plausible meaning using the traditional tools of statutory interpretation. That is what we have done today.

* * *

For the foregoing reasons, we hold that the National Childhood Vaccine Injury Act preempts all design-defect claims against vaccine manufacturers brought by plaintiffs who seek compensation for injury or death caused by vaccine side effects. The judgment of the Court of Appeals is affirmed.

It is so ordered.

JUSTICE KAGAN took no part in the consideration or decision of this case.

SUPREME COURT OF THE UNITED STATES

No. 09–152

RUSSELL BRUESEWITZ, ET AL., PETITIONERS *v.* WYETH LLC, FKA WYETH, INC., FKA WYETH LABORATORIES, ET AL.

ON WRIT OF CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE THIRD CIRCUIT

[February 22, 2011]

JUSTICE BREYER, concurring.

I join the Court's judgment and opinion. In my view, the Court has the better of the purely textual argument. But the textual question considered alone is a close one. Hence, like the dissent, I would look to other sources, including legislative history, statutory purpose, and the views of the federal administrative agency, here supported by expert medical opinion. Unlike the dissent, however, I believe these other sources reinforce the Court's conclusion.

I

House Committee Report 99–908 contains an "authoritative" account of Congress' intent in drafting the pre-emption clause of the National Childhood Vaccine Injury Act of 1986 (NCVIA or Act). See *Garcia* v. *United States*, 469 U. S. 70, 76 (1984) ("[T]he authoritative source for finding the Legislature's intent lies in the Committee Reports on the bill"). That Report says that, "if" vaccine-injured persons

> "cannot demonstrate under applicable law either that a vaccine was improperly prepared or that it was accompanied by improper directions or inadequate warnings [they] should pursue recompense in the

> compensation system, not the tort system." H. R. Rep. No. 99–908, pt. 1, p. 24 (1986) (hereinafter H. R. Rep.).

The Report lists two specific kinds of tort suits that the clause does not pre-empt (suits based on improper manufacturing and improper labeling), while going on to state that compensation for other tort claims, *e.g.*, design-defect claims, lies in "the [NCVIA's no-fault] compensation system, not the tort system." *Ibid.*

The strongest contrary argument rests upon the Report's earlier description of the statute as "set[ting] forth the principle contained in Comment k" (of the Restatement Second of Torts' *strict liability* section, 402A) that "a vaccine manufacturer should not be liable for injuries or deaths resulting from *unavoidable* side effects." *Id.*, at 23 (emphasis added). But the appearance of the word "unavoidable" in this last-mentioned sentence cannot provide petitioners with much help. That is because nothing in the Report suggests that the statute means the word "unavoidable" to summon up an otherwise unmentioned third exception encompassing suits based on design defects. Nor can the Report's reference to comment *k* fill the gap. The Report itself refers, not to comment *k*'s details, but only to its "*principle,*" namely, that vaccine manufacturers should *not* be held liable for unavoidable injuries. It says nothing at all about who—judge, jury, or federal safety agency—should decide whether a safer vaccine could have been designed. Indeed, at the time Congress wrote this Report, different state courts had come to very different conclusions about that matter. See Cupp, Rethinking Conscious Design Liability for Prescription Drugs: The *Restatement (Third)* Standard Versus a Negligence Approach, 63 Geo. Wash. L. Rev. 76, 79 (1994–1995) ("[C]ourts [had] adopted a broad range of conflicting interpretations" of comment *k*). Neither the word "unavoid-

able" nor the phrase "the principle of Comment k" tells us which courts' view Congress intended to adopt. Silence cannot tell us to follow those States where juries decided the design-defect question.

II

The legislative history describes the statute more generally as trying to protect the lives of children, in part by ending "the instability and unpredictability of the childhood vaccine market." H. R. Rep., at 7; see *ante,* at 2–3. As the Committee Report makes clear, routine vaccination is "one of the most spectacularly effective public health initiatives this country has ever undertaken." H. R. Rep., at 4. Before the development of routine whooping cough vaccination, for example, "nearly all children" in the United States caught the disease and more than 4,000 people died annually, most of them infants. U. S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, What Would Happen if We Stopped Vaccinations? http://www.cdc.gov/vaccines/vac-gen/whatifstop.htm (all Internet materials as visited Feb. 17, 2011, and available in Clerk of Court's case file); Preventing Tetanus, Diphtheria, and Pertussis Among Adolescents: Use of Tetanus Toxoid, Reduced Diptheria Toxoid and Acellular Pertussis Vaccines, 55 Morbidity and Mortality Weekly Report, No. RR–3, p. 2 (Mar. 24, 2006) (hereinafter Preventing Tetanus) (statistics for 1934–1943), http://www.cdc.gov/mmwr/PDF/rr/rr5503.pdf; U. S. Dept. of Health and Human Services, Centers for Disease Control and Prevention, Epidemiology and Prevention of Vaccine-Preventable Diseases 200 (11th ed. rev. May 2009). After vaccination became common, the number of annual cases of whooping cough declined from over 200,000 to about 2,300, and the number of deaths from about 4,000 to about 12. Preventing Tetanus 2; Childhood Immunizations, House Committee on Energy and Com-

merce, 99th Cong., 2d Sess., 10 (Comm. Print 1986) (hereinafter Childhood Immunizations).

But these gains are fragile; "[t]he causative agents for these preventable childhood illnesses are ever present in the environment, waiting for the opportunity to attack the unprotected individual." Hearing on S. 827 before the Senate Committee on Labor and Human Resources, 99th Cong., 2d Sess., pt. 2, pp. 20–21 (1985) (hereinafter Hearings) (testimony of the American Academy of Pediatrics); see California Dept. of Public Health, Pertussis Report (Jan. 7, 2011), www.cdph.ca.gov/programs/immunize/Documents/PertussisReport2011–01–07.pdf (In 2010, 8,383 people in California caught whooping cough, and 10 infants died). Even a brief period when vaccination programs are disrupted can lead to children's deaths. Hearings 20–21; see Gangarosa et al., Impact of Anti-Vaccine Movements on Pertussis Control: The Untold Story, 351 Lancet 356–361 (Jan. 31, 1998) (when vaccination programs are disrupted, the number of cases of whooping cough skyrockets, increasing by orders of magnitude).

In considering the NCVIA, Congress found that a sharp increase in tort suits brought against whooping cough and other vaccine manufacturers between 1980 and 1985 had "prompted manufacturers to question their continued participation in the vaccine market." H. R. Rep., at 4; Childhood Immunizations 85–86. Indeed, two whooping cough vaccine manufacturers withdrew from the market, and other vaccine manufacturers, "fac[ing] great difficulty in obtaining [product liability] insurance," told Congress that they were considering "a similar course of action." H. R. Rep., at 4; Childhood Immunizations 68–70. The Committee Report explains that, since there were only one or two manufacturers of many childhood vaccines, "[t]he loss of any of the existing manufacturers of childhood vaccines . . . could create a genuine public health hazard"; it "would present the very real possibility of vaccine short-

ages, and, in turn, increasing numbers of unimmunized children, and, perhaps, a resurgence of preventable diseases." H. R. Rep., at 5. At the same time, Congress sought to provide generous compensation to those whom vaccines injured—as determined by an expert compensation program. *Id.,* at 5, 24.

Given these broad general purposes, to read the preemption clause as preserving design-defect suits seems anomalous. The Department of Health and Human Services (HHS) decides when a vaccine is safe enough to be licensed and which licensed vaccines, with which associated injuries, should be placed on the Vaccine Injury Table. 42 U. S. C. §300aa–14; *ante,* at 3–4; A Comprehensive Review of Federal Vaccine Safety Programs and Public Health Activities 13–15, 32–34 (Dec. 2008), http://www.hhs.gov/nvpo/nvac/documents/vaccine-safety-review.pdf. A special master in the Act's compensation program determines whether someone has suffered an injury listed on the Injury Table and, if not, whether the vaccine nonetheless caused the injury. *Ante,* at 3–4; §300aa–13. To allow a jury in effect to second-guess those determinations is to substitute less expert for more expert judgment, thereby threatening manufacturers with liability (indeed, strict liability) in instances where any conflict between experts and nonexperts is likely to be particularly severe—instances where Congress intended the contrary. That is because potential tort plaintiffs are unlikely to bring suit unless the specialized compensation program has determined that they are not entitled to compensation (say, because it concludes that the vaccine did not cause the injury). Brief for United States as *Amicus Curiae* 28 ("99.8% of successful Compensation Program claimants have accepted their awards, foregoing any tort remedies against vaccine manufacturers"). It is difficult to reconcile these potential conflicts and the resulting tort liabilities with a statute that seeks to diminish

manufacturers' product liability while simultaneously augmenting the role of experts in making compensation decisions.

III

The United States, reflecting the views of HHS, urges the Court to read the Act as I and the majority would do. It notes that the compensation program's listed vaccines have survived rigorous administrative safety review. It says that to read the Act as permitting design-defect lawsuits could lead to a recurrence of "exactly the crisis that precipitated the Act," namely withdrawals of vaccines or vaccine manufacturers from the market, "disserv[ing] the Act's central purposes," and hampering the ability of the agency's "expert regulators, in conjunction with the medical community, [to] control the availability and withdrawal of a given vaccine." Brief for United States as *Amicus Curiae* 30, 31.

The United States is supported in this claim by leading public health organizations, including the American Academy of Pediatrics, the American Academy of Family Physicians, the American College of Preventive Medicine, the American Public Health Association, the American Medical Association, the March of Dimes Foundation, the Pediatric Infectious Diseases Society, and 15 other similar organizations. Brief for American Academy of Pediatrics et al. as *Amici Curiae* (hereinafter AAP Brief). The American Academy of Pediatrics has also supported the retention of vaccine manufacturer tort liability (provided that federal law structured state-law liability conditions in ways that would take proper account of federal agency views about safety). Hearings 14–15. But it nonetheless tells us here, in respect to the specific question before us, that the petitioners' interpretation of the Act would undermine its basic purposes by threatening to "halt the future production and development of childhood vaccines

in this country," *i.e.,* by "threaten[ing] a resurgence of the very problems which . . . caused Congress to intervene" by enacting this statute. AAP Brief 24 (internal quotation marks omitted).

I would give significant weight to the views of HHS. The law charges HHS with responsibility for overseeing vaccine production and safety. It is "likely to have a thorough understanding" of the complicated and technical subject matter of immunization policy, and it is comparatively more "qualified to comprehend the likely impact of state requirements." *Geier* v. *American Honda Motor Co., Inc.*, 529 U. S. 861, 883 (2000) (internal quotation marks omitted); see *Medtronic, Inc.* v. *Lohr*, 518 U. S. 470, 506 (1996) (BREYER, J., concurring in part and concurring in judgment) (the agency is in the best position to determine "whether (or the extent to which) state requirements may interfere with federal objectives"). HHS's position is particularly persuasive here because expert public health organizations support its views and the matter concerns a medical and scientific question of great importance: how best to save the lives of children. See *Skidmore* v. *Swift & Co.*, 323 U. S. 134 (1944).

In sum, congressional reports and history, the statute's basic purpose as revealed by that history, and the views of the expert agency along with those of relevant medical and scientific associations, all support the Court's conclusions. I consequently agree with the Court.

SUPREME COURT OF THE UNITED STATES

No. 09–152

RUSSELL BRUESEWITZ, ET AL., PETITIONERS *v.* WYETH LLC, FKA WYETH, INC., FKA WYETH LABORATORIES, ET AL.

ON WRIT OF CERTIORARI TO THE UNITED STATES COURT OF APPEALS FOR THE THIRD CIRCUIT

[February 22, 2011]

JUSTICE SOTOMAYOR, with whom JUSTICE GINSBURG joins, dissenting.

Vaccine manufacturers have long been subject to a legal duty, rooted in basic principles of products liability law, to improve the designs of their vaccines in light of advances in science and technology. Until today, that duty was enforceable through a traditional state-law tort action for defective design. In holding that §22(b)(1) of the National Childhood Vaccine Injury Act of 1986 (Vaccine Act or Act), 42 U. S. C. §300aa–22(b)(1), pre-empts all design defect claims for injuries stemming from vaccines covered under the Act, the Court imposes its own bare policy preference over the considered judgment of Congress. In doing so, the Court excises 13 words from the statutory text, misconstrues the Act's legislative history, and disturbs the careful balance Congress struck between compensating vaccine-injured children and stabilizing the childhood vaccine market. Its decision leaves a regulatory vacuum in which no one ensures that vaccine manufacturers adequately take account of scientific and technological advancements when designing or distributing their products. Because nothing in the text, structure, or legislative history of the Vaccine Act remotely suggests that Congress intended such a result, I respectfully dissent.

I

A

Section 22 of the Vaccine Act provides "[s]tandards of responsibility" to govern civil actions against vaccine manufacturers. 42 U. S. C. §300aa–22. Section 22(a) sets forth the "[g]eneral rule" that "State law shall apply to a civil action brought for damages for a vaccine-related injury or death." §300aa–22(a). This baseline rule that state law applies is subject to three narrow exceptions, one of which, §22(b)(1), is at issue in this case. Section 22(b)(1) provides:

> "No vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after October 1, 1988, if the injury or death resulted from side effects that were unavoidable even though the vaccine was properly prepared and was accompanied by proper directions and warnings." §300aa–22(b)(1).

The provision contains two key clauses: "if the injury or death resulted from side effects that were unavoidable" (the "if" clause), and "even though the vaccine was properly prepared and was accompanied by proper directions and warnings" (the "even though" clause).

Blackletter products liability law generally recognizes three different types of product defects: design defects, manufacturing defects, and labeling defects (*e.g.*, failure to warn).[1] The reference in the "even though" clause to a "properly prepared" vaccine "accompanied by proper directions and warnings" is an obvious reference to two such defects—manufacturing and labeling defects. The plain terms of the "even though" clause thus indicate that

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[1] W. Keeton, D. Dobbs, R. Keeton, & D. Owen, Prosser and Keeton on Law of Torts 695 (5th ed. 1984).

§22(b)(1) applies only where neither kind of defect is present. Because §22(b)(1) is invoked by vaccine manufacturers as a defense to tort liability, it follows that the "even though" clause requires a vaccine manufacturer in each civil action to demonstrate that its vaccine is free from manufacturing and labeling defects to fall within the liability exemption of §22(b)(1).[2]

Given that the "even though" clause requires the absence of manufacturing and labeling defects, the "if" clause's reference to "side effects that were unavoidable" must refer to side effects caused by something other than manufacturing and labeling defects. The only remaining kind of product defect recognized under traditional products liability law is a design defect. Thus, "side effects that were unavoidable" must refer to side effects caused by a vaccine's *design* that were "unavoidable." Because §22(b)(1) uses the conditional term "if," moreover, the text plainly implies that some side effects stemming from a vaccine's design are "unavoidable," while others are avoidable. See Webster's Third New International Dictionary 1124 (2002) ("if" means "in the event that," "so long as," or "on condition that"). Accordingly, because the "if" clause (like the "even though" clause) sets forth a condition to invoke §22(b)(1)'s defense to tort liability, Congress must also have intended a vaccine manufacturer to demonstrate in each civil action that the particular side effects of a vaccine's design were "unavoidable."

Congress' use of conditional "if" clauses in two other provisions of the Vaccine Act supports the conclusion that §22(b)(1) requires an inquiry in each case in which a manufacturer seeks to invoke the provision's exception to

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[2] See *Silkwood* v. *Kerr-McGee Corp.*, 464 U. S. 238, 255 (1984); *Brown* v. *Earthboard Sports USA, Inc.*, 481 F. 3d 901, 912 (CA6 2007) ("'[F]ederal preemption is an affirmative defense upon which the defendants bear the burden of proof'" (quoting *Fifth Third Bank* v. *CSX Corp.*, 415 F. 3d 741, 745 (CA7 2005))).

state tort liability. In §22(b)(2), Congress created a presumption that, for purposes of §22(b)(1), "a vaccine shall be presumed to be accompanied by proper directions and warnings if the vaccine manufacturer shows that it complied in all material respects with" federal labeling requirements. 42 U. S. C. §300aa–22(b)(2). Similarly, in §23(d)(2), Congress created an exemption from punitive damages "[i]f . . . the manufacturer shows that it complied, in all material respects," with applicable federal laws, unless it engages in "fraud," "intentional and wrongful withholding of information" from federal regulators, or "other criminal or illegal activity." §300aa–23(d)(2). It would be highly anomalous for Congress to use a conditional "if" clause in §§22(b)(2) and 23(d)(2) to require a specific inquiry in each case while using the same conditional "if" clause in §22(b)(1) to denote a categorical exemption from liability. Cf. *Erlenbaugh* v. *United States*, 409 U. S. 239, 243 (1972) ("[A] legislative body generally uses a particular word with a consistent meaning in a given context").

Indeed, when Congress intends to pre-empt design defect claims categorically, it does so using categorical (*e.g.*, "all") and/or declarative language (*e.g.*, "shall"), rather than a conditional term ("if"). For example, in a related context, Congress has authorized the Secretary of Health and Human Services to designate a vaccine designed to prevent a pandemic or epidemic as a "covered countermeasure." 42 U. S. C. §§247d–6d(b), (i)(1), (i)(7)(A)(i). With respect to such "covered countermeasure[s]," Congress provided that subject to certain exceptions, "a covered person *shall* be immune from suit and liability under Federal and State law with respect to *all* claims for loss caused by, arising out of, relating to, or resulting from the administration to or the use by an individual of a covered countermeasure," §247d–6d(a)(1) (emphasis added), including specifically claims relating to

"the design" of the countermeasure, §247d–6d(a)(2)(B).

The plain text and structure of the Vaccine Act thus compel the conclusion that §22(b)(1) pre-empts some—but not all—design defect claims. Contrary to the majority's and respondent's categorical reading, petitioners correctly contend that, where a plaintiff has proved that she has suffered an injury resulting from a side effect caused by a vaccine's design, a vaccine manufacturer may invoke §22(b)(1)'s liability exemption only if it demonstrates that the side effect stemming from the particular vaccine's design is "unavoidable," and that the vaccine is otherwise free from manufacturing and labeling defects.[3]

B

The legislative history confirms petitioners' interpretation of §22(b)(1) and sheds further light on its pre-emptive scope. The House Energy and Commerce Committee Report accompanying the Vaccine Act, H. R. Rep. No. 99–908, pt. 1 (1986) (hereinafter 1986 Report), explains in relevant part:

> "*Subsection (b)—Unavoidable Adverse Side Effects; Direct Warnings*.—This provision sets forth the principle contained in Comment K of Section 402A of the Restatement of Torts (Second) that a vaccine manufacturer should not be liable for injuries or deaths resulting from unavoidable side effects even though the vaccine was properly prepared and accompanied by proper directions and warnings.
>
> "The Committee has set forth Comment K in this bill because it intends that the principle in Comment K regarding 'unavoidably unsafe' products, i.e., those products which in the present state of human skill and knowledge cannot be made safe, apply to the vac-

[3] This leaves the question of what precisely §22(b)(1) means by "unavoidable" side effects, which I address in the next section.

> cines covered in the bill and that such products not be the subject of liability in the tort system." *Id.*, at 25–26.

The Report expressly adopts comment *k* of §402A of the Restatement of Torts (Second) (1963–1964) (hereinafter Restatement), which provides that "unavoidably unsafe" products—*i.e.*, those that "in the present state of human knowledge, are quite incapable of being made safe for their intended and ordinary use"—are not defective.[4] As "[a]n outstanding example" of an "[u]navoidably unsafe" product, comment *k* cites "the vaccine for the Pasteur treatment of rabies, which not uncommonly leads to very serious and damaging consequences when it is injected";

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[4] Comment *k* provides as follows:

"*Unavoidably unsafe products*. There are some products which, in the present state of human knowledge, are quite incapable of being made safe for their intended and ordinary use. These are especially common in the field of drugs. An outstanding example is the vaccine for the Pasteur treatment of rabies, which not uncommonly leads to very serious and damaging consequences when it is injected. Since the disease itself invariably leads to a dreadful death, both the marketing and the use of the vaccine are fully justified, notwithstanding the unavoidable high degree of risk which they involve. Such a product, properly prepared, and accompanied by proper directions and warning, is not defective, nor is it *unreasonably* dangerous. The same is true of many other drugs, vaccines, and the like, many of which for this very reason cannot legally be sold except to physicians, or under the prescription of a physician. It is also true in particular of many new or experimental drugs as to which, because of lack of time and opportunity for sufficient medical experience, there can be no assurance of safety, or perhaps even of purity of ingredients, but such experience as there is justifies the marketing and use of the drug notwithstanding a medically recognizable risk. The seller of such products, again with the qualification that they are properly prepared and marketed, and proper warning is given, where the situation calls for it, is not to be held to strict liability for unfortunate consequences attending their use, merely because he has undertaken to supply the public with an apparently useful and desirable product, attended with a known but apparently reasonable risk." Restatement 353–354.

"[s]ince the disease itself invariably leads to a dreadful death, both the marketing and the use of the vaccine are fully justified, notwithstanding the unavoidable high degree of risk which they involve." *Id.*, at 353. Comment *k* thus provides that "seller[s]" of "[u]navoidably unsafe" products are "not to be held to strict liability" provided that such products "are properly prepared and marketed, and proper warning is given." *Ibid.*

As the 1986 Report explains, Congress intended that the "principle in Comment K regarding 'unavoidably unsafe' products" apply to the vaccines covered in the bill. 1986 Report 26. That intent, in turn, is manifested in the plain text of §22(b)(1)—in particular, Congress' use of the word "unavoidable," as well as the phrases "properly prepared" and "accompanied by proper directions and warnings," which were taken nearly verbatim from comment *k*. 42 U. S. C. §300aa–22(b)(1); see Restatement 353–354 ("Such a[n unavoidably unsafe] product, properly prepared, and accompanied by proper directions and warning, is not defective"). By the time of the Vaccine Act's enactment in 1986, numerous state and federal courts had interpreted comment *k* to mean that a product is "unavoidably unsafe" when, given proper manufacture and labeling, no feasible alternative design would reduce the safety risks without compromising the product's cost and utility.[5] Given Con-

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[5] See, *e.g.*, *Smith ex rel. Smith* v. *Wyeth Labs., Inc.*, No. Civ. A 84–2002, 1986 WL 720792, *5 (SD W. Va., Aug. 21, 1986) ("[A] prescription drug is not 'unavoidably unsafe' when its dangers can be eliminated through design changes that do not unduly affect its cost or utility"); *Kearl* v. *Lederle Labs.*, 172 Cal. App. 3d 812, 830, 218 Cal. Rptr. 453, 464 (1985) ("unavoidability" turns on "(i) whether the product was designed to minimize—to the extent scientifically knowable at the time it was distributed—the risk inherent in the product, and (ii) the availability . . . of any alternative product that would have *as effectively* accomplished the *full intended purpose* of the subject product"), disapproved in part by *Brown* v. *Superior Ct.*, 44 Cal. 3d 1049, 751 P. 2d 470 (1988); *Belle Bonfils Memorial Blood Bank* v. *Hansen*, 665 P. 2d 118,

gress' expressed intent to codify the "principle in Comment K," 1986 Report 26, the term "unavoidable" in §22(b)(1) is best understood as a term of art, which incorporates the commonly understood meaning of "unavoidably unsafe" products under comment *k* at the time of the Act's enactment in 1986. See *McDermott Int'l, Inc.* v. *Wilander*, 498 U. S. 337, 342 (1991) ("[W]e assume that when a statute uses . . . a term [of art], Congress intended it to have its established meaning"); *Morissette* v. *United States*, 342 U. S. 246, 263 (1952) (same).[6] Similarly, courts applying

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122 (Colo. 1983) ("[A]pplicability of comment k . . . depends upon the coexistence of several factors," including that "the product's benefits must not be achievable in another manner; and the risk must be unavoidable under the present state of knowledge"); see also 1 L. Frumer & M. Friedman, Products Liability §§8.07[1]–[2], pp. 8–277 to 8–278 (2010) (comment *k* applies "only to defects in design," and there "must be no feasible alternative design which on balance accomplishes the subject product's purpose with a lesser risk" (internal quotation marks omitted)). To be sure, a number of courts at the time of the Vaccine Act's enactment had interpreted comment *k* to preclude design defect claims categorically for certain kinds of products, see *Hill* v. *Searle Labs.*, 884 F. 2d 1064, 1068 (CA8 1989) (collecting cases), but as indicated by the sources cited above, the courts that had construed comment *k* to apply on a case-specific basis generally agreed on the basic elements of what constituted an "unavoidably unsafe" product. See also n. 8, *infra*. The majority's suggestion that "judges who must rule on motions to dismiss, motions for summary judgment, and motions for judgment as a matter of law" are incapable of adjudicating claims alleging "unavoidable" side effects, *ante*, at 7–8, n. 35, is thus belied by the experience of the many courts that had adjudicated such claims for years by the time of the Vaccine Act's enactment.

[6]The majority refuses to recognize that "unavoidable" is a term of art derived from comment *k*, suggesting that "'[u]navoidable' is hardly a rarely used word." *Ante*, at 10. In fact, however, "unavoidable" is an extremely rare word in the relevant context. It appears exactly *once* (*i.e.*, in §300aa–22(b)(1)) in the entirety of Title 42 of the U. S. Code ("Public Health and Welfare"), which governs, *inter alia*, Social Security, see 42 U. S. C. §301 *et seq.*, Medicare, see §1395 *et seq.*, and several other of the Federal Government's largest entitlement programs. The singular rarity in which Congress used the term supports the conclu-

comment *k* had long required manufacturers invoking the defense to demonstrate that their products were not only "unavoidably unsafe" but also properly manufactured and labeled.[7] By requiring "prope[r] prepar[ation]" and "proper directions and warnings" in §22(b)(1), Congress plainly intended to incorporate these additional comment *k* requirements.

The 1986 Report thus confirms petitioners' interpretation of §22(b)(1). The Report makes clear that "side effects that were unavoidable" in §22(b)(1) refers to side effects stemming from a vaccine's design that were "unavoidable." By explaining what Congress meant by the term "unavoidable," moreover, the Report also confirms that whether a side effect is "unavoidable" for purposes of §22(b)(1) involves a specific inquiry in each case as to whether the vaccine "in the present state of human skill and knowledge cannot be made safe," 1986 Report 26—*i.e.*, whether a feasible alternative design existed that would have eliminated the adverse side effects of the vaccine without compromising its cost and utility. See Brief for Kenneth W. Starr et al. as *Amici Curiae* 14–15 ("If a particular plaintiff could show that her injury at issue was avoidable . . . through the use of a feasible alternative design for a specific vaccine, then she would satisfy the plain language of the statute, because she would have demonstrated that the side effects were *not* unavoidable"). Finally, the Report confirms that the "even though" clause is properly read to establish two additional prerequisites—proper manufacturing and proper labeling—to qualify for

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sion that "unavoidable" is a term of art.

[7] See, *e.g.*, *Brochu* v. *Ortho Pharmaceutical Corp.*, 642 F. 2d 652, 657 (CA1 1981); *Needham* v. *White Labs., Inc.*, 639 F. 2d 394, 402 (CA7 1981); *Reyes* v. *Wyeth Labs.*, 498 F. 2d 1264, 1274–1275 (CA5 1974); *Davis* v. *Wyeth Labs.*, 399 F. 2d 121, 127–129 (CA9 1968); *Feldman* v. *Lederle Labs.*, 97 N. J. 429, 448, 479 A. 2d 374, 384 (1984); see also *Toner* v. *Lederle Labs.*, 112 Idaho 328, 336, 732 P. 2d 297, 305 (1987).

§22(b)(1)'s liability exemption.[8]

In addition to the 1986 Report, one other piece of the Act's legislative history provides further confirmation of the petitioners' textual reading of §22(b)(1). When Congress enacted the Vaccine Act in 1986, it did not initially include a source of payment for the no-fault compensation program the Act established. The Act thus "made the compensation program and accompanying tort reforms contingent on the enactment of a tax to provide funding

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[8] Respondent suggests an alternative reading of the 1986 Report. According to respondent, "the principle in Comment K" is simply that of nonliability for "unavoidably unsafe" products, and thus Congress' stated intent in the 1986 Report to apply the "principle in Comment K" to "the vaccines covered in the bill" means that Congress viewed the covered vaccines as a class to be "'unavoidably unsafe.'" 1986 Report 25–26; Brief for Respondent 42. The concurrence makes a similar argument. *Ante*, at 1–2 (opinion of BREYER, J.). This interpretation finds some support in the 1986 Report, which states that "if [injured individuals] cannot demonstrate under applicable law either that a vaccine was improperly prepared or that it was accompanied by improper directions or inadequate warnings [they] should pursue recompense in the compensation system, not the tort system." 1986 Report 26. It also finds some support in the pre-Vaccine Act case law, which reflected considerable disagreement in the courts over "whether comment k applies to pharmaceutical products across the board or only on a case-by-case basis." Ausness, Unavoidably Unsafe Products and Strict Products Liability: What Liability Rule Should be Applied to the Sellers of Pharmaceutical Products? 78 Ky. L. J. 705, 708, and n. 11 (1989–1990) (collecting cases). This interpretation, however, is undermined by the fact that Congress has never directed the Food and Drug Administration (FDA) or any other federal agency to review vaccines for optimal vaccine design, see *infra*, at 20–22, and n. 19, and thus it seems highly unlikely that Congress intended to eliminate the traditional mechanism for such review (*i.e.*, design defect liability), particularly given its express retention of state tort law in the Vaccine Act, see 42 U. S. C. §300aa–22(a). In any event, to the extent there is ambiguity as to how precisely Congress intended the "principle in Comment K" to apply to the covered vaccines, that ambiguity is explicitly resolved in petitioners' favor by the 1987 House Energy and Commerce Committee Report, H. R. Rep. No. 100–391, pt. 1, pp. 690–691 (hereinafter 1987 Report). See *infra* this page and 11–12.

for the compensation." 1987 Report 690. In 1987, Congress passed legislation to fund the compensation program. The House Energy and Commerce Committee Report[9] accompanying that legislation specifically stated that "the codification of Comment (k) of The Restatement (Second) of Torts was not intended to decide as a matter of law the circumstances in which a vaccine should be deemed unavoidably unsafe." *Id.*, at 691. The Committee noted that "[a]n amendment to establish . . . that a manufacturer's failure to develop [a] safer vaccine was not grounds for liability was rejected by the Committee during its original consideration of the Act." *Ibid.* In light of that rejection, the Committee emphasized that "there should be no misunderstanding that the Act undertook to decide as a matter of law whether vaccines were unavoidably unsafe or not," and that "[t]his question is left to the courts to determine in accordance with applicable law." *Ibid.*

To be sure, postenactment legislative history created by a subsequent Congress is ordinarily a hazardous basis from which to infer the intent of the enacting Congress. See *Sullivan* v. *Finkelstein*, 496 U. S. 617, 631–632 (1990) (SCALIA, J., concurring in part). But unlike ordinary postenactment legislative history, which is justifiably given little or no weight, the 1987 Report reflects the intent of the Congress that enacted the funding legislation necessary to give operative effect to the principal provisions of the Vaccine Act, including §22(b)(1).[10] Congress in

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[9] The Third Circuit's opinion below expressed uncertainty as to whether the 1987 Report was authored by the House Budget Committee or the House Energy and Commerce Committee. See 561 F. 3d 233, 250 (2009). As petitioners explain, although the Budget Committee compiled and issued the Report, the Energy and Commerce Committee wrote and approved the relevant language. Title IV of the Report, entitled "Committee on Energy and Commerce," comprises "two Committee Prints approved by the Committee on Energy and Commerce for inclusion in the forthcoming reconciliation bill." 1987 Report 377, 380.

[10] The majority suggests that the 1987 legislation creating the fund-

1987 had a number of options before it, including adopting an entirely different compensation scheme, as the Reagan administration was proposing;[11] establishing different limitations on tort liability, including eliminating design defect liability, as pharmaceutical industry leaders were advocating;[12] or not funding the compensation program at all, which would have effectively nullified the relevant portions of the Act. Because the tort reforms in the 1986 Act, including §22(b)(1), had no operative legal effect unless and until Congress provided funding for the compensation program, the views of the Congress that enacted that funding legislation are a proper and, indeed, authoritative guide to the meaning of §22(b)(1). Those views, as reflected in the 1987 Report, provide unequivocal confir-

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ing mechanism is akin to appropriations legislation and that giving weight to the legislative history of such legislation "would set a dangerous precedent." *Ante*, at 18. The difference, of course, is that appropriations legislation ordinarily funds congressional enactments that already have operative legal effect; in contrast, operation of the tort reforms in the 1986 Act, including §22(b)(1), was expressly conditioned on the enactment of a separate tax to fund the compensation program. See §323(a), 100 Stat. 3784. Accordingly, this Court's general reluctance to view appropriations legislation as modifying substantive legislation, see, *e.g.*, *TVA* v. *Hill*, 437 U. S. 153, 190 (1978), has no bearing here.

[11] See 1987 Report 700 (describing the administration's alternative proposal).

[12] See, *e.g.*, Hearings on Funding of the Childhood Vaccine Program before the Subcommittee on Select Revenue Measures of the House Committee on Ways and Means, 100th Cong., 1st Sess., 85 (1987) ("[T]he liability provisions of the 1986 Act should be amended to assure that manufacturers will not be found liable in the tort system if they have fully complied with applicable government regulations. In particular, manufacturers should not face liability under a 'design defect' theory in cases where plaintiffs challenge the decisions of public health authorities and federal regulators that the licensed vaccines are the best available way to protect children from deadly diseases" (statement of Robert B. Johnson, President, Lederle Laboratories Division, American Cyanamid Co.)).

mation of petitioners' reading of §22(b)(1).

In sum, the text, structure, and legislative history of the Vaccine Act are fully consistent with petitioners' reading of §22(b)(1). Accordingly, I believe §22(b)(1) exempts vaccine manufacturers from tort liability only upon a showing by the manufacturer in each case that the vaccine was properly manufactured and labeled, and that the side effects stemming from the vaccine's design could not have been prevented by a feasible alternative design that would have eliminated the adverse side effects without compromising the vaccine's cost and utility.

II

In contrast to the interpretation of §22(b)(1) set forth above, the majority's interpretation does considerable violence to the statutory text, misconstrues the legislative history, and draws the wrong conclusions from the structure of the Vaccine Act and the broader federal scheme regulating vaccines.

A

As a textual matter, the majority's interpretation of §22(b)(1) is fundamentally flawed in three central respects. First, the majority's categorical reading rests on a faulty and untenable premise. Second, its reading functionally excises 13 words from the statutory text, including the key term "unavoidable." And third, the majority entirely ignores the Vaccine Act's default rule preserving state tort law.

To begin, the majority states that "[a] side effect of a vaccine could *always* have been avoidable by use of a differently designed vaccine not containing the harmful element." *Ante*, at 7. From that premise, the majority concludes that the statute must mean that "the *design* of the vaccine is a given, not subject to question in the tort action," because construing the statute otherwise would

render §22(b)(1) a nullity. *Ibid.* A tort claimant, according to the majority, will always be able to point to a differently designed vaccine not containing the "harmful element," and if that were sufficient to show that a vaccine's side effects were not "unavoidable," the statute would preempt nothing.

The starting premise of the majority's interpretation, however, is fatally flawed. Although in the most literal sense, as the majority notes, a side effect can always be avoided "by use of a differently designed vaccine not containing the harmful element," *ibid.*, this interpretation of "unavoidable" would effectively read the term out of the statute, and Congress could not have intended that result. Indeed, §22(b)(1) specifically uses the conditional phrase "if the injury or death resulted from side effects that were unavoidable," which plainly indicates that Congress contemplated that there would be some instances in which a vaccine's side effects are "unavoidable" and other instances in which they are not. See *supra*, at 3. The majority's premise that a vaccine's side effects can always be "avoid[ed] by use of a differently designed vaccine not containing the harmful element," *ante*, at 7, entirely ignores the fact that removing the "harmful element" will often result in a less effective (or entirely ineffective) vaccine. A vaccine, by its nature, ordinarily employs a killed or weakened form of a bacteria or virus to stimulate antibody production;[13] removing that bacteria or virus might remove the "harmful element," but it would also necessarily render the vaccine inert. As explained above, the legislative history of the Vaccine Act and the cases interpreting comment *k* make clear that a side effect is

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[13] See American Academy of Pediatrics, Questions and Answers about Vaccine Ingredients (Oct. 2008), http://www.aap.org/immunization/families/faq/Vaccineingredients.pdf (all Internet materials as visited Feb. 18, 2011, and available in Clerk of Court's case file).

"unavoidable" for purposes of §22(b)(1) only where there is no feasible alternative design that would eliminate the side effect of the vaccine without compromising its cost and utility. See *supra*, at 7. The majority's premise—that side effects stemming from a vaccine's design are always avoidable—is thus belied by the statutory text and legislative history of §22(b)(1). And because its starting premise is invalid, its conclusion—that the design of a vaccine is not subject to challenge in a tort action—is also necessarily invalid.

The majority's reading suffers from an even more fundamental defect. If Congress intended to exempt vaccine manufacturers categorically from all design defect liability, it more logically would have provided: "No vaccine manufacturer shall be liable in a civil action for damages arising from a vaccine-related injury or death associated with the administration of a vaccine after October 1, 1988, if the vaccine was properly prepared and was accompanied by proper directions and warnings." There would have been no need for Congress to include the additional 13 words "the injury or death resulted from side effects that were unavoidable even though." See *TRW Inc.* v. *Andrews*, 534 U. S. 19, 31 (2001) (noting "cardinal principle of statutory construction that a statute ought, upon the whole, to be so construed that, if it can be prevented, no clause, sentence, or word shall be superfluous, void, or insignificant" (internal quotation marks omitted)).

In *Bates* v. *Dow Agrosciences LLC*, 544 U. S. 431 (2005), this Court considered an analogous situation where an express pre-emption provision stated that certain States "'shall not impose or continue in effect any requirements for labeling or packaging in addition to or different from those required under this subchapter.'" *Id.*, at 436 (quoting 7 U. S. C. §136v(b) (2000 ed.)). The *Bates* Court stated:

> "Conspicuously absent from the submissions by [respondent] and the United States is any plausible alternative interpretation of 'in addition to or different from' that would give that phrase meaning. Instead, they appear to favor reading those words out of the statute, which would leave the following: 'Such State shall not impose or continue in effect any requirements for labeling or packaging.' This amputated version of [the statute] would no doubt have clearly and succinctly commanded the pre-emption of *all* state requirements concerning labeling. That Congress added the remainder of the provision is evidence of its intent to draw a distinction between state labeling requirements that are pre-empted and those that are not." 544 U. S., at 448–449.

As with the statutory interpretation rejected by this Court in *Bates*, the majority's interpretation of §22(b)(1) functionally excises 13 words out of the statute, including the key term "unavoidable." See *Duncan* v. *Walker*, 533 U. S. 167, 174 (2001) ("We are especially unwilling" to treat a statutory term as surplusage "when the term occupies so pivotal a place in the statutory scheme"). Although the resulting "amputated version" of the statutory provision "would no doubt have clearly and succinctly commanded the pre-emption of *all* state" design defect claims, the fact "[t]hat Congress added the remainder of the provision" is strong evidence of its intent not to pre-empt design defect claims categorically. *Bates*, 544 U. S., at 449; see also *American Home Prods. Corp.* v. *Ferrari*, 284 Ga. 384, 393, 668 S. E. 2d 236, 242 (2008) ("'If Congress had intended to deprive injured parties of a long available form of compensation, it surely would have expressed that intent more clearly'" (quoting *Bates*, 544 U. S., at 449)), cert. pending, No. 08–1120.

Strikingly, the majority concedes that its interpretation

renders 13 words of the statute entirely superfluous. See *ante*, at 12 ("The intervening passage ('the injury or death resulted from side effects that were unavoidable even though') is unnecessary. True enough"). Nevertheless, the majority contends that "the rule against giving a portion of text an interpretation which renders it superfluous . . . applies only if verbosity and prolixity can be eliminated by giving the offending passage, or the remainder of the text, a competing interpretation." *Ibid.* According to the majority, petitioners' reading of §22(b)(1) renders the "even though" clause superfluous because, to reach petitioners' desired outcome, "[i]t would suffice to say 'if the injury or death resulted from side effects that were unavoidable'—full stop." *Ibid.* As explained above, however, the "even though" clause establishes two additional prerequisites—proper manufacturing and proper labeling—to qualify for §22(b)(1)'s exemption from liability. Contrary to the majority's contention, then, the "even though" clause serves an important function by limiting the scope of the preemption afforded by the preceding "if" clause.[14]

The majority's only other textual argument is based on

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[14] In this manner, the "even though" clause functions in a "concessive subordinat[ing]" fashion, *ante*, at 11, in accord with normal grammatical usage. According to the majority, however, the "even though" clause "clarifies the word that precedes it" by "delineat[ing]" the conditions that make a side effect "unavoidable" under the statute. *Ante*, at 7. The majority's interpretation hardly treats the clause as "concessive," and indeed strains the meaning of "even though." In the majority's view, proper manufacturing and labeling are the sole prerequisites that render a vaccine's side effects unavoidable. Thus, an injurious side effect is unavoidable *because* the vaccine was properly prepared and labeled, not "even though" it was. The two conjunctions are not equivalent: The sentence "I am happy *even though* it is raining" can hardly be read to mean that "I am happy *because* it is raining." In any event, the more fundamental point is that petitioners' interpretation actually gives meaning to the words "even though," whereas the majority concedes that its interpretation effectively reads those words entirely out of the statute. See *supra* this page.

the *expressio unius*, *exclusio alterius* canon. According to the majority, because blackletter products liability law generally recognizes three different types of product defects, "[i]f all three were intended to be preserved, it would be strange [for Congress] to mention specifically only two"—namely, manufacturing and labeling defects in the "even though" clause—"and leave the third to implication." *Ante*, at 8. The majority's argument, however, ignores that the default rule under the Vaccine Act is that state law is preserved. As explained above, §22(a) expressly provides that the "[g]eneral rule" is that "State law shall apply to a civil action brought for damages for a vaccine-related injury or death." 42 U. S. C. §300aa–22(a). Because §22(a) already preserves state-law design defect claims (to the extent the exemption in §22(b)(1) does not apply), there was no need for Congress separately and expressly to preserve design defect claims in §22(b)(1). Indeed, Congress' principal aim in enacting §22(b)(1) was not to preserve manufacturing and labeling claims (those, too, were already preserved by §22(a)), but rather, to federalize comment *k*-type protection for "unavoidably unsafe" vaccines. The "even though" clause simply functions to limit the applicability of that defense. The lack of express language in §22(b)(1) specifically preserving design defect claims thus cannot fairly be understood as impliedly (and categorically) pre-empting such traditional state tort claims, which had already been preserved by §22(a).[15]

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[15]This Court, moreover, has long operated on "the assumption that the historic police powers of the States are not to be superseded by the Federal Act unless that was the clear and manifest purpose of Congress." *Altria Group, Inc.* v. *Good*, 555 U. S. ___, ___ (2008) (slip op., at 5) (internal quotation marks and alteration omitted). Given the long history of state regulation of vaccines, see Brief for Petitioners 3–6, the presumption provides an additional reason not to read §22(b)(1) as pre-empting all design defect claims, especially given Congress' inclusion of

The majority also suggests that if Congress wished to preserve design defect claims, it could have simply provided that manufacturers would be liable for "defective manufacture, defective directions or warning, and defective design." *Ante*, at 8 (internal quotation marks omitted). Putting aside the fact that §22(a) already preserves design defect claims (to the extent §22(b)(1) does not apply), the majority's proposed solution would not have fully effectuated Congress' intent. As the legislative history makes clear, Congress used the term "unavoidable" to effectuate its intent that the "principle in Comment K regarding 'unavoidably unsafe' products . . . apply to the vaccines covered in the bill." 1986 Report 26; see also 1987 Report 691. At the time of the Vaccine Act's enactment in 1986, at least one State had expressly rejected comment *k*,[16] while many others had not addressed the applicability of comment *k* specifically to vaccines or applied comment *k* to civil actions proceeding on a theory other than strict liability (*e.g.*, negligence[17]). A statute

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an express saving clause in the same statutory section, see 42 U. S. C. §300aa–22(a), and its use of the conditional "if" clause in defining the pre-emptive scope of the provision. See *Bates* v. *Dow Agrosciences LLC*, 544 U. S. 431, 449 (2005) ("In areas of traditional state regulation, we assume that a federal statute has not supplanted state law unless Congress has made such an intention clear and manifest" (internal quotation marks omitted)).

[16] See *Collins* v. *Eli Lilly Co.*, 116 Wis. 2d 166, 197, 342 N. W. 2d 37, 52 (1984) ("We conclude that the rule embodied in comment k is too restrictive and, therefore, not commensurate with strict products liability law in Wisconsin"). *Collins* did, however, "recognize that in some exigent circumstances it may be necessary to place a drug on the market before adequate testing can be done." *Ibid.* It thus adopted a narrower defense (based on "exigent circumstances") than that recognized in other jurisdictions that had expressly adopted comment *k*.

[17] See, *e.g.*, *Kearl*, 172 Cal. App. 3d, at 831, n. 15, 218 Cal. Rptr., at 465, n. 15 ("[T]he unavoidably dangerous product doctrine merely exempts the product from a strict liability design defect analysis; a plaintiff remains free to pursue his design defect theory on the basis of

that simply stated that vaccine manufacturers would be liable for "defective design" would be silent as to the availability of a comment *k*-type defense for "unavoidably unsafe" vaccines, and thus would not have fully achieved Congress' aim of extending greater liability protection to vaccine manufacturers by providing comment *k*-type protection in all civil actions as a matter of federal law.

B

The majority's structural arguments fare no better than its textual ones. The principal thrust of the majority's position is that, since nothing in the Vaccine Act or the FDA's regulations governing vaccines expressly mentions design defects, Congress must have intended to remove issues concerning the design of FDA-licensed vaccines from the tort system. *Ante*, at 13. The flaw in that reasoning, of course, is that the FDA's silence on design defects existed long before the Vaccine Act was enacted. Indeed, the majority itself concedes that the "FDA has never even spelled out in regulations the criteria it uses to decide whether a vaccine is safe and effective for its intended use."[18] *Ibid.* And yet it is undisputed that prior to the Act, vaccine manufacturers had long been subject to liability under state tort law for defective vaccine design. That the Vaccine Act did not itself set forth a comprehensive regulatory scheme with respect to design defects is thus best understood to mean not that Congress suddenly decided to change course *sub silentio* and pre-empt a

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negligence"); *Toner*, 112 Idaho, at 340, 732 P. 2d, at 309–310 ("The authorities universally agree that where a product is deemed unavoidably unsafe, the plaintiff is deprived of the advantage of a strict liability cause of action, but may proceed under a negligence cause of action").

[18] See 42 U. S. C. §262(a)(2)(C)(i)(I) ("The Secretary shall approve a biologics license application . . . on the basis of a demonstration that . . . the biological product that is the subject of the application is safe, pure, and potent").

longstanding, traditional category of state tort law, but rather, that Congress intended to leave the status quo alone (except, of course, with respect to those aspects of state tort law that the Act expressly altered). See 1987 Report 691 ("It is not the Committee's intention to preclude court actions under applicable law. The Committee's intent at the time of considering the Act . . . was . . . to leave otherwise applicable law unaffected, except as expressly altered by the Act").

The majority also suggests that Congress necessarily intended to pre-empt design defect claims since the aim of such tort suits is to promote the development of improved designs and provide compensation for injured individuals, and the Vaccine Act "provides other means for achieving both effects"—most notably through the no-fault compensation program and the National Vaccine Program. *Ante*, at 14, and nn. 57–60 (citing 42 U. S. C. §§300aa–1, 300aa–2(a)(1)–(3), 300aa–3, 300aa–25(b), 300aa–27(a)(1)). But the majority's position elides a significant difference between state tort law and the federal regulatory scheme. Although the Vaccine Act charges the Secretary of Health and Human Services with the obligation to "promote the development of childhood vaccines" and "make or assure improvements in . . . vaccines, and research on vaccines," §300aa–27(a), neither the Act nor any other provision of federal law places a legal *duty* on vaccine manufacturers to improve the design of their vaccines to account for scientific and technological advances. Indeed, the FDA does not condition approval of a vaccine on it being the most optimally designed among reasonably available alternatives, nor does it (or any other federal entity) ensure that licensed vaccines keep pace with technological and scientific advances.[19] Rather, the function of ensuring

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[19] See, *e.g.*, *Hurley* v. *Lederle Labs.*, 863 F. 2d 1173, 1177 (CA5 1988) ("[T]he FDA is a passive agency: it considers whether to approve

that vaccines are optimally designed in light of existing science and technology has traditionally been left to the States through the imposition of damages for design defects. Cf. *Bates*, 544 U. S., at 451 ("'[T]he specter of damage actions may provide manufacturers with added dynamic incentives to continue to keep abreast of all possible injuries stemming from use of their product[s] so as to forestall such actions through product improvement'"); *Wyeth* v. *Levine*, 555 U. S. ___, ___ (2009) (slip op., at 22–

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vaccine designs only if and when manufacturers come forward with a proposal"); *Jones* v. *Lederle Labs.*, 695 F. Supp. 700, 711 (EDNY 1988) ("[T]he agency takes the drugs and manufacturers as it finds them. While its goal is to oversee inoculation with the best possible vaccine, it is limited to reviewing only those drugs submitted by various manufacturers, regardless of their flaws"). Although the FDA has authority under existing regulations to revoke a manufacturer's biologics licenses, that authority can be exercised only where (as relevant here) "[t]he licensed product is not safe and effective for all of its intended uses." 21 CFR §601.5(b)(1)(vi) (2010); see §600.3(p) (defining "safety" as "relative freedom from harmful effect to persons affected, directly or indirectly, by a product when prudently administered, taking into consideration the character of the product in relation to the condition of the recipient at the time"). The regulation does not authorize the FDA to revoke a biologics license for a manufacturer's failure to adopt an optimal vaccine design in light of existing science and technology. See Conk, Is There a Design Defect in the Restatement (Third) of Torts: Products Liability? 109 Yale L. J. 1087, 1128–1129 (1999–2000) ("The FDA does not claim to review products for optimal design . . . . FDA review thus asks less of drug . . . manufacturers than the common law of products liability asks of other kinds of manufacturers"). At oral argument, counsel for *amicus* United States stated that the Centers for Disease Control and Prevention (CDC) routinely performs comparative analyses of vaccines that are already on the market. See Tr. of Oral Arg. 44–45; *id.*, at 52–53 (describing CDC's comparison of Sabin and Salk polio vaccines). Neither the United States nor any of the parties, however, has represented that CDC examines whether a safer alternative vaccine *could have been designed* given practical and scientific limits, the central inquiry in a state tort law action for design defect. CDC does not issue biologics licenses, moreover, and thus has no authority to require a manufacturer to adopt a different vaccine design.

23) (noting that the FDA has "traditionally regarded state law as a complementary form of drug regulation" as "[s]tate tort suits uncover unknown drug hazards and provide incentives for drug manufacturers to disclose safety risks promptly").[20] The importance of the States' traditional regulatory role is only underscored by the unique features of the vaccine market, in which there are "only one or two manufacturers for a majority of the vaccines listed on the routine childhood immunization schedule." Brief for Respondent 55. The normal competitive forces that spur innovation and improvements to existing product lines in other markets thus operate with less force in the vaccine market, particularly for vaccines that have already been released and marketed to the public. Absent a clear statutory mandate to the contrary, there is no reason to think that Congress intended in the vaccine context to eliminate the traditional incentive and deterrence functions served by state tort liability in favor of a federal regulatory scheme providing only carrots and no sticks.[21] See *Levine*, 555 U. S., at ___ (slip op., at 18) ("The

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[20] Indeed, we observed in *Levine* that the FDA is perpetually understaffed and underfunded, see 555 U. S., at ___, n. 11 (slip op., at 22, n. 11), and the agency has been criticized in the past for its slow response in failing to withdraw or warn about potentially dangerous products, see, *e.g.*, L. Leveton, H. Sox, & M. Soto, Institute of Medicine, HIV and the Blood Supply: An Analysis of Crisis Decisionmaking (1995) (criticizing FDA response to transmission of AIDS through blood supply). These practical shortcomings reinforce the conclusion that "state law offers an additional, and important, layer of consumer protection that complements FDA regulation." *Levine*, 555 U. S., at ___ (slip op., at 23).

[21] The majority mischaracterizes my position as expressing a general "skeptic[ism] of preemption unless the congressional substitute operate[s] like the tort system." *Ante*, at 16. Congress could, of course, adopt a regulatory regime that operates differently from state tort systems, and such a difference is not necessarily a reason to question Congress' pre-emptive intent. In the specific context of the Vaccine Act, however, the relevant point is that this Court should not lightly assume

case for federal pre-emption is particularly weak where Congress has indicated its awareness of the operation of state law in a field of federal interest, and has nonetheless decided to stand by both concepts and to tolerate whatever tension there is between them." (internal quotation marks and alteration omitted)).

III

In enacting the Vaccine Act, Congress established a carefully wrought federal scheme that balances the competing interests of vaccine-injured persons and vaccine manufacturers. As the legislative history indicates, the Act addressed "two overriding concerns": "(a) the inadequacy—from both the perspective of vaccine-injured persons as well as vaccine manufacturers—of the current approach to compensating those who have been damaged by a vaccine; and (b) the instability and unpredictability of the childhood vaccine market." 1986 Report 7. When viewed in the context of the Vaccine Act as a whole, §22(b)(1) is just one part of a broader statutory scheme that balances the need for compensating vaccine-injured children with added liability protections for vaccine manufacturers to ensure a stable childhood vaccine market.

The principal innovation of the Act was the creation of the no-fault compensation program—a scheme funded entirely through an excise tax on vaccines.[22] Through that

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that Congress intended *sub silentio* to displace a longstanding species of state tort liability where, as here, Congress specifically included an express saving clause preserving state law, there is a long history of state-law regulation of vaccine design, and pre-emption of state law would leave an important regulatory function—*i.e.*, ensuring optimal vaccine design—entirely unaddressed by the congressional substitute.

[22]The majority's suggestion that "vaccine manufacturers fund from their sales" the compensation program is misleading. *Ante*, at 15. Although the manufacturers nominally pay the tax, the amount of the tax is specifically included in the vaccine price charged to purchasers. See CDC Vaccine Price List (Feb. 15, 2011), http://www.cdc.gov/

program, Congress relieved vaccine manufacturers of the burden of compensating victims of vaccine-related injuries in the vast majority of cases[23]—an extremely significant economic benefit that "functionally creat[es] a valuable insurance policy for vaccine-related injuries." Reply Brief for Petitioners 10. The structure and legislative history, moreover, point clearly to Congress' intention to divert would-be tort claimants into the compensation program, rather than eliminate a longstanding category of traditional tort claims. See 1986 Report 13 ("The Committee anticipates that the speed of the compensation program, the low transaction costs of the system, the no-fault nature of the required findings, and the relative certainty and generosity of the system's awards will divert a significant number of potential plaintiffs from litigation"). Indeed, although complete pre-emption of tort claims would have eliminated the principal source of the "unpredictability" in the vaccine market, Congress specifically chose *not* to pre-empt state tort claims categorically. See 42 U. S. C. §300aa–22(a) (providing as a "[g]eneral rule" that "State law shall apply to a civil action brought for damages for a vaccine-related injury or death"). That decision reflects Congress' recognition that court actions are essential

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vaccines/programs/vfc/cdc-vac-price-list.htm. Accordingly, the only way the vaccine manufacturers can be said to actually "fund" the compensation program is if the cost of the excise tax has an impact on the number of vaccines sold by the vaccine manufacturer. The majority points to no evidence that the excise tax—which ordinarily amounts to 75 cents per dose, 26 U. S. C. §4131(b)—has any impact whatsoever on the demand for vaccines.

[23] See Brief for United States as *Amicus Curiae* 28 ("Department of Justice records indicate that 99.8% of successful Compensation Program claimants have accepted their awards, foregoing any tort remedies against vaccine manufacturers"); S. Plotkin, W. Orenstein, & P. Offit, Vaccines 1673 (5th ed. 2008) (noting that "[v]irtually all . . . petitioners, even those who were not awarded compensation" under the compensation program, choose to accept the program's determination).

because they provide injured persons with significant procedural tools—including, most importantly, civil discovery—that are not available in administrative proceedings under the compensation program. See §§300aa–12(d)(2)(E), (d)(3). Congress thus clearly believed there was still an important function to be played by state tort law.

Instead of eliminating design defect liability entirely, Congress enacted numerous measures to reduce manufacturers' liability exposure, including a limited regulatory compliance presumption of adequate warnings, see §300aa–22(b)(2), elimination of claims based on failure to provide direct warnings to patients, §300aa–22(c), a heightened standard for punitive damages, §300aa–23(d)(2), and, of course, immunity from damages for "unavoidable" side effects, §300aa–22(b)(1). Considered in light of the Vaccine Act as a whole, §22(b)(1)'s exemption from liability for unavoidably unsafe vaccines is just one part of a broader statutory scheme that reflects Congress' careful balance between providing adequate compensation for vaccine-injured children and conferring substantial benefits on vaccine manufacturers to ensure a stable and predictable childhood vaccine supply.

The majority's decision today disturbs that careful balance based on a bare policy preference that it is better "to leave complex epidemiological judgments about vaccine design to the FDA and the National Vaccine Program rather than juries." *Ante*, at 15.[24] To be sure, reasonable minds can disagree about the wisdom of having juries weigh the relative costs and benefits of a particular vaccine design. But whatever the merits of the majority's

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[24] JUSTICE BREYER's separate concurrence is even more explicitly policy driven, reflecting his own preference for the "more expert judgment" of federal agencies over the "less expert" judgment of juries. *Ante*, at 5.

policy preference, the decision to bar all design defect claims against vaccine manufacturers is one that Congress must make, not this Court.[25] By construing §22(b)(1) to

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[25] Respondent notes that there are some 5,000 petitions alleging a causal link between certain vaccines and autism spectrum disorders that are currently pending in an omnibus proceeding in the Court of Federal Claims (Vaccine Court). Brief for Respondent 56–57. According to respondent, a ruling that §22(b)(1) does not pre-empt design defect claims could unleash a "crushing wave" of tort litigation that would bankrupt vaccine manufacturers and deplete vaccine supply. *Id.*, at 28. This concern underlies many of the policy arguments in respondent's brief and appears to underlie the majority and concurring opinions in this case. In the absence of any empirical data, however, the prospect of an onslaught of autism-related tort litigation by claimants denied relief by the Vaccine Court seems wholly speculative. As an initial matter, the special masters in the autism cases have thus far uniformly rejected the alleged causal link between vaccines and autism. See Brief for American Academy of Pediatrics et al. as *Amici Curiae* 20–21, n. 4 (collecting cases). To be sure, those rulings do not necessarily mean that no such causal link exists, cf. Brief for United States as *Amicus Curiae* 29 (noting that injuries have been added to the Vaccine Injury Table for existing vaccines), or that claimants will not ultimately be able to prove such a link in a state tort action, particularly with the added tool of civil discovery. But these rulings do highlight the substantial hurdles to recovery a claimant faces. See *Schafer* v. *American Cyanamid Co.*, 20 F. 3d 1, 5 (CA1 1994) ("[A] petitioner to whom the Vaccine Court gives nothing may see no point in trying to overcome tort law's yet more serious obstacles to recovery"). Trial courts, moreover, have considerable experience in efficiently handling and disposing of meritless products liability claims, and decades of tort litigation (including for design defect) in the prescription-drug context have not led to shortages in prescription drugs. Despite the doomsday predictions of respondent and the various *amici* cited by the concurrence, *ante*, at 6–7, the possibility of a torrent of meritless lawsuits bankrupting manufacturers and causing vaccine shortages seems remote at best. More fundamentally, whatever the merits of these policy arguments, the issue in this case is what Congress has decided, and as to that question, the text, structure, and legislative history compel the conclusion that Congress intended to leave the courthouse doors open for children who have suffered severe injuries from defectively designed vaccines. The majority's policy-driven decision to the contrary usurps Congress' role and deprives such vaccine-injured children of a key remedy that Congress intended them to have.

pre-empt all design defect claims against vaccine manufacturers for covered vaccines, the majority's decision leaves a regulatory vacuum in which no one—neither the FDA nor any other federal agency, nor state and federal juries—ensures that vaccine manufacturers adequately take account of scientific and technological advancements. This concern is especially acute with respect to vaccines that have already been released and marketed to the public. Manufacturers, given the lack of robust competition in the vaccine market, will often have little or no incentive to improve the designs of vaccines that are already generating significant profit margins. Nothing in the text, structure, or legislative history remotely suggests that Congress intended that result.

I respectfully dissent.

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Bruesewitz v. Wyeth's Impact on the Vaccine Safety Debate

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Childhood vaccines are extolled for effective prevention of dangerous diseases. However, a persistent anti-vaccine movement resists vaccination due to real and perceived links between vaccines and adverse health effects, including autism.[3] Closely related to the vaccine safety debate is the policy concern about balancing the need to compensate individuals who are harmed by vaccines and to prevent vaccine manufacturers from exiting the market due to the prospect of unmanageable tort liability. The recent Supreme Court decision in *Bruesewitz v. Wyeth* strikes a balance in favor of shielding vaccine manufacturers from design-defect liability and thus limits the options for claimants of certain vaccine-related injuries to recover compensation.[4]

The *Bruesewitz* decision held that design-defect claims against vaccine manufacturers are preempted under the National Childhood Vaccine Injury Act (NCVIA).[5] Despite the Court's focus on statutory interpretation, the public health policy implications and vaccine safety debate lurked beneath the surface of the Court's reasoning. Although the Court's decision has largely been lauded as a win for public health, some have criticized the decision as creating a dangerous regulatory vacuum for vaccine improvement and monitoring. This decision has significant ramifications for the vaccine compensation system, including the thousands of pending claims asserting a link between vaccines and autism.

The Current Vaccine Injury Compensation Program

In 1986, Congress enacted NCVIA and established the Vaccine Injury Compensation Program (VICP) in response to a destabilized vaccine market caused by manufacturer withdrawal due to increasing tort liability. The VICP is a no-fault program to compensate individuals who experience adverse reactions to vaccination and to protect vaccine manufacturers from certain types of liability to ensure a sufficient production of vaccine.[6] The VICP allows claimants to petition a vaccine court for an award paid from a fund created by excise taxes on vaccines. The vaccine court will issue an award if the adverse reaction is listed on the Vaccine Injury Table, which lists compensable injuries by vaccine type, without the petitioner needing to prove causation or fault. Alternatively, if the vaccine or injury is not included within the table, the vaccine court will issue an award if the claimant proves the vaccine caused the injury. The claimant may decide whether to accept the vaccine court's judgment or file a state tort claim against the manufacturer, unless the claim is preempted by NCVIA. Preempted claims may only be pursued in vaccine court and include claims relating to manufacturing defects, failures to warn and, after *Bruesewitz*, design-defects.

The Case: *Bruesewitz v. Wyeth*

The *Bruesewitz* case was filed by Russell and Robalee Bruesewitz, who claimed that their daughter, Hannah, experienced seizures and suffered permanent disabilities following the administration of a diphtheria-tetanus-pertussis vaccine when she was six months old.[7] Hannah's parents petitioned the vaccine court on her behalf, but they were denied an award.[8] The Bruesewitzes rejected the vaccine court's ruling, and filed a state claim alleging, among other things, that the vaccine manufactured by Lederle Laboratories (later purchased by Wyeth) had a defective design that caused their daughter's disabilities.[9]

The United States Supreme Court ruled that NCVIA preemptively bars all state-law design-defect claims against vaccine manufacturers.[10] Justice Scalia, writing for the majority, relied on a textual analysis of NCVIA's provision that no vaccine manufacturer is liable for a vaccine-related injury "if the injury or death resulted from side effects that were unavoidable even though the vaccine was properly prepared and was accompanied by proper directions and warnings."[11] The Court noted the policy concern of NCVIA to stabilize the market to entice manufacturers to remain in the vaccine business and avert the vaccine shortages seen in the 1980s due to the threat of tort liability.[12] The majority concluded that

allowing design-defect tort claims, "the most speculative and difficult type of products liability claim to litigate," would "hardly coax manufacturers back into the market."[13]

In her dissent, Justice Sotomayor argued that the text of the statute did contemplate design-defect claims because it provided liability protection only for "unavoidable" side effects.[14] Accordingly, the adverse side effects could have been avoided if the vaccine in question had been designed differently. The dissent expressed concern that the majority's decision creates a significant vacuum—the Food and Drug Administration's approval process does not require vaccines to be optimally designed or continuously improved, and state tort liability for design defects has traditionally provided this incentive.[15] The dissent further pointed to the lack of post-approval regulatory oversight and the lack of competition in the vaccine market as exacerbating the regulatory vacuum.[16]

Whether the majority's decision or the dissent's concerns are correct will be determined as the effect of a bar on state design-defect claims against vaccine manufacturers plays out. Regardless, the decision adds a new component to the vaccine safety debate and could affect the large number of current claims asserting that vaccines have caused autism in children.

The Impact

This case has significant ramifications for the approximately 5,000 pending claims in an omnibus proceeding before the vaccine court alleging that childhood vaccines caused autism. The *Bruesewitz* decision will likely restrict many of the claims to vaccine court and foreclose the possibility of a state tort law alternative for claims asserting that a defective design caused autism.

Claims asserting a link between vaccines and autism have not generally been compensated in vaccine court under NCVIA because autism is not listed on the Vaccine Injury Table and due to the lack of credible medical evidence that vaccines cause autism.[17] A vocal anti-vaccine movement still believes that vaccines, particularly the thimerosal-containing measles-mumps-rubella (MMR) vaccine, cause autism despite the lack of medical evidence,[18] likely due to the co-occurrence of the timing of standard vaccine administration and the emergence of symptoms of autism.

Public health officials voice concern over threats to the health of the population as herd immunity to communicable diseases declines with lower rates of vaccination. Recent measles outbreaks demonstrate the potential public health dangers associated with decisions to not vaccinate. An example is the 2008 measles outbreak in San Diego, spreading primarily among unvaccinated schoolchildren and infants too young to be vaccinated.[19] *Bruesewitz* may strengthen liability protections of vaccine manufacturers necessary to maintain vaccine supply, but it does little to combat the problem of declining immunization rates among the anti-vaccine movement.

Generally, the Court's decision has been hailed by public health commentators because it prevents the specter of a similar vaccine supply crisis that led to the passage of NCVIA. The position adopted by the Court was urged by the Department of Health and Human Services, the American Public Health Association, the American Academy of Pediatrics, and many other professional medical associations.[20] Nevertheless, like the dissent, some commentators have expressed concern that vaccine manufacturers will have few incentives to improve their vaccine designs. Both sides, and the Court, seem to recognize that the compensation scheme created by NCVIA was a significant and necessary public health achievement. In preempting state tort liability for design-defect claims, the Court may have been swayed by the success of the vaccine compensation program and the importance of the public health need for a stable vaccine supply.

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3 Donald G. McNeil Jr., *A Multitude of Vaccine Benefits, Yet Controversy Persists*, N. Y. Times, Mar. 28, 2008, http://www.nytimes.com/ref/health/healthguide/esn-vaccinations-ess.html ; Inst. of Medicine, Immunization Safety Review: Vaccines and Autism (2004).

4 *Bruesewitz v. Wyeth, Inc.* , 131 S. Ct. 1068 (2011).

5 *Id*. at 1082; National Childhood Vaccine Injury Act of 1986, 42 U.S.C. §§ 300aa-1 et. seq.

6 National Vaccine Injury Program, Health Resources and Services Administration available at http://www.hrsa.gov/vaccinecompensation/ (last accessed March 15, 2011).

7 *Id*.

8 *Id*. (The Bruesewitzs were awarded attorneys fees and costs by the vaccine court, but elected to pursue their claim in Pennsylvania state court).

9 *Id*.

10 *Id*. at 1082 (6-2 vote, with Justice Kagan sitting out).

11 42 U.S.C. § 300aa-22(b)(1) (2006).

12 *Bruesewitz*, 131 S. Ct. at 1072-73.

13 *Id*. at 1080.

14 *Id*., 131 S. Ct. at 1087 (Sotomayor, J., dissenting).

15 *Id*. at 1100-01 (Sotomayor, J., dissenting).

16 *Id*. at 1100-01 (Sotomayor, J., dissenting).

17 *See, e.g.* , *Cedillo v. Sec'y of Health and Human Services,* 617 F.3d 1328 (Fed. Cir. 2010); *Hazlehurst v. Sec'y of Health and Human Services*, 604 F.3d 1343 (Fed. Cir. 2010).

18 Editorial, *Autism Fraud*, N.Y. Times, January 12, 2011, at A28 (describing the British Medical Journal's finding that Dr. Andrew Wakefield's influential 1998 study finding a link between the MMR vaccine and autism was deliberately fraudulent. A report seven years after the Wakefield study indicated that the twelve original subjects' medical histories had been falsified in order to make vaccines culpable for injuries.)

19 CDC, 57 MMWR 203 (Feb. 29, 2008), http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5708a3.htm .

20 *Bruesewitz* , 131 S. Ct. at 1085 (Breyer, J., concurring).

Vaccine Injury Compensation Program

(VICP)

HRSA
Health Resources & Services Administration

National Vaccine Injury Compensation Program

Vaccines save lives by preventing disease

In fact, the Centers for Disease Control and Prevention (CDC) named immunizations as one of the ten most important public health achievements of the 20th century.

Most people who get vaccines have no serious problems, but like any medicine, they can cause side effects - most of which are rare and mild. In very rare cases, a vaccine can cause a serious problem, such as a severe allergic reaction.

In those instances, the National Vaccine Injury Compensation Program (VICP) provides individuals with an opportunity to file a petition or claim for financial compensation.

The VICP is a no-fault alternative to the traditional legal system for resolving vaccine injury petitions.

The National Childhood Vaccine Injury Act of 1986 created the VICP, which began on October 1, 1988, after a series of lawsuits threatened to cause vaccine shortages and reduce U.S. vaccination rates.

The following three organizations have a role in the VICP.

- The VICP is administered through the Department of Health and Human Services (HHS).
- The Department of Justice (DOJ) represents HHS in Court.
- The U.S. Court of Federal Claims (the Court) makes the final decision regarding whether a petitioner should be compensated.

Any individual, of any age, who received a covered vaccine and believes he or she was injured as a result, can file a petition. Parents, legal guardians and legal representatives can file on behalf of children, disabled adults and individuals who are deceased.

Please note that, with limited exceptions, all petitions must be filed within 3 years after the first symptom of the alleged vaccine injury, or within 2 years of the death and 4 years after the first symptom of the alleged vaccine injury that resulted in death. For information about additional requirements that must be met in order to pursue compensation, visit the VICP website, www.hrsa.gov/vaccinecompensation.

Did you know?

The risk of experiencing a severe allergic reaction from one of these commonly administered vaccines covered by the VICP – MMR, Hepatitis B, Diphtheria, Tetanus, and Pertussis-- *is 1 or less than 1 out of 1 million doses*, according to the CDC.

The Court makes the final decision regarding whether a petitioner should be compensated and the amount of compensation.

For more information about the VICP

Visit the website:
www.hrsa.gov/vaccinecompensation

1-800-338-2382

National Vaccine Injury Compensation Program

Parklawn Building
5600 Fishers Lane
8N146B
Rockville, Maryland 20857

How the claims process works

1. An individual files a petition with the Court. The Court sends a copy of the petition to DOJ and HHS.
2. An HHS healthcare provider reviews the petition, determines if it meets the medical criteria for compensation and makes a preliminary recommendation to DOJ. The government's position is included in DOJ's report, which is submitted to the Court.
3. The report is presented to a court-appointed special master, who decides whether the petitioner should be compensated.
4. The special master's decision may be appealed.
5. Petitioners who reject the decision of the Court (or those who withdraw their claims after certain timelines are met) may file a claim in civil court against the vaccine manufacturer and/or the health care provider who administered the vaccine.

An individual may contact the Court for more information about filing a petition, including the requirements that must be satisfied to pursue compensation. The petition does not have to be filed by a lawyer but most people use a lawyer. If certain requirements are met, the VICP generally will pay lawyer's fees and other legal costs related to the petition, whether or not the petitioner is paid for a vaccine injury or death. Visit the Court's website for a list of attorneys willing to file VICP petitions.

U.S. Court of Federal Claims
717 Madison Place, N.W.
Washington, DC 20005
202-357-6400
www.uscfc.uscourts.gov

Vaccines covered by the VICP

In order for a category of vaccines to be covered by the VICP, the category of the vaccine must be recommended for routine administration to children by the Centers for Disease Control and Prevention and subject to an excise tax. There are no age restrictions on who may file a petition with the VICP. Petitions may be filed on behalf of infants, children and adolescents, or by adults receiving VICP-covered vaccines. The following vaccines are covered by the VICP:

- Diphtheria and Tetanus vaccines (e.g., DTaP, DTP, DT, Td, or TT)
- Pertussis vaccines (e.g., DTP, DTaP, P, Tdap, DTP-Hib)
- Measles, Mumps, and Rubella vaccines (e.g., MMR, MR, M, R)
- Polio vaccines (e.g., OPV or IPV)
- Hepatitis A vaccines (e.g., HAV)
- Hepatitis B vaccines (e.g.,HBV)
- Haemophilus influenza type b polysaccharide conjugate vaccines (e.g., Hib)
- Varicella vaccines (e.g., VZV) [herpes zoster (shingles) vaccine is not covered]
- Rotavirus vaccines (e.g.,RV)
- Pneumococcal conjugate vaccines (e.g., PCV)
- Seasonal influenza vaccines (e.g., IIV3 standard dose, IIV3 high dose, IIV4, RIV3, LAIV3, LAIV4)
- Human Papillomavirus vaccines (e.g., HPV)
- Meningococcal vaccines (e.g., MCV4, MPSV4, recombinant)

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Data & Statistics

The United States has the safest, most effective vaccine supply in history. In the majority of cases, vaccines cause no side effects, however they can occur, as with any medication—but most are mild. Very rarely, people experience more serious side effects, like allergic reactions.
In those instances, the National Vaccine Injury Compensation Program (VICP) allows individuals to file a petition for compensation.

What does it mean to be awarded compensation?
Being awarded compensation for a petition does not necessarily mean that the vaccine caused the alleged injury. In fact:

- Approximately 60 percent of all compensation awarded by the VICP comes as result of a negotiated settlement between the parties in which HHS has not concluded, based upon review of the evidence, that the alleged vaccine(s) caused the alleged injury.
- Attorneys are eligible for reasonable attorneys' fees, whether or not the petitioner is awarded compensation by the Court, if certain minimal requirements are met. In those circumstances, attorneys are paid by the VICP directly. By statute, attorneys may not charge any other fee, including a contingency fee, for his or her services in representing a petitioner in the VICP.

What reasons might a petition result in a negotiated settlement?

- Consideration of prior U.S. Court of Federal Claims decisions, both parties decide to minimize risk of loss through settlement
- A desire to minimize the time and expense of litigating a case
- The desire to resolve a petition quickly

How many petitions have been awarded compensation?
According to the CDC, from 2006 to 2021 over 4 billion doses of covered vaccines were distributed in the U.S. For petitions filed in this time period, 9,914 petitions were adjudicated by the Court, and of those 7,075 were compensated. This means for every 1 million doses of vaccine that were distributed, approximately 1 individual was compensated.

Since 1988, over 25,446 petitions have been filed with the VICP. Over that 30-year time period, 21,527 petitions have been adjudicated, with 9,304 of those determined to be compensable, while 12,223 were dismissed. Total compensation paid over the life of the program is approximately $4.9 billion.

VICP Adjudication Categories, by Alleged Vaccine for Petitions Filed Since the Inclusion of Influenza as an Eligible Vaccine for Filings 01/01/2006 through 12/31/2021

Name of Vaccine Listed First in a Petition (other vaccines may be alleged or basis for compensation)	Number of Doses Distributed in the U.S., 01/01/2006 through 12/31/2021 (Source: CDC)	Compensable Concession	Compensable Court Decision	Compensable Settlement	Compensable Total	Dismissed/Non-Compensable Total	Grand Total
DT	794,777	1	0	5	6	4	10
DTaP	109,991,074	25	23	121	169	136	305
DTaP-Hep B-IPV	79,798,141	7	8	31	46	67	113
DTaP-HIB	1,135,474	0	1	2	3	2	5
DTaP-IPV	31,439,498	0	0	5	5	5	10
DTap-IPV-HIB	74,403,716	4	4	9	17	41	58
DTaP-IPV-HIB-Hep B	464,070	0	0	0	0	0	0
DTP	0	1	1	3	5	4	9
DTP-HIB	0	1	0	2	3	1	4
Hep A-Hep B	17,946,038	3	1	19	23	8	31
Hep B-HIB	4,787,457	1	1	2	4	1	5
Hepatitis A (Hep A)	203,339,060	9	6	50	65	41	106
Hepatitis B (Hep B)	216,772,259	15	12	82	109	97	206
HIB	137,675,315	2	1	13	16	11	27
HPV	132,062,306	29	13	119	161	335	496
Influenza	1,842,400,000	1,607	234	3,326	5,167	914	6,081
IPV	78,237,532	1	1	5	7	5	12
Measles	135,660	1	0	1	2	0	2

Name of Vaccine Listed First in a Petition (other vaccines may be alleged or basis for compensation)	Number of Doses Distributed in the U.S., 01/01/2006 through 12/31/2021 (Source: CDC)	Compensable Concession	Compensable Court Decision	Compensable Settlement	Compensable Total	Dismissed/Non-Compensable Total	Grand Total
Meningococcal	119,054,485	14	5	46	65	27	92
MMR	116,647,585	24	16	100	140	146	286
MMR-Varicella	32,226,723	12	1	14	27	21	48
Mumps	110,749	0	0	0	0	0	0
Nonqualified	0	0	0	3	3	56	59
OPV	0	1	0	0	1	5	6
Pneumococcal Conjugate	269,907,936	43	3	71	117	76	193
Rotavirus	125,787,826	26	4	24	54	21	75
Rubella	422,548	0	1	1	2	0	2
Td	71,408,785	15	6	69	90	29	119
Tdap	294,534,882	192	21	426	639	143	782
Tetanus	3,836,052	18	2	52	72	22	94
Unspecified	0	1	1	5	7	595	602
Varicella	127,901,171	10	7	33	50	26	76
Grand Total	**4,093,221,119**	**2,063**	**373**	**4,639**	**7,075**	**2,839**	**9,914**

Notes on the Adjudication Categories Table

The date range of 01/01/2006 through 12/31/2021 was selected to reflect petitions filed since the inclusion of influenza vaccine in July 2005. Influenza vaccine now is named in the majority of all VICP petitions.

In addition to the first vaccine alleged by a petitioner, which is the vaccine listed in this table, a VICP petition may allege other vaccines, which may form the basis of compensation.

Vaccine doses are self-reported distribution data provided by US-licensed vaccine manufacturers. The data provide an estimate of the annual national distribution and do not represent vaccine administration. In order to maintain confidentiality of an individual manufacturer or brand, the data are presented in an aggregate format by vaccine type. Flu doses are derived from CDC's FluFinder tracking system, which includes data provided to CDC by US-licensed influenza vaccine manufacturers as well as their first line distributors.

"Unspecified" means insufficient information was submitted to make an initial determination. The conceded "unspecified" petition was for multiple unidentified vaccines that caused abscess formation at the vaccination site(s), and the "unspecified" settlements were for multiple vaccines later identified in the Special Masters' decisions

Definitions

Compensable – The injured person who filed a petition was paid money by the VICP. Compensation can be achieved through a concession by the U.S. Department of Health and Human Services (HHS), a decision on the merits of the petition by a special master or a judge of the U.S. Court of Federal Claims (Court), or a settlement between the parties.

- **Concession**: HHS concludes that a petition should be compensated based on a thorough review and analysis of the evidence, including medical records and the scientific and medical literature. The HHS review concludes that the petitioner is entitled to compensation, including a determination either that it is more likely than not that the vaccine caused the injury or the evidence supports fulfillment of the criteria of the Vaccine Injury Table. The Court also determines that the petition should be compensated.
- **Court Decision**: A special master or the court, within the United States Court of Federal Claims, issues a legal decision after weighing the evidence presented by both sides. HHS abides by the ultimate Court decision even if it maintains its position that the petitioner was not entitled to compensation (e.g., that the injury was not caused by the vaccine).
  For injury petitions, compensable court decisions are based in part on one of the following determinations by the court:
  1. The evidence is legally sufficient to show that the vaccine more likely than not caused (or significantly aggravated) the injury; or
  2. The injury is listed on, and meets all of the requirements of, the Vaccine Injury Table, and HHS has not proven that a factor unrelated to the vaccine more likely than not caused or significantly aggravated the injury. An injury listed on the Table and meeting all Table requirements is given the legal presumption of causation. It should be noted that conditions are placed on the Table for both scientific and policy reasons.
- **Settlement**: The petition is resolved via a negotiated settlement between the parties. This settlement is not an admission by the United States or the Secretary of Health and Human Services that the vaccine caused the petitioner's alleged injuries, and, in settled cases, the Court does not determine that the vaccine caused the injury. A settlement therefore cannot be characterized as a decision by HHS or by the Court that the vaccine caused an injury. Petitions may be resolved by settlement for many reasons, including consideration of prior court decisions; a recognition by both parties that there is a risk of loss in proceeding to a decision by the Court making the certainty of settlement more desirable; a desire by both parties to minimize the time and expense associated with litigating a case to conclusion; and a desire by both parties to resolve a case quickly and efficiently.
- **Non-compensable/Dismissed**: The injured person who filed a petition was ultimately not paid money. Non-compensable Court decisions include the following:
  1. The Court determines that the person who filed the petition did not demonstrate that the injury was caused (or significantly aggravated) by a covered vaccine or meet the requirements of the Table (for injuries listed on the Table).
  2. The petition was dismissed for not meeting other statutory requirements (such as not meeting the filing deadline, not receiving a covered vaccine, and not meeting the statute's severity requirement).
  3. The injured person voluntarily withdrew his or her petition.

Petitions Filed, Compensated and Dismissed, by Alleged Vaccine, Since the Beginning of VICP, 10/01/1988 through 10/01/2022

Vaccines	Filed Injury	Filed Death	Filed Total	Compensated	Dismissed
DTaP-IPV	16	0	16	5	4
DT	69	9	78	26	52
DTP	3,288	696	3,984	1,273	2,711
DTP-HIB	20	8	28	7	21
DTaP	480	88	568	251	273
DTaP-Hep B-IPV	98	39	137	46	67
DTaP-HIB	11	1	12	7	4
DTaP-IPV-HIB	53	21	74	17	41
DTaP-IPV-HIB-HEPB	0	0	0	0	0
Td	236	3	239	136	80
Tdap	1,145	8	1,153	635	137
Tetanus	178	3	181	95	49
Hepatitis A (Hep A)	141	7	148	65	43
Hepatitis B (Hep B)	748	62	810	299	445
Hep A-Hep B	45	0	45	23	9
Hep B-HIB	8	0	8	5	3
HIB	50	3	53	22	21
HPV	657	17	674	161	331
Influenza	8,587	223	8,810	5,138	903
IPV	269	14	283	10	271
OPV	282	28	310	158	152
Measles	145	19	164	56	107
Meningococcal	120	3	123	63	27
MMR	1,044	62	1,106	422	606
MMR-Varicella	61	2	63	26	21
MR	15	0	15	6	9
Mumps	10	0	10	1	9
Pertussis	4	3	7	2	5
Pneumococcal Conjugate	319	24	343	120	89
Rotavirus	114	6	120	74	32
Rubella	190	4	194	71	123
Varicella	116	10	126	70	38
Nonqualified[1]	115	13	128	3	122
Unspecified[2]	5,427	9	5,436	11	5,418
Grand Total	**24,061**	**1,385**	**25,446**	**9,304**	**12,223**

[1] Nonqualified petitions are those filed for vaccines not covered under the VICP.
[2] Unspecified petitions are those submitted with insufficient information to make a determination.

Petitions Filed

Fiscal Year	Total
FY 1988	24
FY 1989	148
FY 1990	1,492
FY 1991	2,718
FY 1992	189
FY 1993	140
FY 1994	107
FY 1995	180
FY 1996	84
FY 1997	104
FY 1998	120
FY 1999	411
FY 2000	164
FY 2001	215
FY 2002	958
FY 2003	2,592
FY 2004	1,214
FY 2005	735
FY 2006	325
FY 2007	410
FY 2008	417
FY 2009	397
FY 2010	447
FY 2011	386
FY 2012	402
FY 2013	504
FY 2014	633
FY 2015	803
FY 2016	1,120
FY 2017	1,243
FY 2018	1,238
FY 2019	1,282
FY 2020	1,192
FY 2021	2,057
FY 2022	995
FY 2023	0
Total	**25,446**

Adjudications

Generally, petitions are not adjudicated in the same fiscal year as filed.
On average, it takes 2 to 3 years to adjudicate a petition after it is filed.

Fiscal Year	Compensable	Dismissed	Total
FY 1989	9	12	21
FY 1990	100	33	133
FY 1991	141	447	588
FY 1992	166	487	653
FY 1993	125	588	713
FY 1994	162	446	608
FY 1995	160	575	735
FY 1996	162	408	570
FY 1997	189	198	387
FY 1998	144	181	325
FY 1999	98	139	237
FY 2000	125	104	229
FY 2001	86	88	174
FY 2002	104	104	208
FY 2003	56	100	156
FY 2004	62	247	309
FY 2005	60	229	289
FY 2006	69	193	262
FY 2007	82	136	218
FY 2008	147	151	298
FY 2009	134	257	391
FY 2010	180	330	510
FY 2011	266	1,742	2,008
FY 2012	265	2,533	2,798
FY 2013	369	651	1,020
FY 2014	370	194	564
FY 2015	521	145	666
FY 2016	700	187	887
FY 2017	696	203	899
FY 2018	545	202	747
FY 2019	641	182	823
FY 2020	711	217	928
FY 2021	755	259	1,014
FY 2022	904	255	1,159
FY 2023	0	0	0
Total	**9,304**	**12,223**	**21,527**

Awards Paid

Fiscal Year	Number of Compensated Awards	Petitioners' Award Amount	Attorneys' Fees/Costs Payments	Number of Payments to Attorneys (Dismissed Cases)	Attorneys' Fees/Costs Payments (Dismissed Cases)	Number of Payments to Interim Attorneys'	Interim Attorneys' Fees/Costs Payments	Total Outlays
FY 1989	6	$1,317,654.78	$54,107.14	0	$0.00	0	$0.00	$1,371,761.92
FY 1990	88	$53,252,510.46	$1,379,005.79	4	$57,699.48	0	$0.00	$54,689,215.73
FY 1991	114	$95,980,493.16	$2,364,758.91	30	$496,809.21	0	$0.00	$98,842,061.28
FY 1992	130	$94,538,071.30	$3,001,927.97	118	$1,212,677.14	0	$0.00	$98,752,676.41
FY 1993	162	$119,693,267.87	$3,262,453.06	272	$2,447,273.05	0	$0.00	$125,402,993.98
FY 1994	158	$98,151,900.08	$3,571,179.67	335	$3,166,527.38	0	$0.00	$104,889,607.13
FY 1995	169	$104,085,265.72	$3,652,770.57	221	$2,276,136.32	0	$0.00	$110,014,172.61
FY 1996	163	$100,425,325.22	$3,096,231.96	216	$2,364,122.71	0	$0.00	$105,885,679.89
FY 1997	179	$113,620,171.68	$3,898,284.77	142	$1,879,418.14	0	$0.00	$119,397,874.59
FY 1998	165	$127,546,009.19	$4,002,278.55	121	$1,936,065.50	0	$0.00	$133,484,353.24
FY 1999	96	$95,917,680.51	$2,799,910.85	117	$2,306,957.40	0	$0.00	$101,024,548.76
FY 2000	136	$125,945,195.64	$4,112,369.02	80	$1,724,451.08	0	$0.00	$131,782,015.74
FY 2001	97	$105,878,632.57	$3,373,865.88	57	$2,066,224.67	0	$0.00	$111,318,723.12
FY 2002	80	$59,799,604.39	$2,653,598.89	50	$656,244.79	0	$0.00	$63,109,448.07
FY 2003	65	$82,816,240.07	$3,147,755.12	69	$1,545,654.87	0	$0.00	$87,509,650.06
FY 2004	57	$61,933,764.20	$3,079,328.55	69	$1,198,615.96	0	$0.00	$66,211,708.71
FY 2005	64	$55,065,797.01	$2,694,664.03	71	$1,790,587.29	0	$0.00	$59,551,048.33
FY 2006	68	$48,746,162.74	$2,441,199.02	54	$1,353,632.61	0	$0.00	$52,540,994.37
FY 2007	82	$91,449,433.89	$4,034,154.37	61	$1,692,020.25	0	$0.00	$97,175,608.51
FY 2008	141	$75,716,552.06	$5,191,770.83	74	$2,531,394.20	2	$117,265.31	$83,556,982.40
FY 2009	131	$74,142,490.58	$5,404,711.98	36	$1,557,139.53	28	$4,241,362.55	$85,345,704.64
FY 2010	173	$179,387,341.30	$5,961,744.40	59	$1,933,550.09	22	$1,978,803.88	$189,261,439.67
FY 2011	251	$216,319,428.47	$9,572,042.87	403	$5,589,417.19	28	$2,001,770.91	$233,482,659.44
FY 2012	249	$163,491,998.82	$9,241,427.33	1,020	$8,649,676.56	37	$5,420,257.99	$186,803,360.70
FY 2013	375	$254,666,326.70	$13,543,099.70	704	$7,012,615.42	50	$1,423,851.74	$276,645,893.56
FY 2014	365	$202,084,196.12	$12,161,422.64	508	$6,824,566.68	38	$2,493,460.73	$223,563,646.17
FY 2015	508	$204,137,880.22	$14,464,063.71	118	$3,546,785.14	50	$3,089,497.68	$225,238,226.75

Fiscal Year	Number of Compensated Awards	Petitioners' Award Amount	Attorneys' Fees/Costs Payments	Number of Payments to Attorneys (Dismissed Cases)	Attorneys' Fees/Costs Payments (Dismissed Cases)	Number of Payments to Interim Attorneys'	Interim Attorneys' Fees/Costs Payments	Total Outlays
FY 2016	689	$230,140,251.20	$16,298,140.59	99	$2,741,830.10	58	$3,398,557.26	$252,578,779.15
FY 2017	706	$252,245,932.78	$22,045,785.00	131	$4,439,538.57	52	$3,363,464.24	$282,094,720.59
FY 2018	521	$199,588,007.04	$16,689,908.68	113	$5,151,255.64	57	$4,999,766.30	$226,428,937.66
FY 2019	653	$196,217,707.64	$18,991,247.55	102	$4,792,528.63	65	$5,457,545.23	$225,459,029.05
FY 2020	733	$186,860,677.55	$20,165,188.43	113	$5,747,755.82	76	$5,090,482.24	$217,864,104.04
FY 2021	719	$208,258,401.31	$24,884,274.59	140	$6,942,253.81	53	$4,249,055.37	$244,333,985.08
FY 2022	927	$195,825,045.57	$22,992,062.07	102	$4,868,964.74	56	$6,329,886.09	$230,015,958.47
FY 2023	0	$0.00	$0.00	0	$0.00	0	$0.00	$0.00
Total	9,220	$4,475,245,417.84	$274,226,734.49	5,809	$102,500,389.97	672	$53,655,027.52	$4,905,627,569.82

NOTE: Some previous fiscal year data has been updated as a result of the receipt and entry of data from documents issued by the Court and system updates which included petitioners' costs reimbursements in outlay totals,

"Compensated" are petitions that have been paid as a result of a settlement between parties or a decision made by the U.S. Court of Federal Claims (Court). The # of awards is the number of petitioner awards paid, including the attorneys' fees/costs payments, if made during a fiscal year. However, petitioners' awards and attorneys' fees/costs are not necessarily paid in the same fiscal year as when the petitions/petitions are determined compensable. "Dismissed" includes the # of payments to attorneys and the total amount of payments for attorneys' fees/costs per fiscal year. The VICP will pay attorneys' fees/costs related to the petition, whether or not the petition/petition is awarded compensation by the Court, if certain minimal requirements are met. "Total Outlays" are the total amount of funds expended for compensation and attorneys' fees/costs from the Vaccine Injury Compensation Trust Fund by fiscal year.

Since influenza vaccines (vaccines administered to large numbers of adults each year) were added to the VICP in 2005, many adult petitions related to that vaccine have been filed, thus changing the proportion of children to adults receiving compensation.

HRSA
Health Resources & Services Administration

Data & Statistics

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In those instances, the National Vaccine Injury Compensation Program (VICP) allows individuals to file a petition for compensation.

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- Attorneys are eligible for reasonable attorneys' fees, whether or not the petitioner is awarded compensation by the Court, if certain minimal requirements are met. In those circumstances, attorneys are paid by the VICP directly. By statute, attorneys may not charge any other fee, including a contingency fee, for his or her services in representing a petitioner in the VICP.

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- Consideration of prior U.S. Court of Federal Claims decisions, both parties decide to minimize risk of loss through settlement
- A desire to minimize the time and expense of litigating a case
- The desire to resolve a petition quickly

How many petitions have been awarded compensation?
According to the CDC, from 2006 to 2021 over 6 billion doses of covered vaccines were distributed in the U.S. For petitions filed in this time period, 10,429 petitions were adjudicated by the Court, and of those 7,483 were compensated. This means for every 1 million doses of vaccine that were distributed, approximately 1 individual was compensated.

Since 1988, over 26,046 petitions have been filed with the VICP. Over that 30-year time period, 22,132 petitions have been adjudicated, with 9,738 of those determined to be compensable, while 12,394 were dismissed. Total compensation paid over the life of the program is approximately $4.9 billion.

VICP Adjudication Categories, by Alleged Vaccine for Petitions Filed Since the Inclusion of Influenza as an Eligible Vaccine for Filings 01/01/2006 through 12/31/2021

Name of Vaccine Listed First in a Petition (other vaccines may be alleged or basis for compensation)	Number of Doses Distributed in the U.S., 01/01/2006 through 12/31/2021 (Source: CDC)	Compensable Concession	Compensable Court Decision	Compensable Settlement	Compensable Total	Dismissed/Non-Compensable Total	Grand Total
DT	794,777	1	0	5	6	4	10
DTaP	119,588,927	27	23	125	175	142	317
DTaP-Hep B-IPV	89,690,234	7	8	32	47	67	114
DTaP-HIB	1,135,474	0	1	2	3	2	5
DTaP-IPV	35,717,741	1	0	5	6	5	11
DTaP-IPV-HIB	85,135,961	5	4	9	18	41	59
DTaP-IPV-HIB-Hep B	464,070	0	0	0	0	0	0
DTP	0	1	1	3	5	4	9
DTP-HIB	0	1	0	2	3	1	4
Hep A-Hep B	19,067,612	3	1	19	23	8	31
Hep B-HIB	4,787,457	1	1	2	4	1	5
Hepatitis A (Hep A)	221,388,946	10	6	54	70	42	112
Hepatitis B (Hep B)	238,582,570	15	12	85	112	101	213
HIB	152,436,021	2	1	13	16	11	27
HPV	149,352,148	32	13	119	164	350	514
Influenza	2,231,400,000	1,784	238	3,458	5,481	980	6,461
IPV	83,134,982	1	1	5	7	5	12
Measles	135,660	1	0	2	3	0	3

Name of Vaccine Listed First in a Petition (other vaccines may be alleged or basis for compensation)	Number of Doses Distributed in the U.S., 01/01/2006 through 12/31/2021 (Source: CDC)	Compensable Concession	Compensable Court Decision	Compensable Settlement	Compensable Total	Dismissed/Non-Compensable Total	Grand Total
Meningococcal	140,401,298	16	5	47	68	28	96
MMR	127,871,467	26	16	106	148	149	297
MMR-Varicella	39,223,326	12	1	14	27	21	48
Mumps	110,749	0	0	0	0	0	0
Nonqualified	0	0	0	3	3	55	58
OPV	0	1	0	0	1	5	6
Pneumococcal Conjugate	303,138,568	47	3	77	127	76	203
Rotavirus	1,422,658,212	27	4	25	56	23	79
Rubella	422,548	0	1	1	2	0	2
Td	76,709,653	17	6	70	93	29	122
Tdap	335,133,138	212	22	445	679	150	829
Tetanus	3,836,052	20	2	56	78	22	100
Unspecified	0	1	1	5	7	597	604
Varicella	138,414,086	11	7	33	51	27	78
Grand Total	**6,020,731,677**	**2,282**	**378**	**4,822**	**7,483**	**2,946**	**10,429**

Notes on the Adjudication Categories Table

The date range of 01/01/2006 through 12/31/2021 was selected to reflect petitions filed since the inclusion of influenza vaccine in July 2005. Influenza vaccine now is named in the majority of all VICP petitions.

In addition to the first vaccine alleged by a petitioner, which is the vaccine listed in this table, a VICP petition may allege other vaccines, which may form the basis of compensation. Vaccine doses are self-reported distribution data provided by US-licensed vaccine manufacturers. The data provide an estimate of the annual national distribution and do not represent vaccine administration. In order to maintain confidentiality of an individual manufacturer or brand, the data are presented in an aggregate format by vaccine type. Flu doses are derived from CDC's FluFinder tracking system, which includes data provided to CDC by US-licensed influenza vaccine manufacturers as well as their first line distributors.

"Unspecified" means insufficient information was submitted to make an initial determination. The conceded "unspecified" petition was for multiple unidentified vaccines that caused abscess formation at the vaccination site(s), and the "unspecified" settlements were for multiple vaccines later identified in the Special Masters' decisions

Definitions

Compensable – The injured person who filed a petition was paid money by the VICP. Compensation can be achieved through a concession by the U.S. Department of Health and Human Services (HHS), a decision on the merits of the petition by a special master or a judge of the U.S. Court of Federal Claims (Court), or a settlement between the parties.

- **Concession**: HHS concludes that a petition should be compensated based on a thorough review and analysis of the evidence, including medical records and the scientific and medical literature. The HHS review concludes that the petitioner is entitled to compensation, including a determination either that it is more likely than not that the vaccine caused the injury or the evidence supports fulfillment of the criteria of the Vaccine Injury Table. The Court also determines that the petition should be compensated.
- **Court Decision**: A special master or the court, within the United States Court of Federal Claims, issues a legal decision after weighing the evidence presented by both sides. HHS abides by the ultimate Court decision even if it maintains its position that the petitioner was not entitled to compensation (e.g., that the injury was not caused by the vaccine).
  For injury petitions, compensable court decisions are based in part on one of the following determinations by the court:
  1. The evidence is legally sufficient to show that the vaccine more likely than not caused (or significantly aggravated) the injury; or
  2. The injury is listed on, and meets all of the requirements of, the Vaccine Injury Table, and HHS has not proven that a factor unrelated to the vaccine more likely than not caused or significantly aggravated the injury. An injury listed on the Table and meeting all Table requirements is given the legal presumption of causation. It should be noted that conditions are placed on the Table for both scientific and policy reasons.
- **Settlement**: The petition is resolved via a negotiated settlement between the parties. This settlement is not an admission by the United States or the Secretary of Health and Human Services that the vaccine caused the petitioner's alleged injuries, and, in settled cases, the Court does not determine that the vaccine caused the injury. A settlement therefore cannot be characterized as a decision by HHS or by the Court that the vaccine caused an injury. Petitions may be resolved by settlement for many reasons, including consideration of prior court decisions; a recognition by both parties that there is a risk of loss in proceeding to a decision by the Court making the certainty of settlement more desirable; a desire by both parties to minimize the time and expense associated with litigating a case to conclusion; and a desire by both parties to resolve a case quickly and efficiently.
- **Non-compensable/Dismissed**: The injured person who filed a petition was ultimately not paid money. Non-compensable Court decisions include the following:
  1. The Court determines that the person who filed the petition did not demonstrate that the injury was caused (or significantly aggravated) by a covered vaccine or meet the requirements of the Table (for injuries listed on the Table).
  2. The petition was dismissed for not meeting other statutory requirements (such as not meeting the filing deadline, not receiving a covered vaccine, and not meeting the statute's severity requirement).
  3. The injured person voluntarily withdrew his or her petition.

Petitions Filed, Compensated and Dismissed, by Alleged Vaccine, Since the Beginning of VICP, 10/01/1988 through 04/01/2023

Vaccines	Filed Injury	Filed Death	Filed Total	Compensated	Dismissed
DT	69	9	78	26	52
DTaP	483	88	571	256	277
DTaP-Hep B-IPV	99	40	139	48	68
DTaP-HIB	11	1	12	7	4
DTaP-IPV	16	0	16	6	5
DTaP-IPV-HIB	53	21	74	18	41
DTaP-IPV-HIB-HEPB	0	0	0	0	0
DTP	3,288	696	3,984	1,273	2,711
DTP-HIB	20	8	28	7	21
Hep A-Hep B	45	0	45	23	9
Hep B-HIB	8	0	8	5	3
Hepatitis A (Hep A)	144	7	151	70	45
Hepatitis B (Hep B)	751	62	813	303	448
HIB	50	3	53	23	21
HPV	746	20	766	163	390
Influenza	8,991	232	9,223	5,473	975
IPV	269	14	283	10	271
Measles	145	19	164	57	107
Meningococcal	123	3	126	68	28
MMR	1,046	62	1,108	430	609
MMR-Varicella	62	2	64	26	21
MR	15	0	15	6	9
Mumps	10	0	10	1	9
Nonqualified[1]	118	13	131	3	124
OPV	282	28	310	158	152
Pertussis	4	3	7	2	5
Pneumococcal Conjugate	330	24	354	131	94
Rotavirus	115	6	121	76	33
Rubella	190	4	194	71	123
Td	239	3	242	139	80
Tdap	1,195	8	1,203	676	152
Tetanus	187	3	190	101	49
Unspecified[2]	5,427	9	5,436	11	5,419
Varicella	117	10	127	71	39
Grand Total	**24,648**	**1,398**	**26,046**	**9,738**	**12,394**

[1] Nonqualified petitions are those filed for vaccines not covered under the VICP.
[2] Unspecified petitions are those submitted with insufficient information to make a determination.

Petitions Filed

Fiscal Year	Total
FY 1988	24
FY 1989	148
FY 1990	1,492
FY 1991	2,718
FY 1992	189
FY 1993	140
FY 1994	107
FY 1995	180
FY 1996	84
FY 1997	104
FY 1998	120
FY 1999	411
FY 2000	164
FY 2001	215
FY 2002	958
FY 2003	2,592
FY 2004	1,214
FY 2005	735
FY 2006	325
FY 2007	410
FY 2008	417
FY 2009	397
FY 2010	447
FY 2011	386
FY 2012	402
FY 2013	504
FY 2014	633
FY 2015	803
FY 2016	1,120
FY 2017	1,243
FY 2018	1,238
FY 2019	1,282
FY 2020	1,192
FY 2021	2,057
FY 2022	1,029
FY 2023	566
Total	**26,046**

Adjudications

Generally, petitions are not adjudicated in the same fiscal year as filed.
On average, it takes 2 to 3 years to adjudicate a petition after it is filed.

Fiscal Year	Compensable	Dismissed	Total
FY 1989	9	12	21
FY 1990	100	33	133
FY 1991	141	447	588
FY 1992	166	487	653
FY 1993	125	588	713
FY 1994	162	446	608
FY 1995	160	575	735
FY 1996	162	408	570
FY 1997	189	198	387
FY 1998	144	181	325
FY 1999	98	139	237
FY 2000	125	104	229
FY 2001	86	88	174
FY 2002	104	104	208
FY 2003	56	100	156
FY 2004	62	247	309
FY 2005	60	229	289
FY 2006	69	193	262
FY 2007	82	136	218
FY 2008	147	151	298
FY 2009	134	257	391
FY 2010	180	330	510
FY 2011	266	1,742	2,008
FY 2012	265	2,533	2,798
FY 2013	369	651	1,020
FY 2014	370	194	564
FY 2015	521	146	667
FY 2016	700	187	887
FY 2017	696	203	899
FY 2018	545	202	747
FY 2019	641	182	823
FY 2020	711	217	928
FY 2021	755	259	1,014
FY 2022	939	270	1,209
FY 2023	399	155	554
Total	**9,738**	**12,394**	**22,132**

Awards Paid

Fiscal Year	Number of Compensated Awards	Petitioners' Award Amount	Attorneys' Fees/Costs Payments	Number of Payments to Attorneys (Dismissed Cases)	Attorneys' Fees/Costs Payments (Dismissed Cases)	Number of Payments to Interim Attorneys'	Interim Attorneys' Fees/Costs Payments	Total Outlays
FY 1989	6	$1,317,654.78	$54,107.14	0	$0.00	0	$0.00	$1,371,761.92
FY 1990	88	$53,252,510.46	$1,379,005.79	4	$57,699.48	0	$0.00	$54,689,215.73
FY 1991	114	$95,980,493.16	$2,364,758.91	30	$496,809.21	0	$0.00	$98,842,061.28
FY 1992	130	$94,538,071.30	$3,001,927.97	118	$1,212,677.14	0	$0.00	$98,752,676.41
FY 1993	162	$119,693,267.87	$3,262,453.06	272	$2,447,273.05	0	$0.00	$125,402,993.98
FY 1994	158	$98,151,900.08	$3,571,179.67	335	$3,166,527.38	0	$0.00	$104,889,607.13
FY 1995	169	$104,085,265.72	$3,652,770.57	221	$2,276,136.32	0	$0.00	$110,014,172.61
FY 1996	163	$100,425,325.22	$3,096,231.96	216	$2,364,122.71	0	$0.00	$105,885,679.89
FY 1997	179	$113,620,171.68	$3,898,284.77	142	$1,879,418.14	0	$0.00	$119,397,874.59
FY 1998	165	$127,546,009.19	$4,002,278.55	121	$1,936,065.50	0	$0.00	$133,484,353.24
FY 1999	96	$95,917,680.51	$2,799,910.85	117	$2,306,957.40	0	$0.00	$101,024,548.76
FY 2000	136	$125,945,195.64	$4,112,369.02	80	$1,724,451.08	0	$0.00	$131,782,015.74
FY 2001	97	$105,878,632.57	$3,373,865.88	57	$2,066,224.67	0	$0.00	$111,318,723.12
FY 2002	80	$59,799,604.39	$2,653,598.89	50	$656,244.79	0	$0.00	$63,109,448.07
FY 2003	65	$82,816,240.07	$3,147,755.12	69	$1,545,654.87	0	$0.00	$87,509,650.06
FY 2004	57	$61,933,764.20	$3,079,328.55	69	$1,198,615.96	0	$0.00	$66,211,708.71
FY 2005	64	$55,065,797.01	$2,694,664.03	71	$1,790,587.29	0	$0.00	$59,551,048.33
FY 2006	68	$48,746,162.74	$2,441,199.02	54	$1,353,632.61	0	$0.00	$52,540,994.37
FY 2007	82	$91,449,433.89	$4,034,154.37	61	$1,692,020.25	0	$0.00	$97,175,608.51
FY 2008	141	$75,716,552.06	$5,191,770.83	74	$2,531,394.20	2	$117,265.31	$83,556,982.40
FY 2009	131	$74,142,490.58	$5,404,711.98	36	$1,557,139.53	28	$4,241,362.55	$85,345,704.64
FY 2010	173	$179,387,341.30	$5,961,744.40	59	$1,933,550.09	22	$1,978,803.88	$189,261,439.67
FY 2011	251	$216,319,428.47	$9,572,042.87	403	$5,589,417.19	28	$2,001,770.91	$233,482,659.44
FY 2012	249	$163,491,998.82	$9,241,427.33	1,020	$8,649,676.56	37	$5,420,257.99	$186,803,360.70
FY 2013	375	$254,666,326.70	$13,543,099.70	704	$7,012,615.42	50	$1,423,851.74	$276,645,893.56
FY 2014	365	$202,084,196.12	$12,161,422.64	508	$6,824,566.68	38	$2,493,460.73	$223,563,646.17
FY 2015	508	$204,137,880.22	$14,464,063.71	118	$3,546,785.14	50	$3,089,497.68	$225,238,226.75
FY 2016	689	$230,140,251.20	$16,298,140.59	100	$2,746,864.60	58	$3,398,557.26	$252,583,813.65
FY 2017	706	$252,245,932.78	$22,045,785.00	132	$4,454,379.49	52	$3,363,464.24	$282,109,561.51
FY 2018	521	$199,588,007.04	$16,689,908.68	113	$5,151,255.64	57	$4,999,766.30	$226,428,937.66

Fiscal Year	Number of Compensated Awards	Petitioners' Award Amount	Attorneys' Fees/Costs Payments	Number of Payments to Attorneys (Dismissed Cases)	Attorneys' Fees/Costs Payments (Dismissed Cases)	Number of Payments to Interim Attorneys'	Interim Attorneys' Fees/Costs Payments	Total Outlays
FY 2019	653	$196,217,707.64	$18,991,247.55	103	$5,292,700.23	65	$5,457,545.23	$225,959,200.65
FY 2020	733	$186,860,677.55	$20,165,188.43	113	$5,747,755.82	76	$5,090,482.24	$217,864,104.04
FY 2021	719	$208,258,401.31	$24,884,274.59	140	$6,942,253.81	53	$4,249,055.37	$244,333,985.08
FY 2022	927	$195,693,889.57	$22,992,062.07	102	$4,868,964.74	56	$6,329,886.09	$229,884,802.47
FY 2023	448	$63,197,564.61	$19,407,138.80	67	$3,679,217.62	38	$4,707,892.58	$90,991,813.61
Total	**9,668**	**$4,538,311,826.45**	**$293,633,873.29**	**5,879**	**$106,699,654.61**	**710**	**$58,362,920.10**	**$4,997,008,274.45**

NOTE: Some previous fiscal year data has been updated as a result of the receipt and entry of data from documents issued by the Court and system updates which included petitioners' costs reimbursements in outlay totals,

"Compensated" are petitions that have been paid as a result of a settlement between parties or a decision made by the U.S. Court of Federal Claims (Court). The # of awards is the number of petitioner awards paid, including the attorneys' fees/costs payments, if made during a fiscal year. However, petitioners' awards and attorneys' fees/costs are not necessarily paid in the same fiscal year as when the petitions/petitions are determined compensable. "Dismissed" includes the # of payments to attorneys and the total amount of payments for attorneys' fees/costs per fiscal year. The VICP will pay attorneys' fees/costs related to the petition, whether or not the petition/petition is awarded compensation by the Court, if certain minimal requirements are met. "Total Outlays" are the total amount of funds expended for compensation and attorneys' fees/costs from the Vaccine Injury Compensation Trust Fund by fiscal year.

Since influenza vaccines (vaccines administered to large numbers of adults each year) were added to the VICP in 2005, many adult petitions related to that vaccine have been filed, thus changing the proportion of children to adults receiving compensation.

Vaccine Injury Table

Applies Only to Petitions for Compensation Filed under the National Vaccine Injury Compensation Program on or after March 21, 2017

(a) In accordance with section 312(b) of the National Childhood Vaccine Injury Act of 1986, title III of Public Law 99-660, 100 Stat. 3779 (42 U.S.C. 300aa-1 note) and section 2114(c) of the Public Health Service Act, as amended (PHS Act) (42 U.S.C. 300aa-14(c)), the following is a table of vaccines, the injuries, disabilities, illnesses, conditions, and deaths resulting from the administration of such vaccines, and the time period in which the first symptom or manifestation of onset or of the significant aggravation of such injuries, disabilities, illnesses, conditions, and deaths is to occur after vaccine administration for purposes of receiving compensation under the Program. Paragraph (b) of this section sets forth additional provisions that are not separately listed in this Table but that constitute part of it. Paragraph (c) of this section sets forth the qualifications and aids to interpretation for the terms used in the Table. Conditions and injuries that do not meet the terms of the qualifications and aids to interpretation are not within the Table. Paragraph (d) of this section sets forth a glossary of terms used in paragraph (c).

Vaccine	Illness, disability, injury or condition covered	Time period for first symptom or manifestation of onset or of significant aggravation after vaccine administration
I. Vaccines containing tetanus toxoid (*e.g.,* DTaP, DTP, DT, Td, or TT)	A. Anaphylaxis B. Brachial Neuritis	≤4 hours. 2-28 days (not less than 2 days and not more than 28 days).
	C. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	D. Vasovagal syncope	≤1 hour.
II. Vaccines containing whole cell pertussis bacteria, extracted or partial cell pertussis bacteria, or specific pertussis antigen(s) (*e.g.,* DTP, DTaP, P, DTP-Hib)	A. Anaphylaxis	≤4 hours.
	B. Encephalopathy or encephalitis	≤72 hours.
	C. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	D. Vasovagal syncope	≤1 hour.
III. Vaccines containing measles, mumps, and rubella virus or any of its components (*e.g.,* MMR, MM, MMRV)	A. Anaphylaxis B. Encephalopathy or encephalitis	≤4 hours. 5-15 days (not less than 5 days and not more than 15 days).
	C. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	D. Vasovagal syncope	≤1 hour.

Vaccine	Illness, disability, injury or condition covered	Time period for first symptom or manifestation of onset or of significant aggravation after vaccine administration
IV. Vaccines containing rubella virus (*e.g.,* MMR, MMRV)	A. Chronic arthritis	7-42 days (not less than 7 days and not more than 42 days).
V. Vaccines containing measles virus (*e.g.,* MMR, MM, MMRV)	A. Thrombocytopenic purpura	7-30 days (not less than 7 days and not more than 30 days).
	B. Vaccine-Strain Measles Viral Disease in an immunodeficient recipient	
	—Vaccine-strain virus identified	Not applicable.
	—If strain determination is not done or if laboratory testing is inconclusive	≤12 months.
VI. Vaccines containing polio live virus (OPV)	A. Paralytic Polio	
	—in a non-immunodeficient recipient	≤30 days.
	—in an immunodeficient recipient	≤6 months.
	—in a vaccine associated community case	Not applicable.
	B. Vaccine-Strain Polio Viral Infection	
	—in a non-immunodeficient recipient	≤30 days.
	—in an immunodeficient recipient	≤6 months.
	—in a vaccine associated community case	Not applicable.
VII. Vaccines containing polio inactivated virus (*e.g.,* IPV)	A. Anaphylaxis	≤4 hours.
	B. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	C. Vasovagal syncope	≤1 hour.
VIII. Hepatitis B vaccines	A. Anaphylaxis	≤4 hours.
	B. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	C. Vasovagal syncope	≤1 hour.

Vaccine	Illness, disability, injury or condition covered	Time period for first symptom or manifestation of onset or of significant aggravation after vaccine administration
IX. Haemophilus influenzae type b (Hib) vaccines	A. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	B. Vasovagal syncope	≤1 hour.
X. Varicella vaccines	A. Anaphylaxis	≤4 hours.
	B. Disseminated varicella vaccine-strain viral disease	
	—Vaccine-strain virus identified	Not applicable.
	—If strain determination is not done or if laboratory testing is inconclusive	7-42 days (not less than 7 days and not more than 42 days).
	C. Varicella vaccine-strain viral reactivation	Not applicable.
	D. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	E. Vasovagal syncope	≤1 hour.
XI. Rotavirus vaccines	A. Intussusception	1-21 days (not less than 1 day and not more than 21 days).
XII. Pneumococcal conjugate vaccines	A. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	B. Vasovagal syncope	≤1 hour.
XIII. Hepatitis A vaccines	A. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	B. Vasovagal syncope	≤1 hour.
XIV. Seasonal influenza vaccines	A. Anaphylaxis	≤4 hours.
	B. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	C. Vasovagal syncope	≤1 hour.
	D. Guillain-Barré Syndrome	3-42 days (not less than 3 days and not more than 42 days).
XV. Meningococcal vaccines	A. Anaphylaxis	≤4 hours.
	B. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	C. Vasovagal syncope	≤1 hour.
XVI. Human papillomavirus (HPV) vaccines	A. Anaphylaxis	≤4 hours.

Vaccine	Illness, disability, injury or condition covered	Time period for first symptom or manifestation of onset or of significant aggravation after vaccine administration
	B. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	C. Vasovagal syncope	≤1 hour.
XVII. Any new vaccine recommended by the Centers for Disease Control and Prevention for routine administration to children, after publication by the Secretary of a notice of coverage	A. Shoulder Injury Related to Vaccine Administration	≤48 hours.
	B. Vasovagal syncope	≤1hour.

(b) *Provisions that apply to all conditions listed.* (1) Any acute complication or sequela, including death, of the illness, disability, injury, or condition listed in paragraph (a) of this section (and defined in paragraphs (c) and (d) of this section) qualifies as a Table injury under paragraph (a) except when the definition in paragraph (c) requires exclusion.

(2) In determining whether or not an injury is a condition set forth in paragraph (a) of this section, the Court shall consider the entire medical record.

(3) An idiopathic condition that meets the definition of an illness, disability, injury, or condition set forth in paragraph (c) of this section shall be considered to be a condition set forth in paragraph (a) of this section.

(c) *Qualifications and aids to interpretation.* The following qualifications and aids to interpretation shall apply to, define and describe the scope of, and be read in conjunction with paragraphs (a), (b), and (d) of this section:

(1) *Anaphylaxis.* Anaphylaxis is an acute, severe, and potentially lethal systemic reaction that occurs as a single discrete event with simultaneous involvement of two or more organ systems. Most cases resolve without sequela. Signs and symptoms begin minutes to a few hours after exposure. Death, if it occurs, usually results from airway obstruction caused by laryngeal edema or bronchospasm and may be associated with cardiovascular collapse. Other significant clinical signs and symptoms may include the following: Cyanosis, hypotension, bradycardia, tachycardia, arrhythmia, edema of the pharynx and/or trachea and/or larynx with stridor and dyspnea. There are no specific pathological findings to confirm a diagnosis of anaphylaxis.

(2) *Encephalopathy.* A vaccine recipient shall be considered to have suffered an encephalopathy if an injury meeting the description below of an acute encephalopathy occurs within the applicable time period and results in a chronic encephalopathy, as described in paragraph (d) of this section.

(i) *Acute encephalopathy.* (A) For children less than 18 months of age who present:

(*1*) Without a seizure, an acute encephalopathy is indicated by a significantly decreased level of consciousness that lasts at least 24 hours.

(*2*) Following a seizure, an acute encephalopathy is demonstrated by a significantly decreased level of consciousness that lasts at least 24 hours and cannot be attributed to a postictal state—from a seizure or a medication.

(B) For adults and children 18 months of age or older, an acute encephalopathy is one that persists at least 24 hours and is characterized by at least two of the following:

(*1*) A significant change in mental status that is not medication related (such as a confusional state, delirium, or psychosis);

(*2*) A significantly decreased level of consciousness which is independent of a seizure and cannot be attributed to the effects of medication; and

(*3*) A seizure associated with loss of consciousness.

(C) The following clinical features in themselves do not demonstrate an acute encephalopathy or a significant change in either mental status or level of consciousness: Sleepiness, irritability (fussiness), high-pitched and unusual screaming, poor feeding, persistent inconsolable crying, bulging fontanelle, or symptoms of dementia.

(D) Seizures in themselves are not sufficient to constitute a diagnosis of encephalopathy and in the absence of other evidence of an acute encephalopathy seizures shall not be viewed as the first symptom or manifestation of an acute encephalopathy.

(ii) *Exclusionary criteria for encephalopathy.* Regardless of whether or not the specific cause of the underlying condition, systemic disease, or acute event (including an infectious organism) is known, an encephalopathy shall not be considered to be a condition set forth in the Table if it is shown that the encephalopathy was caused by:

(A) An underlying condition or systemic disease shown to be unrelated to the vaccine (such as malignancy, structural lesion, psychiatric illness, dementia, genetic disorder, prenatal or perinatal central nervous system (CNS) injury); or

(B) An acute event shown to be unrelated to the vaccine such as a head trauma, stroke, transient ischemic attack, complicated migraine, drug use (illicit or prescribed) or an infectious disease.

(3) *Encephalitis.* A vaccine recipient shall be considered to have suffered encephalitis if an injury meeting the description below of acute encephalitis occurs within the applicable time period and results in a chronic encephalopathy, as described in paragraph (d) of this section.

(i) *Acute encephalitis.* Encephalitis is indicated by evidence of neurologic dysfunction, as described in paragraph (c)(3)(i)(A) of this section, plus evidence of an inflammatory process in the brain, as described in paragraph (c)(3)(i)(B) of this section.

(A) Evidence of neurologic dysfunction consists of either:

(*1*) One of the following neurologic findings referable to the CNS: Focal cortical signs (such as aphasia, alexia, agraphia, cortical blindness); cranial nerve abnormalities; visual field defects; abnormal presence of primitive reflexes (such as Babinski's sign or sucking reflex); or cerebellar dysfunction (such as ataxia, dysmetria, or nystagmus); or

(*2*) An acute encephalopathy as set forth in paragraph (c)(2)(i) of this section.

(B) Evidence of an inflammatory process in the brain (central nervous system or CNS inflammation) must include cerebrospinal fluid (CSF) pleocytosis (>5 white blood cells (WBC)/mm^3 in children >2 months of age and adults; >15 WBC/mm3 in children <2 months of age); or at least two of the following:

(*1*) Fever (temperature ≥ 100.4 degrees Fahrenheit);

(*2*) Electroencephalogram findings consistent with encephalitis, such as diffuse or multifocal nonspecific background slowing and periodic discharges; or

(*3*) Neuroimaging findings consistent with encephalitis, which include, but are not limited to brain/spine magnetic resonance imaging (MRI) displaying diffuse or multifocal areas of hyperintense signal on T2-weighted, diffusion-weighted image, or fluid-attenuation inversion recovery sequences.

(ii) *Exclusionary criteria for encephalitis.* Regardless of whether or not the specific cause of the underlying condition, systemic disease, or acute event (including an infectious organism) is known, encephalitis shall not be considered to be a condition set forth in the Table if it is shown that the encephalitis was caused by:

(A) An underlying malignancy that led to a paraneoplastic encephalitis;

(B) An infectious disease associated with encephalitis, including a bacterial, parasitic, fungal or viral illness (such as herpes viruses, adenovirus, enterovirus, West Nile Virus, or human immunodeficiency virus), which may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing; or

(C) Acute disseminated encephalomyelitis (ADEM). Although early ADEM may have laboratory and clinical characteristics similar to acute encephalitis, findings on MRI are distinct with ADEM displaying evidence of acute demyelination (scattered, focal, or multifocal areas of inflammation and demyelination within cerebral subcortical and deep cortical white matter; gray matter involvement may also be seen but is a minor component); or

(D) Other conditions or abnormalities that would explain the vaccine recipient's symptoms.

(4) *Intussusception.* (i) For purposes of paragraph (a) of this section, intussusception means the invagination of a segment of intestine into the next segment of intestine, resulting in bowel obstruction, diminished arterial blood supply, and blockage of the venous blood flow. This is characterized by a sudden onset of abdominal pain that may be manifested by anguished crying, irritability, vomiting, abdominal swelling, and/or passing of stools mixed with blood and mucus.

(ii) For purposes of paragraph (a) of this section, the following shall not be considered to be a Table intussusception:

(A) Onset that occurs with or after the third dose of a vaccine containing rotavirus;

(B) Onset within 14 days after an infectious disease associated with intussusception, including viral disease (such as those secondary to non-enteric or enteric adenovirus, or other enteric viruses such as Enterovirus), enteric bacteria (such as Campylobacter jejuni), or enteric parasites (such as Ascaris lumbricoides), which may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing;

(C) Onset in a person with a preexisting condition identified as the lead point for intussusception such as intestinal masses and cystic structures (such as polyps, tumors, Meckel's diverticulum, lymphoma, or duplication cysts);

(D) Onset in a person with abnormalities of the bowel, including congenital anatomic abnormalities, anatomic changes after abdominal surgery, and other anatomic bowel abnormalities caused by mucosal

hemorrhage, trauma, or abnormal intestinal blood vessels (such as Henoch Scholein purpura, hematoma, or hemangioma); or

(E) Onset in a person with underlying conditions or systemic diseases associated with intussusception (such as cystic fibrosis, celiac disease, or Kawasaki disease).

(5) *Chronic arthritis.* Chronic arthritis is defined as persistent joint swelling with at least two additional manifestations of warmth, tenderness, pain with movement, or limited range of motion, lasting for at least 6 months.

(i) Chronic arthritis may be found in a person with no history in the 3 years prior to vaccination of arthropathy (joint disease) on the basis of:

(A) Medical documentation recorded within 30 days after the onset of objective signs of acute arthritis (joint swelling) that occurred between 7 and 42 days after a rubella vaccination; and

(B) Medical documentation (recorded within 3 years after the onset of acute arthritis) of the persistence of objective signs of intermittent or continuous arthritis for more than 6 months following vaccination; and

(C) Medical documentation of an antibody response to the rubella virus.

(ii) The following shall not be considered as chronic arthritis: Musculoskeletal disorders such as diffuse connective tissue diseases (including but not limited to rheumatoid arthritis, juvenile idiopathic arthritis, systemic lupus erythematosus, systemic sclerosis, mixed connective tissue disease, polymyositis/determatomyositis, fibromyalgia, necrotizing vasculitis and vasculopathies and Sjogren's Syndrome), degenerative joint disease, infectious agents other than rubella (whether by direct invasion or as an immune reaction), metabolic and endocrine diseases, trauma, neoplasms, neuropathic disorders, bone and cartilage disorders, and arthritis associated with ankylosing spondylitis, psoriasis, inflammatory bowel disease, Reiter's Syndrome, blood disorders, or arthralgia (joint pain), or joint stiffness without swelling.

(6) *Brachial neuritis.* This term is defined as dysfunction limited to the upper extremity nerve plexus (*i.e.,* its trunks, divisions, or cords). A deep, steady, often severe aching pain in the shoulder and upper arm usually heralds onset of the condition. The pain is typically followed in days or weeks by weakness in the affected upper extremity muscle groups. Sensory loss may accompany the motor deficits, but is generally a less notable clinical feature. Atrophy of the affected muscles may occur. The neuritis, or plexopathy, may be present on the same side or on the side opposite the injection. It is sometimes bilateral, affecting both upper extremities. A vaccine recipient shall be considered to have suffered brachial neuritis as a Table injury if such recipient manifests all of the following:

(i) Pain in the affected arm and shoulder is a presenting symptom and occurs within the specified time-frame;

(ii) Weakness;

(A) Clinical diagnosis in the absence of nerve conduction and electromyographic studies requires weakness in muscles supplied by more than one peripheral nerve.

(B) Nerve conduction studies (NCS) and electromyographic (EMG) studies localizing the injury to the brachial plexus are required before the diagnosis can be made if weakness is limited to muscles supplied by a single peripheral nerve.

(iii) Motor, sensory, and reflex findings on physical examination and the results of NCS and EMG studies, if performed, must be consistent in confirming that dysfunction is attributable to the brachial plexus; and

(iv) No other condition or abnormality is present that would explain the vaccine recipient's symptoms.

(7) *Thrombocytopenic purpura.* This term is defined by the presence of clinical manifestations, such as petechiae, significant bruising, or spontaneous bleeding, and by a serum platelet count less than 50,000/mm^3 with normal red and white blood cell indices. Thrombocytopenic purpura does not include cases of thrombocytopenia associated with other causes such as hypersplenism, autoimmune disorders (including alloantibodies from previous transfusions) myelodysplasias, lymphoproliferative disorders, congenital thrombocytopenia or hemolytic uremic syndrome. Thrombocytopenic purpura does not include cases of immune (formerly called idiopathic) thrombocytopenic purpura that are mediated, for example, by viral or fungal infections, toxins or drugs. Thrombocytopenic purpura does not include cases of thrombocytopenia associated with disseminated intravascular coagulation, as observed with bacterial and viral infections. Viral infections include, for example, those infections secondary to Epstein Barr virus, cytomegalovirus, hepatitis A and B, human immunodeficiency virus, adenovirus, and dengue virus. An antecedent viral infection may be demonstrated by clinical signs and symptoms and need not be confirmed by culture or serologic testing. However, if culture or serologic testing is performed, and the viral illness is attributed to the vaccine-strain measles virus, the presumption of causation will remain in effect. Bone marrow examination, if performed, must reveal a normal or an increased number of megakaryocytes in an otherwise normal marrow.

(8) *Vaccine-strain measles viral disease.* This term is defined as a measles illness that involves the skin and/or another organ (such as the brain or lungs). Measles virus must be isolated from the affected organ or histopathologic findings characteristic for the disease must be present. Measles viral strain determination may be performed by methods such as polymerase chain reaction test and vaccine-specific monoclonal antibody. If strain determination reveals wild-type measles virus or another, non-vaccine-strain virus, the disease shall not be considered to be a condition set forth in the Table. If strain determination is not done or if the strain cannot be identified, onset of illness in any organ must occur within 12 months after vaccination.

(9) *Vaccine-strain polio viral infection.* This term is defined as a disease caused by poliovirus that is isolated from the affected tissue and should be determined to be the vaccine-strain by oligonucleotide or polymerase chain reaction. Isolation of poliovirus from the stool is not sufficient to establish a tissue specific infection or disease caused by vaccine-strain poliovirus.

(10) *Shoulder injury related to vaccine administration (SIRVA).* SIRVA manifests as shoulder pain and limited range of motion occurring after the administration of a vaccine intended for intramuscular administration in the upper arm. These symptoms are thought to occur as a result of unintended injection of vaccine antigen or trauma from the needle into and around the underlying bursa of the shoulder resulting in an inflammatory reaction. SIRVA is caused by an injury to the musculoskeletal structures of the shoulder (*e.g.* tendons, ligaments, bursae, etc.). SIRVA is not a neurological injury and abnormalities on neurological examination or nerve conduction studies (NCS) and/or electromyographic (EMG) studies would not support SIRVA as a diagnosis (even if the condition causing the neurological abnormality is not known). A vaccine recipient shall be considered to have suffered SIRVA if such recipient manifests all of the following:

(i) No history of pain, inflammation or dysfunction of the affected shoulder prior to intramuscular vaccine administration that would explain the alleged signs, symptoms, examination findings, and/or diagnostic studies occurring after vaccine injection;

(ii) Pain occurs within the specified time-frame;

(iii) Pain and reduced range of motion are limited to the shoulder in which the intramuscular vaccine was administered; and

(iv) No other condition or abnormality is present that would explain the patient's symptoms (*e.g.* NCS/EMG or clinical evidence of radiculopathy, brachial neuritis, mononeuropathies, or any other neuropathy).

(11) *Disseminated varicella vaccine-strain viral disease.* Disseminated varicella vaccine-strain viral disease is defined as a varicella illness that involves the skin beyond the dermatome in which the vaccination was given and/or disease caused by vaccine-strain varicella in another organ. For organs other than the skin, the disease must be demonstrated in the involved organ and not just through mildly abnormal laboratory values. If there is involvement of an organ beyond the skin, and no virus was identified in that organ, the involvement of all organs must occur as part of the same, discrete illness. If strain determination reveals wild-type varicella virus or another, non-vaccine-strain virus, the viral disease shall not be considered to be a condition set forth in the Table. If strain determination is not done or if the strain cannot be identified, onset of illness in any organ must occur 7- 42 days after vaccination.

(12) *Varicella vaccine-strain viral reactivation disease.* Varicella vaccine-strain viral reactivation disease is defined as the presence of the rash of herpes zoster with or without concurrent disease in an organ other than the skin. Zoster, or shingles, is a painful, unilateral, pruritic rash appearing in one or more sensory dermatomes. For organs other than the skin, the disease must be demonstrated in the involved organ and not just through mildly abnormal laboratory values. There must be laboratory confirmation that the vaccine-strain of the varicella virus is present in the skin or in any other involved organ, for example by oligonucleotide or polymerase chain reaction. If strain determination reveals wild-type varicella virus or another, non-vaccine-strain virus, the viral disease shall not be considered to be a condition set forth in the Table.

(13) *Vasovagal syncope.* Vasovagal syncope (also sometimes called neurocardiogenic syncope) means loss of consciousness (fainting) and postural tone caused by a transient decrease in blood flow to the brain occurring after the administration of an injected vaccine. Vasovagal syncope is usually a benign condition but may result in falling and injury with significant sequela. Vasovagal syncope may be preceded by symptoms such as nausea, lightheadedness, diaphoresis, and/or pallor. Vasovagal syncope may be associated with transient seizure-like activity, but recovery of orientation and consciousness generally occurs simultaneously with vasovagal syncope. Loss of consciousness resulting from the following conditions will not be considered vasovagal syncope: organic heart disease, cardiac arrhythmias, transient ischemic attacks, hyperventilation, metabolic conditions, neurological conditions, and seizures. Episodes of recurrent syncope occurring after the applicable time period are not considered to be sequela of an episode of syncope meeting the Table requirements.

(14) *Immunodeficient recipient.* Immunodeficient recipient is defined as an individual with an identified defect in the immunological system which impairs the body's ability to fight infections. The identified defect may be due to an inherited disorder (such as severe combined immunodeficiency resulting in absent T lymphocytes), or an acquired disorder (such as acquired immunodeficiency syndrome resulting from decreased CD4 cell counts). The identified defect must be demonstrated in the medical records, either preceding or postdating vaccination.

(15) *Guillain-Barré Syndrome (GBS).* (i) GBS is an acute monophasic peripheral neuropathy that encompasses a spectrum of four clinicopathological subtypes described below. For each subtype of GBS, the interval between the first appearance of symptoms and the nadir of weakness is between 12 hours and 28 days. This is followed in all subtypes by a clinical plateau with stabilization at the nadir of symptoms, or subsequent improvement without significant relapse. Death may occur without a clinical plateau. Treatment related fluctuations in all subtypes of GBS can occur within 9 weeks of GBS symptom onset and recurrence of symptoms after this time-frame would not be consistent with GBS.

(ii) The most common subtype in North America and Europe, comprising more than 90 percent of cases, is acute inflammatory demyelinating polyneuropathy (AIDP), which has the pathologic and electrodiagnostic features of focal demyelination of motor and sensory peripheral nerves and nerve roots. Another subtype called acute motor axonal neuropathy (AMAN) is generally seen in other parts of the world and is predominated by axonal damage that primarily affects motor nerves. AMAN lacks features of demyelination. Another less common subtype of GBS includes acute motor and sensory neuropathy (AMSAN), which is an axonal form of GBS that is similar to AMAN, but also affects the sensory nerves and roots. AIDP, AMAN, and AMSAN are typically characterized by symmetric motor flaccid weakness, sensory abnormalities, and/or autonomic dysfunction caused by autoimmune damage to peripheral nerves and nerve roots. The diagnosis of AIDP, AMAN, and AMSAN requires:

(A) Bilateral flaccid limb weakness and decreased or absent deep tendon reflexes in weak limbs;

(B) A monophasic illness pattern;

(C) An interval between onset and nadir of weakness between 12 hours and 28 days;

(D) Subsequent clinical plateau (the clinical plateau leads to either stabilization at the nadir of symptoms, or subsequent improvement without significant relapse; however, death may occur without a clinical plateau); and,

(E) The absence of an identified more likely alternative diagnosis.

(iii) Fisher Syndrome (FS), also known as Miller Fisher Syndrome, is a subtype of GBS characterized by ataxia, areflexia, and ophthalmoplegia, and overlap between FS and AIDP may be seen with limb weakness. The diagnosis of FS requires:

(A) Bilateral ophthalmoparesis;

(B) Bilateral reduced or absent tendon reflexes;

(C) Ataxia;

(D) The absence of limb weakness (the presence of limb weakness suggests a diagnosis of AIDP, AMAN, or AMSAN);

(E) A monophasic illness pattern;

(F) An interval between onset and nadir of weakness between 12 hours and 28 days;

(G) Subsequent clinical plateau (the clinical plateau leads to either stabilization at the nadir of symptoms, or subsequent improvement without significant relapse; however, death may occur without a clinical plateau);

(H) No alteration in consciousness;

(I) No corticospinal track signs; and

(J) The absence of an identified more likely alternative diagnosis.

(iv) Evidence that is supportive, but not required, of a diagnosis of all subtypes of GBS includes electrophysiologic findings consistent with GBS or an elevation of cerebral spinal fluid (CSF) protein with

a total CSF white blood cell count below 50 cells per microliter. Both CSF and electrophysiologic studies are frequently normal in the first week of illness in otherwise typical cases of GBS.

(v) To qualify as any subtype of GBS, there must not be a more likely alternative diagnosis for the weakness.

(vi) Exclusionary criteria for the diagnosis of all subtypes of GBS include the ultimate diagnosis of any of the following conditions: chronic immune demyelinating polyradiculopathy (CIDP), carcinomatous meningitis, brain stem encephalitis (other than Bickerstaff brainstem encephalitis), myelitis, spinal cord infarct, spinal cord compression, anterior horn cell diseases such as polio or West Nile virus infection, subacute inflammatory demyelinating polyradiculoneuropathy, multiple sclerosis, cauda equina compression, metabolic conditions such as hypermagnesemia or hypophosphatemia, tick paralysis, heavy metal toxicity (such as arsenic, gold, or thallium), drug-induced neuropathy (such as vincristine, platinum compounds, or nitrofurantoin), porphyria, critical illness neuropathy, vasculitis, diphtheria, myasthenia gravis, organophosphate poisoning, botulism, critical illness myopathy, polymyositis, dermatomyositis, hypokalemia, or hyperkalemia. The above list is not exhaustive.

(d) *Glossary for purposes of paragraph (c) of this section*—(1) *Chronic encephalopathy.* (i) A chronic encephalopathy occurs when a change in mental or neurologic status, first manifested during the applicable Table time period as an acute encephalopathy or encephalitis, persists for at least 6 months from the first symptom or manifestation of onset or of significant aggravation of an acute encephalopathy or encephalitis.

(ii) Individuals who return to their baseline neurologic state, as confirmed by clinical findings, within less than 6 months from the first symptom or manifestation of onset or of significant aggravation of an acute encephalopathy or encephalitis shall not be presumed to have suffered residual neurologic damage from that event; any subsequent chronic encephalopathy shall not be presumed to be a sequela of the acute encephalopathy or encephalitis.

(2) *Injected* refers to the intramuscular, intradermal, or subcutaneous needle administration of a vaccine.

(3) *Sequela* means a condition or event which was actually caused by a condition listed in the Vaccine Injury Table.

(4) *Significantly decreased level of consciousness* is indicated by the presence of one or more of the following clinical signs:

(i) Decreased or absent response to environment (responds, if at all, only to loud voice or painful stimuli);

(ii) Decreased or absent eye contact (does not fix gaze upon family members or other individuals); or

(iii) Inconsistent or absent responses to external stimuli (does not recognize familiar people or things).

(5) *Seizure* includes myoclonic, generalized tonic-clonic (grand mal), and simple and complex partial seizures, but not absence (petit mal), or pseudo seizures. Jerking movements or staring episodes alone are not necessarily an indication of seizure activity.

(e) *Coverage provisions.* (1) Except as provided in paragraph (e)(2), (3), (4), (5), (6), (7), or (8) of this section, this section applies only to petitions for compensation under the program filed with the United States Court of Federal Claims on or after February 21, 2017.

(2) Hepatitis B, Hib, and varicella vaccines (Items VIII, IX, and X of the Table) are included in the Table as of August 6, 1997.

(3) Rotavirus vaccines (Item XI of the Table) are included in the Table as of October 22, 1998.

(4) Pneumococcal conjugate vaccines (Item XII of the Table) are included in the Table as of December 18, 1999.

(5) Hepatitis A vaccines (Item XIII of the Table) are included on the Table as of December 1, 2004.

(6) Trivalent influenza vaccines (Included in item XIV of the Table) are included on the Table as of July 1, 2005. All other seasonal influenza vaccines (Item XIV of the Table) are included on the Table as of November 12, 2013.

(7) Meningococcal vaccines and human papillomavirus vaccines (Items XV and XVI of the Table) are included on the Table as of February 1, 2007.

(8) Other new vaccines (Item XVII of the Table) will be included in the Table as of the effective date of a tax enacted to provide funds for compensation paid with respect to such vaccines. An amendment to this section will be published in the FEDERAL REGISTER to announce the effective date of such a tax.

VACCINE INJURY TABLE & VACCINE INJURY COMPENSATION

AND WHY THE TABLE IS IMPORTANT.

The Vaccine Injury Table includes every compensable vaccination and injury in the VICP.
It's a vital element in determining vaccine injury compensation.

DO YOU HAVE A TABLE INJURY?

See the Vaccine Injury Table in just a few scrolls.

View the Table below for everything you need to know.

VIEW TABLE HERE
(HTTPS://F.HUBSPOTUSERCONTENT20.NET/HUBFS/4412221/IMPORTED%20MEDIA%20AND%20STYLE%20S

WHAT IS THE VACCINE INJURY TABLE?

To bring a claim in the National Vaccine Injury Compensation Program, the injured party must have received one of the types of vaccines listed in the vaccine injury table. The table includes every compensable vaccination. Certain vaccinations are not included. For instance, the Anthrax vaccine and smallpox vaccine are not included in the table largely because it is unavailable to the general public or from your family healthcare provider. It is only administered to certain members of the armed forces or research workers.

HOW THE VACCINE INJURY TABLE WORKS

As you will see in the Table graphic provided at the top of the page, there are two (2) other columns to the right of the “Vaccine” column. The middle column is the “Illness, Disability, Injury or Condition Covered” column, followed by the third and final column "Time Period for First Symptom". The Vaccine Injury Compensation Program includes a provision that can be extremely beneficial to the injured parties. The provision states the court will 'presume that the vaccination caused the injury/condition,' if the injured party:

- Received a vaccine listed in the "*Vaccine*" column, and
- Developed an illness, disability, injury, or condition listed in the middle column, and
- Within the time period listed in the far right column.

Presumption is a legal term that essentially means that if the injured party proves these three (3) things, he or she does not need to prove anything else. The burden is on the defendant, the Department of Health and Human Services Administration, to disprove the claim. It is extraordinarily difficult for HHS to do this. Therefore, table injury claims are generally resolved through settlement fairly quickly.

COVERED VACCINES UNDER THE TABLE

In Column 1 of the Table, the Vaccine Injury Compensation Program currently lists vaccines that are covered. The majority of these vaccines are recommended during the childhood vaccination schedule of which the program was originally created to cover. Below is a list of the aforementioned vaccines:

- Trivalent and Seasonal Influenza vaccines (/flu-shot-injury) (FluMist, a live attenuated influenza virus vaccine; and injectable influenza vaccines FluShield, Fluvirin, Fluzone, and Afluria)
- Tetanus vaccine (/tetanus-shot) (Td)
- Pertussis vaccines (/tdap-dtap-vaccine) (DTP, DTaP, TDaP)
- Measles, Mumps, and Rubella (/mmr-vaccine) virus-containing vaccines in any combination (MMR, MR, M, R)
- Rubella virus-containing vaccines (/mmr-vaccine) (MMR, MR, R)
- Measles virus-containing vaccine (/mmr-vaccine) (MMR)
- Mumps vaccines (/mmr-vaccine) (MMR, MR, M)
- Polio vaccines (https://www.cdc.gov/vaccines/vpd/polio/index.html) (OPV or IPV)
- Hepatitis B vaccine (/hepatitis-b-vaccine) (HBV)
- Haemophilus Influenza Type B Polysaccharide Conjugate vaccine (HIB)
- Varicella (Chickenpox) vaccine (/chicken-pox-vaccine) (VAR)
- Rotavirus vaccine (/rotavirus-vaccine) (Rota Teq)
- Pneumococcal conjugate vaccine (PCV)
- Hepatitis A vaccine (/hepatitis-a-vaccine) (HAV)
- Meningococcal vaccines (/meningitis-vaccine)(MCV4, MPSV4, MenB-FHbp, MenB-4C) which is commonly contained in the following two types of vaccines: Meningococcal Conjugate and Serogroup B Meningococcal
- Human papillomavirus vaccine (HPV) which is commonly contained in the following three types of vaccines: Gardasil, Gardasil 9, and Cervarix

NON-COVERED VACCINES UNDER THE TABLE

- Pneumococcal polysaccharide vaccine (PPSV (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/ppv.html), PPV (https://www.cdc.gov/vaccines/hcp/vis/vis-statements/ppv.html))
- Herpes zoster (shingles) vaccine (Zostavax (https://www.cdc.gov/vaccines/vpd/shingles/hcp/zostavax/recommendations.html), Shingrix (https://www.cdc.gov/vaccines/vpd/shingles/public/shingrix/index.html))
- Anthrax vaccine (BioThrax (https://www.cdc.gov/vaccines/vpd/anthrax/public/index.html))

COVERED VACCINE INJURIES UNDER THE VACCINE INJURY TABLE

In Column 2 of the Table, the VICP states all of the injuries, illnesses, and disabilities that are recognized to be linked with each vaccine. The most common vaccine related injury seen filed in the Vaccine Court is a SIRVA injury, also known as a Shoulder Injury Related to Vaccine Administration. Below is a list of vaccine injuries covered under the table:

- SIRVA Injury (/sirva-injury) (Shoulder Injury Related to Vaccine Administration)

- Frozen Shoulder (/sirva-injury/frozen-shoulder) (Adhesive Capsulitis)
- Shoulder Bursitis (/sirva-injury/bursitis)
- Rotator Cuff (/sirva-injury/rotator-cuff-injury)
- Shoulder Tendonitis (/sirva-injury/tendonitis)
- Ulnar Neuropathy (/sirva-injury/ulnar-neuropathy)
- Guillain-Barre Syndrome ("GBS") (/gbs-after-flu-shot)
- Parsonage-Turner Syndrome ("PTS") (/parsonage-turner-syndrome)
- Encephalitis (/encephalitis)
- Transverse Myelitis (/transverse-myelitis)
- Intussusception (/intussusception)
- Chronic Inflammatory Demyelinating Polyneuropathy ("CIDP") (/cidp)
- Anaphylaxis (/anaphylaxis)
- Acute Disseminated Encephalomyelitis ("ADEM") (/adem)

WHAT IF MY INJURY IS NOT ON THE VACCINE INJURY TABLE?

That being said, it is not always necessary to have a "Table Injury." In fact, most vaccine injuries are not table injuries. If you receive one of the listed vaccines and suffer any injury, severe allergic reaction, or catastrophic health problems, you still may be entitled to compensation. When the injury is not a table injury, it simply means we must prove the injury was caused by the vaccination. For experienced vaccine injury attorneys like our team here at My Vaccine Lawyer, this is a regular occurrence with each and every one of the vaccine injury petitions that we file.

ONSET OF SYMPTOMS FOR A VACCINE INJURY

It is crucial to report your symptoms or adverse reactions to a medical professional or your doctor immediately. Having a strong case in the Vaccine Injury Compensation Program requires meeting criterion in Column 1, Column 2 and Column 3. A symptom or manifestation of an illness must be present following a vaccine administration. This is usually supported when an injured person mentions their symptoms to a medical professional or doctor and noted in their medical record. Each vaccine and linked vaccine injury have different time frame requirements. Below is a table example of a TDaP or DTaP vaccine:

EXAMPLE: FOLLOWING A TETANUS OR PERTUSSIS VACCINE CONTAINING EITHER DTP, DTAP, TDAP, TT OR DPT VACCINE

- If the injury is Anaphylaxis it should manifest less than or equal to four hours;
- If the injury is Brachial Neuritis it should manifest within 2 to 28 days but no more or no less;
- If the injury is SIRVA it should manifest less than or equal to 48 hours;
- If the injury is Syncope it should manifest in less than one hour.

The team at My Vaccine Lawyer has represented over 3,000 clients across the country for every type of vaccine injury and illness listed under the Vaccine Injury Table. Our vaccine injury lawyers are licensed to practice in the United States Court of Federal Claims and have years of experience filing "Table Cases" and "Non-Table Cases." That's right, it is possible to file a vaccine injury claim that does not exactly meet the VICP's criterion. With our team of attorneys and in-house medical experts, we will review your potential at no cost and determine the best course of action in pursuing a vaccine injury claim.

Page last reviewed and updated: March 2, 2022

VACCINE INJURY SETTLEMENTS AND SIRVA SETTLEMENTS

WHAT IS THE AVERAGE VACCINE INJURY PAYOUT?

Vaccine Injury Settlements and SIRVA Settlements in the VICP come from a multi-billion dollar trust fund adjudicated by the U.S. Court of Federal Claims.

ABOUT VACCINE INJURY SETTLEMENTS

⭐Flu Shot - Guillain Barre Syndrome⭐
$2,473,607

⭐TDaP Vaccine - Encephalitis⭐
$4,095,193

⭐Flu Shot - Parsonage Turner Syndrome⭐
$1,233,543

TETANUS SHOT - SHOULDER TENDONITIS

$111,390

A Louisiana woman suffered adhesive capsulitis (frozen shoulder) and tendonitis of the supraspinatus and subscapularis tendons as a result of improper administration of a tetanus shot. The client underwent an MRI which revealed a loose body within the subacromial space of the shoulder. The treating orthopedic surgeon recommended surgery to remove the loose body and repair the scar tissue and tearing.

FLU SHOT - FROZEN SHOULDER

$106,160

An Alabama woman suffered various shoulder injuries after a flu shot. She was diagnosed with severe adhesive capsulitis (frozen shoulder) and treated with steroid medication and physical therapy. When the physical therapy only provided minimal relief, she was forced to undergo a shoulder manipulation under anesthesia, but continued to experience pain and limited range of motion months after the procedure.

HPV VACCINE - TRANSVERSE MYELITIS

$140,000

An Oklahoma child who suffered Transverse Myelitis following an HPV vaccination. Approximately two weeks after receiving the second dose of the Gardasil vaccination, the client was admitted to the emergency room with severe weakness in his left leg. He was later diagnosed with transverse myelitis and treated with steroid medication and physical therapy. Although his symptoms improved with treatment, the client continued to suffer from weakness in his left leg and difficulty walking.

FLU SHOT - GUILLAIN-BARRE SYNDROME

$162,500

A North Carolina man suffered Guillain-Barre Syndrome after a flu shot. About three weeks after receiving the flu shot, the client began experiencing numbness and tingling in both feet. He was transferred to the hospital via ambulance and diagnosed with GBS. He continued to experience neurological symptoms including weakness in his legs and feet for about a year after the vaccination. His treatment was complicated by chemotherapy for lung cancer.

MENINGITIS VACCINE - ROTATOR CUFF INJURY

$125,000

A South Carolina man suffered a torn rotator cuff requiring surgery after a meningitis vaccination. The client was administered a meningitis vaccination while he was recovering from hip surgery in the hospital. By the time he was discharged, he couldn't move his arm. He was later diagnosed with a rotator cuff tear, subacromial impingement and bursitis by his orthopedic surgeon. He underwent a left shoulder arthroscopy with debridement, subacromial decompression and rotator cuff repair, which greatly improved his symptoms.

FLU SHOT - FROZEN SHOULDER

$110,000

A California woman suffered adhesive capsulitis caused by improper administration of a flu shot. The client participated in physical therapy for several weeks. When she did not improve with therapy, her orthopedic surgeon recommended arthroscopic surgery.

TDAP/DTAP SHOT - SHOULDER BURSITIS

$120,000

A Georgia woman who suffered subacromial bursitis and rotator cuff tendonitis following improper administration of a TDaP/DTaP vaccination. The client received a cortisone injection and participated in a six-week physical therapy course. When she did not improve, her orthopedic surgeon recommended a second six-week therapy course and another cortisone injection. Unfortunately all conservative treatment failed, leading to an arthroscopic surgery to repair the damage.

CHICKENPOX VACCINE - ANAPHYLAXIS

$150,203

A Virginia child suffered an anaphylactic reaction to the chickenpox vaccination. The initial reaction occurred within only hours of the vaccination. The auto-immune response led to several food allergies. The client must now carry an Epipen at all times.

FLU SHOT - GUILLAIN-BARRE SYNDROME

$135,000

A Delaware woman suffered Guillain-Barre Syndrome after a flu shot at her local pharmacy. The client's initial symptoms, including numbness and tingling in her arms, started approximately three weeks post-vaccination. Her condition rapidly declined and eventually resulted in respiratory failure and quadriplegia. She was treated with IVIG. Fortunately, after an extended hospital stay and rehabilitation period, the client was able to regain her ability to walk.

FLU SHOT - ROTATOR CUFF INJURY

$115,000

A Colorado woman who suffered a shoulder injury leading to surgery after a flu shot. Following a flu shot, the client developed severe pain and a bump at the site of the injection. She presented to the emergency room and was diagnosed with shoulder pain and cellulitis. When her symptoms failed to resolve on their own, she was later diagnosed with a severely torn rotator cuff by an MRI. She underwent an arthroscopic rotator cuff repair and post-op physical therapy, however, she continued to experience significant pain requiring subsequent cortisone injections.

TDAP/DTAP SHOT - TRANSVERSE MYELITIS

$140,000

A Rhode Island woman who suffered Transverse Myelitis following a Tdap vaccine. About two months after the Tdap vaccination, the client began experiencing numbness and weakness in her feet. She was transported to the hospital and after multiple rounds of diagnostic testing, was diagnosed with transverse myelitis. She initially spent five weeks in the hospital. Following her discharge from the hospital, she spent weeks in outpatient physical therapy. Although she did not lose wages, the injury greatly affected her ability to perform her nursing job. The case was complicated by a two month onset, which is on the very fringe of acceptable onset in the court.

TDAP/DTAP SHOT - SHOULDER BURSITIS

$120,000

A New Hampshire woman suffered a shoulder injury caused by a tetanus-diphtheria-pertussis TDaP/DTaP vaccination. The client was diagnosed with subacromial bursitis. She participated in physical therapy for several weeks, and received a cortisone injection. However, conservative treatment failed and eventually it became necessary to undergo arthroscopic surgery.

FLU SHOT - ROTATOR CUFF INJURY

$110,000

A Kentucky man developed a shoulder injury and had surgery after a flu shot. The client suffered a rotator cuff tear and bursitis following a flu shot. He was treated with physical therapy and a steroid injection but continued to experience pain and limited range of motion. He later underwent an arthroscopic rotator cuff repair and debridement.

TDAP/DTAP SHOT - SHOULDER BURSITIS

$113,382

A Montana woman sustained shoulder injuries from a TDaP/DTaP vaccine. The client developed severe pain and reduced range of motion immediately following a TDaP/DTaP vaccine. She treated with a chiropractor and then an orthopedic surgeon and was diagnosed with bursitis secondary to a TDaP/DTaP vaccine. She subsequently underwent left shoulder arthroscopy with thorough debridement of subacromial space and bursitis. Her compensation package included reimbursement for significant out of pocket medical expenses as she had to pay for the surgery out of pocket.

FLU SHOT - SIRVA INJURY

$162,622

A New Jersey woman suffered a shoulder injury after a flu shot. The client suffered from edema, tendonitis and bursitis after a flu shot was injected too high on her shoulder. The injury caused her to lose her job as a nurse and take a lower paying job. Our vaccine injury lawyers recovered six figures for pain and suffering along with the projected difference in wages until she was 65 years old.

FLU SHOT - SIRVA INJURY

$95,000

A Pennsylvania woman developed a shoulder injury after a flu shot. The client developed immediate pain, numbness and tingling following a flu shot. She was treated by both neurologists and orthopedic doctors with a variety of treatments including medications, injections and physical therapy. After more than a year of symptoms, she began to develop pain in her elbow as a result of overcompensating from the shoulder injury. She was subsequently diagnosed with Lateral Epicondylitis which her treating physicians opined was secondary to the shoulder injury.

FLU SHOT - GUILLAIN-BARRE SYNDROME

$125,000

A New York man developed the Miller Fisher variant of Guillain-Barre syndrome after a flu shot. Approximately three weeks after the flu shot, the client noticed left-sided facial paralysis and difficulty speaking. He was initially diagnosed with bell's palsy. He subsequently developed numbness and weakness in his lower extremities. He underwent a spinal tap which revealed elevated protein levels, prompting his treating neurologist to change the diagnosis to the Miller Fisher variant of GBS. Following treatment, his neurological symptoms mostly resolved but he continued to experience mild facial paralysis.

FLU SHOT - SIRVA INJURY

$135,000

A Colorado woman suffered a shoulder injury after a flu shot. The client suffered from significant bursitis, tendonitis and adhesive capsulitis as the result of a flu shot. She was initially treated with physical therapy and a steroid injection. When conservative treatment failed to relieve her pain, she ultimately underwent a right shoulder arthroscopy, subacromial decompression and debridement.

FLU SHOT - FROZEN SHOULDER

$108,000

A Texas woman suffered adhesive capsulitis and bursitis caused by improper administration of a flu shot. Her shoulder injuries would eventually require surgery followed by a lengthy physical therapy regimen.

FLU SHOT - SHOULDER TENDONITIS

$130,000

A Florida woman suffered a shoulder injury leading to surgery after a flu shot. The client received a flu shot at work and within 24 hours was unable to move her arm. She was diagnosed with bursitis, tendonitis and adhesive capsulitis and treated with physical therapy and a steroid injection. When the treatment failed to provide relief, she was forced to undergo an arthroscopic rotator cuff repair, but continued to experience pain and reduced range of motion.

FLU SHOT - SHOULDER BURSITIS

$135,000

A Tennessee woman suffered a shoulder injury after improper administration of a flu shot at her local pharmacy. Following a shoulder MRI, the client was diagnosed with tendinitis, bursitis, and rotator cuff tear. The client received two cortisone injections to treat the pain. However, after conservative treatment failed she underwent arthroscopic surgery to repair the damage to her shoulder.

TDAP/DTAP SHOT - SHOULDER TENDONITIS

$135,000

A Texas woman suffered shoulder tendonitis, bursitis, impingement syndrome, and a rotator cuff tear caused by improper administration of a tetanus-diphtheria-pertussis (TDaP) vaccination. The shoulder injuries resulted in cortisone injections and a recommendation for arthroscopic surgery.

FLU SHOT - GUILLAIN-BARRE SYNDROME

$148,926

A Wisconsin man suffered from Guillain-Barre Syndrome ("GBS") following a flu shot. About two weeks after the flu shot, the client was admitted to the hospital with extremity weakness and difficulty ambulating. Over the next three months, he underwent multiple rounds of intravenous immunoglobulin ("IVIG") treatment and inpatient physical therapy. He ultimately made a good recovery with only mild ongoing lower extremity weakness.

FLU SHOT - FROZEN SHOULDER

$130,000

A Wisconsin woman suffered adhesive capsulitis (frozen shoulder) requiring surgery after a flu shot. The client suffered from significant bursitis, tendonitis, adhesive capsulitis and a fully torn rotator cuff as the result of a flu shot. She underwent more than 30 physical therapy sessions but her pain and range of motion issues failed to resolve. When conservative treatment failed to relieve her pain, she ultimately underwent a rotator cuff repair. Her settlement package included more than six months of wage loss.

GUILLAIN-BARRÉ SYNDROME TRIGGERED BY VACCINATION

Common Reactions | National Vaccine Injury Compensation Program | Vaccine Injury Case Results and Amounts Won for Clients | Vaccinations

Reviewed By Anne Carrión Toale, Vaccine Injury Attorney

WHAT IS GUILLAIN-BARRÉ SYNDROME?

Guillain-Barré Syndrome (GBS) is a disorder that causes a patient's immune system to go haywire and attack its own peripheral nervous system. GBS is also referred to as Acute Inflammatory Demyelinating Polyneuropathy (AIDP). Symptoms range from a feeling of pins and needles to complete paralysis and can begin anywhere from a few days to several weeks after vaccination. However, symptoms usually peak around one to two weeks after the vaccine is given. The chronic version of GBS is known as Chronic Inflammatory Demyelinating Polyneuropathy or CIDP.

THE INFORMATION ON THIS PAGE DOES NOT APPLY TO COVID-19 VACCINE INJURIES

WE'VE WON MORE THAN $85 MILLION IN GBS AWARDS FOR OUR CLIENTS

VACCINES THAT COMMONLY TRIGGER GUILLAIN BARRÉ SYNDROME

- Influenza Vaccine or Flu Vaccine
- Hepatitis A or Hepatitis B
- Tetanus, Tdap, or DtaP
- Meningococcal (MCV4) Vaccine
- HPV Vaccine
- Varicella or Chickenpox

THE FLU VACCINE AND GBS

Researchers who study vaccine reaction rates find that "GBS is more strongly associated with the flu vaccine" compared to any other type of immunization.

The Journal of the American Medical Association cites Guillain Barré as the most frequent neurological condition reported after getting the flu vaccine.

HPV VACCINE AND GBS

There is evidence that the HPV (human papillomavirus) vaccination can trigger Guillain Barré Syndrome.

The Centers for Disease Control (CDC) says Guillain Barré has been reported after the HPV vaccination.

Other studies show that 72% of patients who reported GBS symptoms after an HPV vaccine experienced those symptoms within 6 weeks after their vaccination.

WHAT TO DO IF YOU HAVE GBS FROM A VACCINE REACTION?

- Contact a lawyer who is experienced in representing patients in front of the United States Court of Federal Claims. Filing a vaccine injury claim is an extremely complex process.
- There are **no legal costs** for an injured patient represented by **mctlaw**. Instead, at the end of the case, our law firm asks the Court for payment of the fees and costs spent representing you.
- This payment is separate from any money you're awarded by the Court. You **never** share any portion of your payout with our law firm.
- Find out more about the legal process of vaccine injury compensation.

For more information, fill out a form **or call our office at** (888) 952-5242 **to speak directly with someone**

FAQ ABOUT THE VACCINE INJURY COMPENSATION PROCESS

Do I Need an Attorney?

Yes. We advise that you DO NOT attempt to represent yourself in the National Vaccine Compensation Program. Hiring a vaccine attorney comes at no cost to you because the Court pays for all legal fees.

How Much Will this Cost Me? Nothing. Here's Why:

There is NO cost to file a claim. **mctlaw** does not charge its clients to represent them in cases brought under the National Vaccine Injury Compensation Program. We do not take a percentage or contingency fee from your financial award. We are paid separately by the Court of Federal Claims at the conclusion of the case**.**

As a service to our clients, our Firm also covers the costs of litigating the case, such as filing fees, expert witness fees, travel expenses, etc.

Do I Need to Hire a Lawyer in My State?

No. The Vaccine Court is located in Washington, DC and covers all vaccine injury claims in the United States. This is not a "local" case that a local personal injury lawyer should handle.

Our attorneys are ready to represent you no matter where you live in the United States and its territories. Our attorneys come to you at or near your home so there is no need for you to travel to our offices in Washington, DC, Sarasota, FL, or Seattle, WA.

What Information Do We Need to Start Building a Case?

First, we need a copy of your vaccine record. This tells us exactly what vaccines you got and when you got them.

Next, we'll ask for copies of all relevant medical records and a list of every doctor or hospital where you've received treatment for your vaccine injury.

We use this information to gather the remaining medical records on file at each location.

We then turn over a copy of your complete and comprehensive medical records to the Court.

How Long Does the Entire Process Take?

This process is designed to be quicker than civil litigation. With some exceptions, it usually is.

A hearing on whether the vaccine caused the injury often occurs within a year. Cases that settle can conclude in as little as a year or two. Other cases, despite our best efforts, can take several years.

How Much is Usually Awarded in these Cases?

Compensation includes monetary damages for pain and suffering, past and future medical expenses, past and future lost wages, and reasonable attorneys' fees and costs. Compensation for pain and suffering is limited to a maximum of $250,000. There is no limit of compensation for medical expenses and lost wages.

HOW OUR VACCINE ATTORNEYS CAN HELP

Mctlaw currently represents hundreds of patients across the United States who are suffering from Guillain-Barre Syndrome triggered by a vaccine reaction.

Our law firm's vaccine attorneys obtain compensation for patients with vaccine injuries by filing a lawsuit in the United States Court of Federal Claims in Washington, D.C. The outcome of the lawsuit determines what kind of compensation a victim may receive.

Very few attorneys are able to practice law before the Federal Vaccine Court. That's why you should hire an attorney with extensive experience in this area of law. These are NOT simple personal injury cases.

OUR GUILLAÍN-BARRE SYNDROME CASE RESULT AMOUNTS

Search

Amount	Vaccine	Link to Court Decision
$147,513	GBS	Case 19-725V
$230,000	GBS, CIDP	Case 19-1574V
$212,705	Influenza	Case 19-0107V
$3,750	Influenza	Case 19-68V
$83,291	Tdap	Case 20-1004V

« ‹ ... 1 2 3 4 5 ... › »

Search

Amount	Vaccine	Link to Court Decision
$117,450	Influenza	Case 20-1198V
$162,260	Influenza	Case 20-1206V
$244,390	Influenza	Case 19-0277V
$137,675	Influenza	Case 20-0334V
$243,207	Influenza	Case 18-1850V
$195,243	Influenza	Case 19-298V
$62,500	TDaP	Case 19-607V
$60,000	TDaP	Case 20-392V
$220,004	Influenza Vaccine	Case 20-0912V
$166,260	Influenza Vaccine	Case 19-0207V
$115,164	Influenza Vaccine	Case 19-971V
$280,447	Influenza Vaccine	Case 19-474V
$166,260	Influenza Vaccine	Case 19-0207V
$300,705	Influenza Vaccine	Case 18-1431V
$60,000	Tdap Vaccine	Case 19-223V

« ‹ ... 1 2 3 4 5 ... › »

WHAT ARE THE SYMPTOMS OF GUILLAIN-BARRÉ SYNDROME (GBS)?

Symptoms of Guillain-Barré Syndrome start out as weakness or tingling in the legs. Sometimes, these sensations then spread to the arms and upper body until the victim becomes paralyzed. In some cases, GBS causes such severe muscle weakness that patients must be put on a ventilator to breathe.

The most common GBS symptoms include:

- Pins and needles feeling in your extremities
- Weakness in your legs that spreads upwards
- Trouble walking or keeping your balance
- Difficulty chewing or swallowing
- Problems breathing

TYPES OF GUILLAIN-BARRÉ VARIATIONS

There are different variants of Guillain-Barré Syndrome (GBS). Some of the most common are:

- Acute Inflammatory Demyelinating Polyneuropathy (AIDP)
- Miller Fisher Syndrome (MFS)
- Acute Motor Axonal Neuropathy (AMAN)
- Acute Motor Sensory Axonal Neuropathy (AMSAN)
- Pharyngeal-Cervical-Brachial Variant
- Acute Panautonomic Neuropathy
- Bickerstaff's Brainstem Encephalitis (BBE)

CONTENT REVIEWED BY ANNE CARRIÓN TOALE – VACCINE INJURY LAWYER

Anne Carrión Toale, Esq. is a vaccine injury attorney at **mctlaw**. Anne helps vaccine injury clients get compensation from the National Vaccine Injury Compensation Program (NVICP) before the Vaccine Court in the United States Court of Federal Claims. Ms. Toale has served as past president of the Vaccine Injured Petitioners Bar Association, where she provided education and assistance to other attorneys throughout the United States practicing in the area of vaccine injury compensation.

SOURCES

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- https://www.ncbi.nlm.nih.gov/pubmed/17101939

mctlaw

Vaccine Adverse Events Reporting System

(VAERS)

VAERS Vaccine Adverse Event Reporting System
www.vaers.hhs.gov

About VAERS

Background and Public Health Importance

Established in 1990, the Vaccine Adverse Event Reporting System (VAERS) is a national early warning system to detect possible safety problems in U.S.-licensed vaccines. VAERS is co-managed by the Centers for Disease Control and Prevention (CDC) and the U.S. Food and Drug Administration (FDA). VAERS accepts and analyzes reports of adverse events (possible side effects) after a person has received a vaccination. Anyone can report an adverse event to VAERS. Healthcare professionals are required to report certain adverse events and vaccine manufacturers are required to report all adverse events that come to their attention.

VAERS is a passive reporting system, meaning it relies on individuals to send in reports of their experiences to CDC and FDA. VAERS is not designed to determine if a vaccine caused a health problem, but is especially useful for detecting unusual or unexpected patterns of adverse event reporting that might indicate a possible safety problem with a vaccine. This way, VAERS can provide CDC and FDA with valuable information that additional work and evaluation is necessary to further assess a possible safety concern.

Objectives of VAERS

The primary objectives of VAERS are to:

- Detect new, unusual, or rare vaccine adverse events;
- Monitor increases in known adverse events;
- Identify potential patient risk factors for particular types of adverse events;
- Assess the safety of newly licensed vaccines;
- Determine and address possible reporting clusters (*e.g., suspected localized [temporally or geographically] or product-/batch-/lot-specific adverse event reporting*);
- Recognize persistent safe-use problems and administration errors;
- Provide a national safety monitoring system that extends to the entire general population for response to public health emergencies, such as a large-scale pandemic influenza vaccination program.

VAERS is co-sponsored by the Centers for Disease Control and Prevention (CDC), and the Food and Drug Administration (FDA), agencies of the U.S. Department of Health and Human Services (HHS).

Centers for Disease Control and Prevention

How to Report Adverse Events to VAERS

There are 2 ways to submit a report to the Vaccine Adverse Event Reporting System (VAERS)

Reporting adverse events to VAERS helps scientist at CDC and FDA keep vaccines safe.

Option 1: Submit a VAERS Report online (Preferred)
The online VAERS Report must be completed and submitted in the same session; it cannot be saved and edited at a later time. Note: sessions time out after 20 minutes of inactivity; no information is saved.

Option 2: Download a Writable PDF Form and upload when ready
The Writable PDF Form can be downloaded and completed electronically on your own time. When ready, return to the VAERS Writable PDF web page (use link above) and follow **Step 2** instructions to upload the form.

More information on reporting an adverse event to VAERS. If you need further assistance, please email info@VAERS.org or call 1-800-822-7967.

What to Report to VAERS

Reporting possible health problems (adverse events) after vaccination to VAERS provides valuable information. These reports help CDC and FDA detect new or unusual adverse events that could indicate a problem with a vaccine. If it looks as though a vaccine might be causing a problem, FDA and CDC will investigate further and take action if needed.

Everyone is encouraged to report possible adverse events after vaccination to VAERS, even if they are not sure whether the vaccine caused the problem. In general, **you should report any side effect or health problem after vaccination that is concerning to you.**

Under the National Childhood Vaccine Injury Act (NCVIA), healthcare providers are **required by law** to report to VAERS:

- Any adverse event listed in the VAERS Table of Reportable Events Following Vaccination [PDF – 5 Pages] that occurs within the specified time period after vaccinations
- An adverse event listed by the vaccine manufacturer as a contraindication to further doses of the vaccine

Healthcare providers are strongly **encouraged** to report to VAERS:

- Any adverse event that occurs after the administration of a vaccine licensed in the United States, whether it is or is not clear that a vaccine caused the adverse event
- Vaccine administration errors

Vaccine manufacturers are required to report to VAERS all adverse events that come to their attention.

VAERS Reporting Requirements for COVID-19 Vaccines (Updated 3/13/2023)

As of August 2022, there are four vaccines available to protect against COVID-19 disease:

- Pfizer-BioNTech COVID-19 Vaccine (Comirnaty®)
- Moderna COVID-19 Vaccine (Spikevax®)
- Johnson & Johnson's Janssen COVID-19 Vaccine
- Novavax COVID-19 Vaccine, Adjuvanted

The Vaccine Adverse Event Reporting System (VAERS) is a national early warning system to detect possible safety problems in vaccines used in the United States. VAERS accepts and analyzes reports of adverse events (AEs) after a person has received a vaccination. Anyone can report an adverse event to VAERS. Healthcare professionals are required to report certain adverse events and vaccine manufacturers are required to report all adverse events that come to their attention.

The reporting requirements for COVID-19 vaccines are the same for those authorized under emergency use (EUA) or approved under a Biologics License Application (BLA).

Healthcare providers who administer COVID-19 vaccines are required to report the following to VAERS:

- Vaccine administration errors whether or not associated with an adverse event (AE).
  - If the incorrect mRNA COVID-19 vaccine product was inadvertently administered for a second dose in a 2-dose series, VAERS reporting is required.
  - If a different product from the primary series is inadvertently administered for the additional or booster (third dose), VAERS reporting is required.
  - VAERS reporting is not required for the following situations:
    - If a mixed series is given intentionally (e.g., due to hypersensitivity to a vaccine ingredient)
    - Mixing and matching of booster doses intentionally (as of October 21, 2021, mixing and matching of booster doses is allowed)

- Serious AEs regardless of causality. Serious AEs per FDA are defined as:
  1. Death
  2. A life-threatening AE
  3. Inpatient hospitalization or prolongation of existing hospitalization
  4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
  5. A congenital anomaly/birth defect
  6. An important medical event that based on appropriate medical judgement may jeopardize the individual and may require medical or surgical intervention to prevent one of the outcomes listed above
- Cases of myocarditis after a Pfizer-BioNTech, Moderna, Novavax, or Janssen COVID-19 vaccine
- Cases of pericarditis after a Pfizer-BioNTech, Moderna, Novavax, or Janssen COVID-19 vaccine
- Cases of Multisystem Inflammatory Syndrome in children and adults
- Cases of COVID-19 that result in hospitalization or death

> Healthcare providers are encouraged to report to VAERS any additional clinically significant AEs following vaccination, even if unsure whether the vaccine caused the event.
>
> Also report any additional selected AEs and/or any revised safety reporting requirements per FDA's conditions of authorized use of vaccine(s) throughout the duration of any COVID-19 vaccine's Emergency Use Authorization (EUA) or as outlined in the Fact Sheet for Healthcare Providers for any approved COVID-19 vaccine.

VAERS Reporting Requirements for Monkeypox vaccines

> The vaccination provider must report all serious* adverse events following administration of JYNNEOS or ACAM2000 vaccine and vaccine administration errors to the Vaccine Adverse Event Reporting System (VAERS) by submitting online at https://vaers.hhs.gov/reportevent.html .
>
> The vaccination provider is responsible for mandatory reporting of the following listed events following JYNNEOS or ACAM2000 vaccination to VAERS:
>
> - Vaccine administration errors, whether or not associated with an adverse event
> - Serious* adverse events (irrespective of attribution to vaccination)
> - Cases of cardiac events, including myocarditis and pericarditis
> - Cases of thromboembolic events and neurovascular events
>
> *Serious adverse events are defined as:
>
> - Death
> - A life-threatening adverse event
> - Inpatient hospitalization or prolongation of existing hospitalization
> - A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
> - A congenital anomaly/birth defect
> - An important medical event that based on appropriate medical judgement may jeopardize the individual and may require medical or surgical intervention to prevent one of the outcomes listed above
>
> Providers are encouraged to also report to VAERS any additional clinically significant AEs following vaccination, even if they are not sure if vaccination caused the event.
>
> As of August 9, 2022, FDA issued an Emergency Use Authorization (EUA) for JYNNEOS monkeypox vaccine. It authorizes the vaccine to be administered in one of two ways:
>
> 1. Intradermally (between the layers of the skin) on the inner aspect of the forearm, and
> 2. Subcutaneously (under the skin) in the upper arm above the elbow.
>
> These are considered routes of vaccination. When submitting a VAERS report, ensure that you document the **Route** in **Section 17** of the VAERS form, by choosing "intradermal" or "subcutaneous" from the selection menu.

Who can report to VAERS

CDC and FDA encourage anyone who experiences (or is made aware) of an adverse event after vaccination to report it to VAERS, even if they are not sure the vaccine caused the problem:

- Patients
- Parents/family member
- Caregivers
- Those who administer vaccines
- Healthcare providers
- Vaccine manufacturers

Reporting to VAERS helps CDC and FDA scientists keep vaccines safe.

What happens after a report is submitted

Which adverse events are considered "serious"?

By the Code of Federal Regulations (CFR) Title 21, an adverse event is defined as serious if it involves any of the following outcomes:

- Death
- A life-threatening adverse event
- A persistent or significant disability or incapacity
- A congenital anomaly or birth defect
- Hospitalization, or prolongation of existing hospitalization

Learn more about adverse events.

Each VAERS report is assigned a VAERS identification number. This number can be used to provide additional information about the report to VAERS, if necessary. VAERS will send the identification number to the reporting individual in a confirmation letter (electronically or by mail, depending on communications preferences listed on the original report).

Other than the confirmation letter, **VAERS will only reach out to the reporting individual for additional information if "essential fields" of the VAERS form are not filled out. VAERS will not contact the reporting individual by phone for follow-up.** Additional information requests are sent electronically or by mail and will explain what information is missing from the report and how the reporter can update it.

The VAERS program follows up on reports classified as serious by attempting to obtain medical records to better understand the event. These requests for medical records are made directly to health institutions or public health authorities that create and maintain medical records. The medical records are added to the permanent record under the VAERS ID, compliant with privacy standards.

Last Reviewed: March 13, 2023

Grant Final Report

Grant ID: R18 HS 017045

Electronic Support for Public Health–Vaccine Adverse Event Reporting System (ESP:VAERS)

Inclusive dates: 12/01/07 - 09/30/10

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Submitted to:
The Agency for Healthcare Research and Quality (AHRQ)
U.S. Department of Health and Human Services
540 Gaither Road
Rockville, MD 20850
www.ahrq.gov

Abstract

Purpose: To develop and disseminate HIT evidence and evidence-based tools to improve healthcare decision making through the use of integrated data and knowledge management.

Scope: To create a generalizable system to facilitate detection and clinician reporting of vaccine adverse events, in order to improve the safety of national vaccination programs.

Methods: Electronic medical records available from all ambulatory care encounters in a large multi-specialty practice were used. Every patient receiving a vaccine was automatically identified, and for the next 30 days, their health care diagnostic codes, laboratory tests, and medication prescriptions were evaluated for values suggestive of an adverse event.

Results: Restructuring at CDC and consequent delays in terms of decision making have made it challenging despite best efforts to move forward with discussions regarding the evaluation of ESP:VAERS performance in a randomized trial and comparison of ESP:VAERS performance to existing VAERS and Vaccine Safety Datalink data. However, Preliminary data were collected and analyzed and this initiative has been presented at a number of national symposia.

Key Words: electronic health records, vaccinations, adverse event reporting

Final Report

Purpose

This research project was funded to improve the quality of vaccination programs by improving the quality of physician adverse vaccine event detection and reporting to the national Vaccine Adverse Event Reporting System (VAERS), via the following aims:

Aim 1. Identify required data elements, and develop systems to monitor ambulatory care electronic medical records for adverse events following vaccine administration.

Aim 2. Prepare, and securely submit clinician approved, electronic reports to the national Vaccine Adverse Event Reporting System (VAERS).

Aim 3. Comprehensively evaluate ESP:VAERS performance in a randomized trial, and in comparison to existing VAERS and Vaccine Safety Datalink data.

Aim 4. Distribute documentation and application software developed and refined in Aims 1 and 2 that are portable to other ambulatory care settings and to other EMR systems.

Scope

Public and professional confidence in vaccination depends on reliable postmarketing surveillance systems to ensure that rare and unexpected adverse effects are rapidly identified. The goal of this project is to improve the quality of vaccination programs by improving the quality of physician adverse vaccine event detection and reporting to the national Vaccine Adverse Event Reporting System (VAERS). This project is serving as an extension of the Electronic Support for Public Health (ESP) project, an automated system using electronic health record (EHR) data to detect and securely report cases of certain diseases to a local public health authority. ESP provides a ready-made platform for automatically converting clinical, laboratory, prescription, and demographic data from almost any EHR system into database tables on a completely independent server, physically located and secured by the same logical and physical security as the EHR data itself. The ESP:VAERS project developed criteria and algorithms to identify important adverse events related to vaccinations in ambulatory care EHR data, and made attempts at formatting and securely sending electronic VAERS reports directly to the Centers for Disease Control and Prevention (CDC).

Patient data were available from Epic System's Certification Commission for Health Information Technology-certified EpicCare system at all ambulatory care encounters within Atrius Health, a large multispecialty group practice with over 35 facilities. Every patient receiving a vaccine was automatically identified, and for the next 30 days, their health care diagnostic codes, laboratory tests, and medication prescriptions are evaluated for values

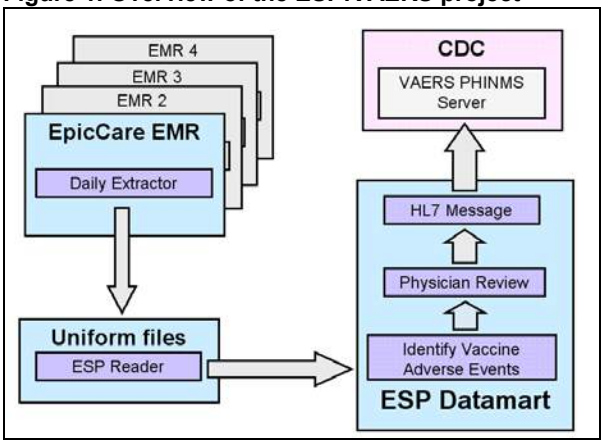
suggestive of an adverse vaccine event. When a possible adverse event was detected, it was recorded, and the appropriate clinician was to be notified electronically.

Clinicians in-basket messaging was designed to provide a preview a pre-populated report with information from the EHR about the patient, including vaccine type, lot number, and possible adverse effect, to inform their clinical judgment regarding whether they wish to send a report to VAERS. Clinicians would then have the option of adding free-text comments to pre-populated VAERS reports or to document their decision not to send a report. The CDC's Public Health Information Network Messaging System (PHIN-MS) software was installed within the facilities so that the approved reports could be securely transferred to VAERS as electronic messages in an interoperable health data exchange format using Health Level 7 (HL7).

Methods

The goal of Aim 1: *Identify required data elements, and develop systems to monitor ambulatory care electronic medical records for adverse events following vaccine administration*, and Aim 2: *Prepare, and securely submit clinician approved, electronic reports to the national Vaccine Adverse Event Reporting System (VAERS)*, was to construct the below flow of data in order to support the first two Aims:

Figure 1. Overview of the ESP:VAERS project



Existing and functioning ESP components are shown on the left, and Aims 1 and 2 on the right. ESP:VAERS flags every vaccinated patient, and prospectively accumulate that patient's diagnostic codes, laboratory tests, allergy lists, vital signs, and medication prescriptions. A main component of Aim 1 was to *Develop AE criteria to assess these parameters for new or abnormal values that might be suggestive of an adverse effect.* A reporting protocol & corresponding algorithms were developed to detect potential adverse event cases using diagnostic codes, and methods were tested to identify prescriptions or abnormal laboratory values that might be suggestive of an adverse effect. These algorithms were designed to seek both expected and unexpected adverse effects.

This reporting protocol was approved by both internal & external partners. We initially prepared a draft document describing the elements, algorithms, interval of interest after vaccination, and actions for broad classes of post-vaccination events, including those to be reported immediately without delay (such as acute anaphylactic reaction following vaccination), those never to be reported (such as routine check-ups following vaccination) and those to be reported at the discretion and with additional information from the attending physician through a feedback mechanism. The draft was then widely circulated as an initial / working draft for comment by relevant staff in the CDC and among our clinical colleagues at Atrius. In addition to review by the internal CDC Brighton Collaboration liaison, this protocol has also received review & comment via the CDC's Clinical Immunization Safety Assessment (CISA) Network.

The goal of Aim 2 was the *Development of HL7 messages code for ESP:VAERS to ensure secure transmission to CDC via PHIN-MS*. The HL7 specification describing the elements for an electronic message to be submitted to Constella, the consultants engaged by CDC for this project was implemented. Synthetic and real test data was been generated and transmitted between Harvard and Constella. However, real data transmissions of non-physician approved reports to the CDC was unable to commence, as by the end of this project, the CDC had yet to respond to multiple requests to partner for this activity.

The goal of Aim 3 was to *Comprehensively evaluate ESP:VAERS performance in a randomized trial, and in comparison to existing VAERS and Vaccine Safety Datalink data.*

We had initially planned to evaluate the system by comparing adverse event findings to those in the Vaccine Safety Datalink project—a collaborative effort between CDC's Immunization Safety Office and eight large managed care organizations. Through a randomized trial, we would also test the hypothesis that the combination of secure, computer-assisted, clinician-approved, adverse event detection, and automated electronic reporting will substantially increase the number, completeness, validity, and timeliness of physician-approved case reports to VAERS compared to the existing spontaneous reporting system; however, due to restructuring at CDC and consequent delays in terms of decision making, it became impossible to move forward with discussions regarding the evaluation of ESP:VAERS performance in a randomized trial, and compare ESP:VAERS performance to existing VAERS and Vaccine Safety Datalink data. Therefore, the components under this particular Aim were not achieved.

Aim 4 *Distribution of documentation and application software developed and refined in Aims 1 and 2 that are portable to other ambulatory care settings and to other EMR systems* has been successfully completed. Functioning source code is available to share under an approved open source license. ESP:VAERS source code is available as part of the ESP source code distribution. It is licensed under the LGPL, an open source license compatible with commercial use. We have added the ESP:VAERS code, HL7 and other specifications and documentation to the existing ESP web documentation and distribution resource center http://esphealth.org, specifically, the Subversion repository available at:
http://esphealth.org/trac/ESP/wiki/ESPVAERS.

Results

Preliminary data were collected from June 2006 through October 2009 on 715,000 patients, and 1.4 million doses (of 45 different vaccines) were given to 376,452 individuals. Of these doses, 35,570 possible reactions (2.6 percent of vaccinations) were identified. This is an average of 890 possible events, an average of 1.3 events per clinician, per month. These data were presented at the 2009 AMIA conference.

In addition, ESP:VAERS investigators participated on a panel to explore the perspective of clinicians, electronic health record (EHR) vendors, the pharmaceutical industry, and the FDA towards systems that use proactive, automated adverse event reporting.

Adverse events from drugs and vaccines are common, but underreported. Although 25% of ambulatory patients experience an adverse drug event, less than 0.3% of all adverse drug events and 1-13% of serious events are reported to the Food and Drug Administration (FDA). Likewise, fewer than 1% of vaccine adverse events are reported. Low reporting rates preclude or slow the identification of "problem" drugs and vaccines that endanger public health. New surveillance methods for drug and vaccine adverse effects are needed. Barriers to reporting include a lack of clinician awareness, uncertainty about when and what to report, as well as the burdens of reporting: reporting is not part of clinicians' usual workflow, takes time, and is duplicative. Proactive, spontaneous, automated adverse event reporting imbedded within EHRs and other information systems has the potential to speed the identification of problems with new drugs and more careful quantification of the risks of older drugs.

Unfortunately, there was never an opportunity to perform system performance assessments because the necessary CDC contacts were no longer available and the CDC consultants responsible for receiving data were no longer responsive to our multiple requests to proceed with testing and evaluation.

Inclusion of AHRQ Priority Populations

The focus of our project was the Atrius Health (formerly HealthOne) provider & patient community. This community serves several AHRQ inclusion populations, specifically low-income and minority populations in primarily urban settings.

Atruis currently employs approximately 700 physicians to serve 500,000 patients at more than 18 office sites spread throughout the greater Metropolitan Boston area. The majority of Atruis physicians are primary care internal medicine physicians or pediatricians but the network also includes physicians from every major specialty.

The entire adult and pediatric population served by Atruis was included in our adverse event surveillance system (ESP:VAERS). Atruis serves a full spectrum of patients that reflects the broad diversity of Eastern Massachusetts. A recent analysis suggests that the population served by Atruis is 56% female, 16.6% African American, 4% Hispanic. The prevalence of type 2 diabetes in the adult population is 5.7%. About a quarter of the Atruis population is under age 18.

List of Publications and Products

ESP:VAERS [source code available as part of the ESP source code distribution]. Licensed under the GNU Lesser General Public License (LGPL), an open source license compatible with commercial use. Freely available under an approved open source license at: http://esphealth.org.

Lazarus, R, Klompas M, Hou X, Campion FX, Dunn J, Platt R. Automated Electronic Detection & Reporting of Adverse Events Following Vaccination: ESP:VAERS. The CDC Vaccine Safety Datalink (VSD) Annual Meeting. Atlanta, GA; April, 2008.

Lazarus R, Klompas M Automated vaccine adverse event detection and reporting from electronic medical records. CDC Public Health Informatics Network (PHIN) Conference August 27, 2008.

Klompas M, Lazarus R ESP:VAERS Presented at the American Medical Informatics Association Annual Symposium; 2009 November 17th.

Lazarus R, Klompas M, Kruskal B, Platt R Temporal patterns of fever following immunization in ambulatory care data identified by ESP:VAERS Presented at the American Medical Informatics Association Annual Symposium; 2009 November 14–18: San Francisco, CA.

Linder J, Klompas M, Cass B, et al. Spontaneous Electronic Adverse Event Reporting: Perspectives from Clinicians, EHR Vendors, Biopharma, and the FDA. Presented at the American Medical Informatics Association Annual Symposium; 2009 November 14–18: San Francisco, CA.

12 Adverse Event Detection, Processing, and Reporting

1. Introduction

Registries that collect information on specific drugs and medical devices need to anticipate the need for adverse event (AE) detection, processing, and reporting. This chapter addresses the identification, processing, and reporting of AEs detected in situations in which a registry has contact with individual patients. This document is not a formal regulatory or legal document; therefore, any information or suggestions presented herein do not supersede, replace, or otherwise interpret Federal guidance documents that touch on these subjects. Registry sponsors are encouraged to discuss plans for AE collection and processing with local health authorities when planning a registry.

This chapter primarily focuses on AEs related to pharmaceutical products. Medical devices are significantly different from pharmaceutical products in the manner in which AEs and product problems (complaints) present themselves, in the etiology of their occurrence, and in the regulation governing the defining and reporting of these occurrences, as well as postapproval study requirements. Other sources provide more information about defining and reporting device-related AEs and product problems, and about postmarketing studies (including those involving registries).[1-3]

2. Identifying and Reporting Adverse Drug Events

The U.S. Food and Drug Administration (FDA) defines an adverse drug experience as any AE associated with the use of a drug in humans, whether or not considered drug related,[4] while the International Conference on Harmonisation (ICH) guideline ICH E2A similarly defines an AE as an untoward medical occurrence in a patient administered a pharmaceutical product, whether or not the occurrence is related to or considered to have a causal relationship with the treatment.[5]

For marketed products regulated by FDA, AEs are categorized for reporting purposes according to the seriousness and expectedness of the event (i.e., whether the event was previously observed and included in local product labeling), as it is presumed that all spontaneously reported events are potentially related to the product for the purposes of FDA reporting. Prior to marketing approval, relatedness is an additional determinant for reporting events occurring during clinical trials or preclinical studies associated with investigational new drugs and biologics. For AEs occurring in postapproval studies and reported during planned contacts and active solicitation of information from patients, as when registries collect data regarding one or more FDA-approved products,[6, 7] the requirements for mandatory reporting also include whether there is a reasonable possibility that the drug caused the adverse experience.[4] For registries that do not actively solicit AEs, incidentally reported events (e.g., those reported during clinician or consumer contact for another purpose) should typically be handled and evaluated as spontaneously reported events.

The medical device reporting regulations differ from those for drugs and biologics in that reportable events include both AEs and problems with the device itself.[8] Medical device reporting is required for incidents in which the device may have caused or contributed to a death or serious injury, or may have malfunctioned and would likely cause or contribute to death or serious injury if the malfunction were to recur.[9]

Most registries have the opportunity to identify and capture information on AEs for biopharmaceutical products and/or medical devices. With the passing of the FDA Amendments Act in September 2007 and the increased emphasis on ongoing monitoring of safety profiles, evaluation of risks unknown at the time of product approval, and proactive detection of potential safety issues, registries increasingly continue to be used to fulfill safety-related objectives.[10] Although no

regulations in the United States specifically compel registries to capture and process AE reports (aside from reporting requirements for registries that are sponsored by regulated industries), there is an implicit requirement from the perspective of systematic data collection and promoting public health: any individual who believes a serious risk may be associated with exposure to a medical product should be encouraged to report this AE either to the product sponsor or directly to FDA. The FDA maintains MedWatch*, a Web-based reporting system that allows consumers and health professionals to voluntarily report serious adverse events and other serious problems that they suspect are associated with the use of an FDA-regulated product.[11] *Note: Adverse events which are potentially vaccine-related should be reported to VAERS.

The minimum dataset required to consider information as a reportable AE is indeed minimal, namely (1) an identifiable patient, (2) an identifiable reporter, (3) product exposure, and (4) an event. However, in addition to direct data collection, AEs can be detected through retrospective analysis of a population database, where direct patient or health care provider contact does not occur. Patient interactions include clinical interactions and data collection by phone, Internet, or other means; perusal of electronic medical records or insurance claims data would not be considered direct patient interaction. Reporting is rarely required for individual AEs observed in aggregate population data, since there is no direct patient interaction where an association might be suggested or inferred. Nevertheless, if aggregate or epidemiologic analyses suggest that an AE is associated with exposure to a drug or medical product, it is desirable that the minimum dataset information be forwarded to the manufacturer of the product, who will determine any need for, and timing of, reporting of study results to the relevant regulatory authorities.

Figure 12–1 provides a broad overview of the reporting requirements for AEs and shows how the reporting differs according to whether the registry has direct patient interaction, and whether it receives sponsorship and/or financial support from a regulated industry.[12] These industries may include entities with products subject to FDA regulation, including products with FDA approval, an FDA-granted license, and investigational products; and other entities such as manufacturers, user facilities, and distributors.

Figure 12–1

Best practices for adverse event reporting to FDA by registries of postmarket products. AE = adverse event; SAE = serious adverse event; FDA = U.S. Food and Drug Administration; PSUR = periodic safety update report.

All AE reporting begins with a suspicion by the physician (or responsible person who obtains or receives information) that a patient exposed to a medicinal product has experienced some AE and that the event has a reasonable possibility of being causally related to the product being used; this is referred to as the "becoming aware" principle. Some registries also collect and record AEs reported directly by the patients or their caregivers. It is important to develop a plan for detecting, processing, and reporting AEs for any registry that has direct patient contact. If the registry receives sponsorship in whole or part from a regulated industry (for drugs or devices), the sponsor has mandated reporting requirements, including stringent timelines. AE reporting requirements for registry sponsors are discussed later in this chapter.

Prior to registry launch, the process for detecting and reporting AEs should be established in collaboration with the sponsor and any oversight committees. (See Chapter 2.) Once the plans have been developed, the registry operator or sponsor should provide training to the physicians or other responsible parties (referred to as "sites" hereafter) on how to identify AEs and to whom they should be reported. AE reporting is based on categorization of the AE according to the seriousness of the event,

its expectedness based on product labeling, and presumed causality or possible association with use of the product, as follows:

- *Seriousness*: Serious AEs (SAEs) include events that result in death, are life threatening (an event in which the patient was at risk of death at the time of the event), require or prolong inpatient hospitalization, result in persistent or significant disability or incapacity, or result in a congenital anomaly. Important medical events may also be considered serious when, based on medical judgment, they may jeopardize the person exposed and may require medical or surgical intervention to prevent one of the outcomes listed above (e.g., death or prolonged hospitalization).
- *Expectedness*: All AEs that are previously unobserved or undocumented are referred to as "unexpected," in that their nature and severity are not consistent with information provided in the relevant product information (e.g., approved professional package insert or product label). Determination of expectedness is made by the sponsor on a case-by-case basis. Expected events typically do not require expedited reporting to the regulatory authorities.
- *Relatedness*: Relatedness is a term intended to indicate that a determination has been made that the event had a reasonable possibility of being related to exposure to the product. This assessment of causality may be based on factors such as biological plausibility, prior experience with the product, and temporal relationship between product exposure and onset of the event, as well as dechallenge (discontinuation of the product to determine if the AE resolves) and rechallenge (reintroduction of the product to determine if the AE recurs). Many terms and scales are used to describe the degree of causality, including terms such as certainly, definitely, probably, possibly, or likely related or not related, but there is no standard nomenclature.[13] All spontaneous reports have an implied causal relationship as per regulatory guidance, regardless of the reporter's assessment.

The registry may use forms such as a structured questionnaire or an AE case report form to collect the information from providers or patients. When solicitation of AEs is not prespecified in the registry's operating plans, the registry may permit AE detection by asking general questions to solicit events, such as "Have you had any problems since your last visit or since we last spoke?" and then following up any such reports with probes as to what happened, diagnoses, and other documentation. This practice is not required.

3. Collecting AE Data in a Registry

There are two key considerations regarding AE collection as part of a registry: (1) what data need to be collected to meet the registry's safety-related objectives, and (2) what processes need to be in place to ensure that the registry is in compliance with regulations regarding expedited and periodic AE event reporting, if applicable. The data fields needed for the purpose of analysis by the registry may be minimal (e.g., event and onset date), whereas a complete SAE form for a subset of events reported to the registry may be sought to fulfill the sponsor's reporting requirements. Due to the nature of registries, the goal of collecting enough data to meet the registry's objectives must constantly be balanced with the burden on sites. To this end, the processes for AE reporting should be streamlined as much as possible.

The collection of AE data by a registry is generally either intentionally solicited (meaning that the data are part of the uniform collection of information in the registry) or unsolicited (meaning that the AE information is volunteered or noted in an unsolicited manner and not as a required data element through a case report form). As described further below, it is good practice for a registry to specify when and how AE information (and any other events of special interest) should and should not be solicited from patients by a site and, if that information has been obtained, how and when the site should inform the appropriate persons.

While an AE may be reported to the manufacturer, to FDA (e.g., via MedWatch), or to the registry itself (and then from the registry to the manufacturer), it is strongly encouraged that the protocol describe the procedures that should be followed, and that the sites be trained in these procedures as well as in their general obligations and the relevant public health considerations. A separate safety reporting plan that fully identifies the responsible parties and describes the operational considerations may also be considered to ensure that potentially reportable information is evaluated in an appropriate timeframe, and, for manufacturer-sponsored registries, in accordance with any applicable standard operating procedures. This type of plan also should describe how deviations or systemic failures in detection and reporting processes will be identified, addressed, and considered for corrective action.

Determining whether a registry should use a case report form to collect AEs should be based on the principles described in Chapter 4, which refer to the scientific importance of the information for evaluating the specified outcomes of interest. This may mean that all, some, or no AEs are collected on the case report forms. However, if some AEs are collected in an intentional, solicited manner (e.g., routine collection of a primary or secondary outcome via an AE case report form) and others come to the registry's attention in an unsolicited, "spontaneous" way (e.g., when an AE is reported in the course of a registry contact, such as a call to the sponsor or to registry support staff), then from a practical perspective it is even more important to have a clear process, so that AEs that require reporting are identified. In this scenario, one best practice that is often used in electronic registry studies is to have a notification sent promptly to the sponsor's safety group when a case report form is submitted that contains specific or potential information indicating that a serious AE has occurred. This process allows for rapid followup by the sponsor, as needed.

4. AE Reporting by the Registry

Once suspicion has been aroused that an unexpected serious event has a reasonable possibility of being causally related to a drug, the AE should be reported to FDA through MedWatch, to the company that manufactures the product, or to the registry coordinating center. (See Chapter 11.) A system should be developed such that all appropriate events are captured and duplicate reporting is avoided to the extent possible. Generally, AE reports are submitted directly by the site or by the registry to the manufacturer, since they are often most efficient at evaluating, processing, and reporting for regulatory purposes within the required time periods. Alternatively, sites could be instructed to report AEs directly to FDA according to their normal practices for marketed products; however, this often means that the companies are not notified of the AE and are not able to follow up or evaluate the event in the context of their safety database. In fact, companies are not necessarily notified by FDA if an AE report comes directly to FDA, since only certain reports are shared with industry, and reporters have an option to request that the information not be shared directly with the company.[14] When sites report AEs directly to FDA, this process can also lead to inadvertent duplication of information for events recorded both by the registry and the company.

Systematic collection of all AEs provides a unique resource of consistent and contemporaneously collected comparison information that can be used at a later date to conduct epidemiologic assessments. Ideally, the practice for handling AEs and SAEs should be applied to all treatments (including comparators) recorded in the registry, so that all subjects are treated similarly. In fact, a strong advantage of registries with systematic data collection and internal comparators is that they provide both numerators and denominators for safety events; thus, reporting of comparative known AE rates in the context of a safety evaluation provides valuable information on real-world performance. The contrast with comparators helps promote clarity about whether the observed effects are unique to the product, unique to a class, or common to the condition being treated. Reporting AEs without denominator information is less useful from a surveillance perspective since events rates cannot be calculated without both numerators and denominators. The reliability of the denominator should always be judged, however, by considering the likelihood that all events were reported appropriately.

For postapproval registries not financially supported by pharmaceutical companies, health care providers at registry sites should be instructed that if they suspect or otherwise become aware of a serious AE that has a reasonable possibility of being causally related to a drug or product, they should report the event directly to the product manufacturer (who must then report to FDA under regulation) or to FDA's MedWatch program (or local health authority if the study is conducted outside of the United States). Reporting can be facilitated by providing the MedWatch Form 3500,[15] information regarding the process for submission, and MedWatch contact information.

For registries that are sponsored or financially supported in full or in part by a regulated industry and that study a single product, the most efficient monitoring system to avoid duplicate reporting is one in which all physicians participating in the registry report all AEs (or SAEs only) directly to the sponsor or centralized designated responsible personnel, who then reports to the regulatory authorities. However, when products other than those exclusively manufactured by the sponsor are involved, including other treatments, sponsors will need to determine how to process AE reports received for these other products. Since sponsors are not obligated to report AEs for their competitors, it is good practice from a public health perspective to specify how the site should address those AEs (e.g., whether to report directly to the other product's manufacturer or to FDA). Options for the sponsor include (1) recommending that sites report the AEs of comparators directly to the manufacturer or to FDA; (2) collecting all AEs and forwarding the AE report directly to the comparator's manufacturer (who would then, in turn, report to FDA); and (3) actually reporting the AE for the comparator product directly to FDA. As standard practice in pharmacovigilance, many sponsors report events potentially associated with another manufacturer's drug to that manufacturer's safety department as a courtesy, rather than report events directly to FDA, and choose to continue that practice when conducting a registry or other observational study.

Some disease registries are not focused on a specific product, but rather on conducting natural history studies or evaluating treatment patterns and outcomes in a particular patient population prior to marketing approval of the sponsor's product. In these situations, it is recommended that sites follow their own standard practices for spontaneous AE reporting, including reporting any events associated with a product known to be manufactured by the sponsor.

In most circumstances in which a serious drug-associated AE is suspected, sites are encouraged to submit supportive data to sponsors, such as laboratory values, vital signs, and examination results, along with the SAE report form. If the event is determined to be an AE, the sponsor will include it in the safety database, evaluate it internally, and transfer the AE report to the regulatory authorities if required. It should be noted that the regulations represent minimum requirements for compliance; special circumstances for a particular product may result in additional events being reportable (e.g., expected events of particular interest to regulators). It should not be expected that registry participants be aware of all the reporting nuances associated with a particular product. To the extent possible, guidance on reporting events of special interest should be provided in the protocol and in any safety training.

If an external party manages a registry, SAEs should be submitted to the sponsors as quickly as possible after the registry becomes aware of the event. In this situation, the registry is an agent of the sponsor, and FDA's 15-calendar-day reporting requirement starts as soon as the event has come to the attention of the registry. (See Section 7 below.) This submission can be accomplished by phone or fax, or by means of automated rules built into the vehicle used for data collection (such as automatic triggers that can be designed into electronic data capture programs). For direct regulatory submissions, the MedWatch Form 3500A[16] should be used for postapproval reporting for drugs and therapeutic biologics unless other means of submission are agreed upon. For vaccines, the Vaccine Adverse Event Reporting System should be consulted.[17] Foreign events may be submitted on a CIOMS form (the World Health Organization's Council for International Organizations of Medical Sciences),[8, 18, 19] or a letter can be generated that includes the relevant information in narrative format.

5. Coding

Coding AEs into a standard nomenclature should be done by trained experts to ensure accuracy and consistency. Reporters, patients, health care providers, and registry personnel should do their best to capture the primary data clearly, completely, and in as "natural" clinical language as possible. Since reporters may use different verbatim terms to describe the same event, it is recommended that sponsors apply coding conventions to code the verbatim terms. The Medical Dictionary for Regulatory Activities (MedDRA®) is customarily used throughout the product development cycle and as part of pharmacovigilance; however, other coding systems are also used. For example, SNOMED-CT (Systematized Nomenclature of Medicine-Clinical Terms) is used instead of MedDRA in some electronic health records. Coding the different verbatim language to preferred terms allows similar events to be appropriately grouped, creates consistency among the terms for evaluation, and maximizes the likelihood that safety signals will be detected.

Sponsors or their designees should review the accuracy of the coding of verbatim AEs into appropriate terms. If coding is performed by someone other than the sponsor, any applicable coding conventions associated with the underlying condition or product should be shared. Review of the coding process should focus on terms that do not accurately communicate the severity or magnitude of the AE or possibly mischaracterize the AE. Review of the coded terms compared with reported verbatim terms should be performed in order to ensure consistency and accuracy of the AE reporting and to minimize variability of coding of similar AE terms. Attention to consistency is especially important, as many different individuals may code AEs over time, and this situation contributes to variability in the coding process. In addition to monitoring AEs individually for complete clinical evaluation of the safety data, sponsors should consider grouping and analyzing clinically relevant coded terms that could represent similar toxicities or syndromes. Combining terms may provide a method of detecting less common and serious events that would otherwise be obscured. However, sponsors should be careful when combining related terms to avoid amplifying a weak signal or obscuring important overall findings when grouping is overly broad. In addition to monitoring individual AEs, sites and registry personnel should be attentive to toxicities that may cluster into syndromes.

6. Adverse Event Management

In some cases, such as when a safety registry is created as a condition of regulatory approval, a data safety monitoring board (DSMB), data monitoring committee (DMC), or adjudication committee may be established with the primary role of periodically reviewing the data as they are generated by the registry. Such activities are generally discussed directly with the regulatory authorities, such as FDA. These authorities are typically involved in the design and critique of protocols for postapproval studies. Ultimately, registry planning and the registry protocol should anticipate and clearly delineate the roles, responsibilities, processes, forms, and lines of communication for AE reporting for sites, registry personnel, the DSMB, DMC, or adjudication committee if one exists, and the sponsoring organization. Documentation should be provided for definitions and approaches to determining what is considered unexpected and possibly related to drug or device exposure. The management of AE reporting should be clearly specified in the registry protocol, including explanations of the roles, responsibilities, processes, and methods for handling AE reports by the various parties conducting the registry, and for performing followup activities with the site to ensure that complete information is obtained. Sponsors who are stakeholders in a registry should have a representative of their internal drug safety or pharmacovigilance group participate in the design and review of the registry protocol and have a role in the data collection and reporting process (discussed in Chapter 2) to facilitate appropriate and timely reporting and communication.

For postapproval studies financially sponsored by manufacturers, the overall company AE monitoring systems are usually operated by personnel experienced in drug safety (also referred to as pharmacovigilance, regulatory safety, product safety, and safety and risk management). If sites need to report or discuss an AE, they can call the contact number provided for the registry, and are then prompted to press a number if reporting an AE. This number then transfers them to drug safety surveillance so that they can interact directly with personnel in this division and bypass the registry coordinating group. These calls may or may not be tracked by the registry. Alternatively, the registry system can provide instructions to the site on how to report AEs directly to the sponsor's drug safety surveillance division. By this method, the sponsor provides a separate contact number for AE reporting (independent of the registry support staff) that places the site in direct contact with drug safety personnel. This process minimizes the possibility of duplicate AE reports and the potentially complicated reconciliation of two different systems collecting AE information. Use of this process is critical when dealing with products that are available via a registry system as well as outside of a registry system, and it allows sites to have one designated drug safety representative for interaction.

Sponsors of registries designed specifically for surveillance of product safety are strongly encouraged to hold discussions with the regulatory authorities when considering the design of the AE monitoring system. These discussions should be focused on the purpose of the registry, the “best fit” model for AE monitoring, and the timing of routine registry updates. With respect to internal operations chosen by the sponsor to support the requirements of an AE monitoring system, anecdotal feedback suggests that health authorities expect compliance with the agreed-upon requirements. Details regarding implementation are the responsibility of the sponsor.

It should also be noted that FDA's Proposed Rule for Safety Reporting Requirements for Human Drug and Biologics Products (68 FR 12406, March 14, 2003) suggests that the responsible point of contact for FDA should be provided for all expedited and periodic AE reports, and preferably, this individual should be a licensed physician. Although this proposed rule has never been finalized, the principle is similar to the Qualified Person for Pharmacovigilance (QPPV) in Europe, whereby a specific, qualified individual is identified to provide responses to health authorities, upon request, including those regarding AEs reported via the registry system. Updated pharmacovigilance regulations issued by the European Medicines Agency are expected to be implemented in July 2012.[20, 21]

7. Adverse Event Required Reporting for Registry Sponsors

The reporting requirements of the sponsor directly affect how registries should collect and report AEs. Sponsors that are regulated industries are subject to the requirements shown in Table 12–1. ICH guidelines describe standards for expedited reporting[5, 22] and provide recommendations for periodic safety update reports[23] that are generally accepted globally.

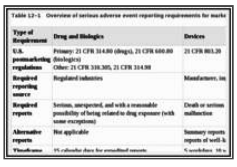


Table 12–1

Overview of serious adverse event reporting requirements for marketed products.

Requirements for regulated industries that sponsor or financially support a registry include expedited reporting of serious and unexpected AEs made known to them via spontaneous reports. For registries, the 15-calendar-day notification applies if the regulated industry believes there is a reasonable possibility that the unexpected SAE was causally related to product exposure. Best practices for international reporting are that all “affiliates” of a sponsor report serious, unexpected, and

possibly related events to the sponsor in a timely fashion, ideally within 2 calendar days; this allows the sponsor, in turn, to complete notification to the responsible regulatory authority within a total of 15 calendar days. Events that do not meet the requirements of expedited reporting (such as nonserious events or serious events considered expected or not related) may require submission through inclusion in an appropriate safety update, such as the New Drug Application or Biologic Licensing Application Annual Report, Periodic Report, or Periodic Safety Update Report, as applicable.[4] In many cases, sponsors are also required to provide registry safety updates to the health authority. Thus, sponsors may coordinate registry safety updates (i.e., determining the date for creating the dataset—the data cutoff date) with the timing of the New Drug Application Annual Report, Periodic Report, Periodic Safety Update Report, or other agreed-upon periodic reporting format. Devices, however, have different reporting requirements (see http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm). In any event, sponsors should discuss safety reporting requirements for their specific registries with the applicable health authorities (such as FDA and European Medicines Agency) before finalizing their registry protocol.

In some cases, a registry sponsor may encourage sites to systematically report all potential SAEs to the sponsor. Given the potential for various assessments by different sites of the seriousness and relatedness of a particular AE—and therefore, inconsistency across sites in the evaluation of a particular AE—this method has certain advantages. In addition, expectedness is not always a straightforward assessment, and the expectedness of events can have significant variability depending on the local approved product labeling. For this reason, it is important that this determination be made by the sponsor and not the reporter of the event. Although this approach may result in substantially greater demands on the sponsor to evaluate all reports, it helps ensure compliance and avoid underreporting. Furthermore, sponsors must make their own assessments regarding the causality of individual solicited events. This requirement typically does not affect the need for reporting, but allows the sponsor to provide its own evaluation in the full context of the safety database. For these reasons, planning for high-quality and consistent training in AE reporting requirements across sites is the preferred approach for a patient registry.

Regardless of who assesses presumed relatedness, sponsors should be prepared to manage the increased volume of AE reports, and sponsors' registry staff should be trained to understand company policy and regulations on AE reporting in order to ensure compliance with local regulations. This training includes the ability to identify and evaluate the attributes of each AE and determine whether the AE should be reported to the health authority in keeping with local regulation. Sponsors are encouraged to appoint a health care practitioner to this role in order to ensure appropriate assessment of the characteristics of an AE.

When biopharmaceutical or device companies are not sponsoring, financially supporting, or participating in a registry in any way, AE reporting is dependent upon the “become aware” principle. If any agent or employee of the company receives information regarding an AE report, the agent or employee must document receipt and comply with internal company policy and regulatory requirements regarding AE reporting, to ensure compliance with applicable drug and device regulations.

8. Special Case: Risk Evaluation and Mitigation Strategies (REMS)

Under the FDA Amendments Act (2007), FDA established a legally enforceable new framework for risk management of products with known safety concerns, called Risk Evaluation and Mitigation Strategies (REMS).[6, 10, 24] The purpose of REMS is to ensure that the benefits of a particular drug outweigh the risks. New REMS programs can be imposed by FDA during clinical development, as part of the approval process, or at any time postapproval, should a new safety signal be identified. Although each REMS is customized depending on the product and associated safety issues, potential components include some combination of—

- A medication guide and/or patient package insert. Medication guides are informational packets distributed with some prescription drugs, which provide important information to patients about possible side effects and drug-drug interactions. The FDA has indicated the situations in which a medication guide is required to be available and distributed to patients.[25] A medication guide alone can and frequently does constitute a REMS.
- A communication plan that specifies targeted education and outreach for physicians, pharmacists, and patients.
- Elements to assure safe use (ETASU), in some cases. ETASU may include restriction of prescribing to health care providers with particular training, experience, and certification; dispensing of the drug in restricted settings; documentation of safe use conditions (such as laboratory results or specific patient monitoring); and registries.[24]

Unlike the less structured disease or exposure registries discussed above, a restricted-access system associated with an ETASU is designed for approved products that have particular risk-benefit profiles that require more careful controls. The purpose of ETASU is to mitigate a certain known drug-associated risk by ensuring that product access is tightly linked to some preventive and/or monitoring measure. Examples include systems that monitor laboratory values, such as white blood cell counts during clozapine administration to prevent severe leucopenia, or routine pregnancy testing during thalidomide administration to prevent in utero exposure of this known teratogenic compound. When these programs include registries, the registries often prospectively collect a battery of information using standardized instruments.

Data collection under ETASU may carry special AE reporting requirements, and as a result of the extensive contact with a variety of potential sources of safety information (e.g., pharmacists and patients), care should be taken to identify all possible routes of reporting. If special requirements exist, they should be made explicit in the registry protocol, with clear definitions of roles, responsibilities, and processes. Training of involved health care providers, such as physicians, nurses, and pharmacists, can be undertaken with written instructions, via telephone or with face-to-face counseling. Training of these health care providers should also extend beyond AE reporting to the specific requirements of the program in question. Such training may include the intended use and associated risk of the product, appropriate patient enrollment, and specific patient monitoring requirements, including guidelines for product discontinuation and management of AEs, as well as topics to cover during comprehensive counseling of patients. The objectives of the ETASU system and overall REMS should be clearly stated (e.g., prevention of in utero exposure during therapy via routine pregnancy testing), and registration forms that document the physician's and pharmacist's attestation of their commitment to requirements of the patient registry system should be completed prior to prescribing or dispensing the product.

9. Reporting Breaches of Confidentiality or Other Risks

In addition to addressing regulatory responsibilities for reporting adverse events, registries must also understand regulatory and ethical requirements and expectations regarding breaches of confidentiality or the reporting of other risks to patients that may arise during the course of a registry. The Health Information Technology for Economic and Clinical Health Act (HITECH Act) requires HIPAA-covered entities (entities covered by the Health Insurance Portability and Accountability Act of 1996) and their business associates to provide notification following a breach of unsecured protected health information.[26] See Chapter 7 for a detailed discussion of the HITECH Act. State breach notification laws may also apply to registry data.

Beyond these legal requirements, registries should establish clear notification procedures for breaches of confidentiality or other risks that become known during the course of the registry, whether or not they are governed by HIPAA or subject to State laws.

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VACCINE SCHEDULE & INSERTS

CDC Vaccine Schedule

Recommended Child and Adolescent Immunization Schedule
for ages 18 years or younger

UNITED STATES
2022

Vaccines in the Child and Adolescent Immunization Schedule*

Vaccine	Abbreviation(s)	Trade name(s)
Dengue vaccine	**DEN4CYD**	Dengvaxia®
Diphtheria, tetanus, and acellular pertussis vaccine	**DTaP**	Daptacel® Infanrix®
Diphtheria, tetanus vaccine	**DT**	No trade name
Haemophilus influenzae type b vaccine	**Hib (PRP-T)** **Hib (PRP-OMP)**	ActHIB® Hiberix® PedvaxHIB®
Hepatitis A vaccine	**HepA**	Havrix® Vaqta®
Hepatitis B vaccine	**HepB**	Engerix-B® Recombivax HB®
Human papillomavirus vaccine	**HPV**	Gardasil 9®
Influenza vaccine (inactivated)	**IIV4**	Multiple
Influenza vaccine (live, attenuated)	**LAIV4**	FluMist® Quadrivalent
Measles, mumps, and rubella vaccine	**MMR**	M-M-R II®
Meningococcal serogroups A, C, W, Y vaccine	**MenACWY-D**	Menactra®
	MenACWY-CRM	Menveo®
	MenACWY-TT	MenQuadfi®
Meningococcal serogroup B vaccine	**MenB-4C**	Bexsero®
	MenB-FHbp	Trumenba®
Pneumococcal 13-valent conjugate vaccine	**PCV13**	Prevnar 13®
Pneumococcal 23-valent polysaccharide vaccine	**PPSV23**	Pneumovax 23®
Poliovirus vaccine (inactivated)	**IPV**	IPOL®
Rotavirus vaccine	**RV1** **RV5**	Rotarix® RotaTeq®
Tetanus, diphtheria, and acellular pertussis vaccine	**Tdap**	Adacel® Boostrix®
Tetanus and diphtheria vaccine	**Td**	Tenivac® Tdvax™
Varicella vaccine	**VAR**	Varivax®
Combination vaccines *(use combination vaccines instead of separate injections when appropriate)*		
DTaP, hepatitis B, and inactivated poliovirus vaccine	**DTaP-HepB-IPV**	Pediarix®
DTaP, inactivated poliovirus, and *Haemophilus influenzae* type b vaccine	**DTaP-IPV/Hib**	Pentacel®
DTaP and inactivated poliovirus vaccine	**DTaP-IPV**	Kinrix® Quadracel®
DTaP, inactivated poliovirus, *Haemophilus influenzae* type b, and hepatitis B vaccine	**DTaP-IPV-Hib-HepB**	Vaxelis®
Measles, mumps, rubella, and varicella vaccine	**MMRV**	ProQuad®

*Administer recommended vaccines if immunization history is incomplete or unknown. Do not restart or add doses to vaccine series for extended intervals between doses. When a vaccine is not administered at the recommended age, administer at a subsequent visit. The use of trade names is for identification purposes only and does not imply endorsement by the ACIP or CDC.

How to use the child and adolescent immunization schedule

1 Determine recommended vaccine by age **(Table 1)**

2 Determine recommended interval for catch-up vaccination **(Table 2)**

3 Assess need for additional recommended vaccines by medical condition or other indication **(Table 3)**

4 Review vaccine types, frequencies, intervals, and considerations for special situations **(Notes)**

5 Review contraindications and precautions for vaccine types **(Appendix)**

Recommended by the Advisory Committee on Immunization Practices (www.cdc.gov/vaccines/acip) and approved by the Centers for Disease Control and Prevention (www.cdc.gov), American Academy of Pediatrics (www.aap.org), American Academy of Family Physicians (www.aafp.org), American College of Obstetricians and Gynecologists (www.acog.org), American College of Nurse-Midwives (www.midwife.org), American Academy of Physician Associates (www.aapa.org), and National Association of Pediatric Nurse Practitioners (www.napnap.org).

Report

- Suspected cases of reportable vaccine-preventable diseases or outbreaks to your state or local health department
- Clinically significant adverse events to the Vaccine Adverse Event Reporting System (VAERS) at www.vaers.hhs.gov or 800-822-7967

Questions or comments

Contact www.cdc.gov/cdc-info or 800-CDC-INFO (800-232-4636), in English or Spanish, 8 a.m.–8 p.m. ET, Monday through Friday, excluding holidays

Download the CDC Vaccine Schedules app for providers at www.cdc.gov/vaccines/schedules/hcp/schedule-app.html

Helpful information

- Complete Advisory Committee on Immunization Practices (ACIP) recommendations: www.cdc.gov/vaccines/hcp/acip-recs/index.html
- *General Best Practice Guidelines for Immunization* (including contraindications and precautions): www.cdc.gov/vaccines/hcp/acip-recs/general-recs/index.html
- Vaccine information statements: www.cdc.gov/vaccines/hcp/vis/index.html
- Manual for the Surveillance of Vaccine-Preventable Diseases (including case identification and outbreak response): www.cdc.gov/vaccines/pubs/surv-manual
- ACIP Shared Clinical Decision-Making Recommendations www.cdc.gov/vaccines/acip/acip-scdm-faqs.html

Scan QR code for access to online schedule

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

CS310020-A

Table 1 Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2022

These recommendations must be read with the notes that follow. For those who fall behind or start late, provide catch-up vaccination at the earliest opportunity as indicated by the green bars.
To determine minimum intervals between doses, see the catch-up schedule (Table 2).

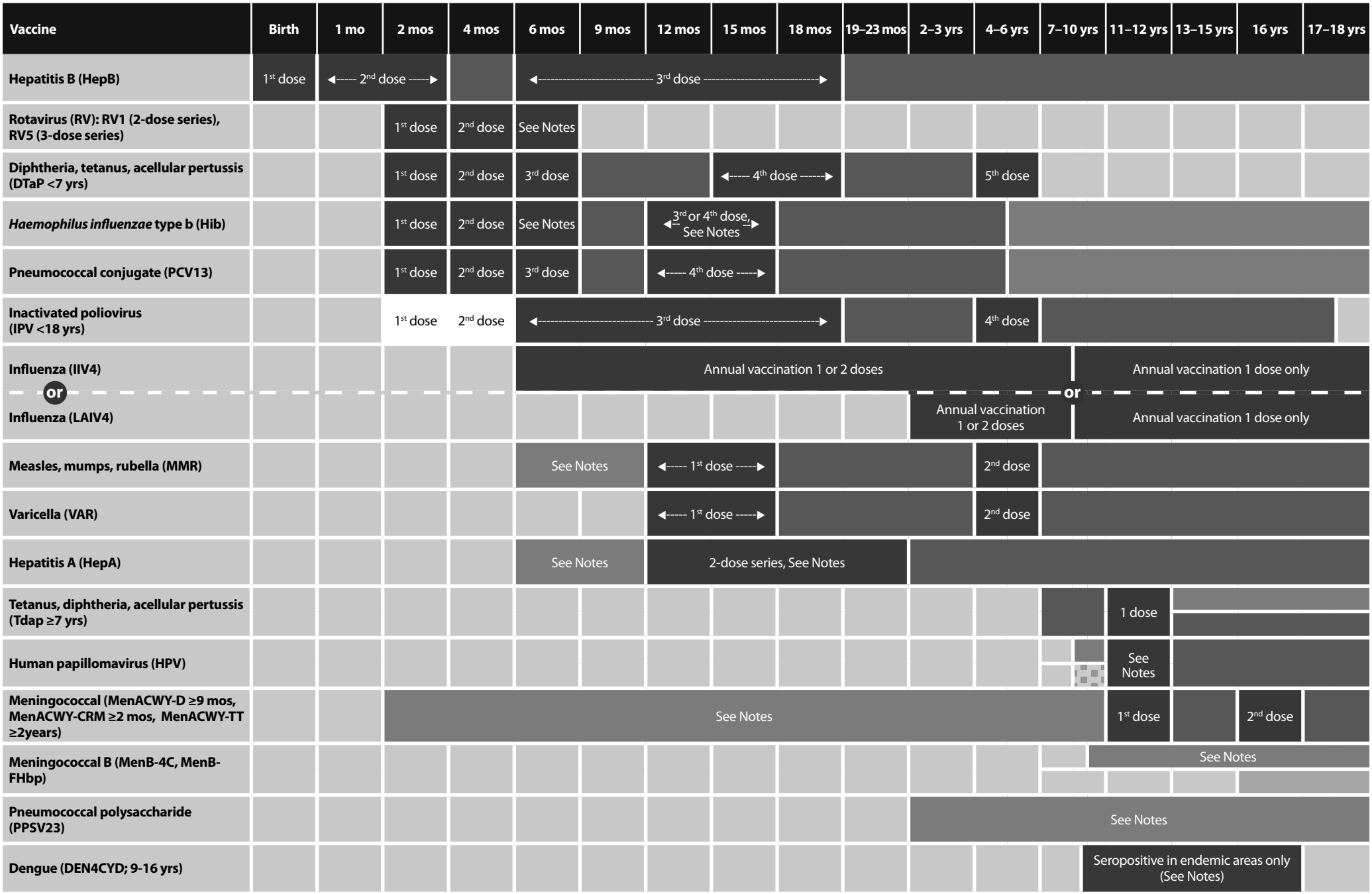

Table 2 Recommended Catch-up Immunization Schedule for Children and Adolescents Who Start Late or Who Are More than 1 Month Behind, United States, 2022

The table below provides catch-up schedules and minimum intervals between doses for children whose vaccinations have been delayed. A vaccine series does not need to be restarted, regardless of the time that has elapsed between doses. Use the section appropriate for the child's age. **Always use this table in conjunction with Table 1 and the Notes that follow.**

Vaccine	Minimum Age for Dose 1	Minimum Interval Between Doses			
		Dose 1 to Dose 2	Dose 2 to Dose 3	Dose 3 to Dose 4	Dose 4 to Dose 5
Children age 4 months through 6 years					
Hepatitis B	Birth	**4 weeks**	**8 weeks *and* at least 16 weeks after first dose** minimum age for the final dose is 24 weeks		
Rotavirus	6 weeks Maximum age for first dose is 14 weeks, 6 days.	**4 weeks**	**4 weeks** maximum age for final dose is 8 months, 0 days		
Diphtheria, tetanus, and acellular pertussis	6 weeks	**4 weeks**	**4 weeks**	**6 months**	**6 months**
Haemophilus influenzae type b	6 weeks	**No further doses needed** if first dose was administered at age 15 months or older. **4 weeks** if first dose was administered before the 1st birthday. **8 weeks (as final dose)** if first dose was administered at age 12 through 14 months.	**No further doses needed** if previous dose was administered at age 15 months or older **4 weeks** if current age is younger than 12 months ***and*** first dose was administered at younger than age 7 months ***and*** at least 1 previous dose was PRP-T (ActHib®, Pentacel®, Hiberix®), Vaxelis® or unknown **8 weeks *and* age 12 through 59 months (as final dose)** if current age is younger than 12 months ***and*** first dose was administered at age 7 through 11 months; OR if current age is 12 through 59 months ***and*** first dose was administered before the 1st birthday ***and*** second dose was administered at younger than 15 months; OR if both doses were PedvaxHIB® and were administered before the 1st birthday	**8 weeks (as final dose)** This dose only necessary for children age 12 through 59 months who received 3 doses before the 1st birthday.	
Pneumococcal conjugate	6 weeks	**No further doses needed** for healthy children if first dose was administered at age 24 months or older **4 weeks** if first dose was administered before the 1st birthday **8 weeks (as final dose for healthy children)** if first dose was administered at the 1st birthday or after	**No further doses needed** for healthy children if previous dose was administered at age 24 months or older **4 weeks** if current age is younger than 12 months and previous dose was administered at <7 months old **8 weeks (as final dose for healthy children)** if previous dose was administered between 7–11 months (wait until at least 12 months old); OR if current age is 12 months or older and at least 1 dose was administered before age 12 months	**8 weeks (as final dose)** This dose only necessary for children age 12 through 59 months who received 3 doses before age 12 months or for children at high risk who received 3 doses at any age.	
Inactivated poliovirus	6 weeks	**4 weeks**	**4 weeks** if current age is <4 years **6 months (as final dose)** if current age is 4 years or older	**6 months (minimum age 4 years for final dose)**	
Measles, mumps, rubella	12 months	**4 weeks**			
Varicella	12 months	**3 months**			
Hepatitis A	12 months	**6 months**			
Meningococcal ACWY	2 months MenACWY-CRM 9 months MenACWY-D 2 years MenACWY-TT	**8 weeks**	See Notes	See Notes	
Children and adolescents age 7 through 18 years					
Meningococcal ACWY	Not applicable (N/A)	**8 weeks**			
Tetanus, diphtheria; tetanus, diphtheria, and acellular pertussis	7 years	**4 weeks**	**4 weeks** if first dose of DTaP/DT was administered before the 1st birthday **6 months (as final dose)** if first dose of DTaP/DT or Tdap/Td was administered at or after the 1st birthday	**6 months** if first dose of DTaP/DT was administered before the 1st birthday	
Human papillomavirus	9 years	**Routine dosing intervals are recommended.**			
Hepatitis A	N/A	**6 months**			
Hepatitis B	N/A	**4 weeks**	**8 weeks *and* at least 16 weeks after first dose**		
Inactivated poliovirus	N/A	**4 weeks**	**6 months** A fourth dose is not necessary if the third dose was administered at age 4 years or older and at least 6 months after the previous dose.	A fourth dose of IPV is indicated if all previous doses were administered at <4 years or if the third dose was administered <6 months after the second dose.	
Measles, mumps, rubella	N/A	**4 weeks**			
Varicella	N/A	**3 months** if younger than age 13 years. **4 weeks** if age 13 years or older			
Dengue	9 years	**6 months**	6 months		

Table 3

Recommended Child and Adolescent Immunization Schedule by Medical Indication, United States, 2022

Always use this table in conjunction with Table 1 and the Notes that follow.

VACCINE	INDICATION									
	Pregnancy	Immunocompromised status (excluding HIV infection)	HIV infection CD4+ count[1]		Kidney failure, end-stage renal disease, or on hemodialysis	Heart disease or chronic lung disease	CSF leak or cochlear implant	Asplenia or persistent complement component deficiencies	Chronic liver disease	Diabetes
			<15% or total CD4 cell count of <200/mm³	≥15% and total CD4 cell count of ≥200/mm³						
Hepatitis B										
Rotavirus		SCID[2]								
Diphtheria, tetanus, and acellular pertussis (DTaP)										
Haemophilus influenzae type b										
Pneumococcal conjugate										
Inactivated poliovirus										
Influenza (IIV4)										
or										
Influenza (LAIV4)						Asthma, wheezing: 2–4yrs[3]				
Measles, mumps, rubella	*									
Varicella	*									
Hepatitis A										
Tetanus, diphtheria, and acellular pertussis (Tdap)										
Human papillomavirus	*									
Meningococcal ACWY										
Meningococcal B										
Pneumococcal polysaccharide										
Dengue										

- Vaccination according to the routine schedule recommended
- Recommended for persons with an additional risk factor for which the vaccine would be indicated
- Vaccination is recommended, and additional doses may be necessary based on medical condition or vaccine. See Notes.
- Precaution—vaccine might be indicated if benefit of protection outweighs risk of adverse reaction
- Contraindicated or not recommended—vaccine should not be administered
- No recommendation/not applicable

*Vaccinate after pregnancy

1 For additional information regarding HIV laboratory parameters and use of live vaccines, see the *General Best Practice Guidelines for Immunization*, "Altered Immunocompetence," at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/immunocompetence.html and Table 4-1 (footnote J) at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html.
2 Severe Combined Immunodeficiency
3 LAIV4 contraindicated for children 2–4 years of age with asthma or wheezing during the preceding 12 months

Notes

Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2022

For vaccination recommendations for persons ages 19 years or older, see the Recommended Adult Immunization Schedule, 2022.

Additional information

COVID-19 Vaccination

COVID-19 vaccines are recommended for use within the scope of the Emergency Use Authorization or Biologics License Application for the particular vaccine. ACIP recommendations for the use of COVID-19 vaccines can be found at www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/covid-19.html**.**

CDC's interim clinical considerations for use of COVID-19 vaccines can be found at www.cdc.gov/vaccines/covid-19/clinical-considerations/covid-19-vaccines-us.html**.**

- Consult relevant ACIP statements for detailed recommendations at www.cdc.gov/vaccines/hcp/acip-recs/index.html.
- For calculating intervals between doses, 4 weeks = 28 days. Intervals of ≥4 months are determined by calendar months.
- Within a number range (e.g., 12–18), a dash (–) should be read as "through."
- Vaccine doses administered ≤4 days before the minimum age or interval are considered valid. Doses of any vaccine administered ≥5 days earlier than the minimum age or minimum interval should not be counted as valid and should be repeated as age appropriate. **The repeat dose should be spaced after the invalid dose by the recommended minimum interval.** For further details, see Table 3-1, Recommended and minimum ages and intervals between vaccine doses, in *General Best Practice Guidelines for Immunization* at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/timing.html.
- Information on travel vaccination requirements and recommendations is available at www.cdc.gov/travel/.
- For vaccination of persons with immunodeficiencies, see Table 8-1, Vaccination of persons with primary and secondary immunodeficiencies, in *General Best Practice Guidelines for Immunization* at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/immunocompetence.html, and Immunization in Special Clinical Circumstances (In: Kimberlin DW, Brady MT, Jackson MA, Long SS, eds. *Red Book: 2018 Report of the Committee on Infectious Diseases*. 31st ed. Itasca, IL: American Academy of Pediatrics; 2018:67–111).
- For information about vaccination in the setting of a vaccine-preventable disease outbreak, contact your state or local health department.
- The National Vaccine Injury Compensation Program (VICP) is a no-fault alternative to the traditional legal system for resolving vaccine injury claims. All routine child and adolescent vaccines are covered by VICP except for pneumococcal polysaccharide vaccine (PPSV23). For more information, see www.hrsa.gov/vaccinecompensation/index.html.

Dengue vaccination (minimum age: 9 years)

Routine vaccination

- Age 9–16 years living in dengue endemic areas **AND** have laboratory confirmation of previous dengue infection
  - 3-dose series administered at 0, 6, and 12 months
- Endemic areas include Puerto Rico, American Samoa, US Virgin Islands, Federated States of Micronesia, Republic of Marshall Islands, and the Republic of Palau. For updated guidance on dengue endemic areas and pre-vaccination laboratory testing see www.cdc.gov/mmwr/volumes/70/rr/rr7006a1.htm?s_cid=rr7006a1_w and www.cdc.gov/dengue/vaccine/hcp/index.html

Diphtheria, tetanus, and pertussis (DTaP) vaccination (minimum age: 6 weeks [4 years for Kinrix® or Quadracel®])

Routine vaccination

- 5-dose series at age 2, 4, 6, 15–18 months, 4–6 years
  - **Prospectively:** Dose 4 may be administered as early as age 12 months if at least 6 months have elapsed since dose 3.
  - **Retrospectively:** A 4th dose that was inadvertently administered as early as age 12 months may be counted if at least 4 months have elapsed since dose 3.

Catch-up vaccination

- Dose 5 is not necessary if dose 4 was administered at age 4 years or older and at least 6 months after dose 3.
- For other catch-up guidance, see Table 2.

Special situations

- Wound management in children less than age 7 years with history of 3 or more doses of tetanus-toxoid-containing vaccine: For all wounds except clean and minor wounds, administer DTaP if more than 5 years since last dose of tetanus-toxoid-containing vaccine. For detailed information, see www.cdc.gov/mmwr/volumes/67/rr/rr6702a1.htm.

Haemophilus influenzae type b vaccination (minimum age: 6 weeks)

Routine vaccination

- **ActHIB®, Hiberix®, Pentacel®, or Vaxelis®:** 4-dose series (3 dose primary series at age 2, 4, and 6 months, followed by a booster dose* at age 12–15 months)
  - *Vaxelis® is not recommended for use as a booster dose. A different Hib-containing vaccine should be used for the booster dose.
- **PedvaxHIB®:** 3-dose series (2-dose primary series at age 2 and 4 months, followed by a booster dose at age 12–15 months)

Catch-up vaccination

- **Dose 1 at age 7–11 months:** Administer dose 2 at least 4 weeks later and dose 3 (final dose) at age 12–15 months or 8 weeks after dose 2 (whichever is later).
- **Dose 1 at age 12–14 months:** Administer dose 2 (final dose) at least 8 weeks after dose 1.
- **Dose 1 before age 12 months and dose 2 before age 15 months:** Administer dose 3 (final dose) at least 8 weeks after dose 2.
- **2 doses of PedvaxHIB® before age 12 months:** Administer dose 3 (final dose) at 12–59 months and at least 8 weeks after dose 2.
- **1 dose administered at age 15 months or older:** No further doses needed
- **Unvaccinated at age 15–59 months:** Administer 1 dose.
- **Previously unvaccinated children age 60 months or older who are not considered high risk:** Do not require catch-up vaccination

For other catch-up guidance, see Table 2. Vaxelis® can be used for catch-up vaccination in children less than age 5 years. Follow the catch-up schedule even if Vaxelis® is used for one or more doses. For detailed information on use of Vaxelis® see www.cdc.gov/mmwr/volumes/69/wr/mm6905a5.htm.

Special situations

- **Chemotherapy or radiation treatment:**
  Age 12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Doses administered within 14 days of starting therapy or during therapy should be repeated at least 3 months after therapy completion.
- **Hematopoietic stem cell transplant (HSCT):**
  - 3-dose series 4 weeks apart starting 6 to 12 months after successful transplant, regardless of Hib vaccination history
- **Anatomic or functional asplenia (including sickle cell disease):**
  Age 12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Unvaccinated persons age 5 years or older*
  - 1 dose
- **Elective splenectomy:**
  Unvaccinated persons age 15 months or older*
  - 1 dose (preferably at least 14 days before procedure)
- **HIV infection:**
  Age 12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

  Unvaccinated persons age 5–18 years*
  - 1 dose
- **Immunoglobulin deficiency, early component complement deficiency:**
  Age 12–59 months
  - Unvaccinated or only 1 dose before age 12 months: 2 doses, 8 weeks apart
  - 2 or more doses before age 12 months: 1 dose at least 8 weeks after previous dose

**Unvaccinated* = Less than routine series (through age 14 months) OR no doses (age 15 months or older)*

Notes

Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2022

Hepatitis A vaccination
(minimum age: 12 months for routine vaccination)

Routine vaccination
- 2-dose series (minimum interval: 6 months) at age 12–23 months

Catch-up vaccination
- Unvaccinated persons through age 18 years should complete a 2-dose series (minimum interval: 6 months).
- Persons who previously received 1 dose at age 12 months or older should receive dose 2 at least 6 months after dose 1.
- Adolescents age 18 years or older may receive the combined HepA and HepB vaccine, **Twinrix®**, as a 3-dose series (0, 1, and 6 months) or 4-dose series (3 doses at 0, 7, and 21–30 days, followed by a booster dose at 12 months).

International travel
- Persons traveling to or working in countries with high or intermediate endemic hepatitis A (www.cdc.gov/travel/):
  - **Infants age 6–11 months**: 1 dose before departure; revaccinate with 2 doses, separated by at least 6 months, between age 12–23 months.
  - **Unvaccinated age 12 months or older**: Administer dose 1 as soon as travel is considered.

Hepatitis B vaccination
(minimum age: birth)

Birth dose (monovalent HepB vaccine only)
- **Mother is HBsAg-negative:**
  - **All** medically stable infants ≥2,000 grams: 1 dose within 24 hours of birth
  - Infants <2,000 grams: Administer 1 dose at chronological age 1 month or hospital discharge (whichever is earlier and even if weight is still <2,000 grams).
- **Mother is HBsAg-positive:**
  - Administer **HepB vaccine** and **hepatitis B immune globulin (HBIG)** (in separate limbs) within 12 hours of birth, regardless of birth weight. For infants <2,000 grams, administer 3 additional doses of vaccine (total of 4 doses) beginning at age 1 month.
  - Test for HBsAg and anti-HBs at age 9–12 months. If HepB series is delayed, test 1–2 months after final dose.
- **Mother's HBsAg status is unknown:**
  - Administer **HepB vaccine** within 12 hours of birth, regardless of birth weight.
  - For infants <2,000 grams, administer **HBIG** in addition to HepB vaccine (in separate limbs) within 12 hours of birth. Administer 3 additional doses of vaccine (total of 4 doses) beginning at age 1 month.
  - Determine mother's HBsAg status as soon as possible. If mother is HBsAg-positive, administer **HBIG** to infants ≥2,000 grams as soon as possible, but no later than 7 days of age.

Routine series
- 3-dose series at age 0, 1–2, 6–18 months (use monovalent HepB vaccine for doses administered before age 6 weeks)
- Infants who did not receive a birth dose should begin the series as soon as feasible (see Table 2).
- Administration of **4 doses** is permitted when a combination vaccine containing HepB is used after the birth dose.
- **Minimum age** for the final (3rd or 4th) dose: 24 weeks
- **Minimum intervals:** dose 1 to dose 2: 4 weeks / dose 2 to dose 3: 8 weeks / dose 1 to dose 3: 16 weeks (when 4 doses are administered, substitute "dose 4" for "dose 3" in these calculations)

Catch-up vaccination
- Unvaccinated persons should complete a 3-dose series at 0, 1–2, 6 months.
- Adolescents age 11–15 years may use an alternative 2-dose schedule with at least 4 months between doses (adult formulation **Recombivax HB®** only).
- Adolescents age 18 years or older may receive a 2-dose series of HepB **(Heplisav-B®)** at least 4 weeks apart.
- Adolescents age 18 years or older may receive the combined HepA and HepB vaccine, **Twinrix®**, as a 3-dose series (0, 1, and 6 months) or 4-dose series (3 doses at 0, 7, and 21–30 days, followed by a booster dose at 12 months).
- For other catch-up guidance, see Table 2.

Special situations
- Revaccination is not generally recommended for persons with a normal immune status who were vaccinated as infants, children, adolescents, or adults.
- **Post-vaccination serology testing and revaccination** (if anti-HBs < 10mIU/mL) is recommended for certain populations, including:
  - **Infants born to HBsAg-positive mothers**
  - **Hemodialysis patients**
  - **Other immunocompromised persons**

For detailed revaccination recommendations, see www.cdc.gov/vaccines/hcp/acip-recs/vacc-specific/hepb.html.

Human papillomavirus vaccination
(minimum age: 9 years)

Routine and catch-up vaccination
- HPV vaccination routinely recommended at **age 11–12 years (can start at age 9 years)** and catch-up HPV vaccination recommended for all persons through age 18 years if not adequately vaccinated
- 2- or 3-dose series depending on age at initial vaccination:
  - **Age 9–14 years at initial vaccination**: 2-dose series at 0, 6–12 months (minimum interval: 5 months; repeat dose if administered too soon)
  - **Age 15 years or older at initial vaccination**: 3-dose series at 0, 1–2 months, 6 months (minimum intervals: dose 1 to dose 2: 4 weeks / dose 2 to dose 3: 12 weeks / dose 1 to dose 3: 5 months; repeat dose if administered too soon)
- **Interrupted schedules:** If vaccination schedule is interrupted, the series does not need to be restarted.
- No additional dose recommended when any HPV vaccine series has been completed using the recommended dosing intervals.

Special situations
- **Immunocompromising conditions, including HIV infection:** 3-dose series, even for those who initiate vaccination at age 9 through 14 years.
- **History of sexual abuse or assault:** Start at age 9 years.
- **Pregnancy:** Pregnancy testing not needed before vaccination; HPV vaccination not recommended until after pregnancy; no intervention needed if vaccinated while pregnant

Influenza vaccination
(minimum age: 6 months [IIV], 2 years [LAIV4], 18 years [recombinant influenza vaccine, RIV4])

Routine vaccination
- Use any influenza vaccine appropriate for age and health status annually:
  - 2 doses, separated by at least 4 weeks, for **children age 6 months–8 years** who have received fewer than 2 influenza vaccine doses before July 1, 2021, or whose influenza vaccination history is unknown (administer dose 2 even if the child turns 9 between receipt of dose 1 and dose 2)
  - 1 dose for **children age 6 months–8 years** who have received at least 2 influenza vaccine doses before July 1, 2021
  - 1 dose for **all persons age 9 years or older**
- For the 2021-2022 season, see www.cdc.gov/mmwr/volumes/70/rr/rr7005a1.htm.
- For the 2022–23 season, see the 2022–23 ACIP influenza vaccine recommendations.

Special situations
- **Egg allergy, hives only**: Any influenza vaccine appropriate for age and health status annually
- **Egg allergy with symptoms other than hives** (e.g., angioedema, respiratory distress) or required epinephrine or another emergency medical intervention: see Appendix listing contraindications and precautions
- **Severe allergic reaction (e.g., anaphylaxis) to a vaccine component or a previous dose of any influenza vaccine:** see Appendix listing contraindications and precautions

Measles, mumps, and rubella vaccination
(minimum age: 12 months for routine vaccination)

Routine vaccination
- 2-dose series at age 12–15 months, age 4–6 years
- MMR or MMRV may be administered

Note: For dose 1 in children age 12–47 months, it is recommended to administer MMR and varicella vaccines separately. MMRV may be used if parents or caregivers express a preference.

Catch-up vaccination
- Unvaccinated children and adolescents: 2-dose series at least 4 weeks apart
- The maximum age for use of MMRV is 12 years.
- Minimum interval between MMRV doses: 3 months

Special situations
International travel
- **Infants age 6–11 months:** 1 dose before departure; revaccinate with 2-dose series at age 12–15 months (12 months for children in high-risk areas) and dose 2 as early as 4 weeks later.
- **Unvaccinated children age 12 months or older:** 2-dose series at least 4 weeks apart before departure

Notes

Meningococcal serogroup A,C,W,Y vaccination
(minimum age: 2 months [MenACWY-CRM, Menveo], 9 months [MenACWY-D, Menactra], 2 years [MenACWY-TT, MenQuadfi])

Routine vaccination
- 2-dose series at age 11–12 years; 16 years

Catch-up vaccination
- Age 13–15 years: 1 dose now and booster at age 16–18 years (minimum interval: 8 weeks)
- Age 16–18 years: 1 dose

Special situations
Anatomic or functional asplenia (including sickle cell disease), HIV infection, persistent complement component deficiency, complement inhibitor (e.g., eculizumab, ravulizumab) use:
- **Menveo**
  - Dose 1 at age 2 months: 4-dose series (additional 3 doses at age 4, 6 and 12 months)
  - Dose 1 at age 3–6 months: 3- or 4- dose series (dose 2 [and dose 3 if applicable] at least 8 weeks after previous dose until a dose is received at age 7 months or older, followed by an additional dose at least 12 weeks later and after age 12 months)
  - Dose 1 at age 7–23 months: 2-dose series (dose 2 at least 12 weeks after dose 1 and after age 12 months)
  - Dose 1 at age 24 months or older: 2-dose series at least 8 weeks apart
- **Menactra**
  - **Persistent complement component deficiency or complement inhibitor use:**
    - Age 9–23 months: 2-dose series at least 12 weeks apart
    - Age 24 months or older: 2-dose series at least 8 weeks apart
  - **Anatomic or functional asplenia, sickle cell disease, or HIV infection:**
    - Age 9–23 months: Not recommended
    - Age 24 months or older: 2-dose series at least 8 weeks apart
    - **Menactra®** must be administered at least 4 weeks after completion of PCV13 series.
- **MenQuadfi®**
  - Dose 1 at age 24 months or older: 2-dose series at least 8 weeks apart

Travel in countries with hyperendemic or epidemic meningococcal disease, including countries in the African meningitis belt or during the Hajj (www.cdc.gov/travel/)**:**
- Children less than age 24 months:
  - **Menveo® (age 2–23 months)**
    - Dose 1 at age 2 months: 4-dose series (additional 3 doses at age 4, 6 and 12 months)
    - Dose 1 at age 3–6 months: 3- or 4- dose series (dose 2 [and dose 3 if applicable] at least 8 weeks after previous dose until a dose is received at age 7 months or older, followed by an additional dose at least 12 weeks later and after age 12 months)
    - Dose 1 at age 7–23 months: 2-dose series (dose 2 at least 12 weeks after dose 1 and after age 12 months)
  - **Menactra® (age 9–23 months)**
    - 2-dose series (dose 2 at least 12 weeks after dose 1; dose 2 may be administered as early as 8 weeks after dose 1 in travelers)
- Children age 2 years or older: 1 dose Menveo®, Menactra®, or MenQuadfi®

First-year college students who live in residential housing (if not previously vaccinated at age 16 years or older) or military recruits:
- 1 dose **Menveo®, Menactra®,** or **MenQuadfi®**

Adolescent vaccination of children who received MenACWY prior to age 10 years:
- **Children for whom boosters are recommended** because of an ongoing increased risk of meningococcal disease (e.g., those with complement deficiency, HIV, or asplenia): Follow the booster schedule for persons at increased risk.
- **Children for whom boosters are not recommended** (e.g., a healthy child who received a single dose for travel to a country where meningococcal disease is endemic): Administer MenACWY according to the recommended adolescent schedule with dose 1 at age 11–12 years and dose 2 at age 16 years.

Note: Menactra® should be administered either before or at the same time as DTaP. MenACWY vaccines may be administered simultaneously with MenB vaccines if indicated, but at a different anatomic site, if feasible.

For MenACWY **booster dose recommendations** for groups listed under "Special situations" and in an outbreak setting and additional meningococcal vaccination information, see www.cdc.gov/mmwr/volumes/69/rr/rr6909a1.htm.

Meningococcal serogroup B vaccination
(minimum age: 10 years [MenB-4C, Bexsero®; MenB-FHbp, Trumenba®])

Shared clinical decision-making
- **Adolescents not at increased risk** age 16–23 years (preferred age 16–18 years) based on shared clinical decision-making:
  - **Bexsero®:** 2-dose series at least 1 month apart
  - **Trumenba®:** 2-dose series at least 6 months apart; if dose 2 is administered earlier than 6 months, administer a 3rd dose at least 4 months after dose 2.

Special situations
Anatomic or functional asplenia (including sickle cell disease), persistent complement component deficiency, complement inhibitor (e.g., eculizumab, ravulizumab) use:
- **Bexsero®:** 2-dose series at least 1 month apart
- **Trumenba®:** 3-dose series at 0, 1–2, 6 months

Note: Bexsero® and **Trumenba®** are not interchangeable; the same product should be used for all doses in a series.

For MenB **booster dose recommendations** for groups listed under "Special situations" and in an outbreak setting and additional meningococcal vaccination information, see www.cdc.gov/mmwr/volumes/69/rr/rr6909a1.htm.

Pneumococcal vaccination
(minimum age: 6 weeks [PCV13], 2 years [PPSV23])

Routine vaccination with PCV13
- 4-dose series at age 2, 4, 6, 12–15 months

Catch-up vaccination with PCV13
- 1 dose for healthy children age 24–59 months with any incomplete* PCV13 series
- For other catch-up guidance, see Table 2.

Special situations
Underlying conditions below: When both PCV13 and PPSV23 are indicated, administer PCV13 first. PCV13 and PPSV23 should not be administered during same visit.

Chronic heart disease (particularly cyanotic congenital heart disease and cardiac failure); chronic lung disease (including asthma treated with high-dose, oral corticosteroids); diabetes mellitus:

Age 2–5 years
- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after completing all recommended PCV13 doses)

Age 6–18 years
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after completing all recommended PCV13 doses)

Cerebrospinal fluid leak, cochlear implant:

Age 2–5 years
- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

Age 6–18 years
- No history of either PCV13 or PPSV23: 1 dose PCV13, 1 dose PPSV23 at least 8 weeks later
- Any PCV13 but no PPSV23: 1 dose PPSV23 at least 8 weeks after the most recent dose of PCV13
- PPSV23 but no PCV13: 1 dose PCV13 at least 8 weeks after the most recent dose of PPSV23

Sickle cell disease and other hemoglobinopathies; anatomic or functional asplenia; congenital or acquired immunodeficiency; HIV infection; chronic renal failure; nephrotic syndrome; malignant neoplasms, leukemias, lymphomas, Hodgkin disease, and other diseases associated with treatment with immunosuppressive drugs or radiation therapy; solid organ transplantation; multiple myeloma:

Age 2–5 years
- Any incomplete* series with:
  - 3 PCV13 doses: 1 dose PCV13 (at least 8 weeks after any prior PCV13 dose)
  - Less than 3 PCV13 doses: 2 doses PCV13 (8 weeks after the most recent dose and administered 8 weeks apart)
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose) and a dose 2 of PPSV23 5 years later

Age 6–18 years
- No history of either PCV13 or PPSV23: 1 dose PCV13, 2 doses PPSV23 (dose 1 of PPSV23 administered 8 weeks after PCV13 and dose 2 of PPSV23 administered at least 5 years after dose 1 of PPSV23)
- Any PCV13 but no PPSV23: 2 doses PPSV23 (dose 1 of PPSV23 administered 8 weeks after the most recent dose of PCV13 and dose 2 of PPSV23 administered at least 5 years after dose 1 of PPSV23)
- PPSV23 but no PCV13: 1 dose PCV13 at least 8 weeks after the most recent PPSV23 dose and a dose 2 of PPSV23 administered 5 years after dose 1 of PPSV23 and at least 8 weeks after a dose of PCV13

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Chronic liver disease, alcoholism:
Age 6–18 years
- No history of PPSV23: 1 dose PPSV23 (at least 8 weeks after any prior PCV13 dose)

**Incomplete series* = Not having received all doses in either the recommended series or an age-appropriate catch-up series See Tables 8, 9, and 11 in the ACIP pneumococcal vaccine recommendations (www.cdc.gov/mmwr/pdf/rr/rr5911.pdf) for complete schedule details.

Poliovirus vaccination
(minimum age: 6 weeks)

Routine vaccination
- 4-dose series at ages 2, 4, 6–18 months, 4–6 years; administer the final dose on or after age 4 years and at least 6 months after the previous dose.
- 4 or more doses of IPV can be administered before age 4 years when a combination vaccine containing IPV is used. However, a dose is still recommended on or after age 4 years and at least 6 months after the previous dose.

Catch-up vaccination
- In the first 6 months of life, use minimum ages and intervals only for travel to a polio-endemic region or during an outbreak.
- IPV is not routinely recommended for U.S. residents age 18 years or older.

Series containing oral polio vaccine (OPV), either mixed OPV-IPV or OPV-only series:
- Total number of doses needed to complete the series is the same as that recommended for the U.S. IPV schedule. See www.cdc.gov/mmwr/volumes/66/wr/mm6601a6.htm?s_%20cid=mm6601a6_w.
- Only trivalent OPV (tOPV) counts toward the U.S. vaccination requirements.
  - Doses of OPV administered before April 1, 2016, should be counted (unless specifically noted as administered during a campaign).
  - Doses of OPV administered on or after April 1, 2016, should not be counted.
  - For guidance to assess doses documented as "OPV," see www.cdc.gov/mmwr/volumes/66/wr/mm6606a7.htm?s_cid=mm6606a7_w.
- For other catch-up guidance, see Table 2.

Rotavirus vaccination
(minimum age: 6 weeks)

Routine vaccination
- **Rotarix®:** 2-dose series at age 2 and 4 months
- **RotaTeq®:** 3-dose series at age 2, 4, and 6 months
- If any dose in the series is either **RotaTeq®** or unknown, default to 3-dose series.

Catch-up vaccination
- Do not start the series on or after age 15 weeks, 0 days.
- The maximum age for the final dose is 8 months, 0 days.
- For other catch-up guidance, see Table 2.

Tetanus, diphtheria, and pertussis (Tdap) vaccination
(minimum age: 11 years for routine vaccination, 7 years for catch-up vaccination)

Routine vaccination
- **Adolescents age 11–12 years:** 1 dose Tdap
- **Pregnancy:** 1 dose Tdap during each pregnancy, preferably in early part of gestational weeks 27–36.
- Tdap may be administered regardless of the interval since the last tetanus- and diphtheria-toxoid-containing vaccine.

Catch-up vaccination
- **Adolescents age 13–18 years who have not received Tdap:** 1 dose Tdap, then Td or Tdap booster every 10 years
- **Persons age 7–18 years not fully vaccinated* with DTaP:** 1 dose Tdap as part of the catch-up series (preferably the first dose); if additional doses are needed, use Td or Tdap.
- **Tdap administered at age 7–10 years:**
  - **Children age 7–9 years** who receive Tdap should receive the routine Tdap dose at age 11–12 years.
  - **Children age 10 years** who receive Tdap do not need the routine Tdap dose at age 11–12 years.
- **DTaP inadvertently administered on or after age 7 years**:
  - **Children age 7–9 years**: DTaP may count as part of catch-up series. Administer routine Tdap dose at age 11–12 years.
  - **Children age 10–18 years**: Count dose of DTaP as the adolescent Tdap booster.
- For other catch-up guidance, see Table 2.

Special situations
- **Wound management** in persons age 7 years or older with history of 3 or more doses of tetanus-toxoid-containing vaccine: For clean and minor wounds, administer Tdap or Td if more than 10 years since last dose of tetanus-toxoid-containing vaccine; for all other wounds, administer Tdap or Td if more than 5 years since last dose of tetanus-toxoid-containing vaccine. Tdap is preferred for persons age 11 years or older who have not previously received Tdap or whose Tdap history is unknown. If a tetanus-toxoid-containing vaccine is indicated for a pregnant adolescent, use Tdap.
- For detailed information, see www.cdc.gov/mmwr/volumes/69/wr/mm6903a5.htm.

**Fully vaccinated* = 5 valid doses of DTaP OR 4 valid doses of DTaP if dose 4 was administered at age 4 years or older

Varicella vaccination
(minimum age: 12 months)

Routine vaccination
- 2-dose series at age 12–15 months, 4–6 years
- VAR or MMRV may be administered*
- Dose 2 may be administered as early as 3 months after dose 1 (a dose inadvertently administered after at least 4weeks may be counted as valid)

****Note:*** For dose 1 in children age 12–47 months, it is recommended to administer MMR and varicella vaccines separately. MMRV may be used if parents or caregivers express a preference.

Catch-up vaccination
- Ensure persons age 7–18 years without evidence of immunity (see *MMWR* at www.cdc.gov/mmwr/pdf/rr/rr5604.pdf) have a 2-dose series:
  - **Age 7–12 years**: routine interval: 3 months (a dose inadvertently administered after at least 4 weeks may be counted as valid)
  - **Age 13 years and older**: routine interval: 4–8 weeks (minimum interval: 4 weeks)
  - The maximum age for use of MMRV is 12 years.

Appendix Recommended Child and Adolescent Immunization Schedule for ages 18 years or younger, United States, 2022

Guide to Contraindications and Precautions to Commonly Used Vaccines

Adapted from Table 4-1 in Advisory Committee on Immunization Practices (ACIP) General Best Practice Guidelines for Immunization: Contraindication and Precautions available at www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html *and ACIP's Recommendations for the Prevention and Control of 2021-22 seasonal influenza with Vaccines available at* www.cdc.gov/mmwr/volumes/70/rr/rr7005a1.htm.

Interim clinical considerations for use of COVID-19 vaccines including contraindications and precautions can be found at
www.cdc.gov/vaccines/covid-19/clinical-considerations/covid-19-vaccines-us.html

Vaccine	Contraindications[1]	Precautions[2]
Influenza, egg-based, inactivated injectable (IIV4)	• Severe allergic reaction (e.g., anaphylaxis) after previous dose of any influenza vaccine (i.e., any egg-based IIV, ccIIV, RIV, or LAIV of any valency) • Severe allergic reaction (e.g., anaphylaxis) to any vaccine component[3] (excluding egg)	• Guillain-Barré syndrome (GBS) within 6 weeks after a previous dose of any type of influenza vaccine • Persons with egg allergy with symptoms other than hives (e.g., angioedema, respiratory distress) or required epinephrine or another emergency medical intervention: Any influenza vaccine appropriate for age and health status may be administered. If using egg-based IIV4, administer in medical setting under supervision of health care provider who can recognize and manage severe allergic reactions. May consult an allergist. • Moderate or severe acute illness with or without fever
Influenza, cell culture-based inactivated injectable [(ccIIV4), Flucelvax® Quadrivalent]	• Severe allergic reaction (e.g., anaphylaxis) to any ccIIV of any valency, or to any component[3] of ccIIV4	• Guillain-Barré syndrome (GBS) within 6 weeks after a previous dose of any type of influenza vaccine • Persons with a history of severe allergic reaction (e.g., anaphylaxis) after a previous dose of any egg-based IIV, RIV, or LAIV of any valency. If using ccIV4, administer in medical setting under supervision of health care provider who can recognize and manage severe allergic reactions. May consult an allergist. • Moderate or severe acute illness with or without fever
Influenza, recombinant injectable [(RIV4), Flublok® Quadrivalent]	• Severe allergic reaction (e.g., anaphylaxis) to any RIV of any valency, or to any component[3] of RIV4	• Guillain-Barré syndrome (GBS) within 6 weeks after a previous dose of any type of influenza vaccine • Persons with a history of severe allergic reaction (e.g., anaphylaxis) after a previous dose of any egg- based IIV, ccIIV, or LAIV of any valency. If using RIV4, administer in medical setting under supervision of health care provider who can recognize and manage severe allergic reactions. May consult an allergist. • Moderate or severe acute illness with or without fever
Influenza, live attenuated [LAIV4, Flumist® Quadrivalent]	• Severe allergic reaction (e.g., anaphylaxis) after previous dose of any influenza vaccine (i.e., any egg-based IIV, ccIIV, RIV, or LAIV of any valency) • Severe allergic reaction (e.g., anaphylaxis) to any vaccine component[3] (excluding egg) • Children age 2 – 4 years with a history of asthma or wheezing • Anatomic or functional asplenia • Immunocompromised due to any cause including, but not limited to, medications and HIV infection • Close contacts or caregivers of severely immunosuppressed persons who require a protected environment • Pregnancy • Cochlear implant • Active communication between the cerebrospinal fluid (CSF) and the oropharynx, nasopharynx, nose, ear or any other cranial CSF leak • Children and adolescents receiving aspirin or salicylate-containing medications • Received influenza antiviral medications oseltamivir or zanamivir within the previous 48 hours, peramivir within the previous 5 days, or baloxavir within the previous 17 days	• Guillain-Barré syndrome (GBS) within 6 weeks after a previous dose of any type of influenza vaccine • Asthma in persons aged 5 years old or older • Persons with egg allergy with symptoms other than hives (e.g., angioedema, respiratory distress) or required epinephrine or another emergency medical intervention: Any influenza vaccine appropriate for age and health status may be administered. If using LAIV4 (which is egg based), administer in medical setting under supervision of health care provider who can recognize and manage severe allergic reactions. May consult an allergist. • Persons with underlying medical conditions (other than those listed under contraindications) that might predispose to complications after wild-type influenza virus infection [e.g., chronic pulmonary, cardiovascular (except isolated hypertension), renal, hepatic, neurologic, hematologic, or metabolic disorders (including diabetes mellitus)] • Moderate or severe acute illness with or without fever

1. When a contraindication is present, a vaccine should NOT be administered. Kroger A, Bahta L, Hunter P. ACIP General Best Practice Guidelines for Immunization. www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html
2. When a precaution is present, vaccination should generally be deferred but might be indicated if the benefit of protection from the vaccine outweighs the risk for an adverse reaction. Kroger A, Bahta L, Hunter P. ACIP General Best Practice Guidelines for Immunization. www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html
3. Vaccination providers should check FDA-approved prescribing information for the most complete and updated information, including contraindications, warnings, and precautions. Package inserts for U.S.-licensed vaccines are available at www.fda.gov/vaccines-blood-biologics/approved-products/vaccines-licensed-use-united-states

Appendix

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Vaccine	Contraindications[1]	Precautions[2]
Dengue (DEN4CYD)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • Severe immunodeficiency (e.g., hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, long- term immunosuppressive therapy or patients with HIV infection who are severely immunocompromised)	• Pregnancy • HIV infection without evidence of severe immunosuppression • Moderate or severe acute illness with or without fever
Diphtheria, tetanus, pertussis (DTaP) Tetanus, diphtheria (DT)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • For DTaP only: Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) not attributable to another identifiable cause within 7 days of administration of previous dose of DTP or DTaP	• Guillain-Barré syndrome (GBS) within 6 weeks after previous dose of tetanus-toxoid–containing vaccine • History of Arthus-type hypersensitivity reactions after a previous dose of diphtheria-toxoid— containing or tetanus-toxoid– containing vaccine; defer vaccination until at least 10 years have elapsed since the last tetanus-toxoid- containing vaccine • For DTaP only: Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, progressive encephalopathy; defer DTaP until neurologic status clarified and stabilized • Moderate or severe acute illness with or without fever
Haemophilus influenzae type b (Hib)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • For Hiberix, ActHib, and PedvaxHIB only: History of severe allergic reaction to dry natural latex • Less than age 6 weeks	• Moderate or severe acute illness with or without fever
Hepatitis A (HepA)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] including neomycin	• Moderate or severe acute illness with or without fever
Hepatitis B (HepB)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] including yeast • For Heplisav-B only: Pregnancy	• Moderate or severe acute illness with or without fever
Hepatitis A- Hepatitis B vaccine [HepA-HepB, (Twinrix®)]	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] including neomycin and yeast	• Moderate or severe acute illness with or without fever
Human papillomavirus (HPV)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3]	• Moderate or severe acute illness with or without fever
Measles, mumps, rubella (MMR)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • Severe immunodeficiency (e.g., hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, long-term immunosuppressive therapy or patients with HIV infection who are severely immunocompromised) • Pregnancy • Family history of altered immunocompetence, unless verified clinically or by laboratory testing as immunocompetent	• Recent (≤11 months) receipt of antibody-containing blood product (specific interval depends on product) • History of thrombocytopenia or thrombocytopenic purpura • Need for tuberculin skin testing or interferon-gamma release assay (IGRA) testing • Moderate or severe acute illness with or without fever
Meningococcal ACWY (MenACWY) [MenACWY-CRM (Menveo®); MenACWY-D (Menactra®); MenACWY-TT (MenQuadfi®)]	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • For MenACWY-D and Men ACWY-CRM only: severe allergic reaction to any diphtheria toxoid– or CRM197–containing vaccine • For MenACWY-TT only: severe allergic reaction to a tetanus toxoid-containing vaccine	• For MenACWY-CRM only: Preterm birth if less than age 9 months • Moderate or severe acute illness with or without fever
Meningococcal B (MenB) [MenB-4C (Bexsero®); MenB-FHbp (Trumenba®)]	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3]	• Pregnancy • For MenB-4C only: Latex sensitivity • Moderate or severe acute illness with or without fever
Pneumococcal conjugate (PCV13)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • Severe allergic reaction (e.g., anaphylaxis) to any diphtheria-toxoid– containing vaccine or its component[3]	• Moderate or severe acute illness with or without fever
Pneumococcal polysaccharide (PPSV23)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3]	• Moderate or severe acute illness with or without fever
Poliovirus vaccine, inactivated (IPV)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3]	• Pregnancy • Moderate or severe acute illness with or without fever
Rotavirus (RV) [RV1 (Rotarix®), RV5 (RotaTeq®)]	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • Severe combined immunodeficiency (SCID) • History of intussusception	• Altered immunocompetence other than SCID • Chronic gastrointestinal disease • RV1 only: Spina bifida or bladder exstrophy • Moderate or severe acute illness with or without fever
Tetanus, diphtheria, and acellular pertussis (Tdap) Tetanus, diphtheria (Td)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • For Tdap only: Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) not attributable to another identifiable cause within 7 days of administration of previous dose of DTP, DTaP, or Tdap	• Guillain-Barré syndrome (GBS) within 6 weeks after a previous dose of tetanus-toxoid–containing vaccine • History of Arthus-type hypersensitivity reactions after a previous dose of diphtheria-toxoid— containing or tetanus-toxoid– containing vaccine; defer vaccination until at least 10 years have elapsed since the last tetanus-toxoid– containing vaccine • For Tdap only: Progressive or unstable neurological disorder, uncontrolled seizures, or progressive encephalopathy until a treatment regimen has been established and the condition has stabilized • Moderate or severe acute illness with or without fever
Varicella (VAR)	• Severe allergic reaction (e.g., anaphylaxis) after a previous dose or to a vaccine component[3] • Severe immunodeficiency (e.g., hematologic and solid tumors, receipt of chemotherapy, congenital immunodeficiency, long- term immunosuppressive therapy or patients with HIV infection who are severely immunocompromised) • Pregnancy • Family history of altered immunocompetence, unless verified clinically or by laboratory testing as immunocompetent	• Recent (≤11 months) receipt of antibody-containing blood product (specific interval depends on product) • Receipt of specific antiviral drugs (acyclovir, famciclovir, or valacyclovir) 24 hours before vaccination (avoid use of these antiviral drugs for 14 days after vaccination) • Use of aspirin or aspirin-containing products • Moderate or severe acute illness with or without fever

1. When a contraindication is present, a vaccine should NOT be administered. Kroger A, Bahta L, Hunter P. ACIP General Best Practice Guidelines for Immunization. www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html
2. When a precaution is present, vaccination should generally be deferred but might be indicated if the benefit of protection from the vaccine outweighs the risk for an adverse reaction. Kroger A, Bahta L, Hunter P. ACIP General Best Practice Guidelines for Immunization. www.cdc.gov/vaccines/hcp/acip-recs/general-recs/contraindications.html
3. Vaccination providers should check FDA-approved prescribing information for the most complete and updated information, including contraindications, warnings, and precautions. Package inserts for U.S.-licensed vaccines are available at www.fda.gov/vaccines-blood-biologics/approved-products/vaccines-licensed-use-united-states.

All Inserts

Food and Drug Administration

Package Inserts & FDA Product Approvals

Product Approval - Adenovirus

Adenovirus Package Insert

Barr Labs Inc.

Adenovirus Product Approval

Product Approval - Anthrax

BioThrax Package Insert

Emergent BioSolutions

BioThrax Product Approval

Product Approval - BCG Vaccine (tuberculosis)

BCG Package Insert

Organon Teknica (Merck)

BCG Product Approval

Product Approval - Cholera

Vaxchora Package Insert

Emergent BioSolutions

Vaxchora Product Approval

Product Approval - FDA Approval and Emergency Use Authorization (EUA) – COVID-19

Comirnaty Package Insert

Pfizer-BioNTech

Comirnaty Product Approval

EUA Full Prescribing Information

Pfizer-BioNTech

Letter of Authorization

Fact Sheet for Healthcare Providers

Fact Sheets for Patients

EUA Full Prescribing Information

Janssen (Johnson and Johnson)

Letter of Authorization

Fact Sheet for Healthcare Providers

Fact Sheet for Patients

Spikevax Package Insert

Moderna

Spikevax Product Approval

EUA Full Prescribing Information

Moderna

Letter of Authorization

Fact Sheet for Healthcare Providers

Fact Sheets for Patients

EUA Full Prescribing Information

Novavax, Inc

Letter of Authorization

Fact Sheet for Healthcare Providers

Fact Sheets for Patients

Product Approval - Dengue

Dengvaxia Package Insert

Sanofi U.S.

Dengvaxia Product Approval

Product Approval - DT

DT (generic) Package Insert

Sanofi U.S.

DT (generic) Product Approval

Product Approval - DTaP

Daptacel Package Insert
Sanofi U.S.
Daptacel Product Approval

Infanrix Package Insert
GSK
Infanrix Product Approval

Kinrix Package Insert
GSK
Kinrix Product Approval

Pediarix Package Insert
GSK
Pediarix Product Approval

Pentacel Package Insert
Sanofi U.S.
Pentacel Product Approval

Quadracel Package Insert
Sanofi U.S.
Quadracel Product Approval

Vaxelis Package Insert
Sanofi U.S.
Vaxelis Product Approval

Product Approval - Ebola virus disease (EVD)

Ervebo Package Insert
Merck & Co, Inc.
Ervebo Product Approval

Product Approval - *Haemophilus influenzae* type b

ActHIB Package Insert
Sanofi U.S.
ActHIB Product Approval

Hiberix Package Insert
GSK
Hiberix Product Approval

PedvaxHIB Package Insert
Merck & Co., Inc.
PedvaxHIB Product Approval

Pentacel Package Insert
Sanofi U.S.
Pentacel Product Approval

Vaxelis Package Insert
Sanofi U.S.
Vaxelis Product Approval

Product Approval - Hepatitis A

Havrix Package Insert
GSK
Havrix Product Approval

Twinrix Package Insert
GSK
Twinrix Product Approval

Vaqta Package Insert
Merck & Co., Inc.
Vaqta Product Approval

Product Approval - Hepatitis B

Engerix-B Package Insert
GSK
Engerix-B Product Approval

Heplisav-B Package Insert
Dynavax Technologies
Heplisav-B Product Approval

Pediarix Package Insert
GSK
Pediarix Product Approval

PreHevbrio Package Insert
VBI Vaccines Inc.
PreHevbrio Product Approval

Recombivax HB Package Insert
Merck & Co., Inc.
Recombivax HB Product Approval

Twinrix Package Insert
GSK
Twinrix Product Approval

Vaxelis Package Insert
Sanofi U.S.
Vaxelis Product Approval

Product Approval - Human papillomavirus (HPV)

Gardasil 9 Package Insert
Merck & Co., Inc.
Gardasil 9 Product Approval

Product Approval - Influenza

Afluria Quadrivalent Package Insert
Seqirus
Afluria Quadrivalent Product Approval (injectable)

Fluad Quadrivalent Package Insert
Seqirus
Fluad Quadrivalent Product Approval (injectable)

Fluarix Quadrivalent Package Insert
GSK
Fluarix Quadrivalent Product Approval (injectable)

Flublok Quadrivalent Package Insert
Protein Sciences Corporation
Flublok Quadrivalent Product Approval (injectable)

Flucelvax Quadrivalent Package Insert
Seqirus
Flucelvax Quadrivalent Product Approval (injectable)

FluLaval Quadrivalent Package Insert
ID Biomedical Corporation
FluLaval Quadrivalent Product Approval (injectable)

FluMist Quadrivalent Package Insert
MedImmune, Inc.
FluMist Quadrivalent Product Approval (intranasal)

Fluzone High Dose Quadrivalent Package Insert
Sanofi U.S.
Fluzone High Dose Quadrivalent Product Approval (injectable)

Fluzone Quadrivalent Package Insert
Sanofi U.S.
Fluzone Quadrivalent Product Approval (injectable)

Product Approval - Japanese encephalitis

IXIARO Package Insert
Valneva
IXIARO Product Approval

Product Approval - Measles

MMR II Package Insert
Merck & Co., Inc.
MMR II Product Approval

Priorix Package Insert
GSK
Priorix Product Approval

ProQuad Package Insert
Merck & Co., Inc.
ProQuad Product Approval

Product Approval - Meningococcal ACWY

Menactra Package Insert
Sanofi U.S.
Menactra Product Approval

MenQuadfi Package Insert
Sanofi U.S.
MenQuadfi Product Approval

Menveo Package Insert
GSK
Menveo Product Approval

Product Approval - Meningococcal B

Bexsero Package Insert
GSK
Bexsero Product Approval

Trumenba Package Insert
Pfizer
Trumenba Product Approval

Product Approval - Mumps

MMR II Package Insert
Merck & Co.
MMR II Product Approval

Priorix Package Insert
GSK
Priorix Product Approval

ProQuad Package Insert
Merck & Co.
ProQuad Product Approval

Product Approval - Pneumococcal (PCV)

Prevnar 13 Package Insert
Pfizer
Prevnar 13 Product Approval

Vaxneuvance (PCV15) Package Insert
Merck & Co, Inc.
Vaxneuvance (PCV15) Product Approval

Prevnar 20 Package Insert
Pfizer
Prevnar 20 Product Approval

Product Approval - Pneumococcal (PPSV)

Pneumovax 23 Package Insert
Merck & Co., Inc.
Pneumovax 23 Product Approval

Product Approval - Polio (IPV)

IPOL Package Insert
Sanofi U.S.
IPOL Product Approval

Kinrix Package Insert
GSK
Kinrix Product Approval

Pediarix Package Insert
GSK
Pediarix Product Approval

Pentacel Package Insert
Sanofi U.S.
Pentacel Product Approval

Quadracel Package Insert
Sanofi U.S.
Quadracel Product Approval

Vaxelis Package Insert
Sanofi U.S.
Vaxelis Product Approval

Product Approval - Rabies

Imovax Package Insert
Sanofi U.S.
Imovax Product Approval

RabAvert Package Insert
Bavarian Nordic
RabAvert Product Approval

Product Approval - Rotavirus

Rotarix Package Insert
GSK
Rotarix Product Approval

RotaTeq Package Insert
Merck & Co., Inc.
RotaTeq Product Approval

Product Approval - Rubella

MMR II Package Insert
Merck & Co., Inc.
MMR II Product Approval

Priorix Package Insert
GSK
Priorix Product Approval

ProQuad Package Insert
Merck & Co., Inc.
ProQuad Product Approval

Product Approval - Smallpox and Monkeypox

ACAM2000 Package Insert
Emergent Biosolutions
ACAM2000 Product Approval - (smallpox only)

Jynneos Package Insert
Bavarian Nordic A/S
Jynneos Product Approval

EUA Full Prescribing Information
Jynneos
Letter of Authorization
Fact Sheet for Healthcare Providers
Fact Sheet for Patients

Product Approval - Tetanus (Td)

Td (generic) Package Insert
Mass. Biological Lab
Td (generic) Product Approval

Tenivac Package Insert
Sanofi U.S.
Tenivac Product Approval

Product Approval - Tetanus (Tdap)

Adacel Package Insert
Sanofi U.S.
Adacel Product Approval

Boostrix Package Insert
GSK
Boostrix Product Approval

Product Approval – Tick-borne encephalitis

Ticovac Package Insert
Pfizer
Ticovac Product Approval

Product Approval - Typhoid

Typhim Vi Package Insert
Sanofi U.S.
Typhim Vi Product Approval

Vivotif Package Insert
Emergent BioSoulutions
Vivotif Product Approval

Product Approval - Varicella (chickenpox)

Varivax Package Insert
Merck & Co., Inc.
Varivax Product Approval

ProQuad Package Insert
Merck & Co., Inc.
ProQuad Product Approval

Product Approval - Yellow Fever

YF-Vax Package Insert
Sanofi U.S.
YF-Vax Product Approval

Product Approval - Zoster (shingles)

Shingrix Package Insert
GSK
Shingrix Product Approval

This page was updated on October 3, 2022.
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Excerpts below are from the first chapter of **Turtles All The Way Down: Vaccine Science and Myth** (pages 40-82).

The Randomized Controlled Trial (RCT)

In a "simple" vaccine clinical trial (one without a control group, as is the case for trials in Phases 1 and 2), researchers face an inherent difficulty in determining whether a specific condition reported during the trial period is actually caused by the experimental compound or not. If a trial subject experiences a severe and immediate phenomenon following the receipt of the test vaccine, such as fainting or cardiac arrest, it could be reasonably assumed that the recently consumed vaccine was the culprit. When the side effect is less pronounced, or appears days or weeks following vaccine administration, however, the researchers decision is less obvious. For example, if the subject's temperature rises to 103°F less than 48 hours after administration of the test vaccine, the researchers do not have enough information to decide whether this is a true side effect or merely an unfortunate coincidence. One option is to have every participant who experiences a health related condition during the trial undergo a series of in depth medical examinations in order to uncover possible links to the experimental vaccine. This strategy is not feasible or economical, however, if only because the vaccine is new and its specific effect on the human body is virtually unknown. Consequently, such an investigation could prove lengthy, costly, and unlikely to yield conclusive results.

A better option is to conduct an "enhanced" clinical trial - a controlled, randomized, and blinded trial (also known as a randomized controlled trial - RCT. In an RCT, subjects are divided into two groups: the trial group, receiving the test compound, and a control group, receiving a dummy or existing compound (whose efficacy and safety profile is well known). Subjects are randomly assigned to the two groups prior to the start of the trial to ensure that the groups are virtually alike in every relevant characteristic (age, gender, area of residence, demographic status, and so on). The term blinded (or blinding), means that the trial subjects do not know which group they are in and thus do not know whether they will receive the test or dummy compound. In a double blind trial, the researchers also do not know which subjects belong to which group. Thus, prior knowledge of which compound a participant will receive is less likely to influence either subjects or researchers and skew the results of the trial. In a non-blinded trial, subjects who receive the test compound, rather than the dummy one, may complain more about side effects, since they expect them to occur. Similarly, a researcher who knows a particular subject belongs to the control group also knows that any reported side effects are not due to the vaccine and may inadvertently (subconsciously underreport medical conditions occurring during the trial period. Only when the trial is over, after all relevant information has been collected, is the specific compound administered to each of the study subjects revealed, and the researchers, with the complete data in hand, can begin the post-clinical data analysis.

When it comes to pre-licensure testing of drugs, vaccines, and other medical products, RCTs are widely considered the industry's "gold standard". The random distribution of subjects to trial and control groups, as well as the minimization of potential biases through the use of double-blinding, facilitates a reliable and meaningful comparison of trial and control group data. As an example, in a vaccine trial in which the control group is receiving a dummy compound, one can measure the level of antibodies produced in trial group subjects and compare it to that of the control group, thus getting a measure of vaccine efficacy.

Similarly, a researcher could compare the incidence of adverse events following vaccination in the two groups, thus getting an estimation of vaccine safety. The larger the number of trial participants and the better the researchers adhere to RCT standard practices, the more reliable and comprehensive the trial results will be.

Due to the high quality and reliability of RCTs, they are the method designated by regulatory agencies (and accepted by the pharmaceutical industry) for evaluating efficacy and safety of vaccines in Phase 3 clinical trials.

The Control Group in a Clinical Trial

As we have seen, the use of a control group in a clinical trial allows researchers to examine the therapeutic effect of the compound (efficacy) and the rate of adverse events it causes (safety) by comparing outcomes in the trial group with those of the control group. This comparative statistical analysis, then, will be influenced by the nature of the compound the researchers give to the control group.

As a general rule, when deciding upon the type of compound given to the control group in an RCT, there are two options. For a trial of a completely new drug or vaccine, i.e. one which does not have an approved equivalent, the control group should receive an inert compound (placebo) that does not affect the parameters measured in the trial. However, if a proven treatment already exists, it may be unethical to prevent control group participants from receiving it. For example, in trials of new cancer drugs, it is considered unethical to prevent the control group's subjects from receiving an existing drug for their illness. In this scenario, then, the control group would receive the current approved treatment. This practice is also the norm for vaccines even though vaccines are used preventatively (not treatment for an existing condition) and are given to healthy individuals.

If we apply the above guidelines to the clinical trials for the two generations of the Prevnar vaccine,' then the original Prevnar, a new vaccine that had no therapeutic alternative at the time it was developed, should have been tested in an RCT in which the control group received an inert injection as a placebo. In the trials of Prevnar-13, the next-generation vaccine, the control group should have received the (original) Prevnar vaccine, assuming that it would be unethical to deprive that group's subjects of the current Prevnar vaccine's protection, whose efficacy is already proven.

So how do researchers determine the incidence of adverse events associated with the new compound being tested in a controlled clinical trial? By comparing the rate of adverse events observed in the trial group to that of the control group. For example, if in a new vaccine's trial group of 1,000 infants there were 20 cases of high fever, and in the control group (which has the same number of subjects) there were only 10 such cases registered, the results would imply the risk of high fever in the vaccinated is twice as high as in the unvaccinated. In absolute terms, the data shows that the vaccine increases the risk of high fever occurrence from 1 in every 100 infants to 1 in 50.

When the control group's subjects are given a placebo, an inert substance not known to cause high fever, it is assumed that the incidence of high fever recorded for the group represents the background rate (or baseline rate) of the phenomenon. In other words, the background rate is the number of subjects who would experience high fever naturally, regardless of any trial intervention. In our example above, we would assume that 1 in 100 control group subjects developed high fever due to random causes (unrelated to the trial). Since the trial group would likely experience a similar background rate of high fever (1 in 100), any significant deviation from this level should be attributed to the experimental vaccine. It follows, then, that an RCT in which the control group receives an inert placebo is designed to answer the critical question of How many adverse events does the new vaccine cause? Of course, we should keep in mind that trial results are no more than a good estimation. If or when the vaccine is released to the market, the actual reported adverse event rate might deviate significantly from that observed in the clinical trial. Still, the results of RCTs are the best estimate of safety available to science during the vaccine approval process, and in many cases, throughout its lifetime.

In a trial in which the control group receives a different vaccine (as in the trial of Prevnar-13 vs. Prevnar, its predecessor), the results obtained are always relative, answering the question How many more (or less adverse events does the new vaccine cause compared to the current vaccine? For example, if (out of 1,000 subjects) 24 cases of high fever were observed in the trial group, while 20 such cases were reported in the control group, the new vaccine would appear to increase the odds of high fever by 20% (relative to the current vaccine). That is an important piece of information as it reveals how the new-generation vaccine's safety fares against that of its predecessor. However, it is impossible to calculate from a trial such as this one the absolute rate of adverse events caused by the experimental vaccine - that is, the rate of adverse events from vaccinating compared to not vaccinating. The absolute rate could not be calculated because the control group received a compound (the current vaccine) which is not inert (neutral), but rather has side effects of its own. In the above example, 24 cases of high fever were observed in recipients of the new vaccine, and 20 cases in current vaccine recipients. But how many cases would have been reported in trial subjects given a true placebo? This trial cannot answer that question; therefore, the absolute rate of adverse events caused by the new vaccine cannot be calculated from trial data. The new vaccine could be said to cause 24 cases of high fever per 1,000 subjects, but this number would not represent a reliable estimate as it does not take into account the background rate of the phenomenon, which was not measured in the trial.

In order to determine the true rate of adverse events of a new generation vaccine, a three-arm trial must be conducted, combining the two methods described above. In this kind of trial, subjects would be randomly allocated into three groups, one trial and two controls: The trial group would receive the new generation vaccine, the first control group would receive the current vaccine, and the second control group would receive an inert placebo. This trial design is considered to be of excellent quality, as it measures both the absolute rate of adverse events (comparing the new vaccine to the placebo) and the relative rate (comparing the new vaccine to the current vaccine). From a public health perspective, the three-arm trial answers two important questions: (1) How many adverse events does the new vaccine cause when compared to not vaccinating? and (2) How many adverse events does the new vaccine cause when compared to the existing vaccine?

Continuing with our Prevnar example, if the placebo-receiving control group reported, say, 8 high fever cases per 1,000 subjects, then the study would indicate that the new vaccine - which, as we recall, had 24 cases of high fever per 1,000 subjects - increased the risk of high fever by a factor of three (or, put differently, caused 16 more cases per 1,000 subjects), compared to not vaccinating.

Another scenario in which a three-arm trial would be appropriate is re-establishing the safety of a legacy vaccine that was originally tested many years ago. The environment into which today's infants are born may differ significantly in crucial health-related aspects from the environment in which a first-generation vaccine was tested decades ago. For example, the current measles-mumps-rubella-varicella (MMRV) vaccine (ProQuad) is the "grandchild" of the original MMR vaccine, which was tested in the late 1960s. Back then, the vaccine schedule consisted of only the diphtheria-pertussis-tetanus (DPT) and polio vaccines, with the first dose administered at age two months. If ProQuad were clinically tested against the original MMR and proved to have a similar safety profile, could we assume it is safe just because its grandparent vaccine was deemed safe 50 years ago? MMR vaccines are typically administered in the second year of life, after most of the infant vaccine schedule has already been delivered.

If, hypothetically, the MMR's risk of harmful side effects were related to the load of previously administered vaccines, then we could not automatically accept the present safety of the original MIMR. Remember that the MMR was first tested when the vaccine schedule consisted of only two other vaccines. If it were tested today, with many more vaccines on the schedule, some of which are given to pregnant mothers, others to newborns and infants one month of age, would it still be proven safe? And the changing vaccine program is just one aspect of the environment that may affect the safety of a given vaccine. Other factors, such as chemical exposure, changing diets, air pollution, radiation, etc., could also play a role. Therefore, a clinical trial comparing ProQuad to MMR alone is deficient, as it would rely on the presumed safety of a vaccine (MM) that might no longer be safe. Once more, a third group receiving a placebo is the proper solution to the problem.

To summarize, in a clinical trial of an (entirely) new vaccine, the control group should receive a placebo so that the absolute rate of the vaccine's adverse events can be determined. This design does not pose an ethical problem, since the vaccine has no existing alternative. In a trial of a new-generation vaccine, one control group should receive the current vaccine and another should receive a placebo (a three-arm trial).

External Control Group

Another important point to consider is that an RCT control group cannot be replaced with data from another trial, or any other externally calculated background rate. In other words, it is not scientifically valid to draw conclusions by comparing the observed rate of any phenomenon in a randomized controlled trial to the rate reported in another trial or to a rate observed in the general population.

For example, if in a particular vaccine trial the reported incidence of sudden infant death syndrome (SIDS or "crib death?) in the trial group were 0.5% (1 in 200), researchers could not then compare this rate to the background rate of the phenomenon in the population (say 0.8%), thus determining that the vaccine lowered the risk of SIDS. This is because trial participants comprise a subgroup which could possess specific characteristics, known or unknown, which are not representative of the entire population. This could potentially yield trial results that are not comparable to rates in the general population. For example, the proportion of infants participating in a trial whose parents smoke may be much lower than the background rate in the entire population, skewing the incidence of crib death in trial participants in a downward direction. Of course, skewing in the opposite direction is equally possible.

Similarly, there is little scientific merit in comparing results from different clinical trials. For example, no significant insights could be derived from comparing the results of a Prevnar trial carried out in infants from the New York area in 2010 with those of a Prevnar-13 trial conducted in Philadelphia in 2005. This is due to the randomization principle of the Randomized Controlled Trial (RCT), which requires that the trial participants be randomly divided between the trial group and the control group. Obviously, groups whose members were selected at different times and places would not satisfy this requirement. In the above examples, any differences in trial results could be entirely due to dissimilarities between the groups, such as different socioeconomic status, environmental exposures, or behavioral characteristics.

The principle described above is well known to the pharmaceutical industry and it appears in numerous vaccine manufacturers leaflets. For example, the package insert for Glaxo-Smith-Kline's (GSK) hepatitis A vaccine (Havrix) reads: "Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine, and may not reflect the rates observed in practice.

Clinical Trials in Children

Throughout most of the 20th century, the prevailing opinion in the world of medicine was that due to the relative fragility of children (compared to adults), they should be protected from the perils of medical research. The result was a distinct lack of scientific knowledge about the effects of medical interventions (such as medication) on children. Administering medication to children, therefore, was largely a wide-ranging experiment conducted on the public. Circumstances began to change in 1977 when the American Academy of Pediatrics (AAP) published new guidelines regulating the participation of children in clinical trials. In the new guidelines, the AAP said that drugs and vaccines should be tested on the population for which they are intended - in this case, children - and that this requirement is not only ethical, but essential to their health as well.

Over the following decades, various international medical organizations have formulated ethical rules governing the participation of children in clinical trials of drugs and vaccines. According to these rules, children may only be included in experiments intended to achieve an important scientific or public health objective directly related to the health and wellbeing of children. Children should not participate in studies that do not promote such goals, such as studies designed merely to confirm the results of other studies or studies designed to advance scientific knowledge that does not concern children.

In addition, the medical code of ethics states that all parties involved in a trial must carefully weigh the potential benefit to child participants against the potential dangers involved. If the study's children cannot be expected to benefit from the studied intervention, then the intervention's inherent risk must be "minimal', especially if the subject has not consented to participate in the trial (as is the case with infants). For example, if children assigned to the control group of a drug trial were to receive a dummy medication (placebo) and a blood test, then both the medication and the blood draw must present no more than "minimal" risk. And any potential benefit must be substantial enough to justify the intervention's risk. For example, in a trial of a children's cough syrup, the risk associated with the new drug should be relatively low as the potential benefit would be relatively low, while the potential benefit in a trial of a child cancer medication would be significantly higher, thus the risk posed by the drug could be proportionately higher as well.

A more lenient approach holds that even if the experimental procedure has no potential benefit, there may be a "minor increase over minimal risk" if the experiment has the potential for gaining knowledge about the subjects' disorder, that is considered to be of "vital importance". However, even with this approach, the risk associated with the intervention must not exceed the risk a healthy child would face in everyday life and should not cause permanent or irreparable damage. In any case, there must be prior knowledge of the level of risk inherent in the procedure. If the risk is unknown, it cannot be determined to be "a minor increase over minimal risk".

Now that we are familiar with the different clinical phases of the vaccine approval process, the purpose of control groups in randomized controlled trials, and the ethical limitations imposed on children's participation in medical research, we can better examine the deliberately flawed procedure the industry uses to conduct vaccine clinical trials.

A Problem and a Solution

Let's take a moment to examine a hypothetical scenario: A major pharmaceutical company has developed a new drug against a particular medical problem. Following the drug's preliminary trials, the company realizes that the drug is associated with a relatively high incidence of serious side effects that may negatively affect its chances to win FDA approval. Let us suppose that, since the company spent hundreds of millions of dollars developing the drug and the target market segment is worth billions of dollars in sales per year, the company decides to move forward with the licensing process and start a Phase 3 clinical trial.

Given all of the above, what are the company's options, legal and illegal, for ensuring that the trial demonstrates a positive safety profile, thus clearing the way for the drug's approval?

One option is to artificially lower the incidence of adverse events reported in the trial group (the group receiving the new drug), by withholding or modifying data for specific cases. The difficulty with this technique is that for the duration of the trial, because of the enforced double-blinding, researchers do not know which subjects belong to which trial group. Thus, one cannot suppress or dilute reports for a specific group (the trial group, in this case) while leaving those of the other intact. Randomly suppressing reports would not be likely to accomplish the desired effect as the ratio of adverse events between the two groups would probably not change much.

Another theoretical option would be to modify the results following the conclusion of the clinical stage of the trial, at which point the blinding is removed and the data becomes fully available to the researchers. The difficulty with this approach is that falsifying trial data is a criminal offense, which can lead to grave consequences for the company and the researchers themselves, making this an unattractive option.

Another option would be to use various statistical techniques (which will be discussed later in the book), to build a false safety profile for the drug being tested. The difficulty with this approach is that the RCT study design greatly reduces researchers' ability to affect the results since they gain access to the full data set at a time when the data can no longer be altered. With limited ability to control the data, it can be quite difficult to eliminate undesired signals by statistical manipulation while at the same time successfully covering one's tracks.

The last option available to the company wishing to hide their product's undesirable side effects is to design a trial in which the reported rate of adverse events in the control group would likely be very similar to that of the trial group. As described previously, the RCT control group represents the baseline rate to which the trial group is compared. A similar proportion between the two groups would indicate that the adverse events reported in the trial group were the result of "background noise" only and not caused by the experimental drug. This technique has three distinct advantages: (1) It is 100% legal, (2) it is very effective, and, as it turns out, (3) it has the full approval of licensing authorities around the world. As we shall shortly see, this method is exactly the one vaccine manufacturers employ to deliberately obscure the real incidence of vaccine adverse events.

The entire vaccine program is founded upon this deception.

Fake Placebo

It is virtually impossible to state the bottom line of the analysis presented above mildly, so here goes: **Vaccine trials in general, and childhood vaccine trials specifically, are purposely designed to obscure the true incidence of adverse events of the vaccine being tested.**

How do they do this? By using a two-step scheme: First, a new vaccine (one which does not have a predecessor), is always tested in a Phase 3 RCT in which the control group receives another vaccine (or a compound very similar to the experimental vaccine, see explanation below). A new pediatric vaccine is never tested during its formal approval process against a neutral solution (placebo). Comparing a trial group to a control group that was given a compound that is likely to cause a similar rate of adverse events facilitates the formation of a false safety profile. The rate of adverse events of the tested vaccine is said to be similar to the "background rate", hence it is considered safe. The researchers, and the vaccine manufacturer they work for, seem to "forget" that the compound they administered to the control group is a bioactive substance, carrying its own risks and side effects, and hardly represents the baseline or background rate that is essential to an RCT for a new vaccine.

The vaccine is subsequently approved and added to national vaccine programs throughout the world. Then, when the "next generation" vaccine comes along, its pre-licensing clinical trial will always compare the new vaccine to the current vaccine and never to a placebo. Thus, all parties involved ensure that the true rate of vaccine adverse events is never discovered - for either the original or upgraded vaccine - and that rate is never shared with the public, or even the medical world.

The practice of giving a different vaccine to the control group in an RCT of an entirely new vaccine and calling it "placebo" is a deliberate misrepresentation of the term. As explained previously, a placebo is a compound (or procedure) that does not affect the parameters measured in the trial. When testing the efficacy of a new vaccine, researchers measure the level of disease antibodies in both study groups, so the substance given to the control group must not affect that antibody level or the comparison becomes meaningless. For example, in a hypothetical new hepatitis C vaccine trial, it would not make scientific sense to inject the control group subjects with a compound that could increase (or decrease) the subjects' hepatitis C antibodies. Doing so would preclude a valid assessment of the effect of the vaccine on the antibody level, as the substance taken by the controls could have distorted the comparison."

The above analysis holds true for safety testing as well. If the compound given to the control group has its own significant side effects, it cannot be regarded as a true placebo. If the rates of adverse events observed in the trial and control groups appear similar, is it because the experimental vaccine is safe or because the control compound is just as unsafe as the vaccine? It would be impossible to know. Giving the control group an active substance in an RCT intended to test safety would be a bad design decision, then. Yet this is exactly how new vaccine Phase 3 trials are performed: Instead of a placebo, the control group receives a different vaccine, which is certain to cause its own adverse events and can in no way be deemed a neutral substance.

This practice of administering a different vaccine to the control group in a new-vaccine trial has no bearing on efficacy testing: It is highly likely that the control vaccine, which usually targets a different disease, would have no effect on the antibody level of the disease targeted by the test vaccine.

Thus, using our hepatitis C example, if the control group subjects in the vaccine trial were given the Prevnar vaccine, no change in their hepatitis C antibody level would be expected; thus, the true efficacy of the test vaccine could be determined. But this lack of effect is not the case when it comes to safety: Because the Prevnar vaccine has its own side effects, it cannot be considered neutral in this context. Therefore, the true rate of adverse events for the experimental hepatitis C vaccine cannot be determined by comparing it to the rate in the group that received Prevnar since the controls did not receive a neutral compound.

This deliberate distortion of the placebo concept in clinical trials of new vaccines is so prevalent that researchers and vaccine package inserts frequently refer to the bioactive compound given to a control group as "placebo", even when it's clear it is another vaccine or a similar bioactive compound, which in itself is not safety-neutral. Falsely using the term "placebo" allows researchers to conclude that the new compound "was proven safe" because its rate of adverse events was similar to that of placebo - even though the substance the control group received was decidedly not a placebo. For example, in one of the DTaPo vaccine trials, the rate of hospital admissions in the trial group was almost 1 in every 22 subjects. The researchers did not consider this statistic alarming, however, because in the control groups that received different DTP vaccines, the hospitalization rate was similar. 16 Was such a high hospitalization rate in trial participants unrelated to the vaccines used, or were they the main culprit? Only the use of a true placebo control group could answer that question.

No logical explanation can be found for the ubiquitous practice of administering bioactive compounds to control groups in trials of new vaccines other than a desire to conceal the true rate of adverse events of the vaccine. Testing a new vaccine against a placebo in an RCT is the simplest, safest, cheapest and most reliable option. Saline (sterilized salt water), for example, is a safe, reliable, widely available, and inexpensive compound - certainly when compared to a vaccine. Because it does not cause significant adverse events, nor does it produce disease-specific antibodies, it provides a reliable baseline for both safety and efficacy testing and is therefore ideal for control group usage. Calculation of the true rate of adverse events of the test vaccine becomes straightforward and simple. Despite its clear benefits as a placebo, vaccine makers prefer not to use saline in vaccine trials, and the reason for this should be obvious by now.

Mere Coincidence or Deliberately Flawed Design?

As we've clearly illustrated in the preceding sections, not one of the vaccines the CDC recommends all American children receive was tested for safety in a Phase 3 clinical trial where the control group received an inert placebo. All the vaccines reviewed in the preceding pages - of which tens of millions of doses are administered to infants and toddlers in the US every year - were tested in trials which did not include any control group at all, or ones in which the so-called control group received at least one other vaccine.

Is it just coincidence that none of these vaccines has been tested against a true placebo, despite the fact that in many cases doing so would have been easier, cheaper, and yielded more valid results than the testing that was done?

Is it just an accident of fate that the accepted methodology of all childhood vaccine trials obscures the real rate of adverse events of the new vaccine? That seems highly improbable.

As explained at the start of this discussion, testing the safety of a next-generation vaccine against its predecessor is justifiable on ethical grounds: Withholding an existing and proven treatment from control group subjects would be immoral. However, there is no justification for conducting a chain of trials (turtle upon turtle upon turtle) that ultimately stands on nothing but air. Moreover, what possible rationale could justify trials for new vaccines wherein the control groups receive other (sometimes experimental) vaccines? Would a safety trial for a new cigarette have any credibility at all if the "control" group consisted of subjects who smoked a different kind of cigarette?

Whether or not you believe this trial methodology is ethical, its consequence remains the same: The true rate of adverse events of routine childhood vaccines is virtually unknown; therefore, there is no scientific basis for claiming they're safe.

The fact that we don't know how often childhood vaccines hurt the children who receive them casts a dark shadow over the legitimacy of vaccine programs the world over. But that is not all. Even worse, as we shall shortly see, safety trials conducted for some childhood vaccines blatantly and seriously violate the medical code of ethics. In any vaccine clinical trial, a balance must be struck between the vaccine's potential benefits (disease protection) and potential risks (adverse events). When control subjects in vaccine trials receive another type of vaccine, even if it's done in order to obfuscate the real rate of adverse events of the vaccine being tested, the compound they receive is at least of some potential benefit to them. However, in rotavirus vaccine trials this imperative ethical risk-to-benefit balance was blatantly violated.

The Clinical Trials of the Rotavirus Vaccines

Designing clinical trials for the RotaTeq and Rotarix vaccines was particularly challenging for their manufacturers, Merck and GSK, respectively. To begin with, the first rotavirus vaccine brand (RotaShield) was recalled from the market after it was found to significantly increase the risk of intussusception, a highly dangerous condition in infants. This meant that clinical trials for the new rotavirus vaccines had to adhere to higher safety standards. In addition, the companies faced an equally serious problem: With RotaShield off the market, there was no suitable vaccine to give to control group subjects.

A rotavirus vaccine dose, a few drops of an opaque liquid, is consumed orally. Hence, the control group in its clinical trials could not receive a vaccine administered via injection as it would violate the RCT blinding principle. If the trial group were vaccinated orally, while the control group was injected, it would be easy to tell the two groups apart. At the time the rotavirus vaccine trials began, there was no other orally ingested vaccine licensed for use.

The use of the live polio vaccine (OPV), also consumed by mouth, was terminated in Western countries several years earlier.? As a result, there was no oral vaccine available to compare with rotavirus vaccines in clinical trials.

Another option would be to give the control group a few drops of a neutral liquid, such as a solution of sugar or salt water. These compounds are safe, inexpensive and convenient to use - ideal for the purpose of testing the vaccine's efficacy and safety. Because these were entirely new vaccines, which had no alternative, there were no ethical objections to using such a solution.

So, on the one hand, rotavirus vaccine manufacturers did not have a ready-made vaccine available for use in the control group, and on the other, there was no impediment to using a cheap, available and effective substance, such as sugar water. How, then, did they choose to conduct their Phase 3 clinical trials? A preliminary examination of the clinical trial record of the rotavirus vaccine shows that the control groups in the RotaTeq and Rotarix trials received... a placebo! Was this, then, the industry's first breach of the sacred tradition that vaccines never be tested against a true placebo? Were the rotavirus vaccine trials the first to provide reliable and relevant information about the rate of adverse events of a childhood vaccine?

The answer to these questions is, unfortunately, "no and no".

Examining one of the licensing documents submitted to the FDA by GSKs indicates that the placebo received by the control group in the main Rotarix trial (which included approximately 63,000 infants) is nothing but the tested vaccine without its antigenic component. This compound, the vaccine-sans-antigen (sans means without), is well suited for testing the efficacy of the vaccine as it does not produce rotavirus antibodies. However, when it comes to safety, it's a whole different ballgame: The vaccine-sans-antigen is a potentially potent compound whose side effects are likely to be quite similar to those of the vaccine being tested.

And what was the placebo in Merck's RotaTeq vaccine trial? That's difficult to say because Merck deleted its description from the licensing document submitted to the FDA. It appears that the trial's placebo is a trade secret, which implies its contents were very similar to the vaccine's. Further examination of RotaTeg documents supports this hypothesis: In another RotaTeq clinical trial, the control group received the vaccine-sans-antigen, similar to the compound control group subjects received in the Rotarix trial.

The bioactivity of the compounds given to the control groups in rotavirus vaccine trials was seemingly apparent in the rate of adverse events reported in the trials. In the Rotarix trial, about 1 in 30 control group subjects experienced a "severe" medical event (a rate which was even slightly higher than that of the trial group), and a similar proportion of participants was hospitalized. In addition, 16 infants suffered intussusception and 43 died." In the RotaTeq trial, similar rates were recorded in the control group: Serious adverse events were reported in 1 of every 40 subjects, 15 suffered intussusception, and 20 infants died.

Using the word placebo to describe the vaccine-sans-antigen leaves the false impression that it is a safe compound that has no side effects of its own. Formal documents, which reference the rotavirus vaccine trials, rely on the supposed biological neutrality of that "placebo". One example is the Rotarix vaccine package insert, which states in the clause discussing the rate of intussus-ception reported in pre-licensure trials: "No increased risk of intussusception was observed in this clinical trial following administration of ROTARIX when compared with placebo."63 (The trial in question is the same trial referenced above. There are plenty of other examples, too). Nowhere is there any reference to the actual contents of that "placebo".

The rotavirus vaccine makers were evidently able to find a creative solution to the challenge they faced. They gave their trials control groups compounds that were very similar to their vaccines, and, as was no doubt expected, the resultant rates of adverse events were not significantly different from those observed in the trial groups. In future trials of next-generation rotavirus vaccines, GSK and Merck will be able to give their control groups the standard "placebo" - the currently licensed vaccine - whose safety "was already proven" in its pre-licensure trials.

But there's a fly in this sticky ointment.

Unethical Trials

As previously discussed, the ethical standards for using children as subjects in clinical trials are exceptionally high. Clinical trial designers must ensure that planned procedures are balanced with respect to the expected benefit and risk to the participating infant or child. If a child subject is likely to receive no benefit, the potential harm must be "minimal" or only "slightly above the minimum", and by no means permanent or irreparable. In addition, the risks associated with any procedures must be well known in advance.

In stark contrast to the standards above, tens of thousands of infants in the control groups of the rotavirus vaccine trials received compounds that could provide no potential benefit to the recipient yet carried significant risk. Neither GSK's nor Merck's vaccine-sans-antigen could possibly prevent rotavirus as they did not contain the antigenic particles that evoke immune reactions to the virus. On the other hand, these compounds had significant potential to cause harm, as demonstrated in the trials. (Remember, 1 in every 30 or 40 control group subjects experienced a serious adverse event). In addition, the safety profiles for the vaccines-sans-antigens were unknown (and, for all we know, still are) as they were new compounds specifically formulated for the rotavirus trials with no documentation of past safety studies. Hence, the health risks associated with administering them to infants was undetermined.

To sum up, tens of thousands of infants were given an utterly useless compound whose safety was unknown and whose side effects could be (and probably were in some cases) severe and permanent. Thus, the Phase 3 clinical trials of the rotavirus vaccine constitute blatant violations of the medical code of ethics.

This ruthless breach of ethics and morality is highlighted by the fact that there was no scientific justification for giving the vaccine-sans-antigen to the control group other than a malicious intention to conceal the experimental vaccine's true rate of adverse events. Using a real placebo that posed no health risk - a few drops of sugar or salt water - would have cost less and led to more scientifically valid conclusions by enabling straightforward calculations of the true adverse event rates as well as vaccine efficacy.

The manner in which the rotavirus vaccine trials were conducted raises grave questions which should not be directed solely toward the vaccines' manufacturers. The FDA supervises the vaccine approval process, and it is the FDA that approved these trials. The vaccine also received CDC approval and that of other health authorities around the world, even though the vaccine trials unnecessarily endangered tens of thousands of children and may have caused serious harm to hundreds, as well as dozens of needless deaths.

The Declaration of Helsinki is the ethical code governing the conduct of human medical experimentation. The Declaration was formulated for the medical-scientific community by the World Medical Association and is considered the ethical cornerstone of the medical research field. It leaves no doubt as to the ethical violations perpetrated in the rotavirus trials:

> *Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed. When the risks are found to outweigh the potential benefits [...] physicians must assess whether to continue, modify or immediately stop the study.*
>
> *...[A] potential research subject who is incapable of giving informed consent [...] must not be included in a research study that has no likelihood of benefit for them unless [...I the research entails only minimal risk and minimal burden.*

The Nuremberg Code, the medical code of ethics established in the late 1940s to bring Nazi doctors to justice, constitutes the basis of the Declaration of Helsinki. It too underlines the immorality of the rotavirus vaccine trials: "[An] experiment should be so conducted as to avoid all unnecessary physical and mental suffering and injury." A similar conclusion was also reached by a World Health Organization (WHO committee that recently examined placebo use in clinical trials.

Ponder it as you will, you won't find a satisfactory explanation for the way the rotavirus vaccine trials were conducted Other than the malicious desire to assist the manufacturers in obscuring and concealing the vaccines' true adverse event rates. This demonstrates that the public health establishment is willing to go to great lengths to maintain the pretense of vaccine safety; casting aside medical ethics and even fundamental principles of morality in the process.

Summary

Vaccines, as opposed to drugs, are given to healthy babies and thus must meet a particularly high safety standard. Clinical trials of new vaccines must be impeccably designed and performed, thereby providing high-quality, reliable data about the products efficacy, and more importantly, about their safety. Anything less is socially and morally unacceptable.

Vaccine manufacturers and health authorities worldwide frequently assure us, the public, that vaccines are tested at the highest possible level and that the rigorous series of clinical trials they undergo as part of the licensing process ensures that vaccines are truly safe and effective.

These assurances, however, are meaningless at best and deliberately misleading at worst.

As we have seen in this chapter, vaccine trials are designed and performed in such a way as to ensure that the true extent of adverse events is hidden from the public. There is not a single vaccine in the US routine childhood vaccination program whose true rate of adverse events is known. The assertion that vaccines cause serious side effects in "one in a million" vaccinees contradicts the results of numerous clinical trials in which serious adverse events were reported in 1 in 40, 30, or even as few as 20 vaccinated infants. After becoming acquainted with the finer details of vaccine safety trials, hearing the familiar tune of "a similar rate of adverse events was reported in the control group (which received another vaccine or similar compound)" comes off as ludicrous, cynical, and patently immoral.

Current vaccine clinical trial methodology completely invalidates the claims that vaccines are safe and that they are thoroughly and rigorously tested. And pulling out that bogus card completely topples the childhood vaccine program's house of cards, as officials' assurances of vaccine safety rely primarily on deliberately flawed, industry-sponsored clinical trials.

Furthermore, some of the clinical trials that have been conducted for routine childhood vaccines, which were approved by relevant health authorities, blatantly violated the medical code of ethics (the Declaration of Helsinki) and fundamental principles of morality. In these trials, infants in the control groups were given completely useless compounds (an antigen-free vaccine) whose safety was unknown and which had the potential to cause serious and irreversible damage to health, including death.

Any reader looking for a quick and definitive understanding of the truth about vaccine safety - well, you can put this book down right now. You have your answer: The entire vaccine program is based on a deliberate cover-up of true vaccine adverse event rates. This seemingly mighty fortress, carefully constructed over many decades and fortified by countless officials, researchers, and physicians - actually stands on nothing but turtles all the way down.

COVID

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use COMIRNATY safely and effectively. See full prescribing information for COMIRNATY.

COMIRNATY® (COVID-19 Vaccine, mRNA) suspension for injection, for intramuscular use
Initial U.S. Approval: 2021

---------- RECENT MAJOR CHANGES ----------

Indications and Usage (1)	7/2022
Dosage and Administration (2)	8/2022
Dosage and Administration, Preparation for Administration (2.1)	8/2022
Dosage and Administration, Administration Information (2.2)	8/2022

---------- INDICATIONS AND USAGE ----------

COMIRNATY is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 12 years of age and older. (1)

---------- DOSAGE AND ADMINISTRATION ----------

- COMIRNATY supplied in single dose vials or multiple dose vials with gray caps and labels with gray borders MUST NOT be diluted prior to use. (2.1)
- For intramuscular injection only. (2.2)
- COMIRNATY is administered intramuscularly as a series of 2 doses (0.3 mL each) 3 weeks apart. (2.3)

---------- DOSAGE FORMS AND STRENGTHS ----------

Suspension for injection. A single dose is 0.3 mL. (3)

---------- CONTRAINDICATIONS ----------

Known history of a severe allergic reaction (e.g., anaphylaxis) to any component of COMIRNATY. (4)

---------- WARNINGS AND PRECAUTIONS ----------

- Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. (5.2)
- Syncope (fainting) may occur in association with administration of injectable vaccines, including COMIRNATY. Procedures should be in place to avoid injury from fainting. (5.4)

---------- ADVERSE REACTIONS ----------

- In clinical studies of participants 16 through 55 years of age, the most commonly reported adverse reactions (≥10%) were pain at the injection site (88.6%), fatigue (70.1%), headache (64.9%), muscle pain (45.5%), chills (41.5%), joint pain (27.5%), fever (17.8%), and injection site swelling (10.6%). (6.1)
- In clinical studies of participants 56 years of age and older, the most commonly reported adverse reactions (≥10%) were pain at the injection site (78.2%), fatigue (56.9%), headache, (45.9%), muscle pain (32.5%), chills (24.8%), joint pain (21.5%), injection site swelling (11.8%), fever (11.5%), and injection site redness (10.4%). (6.1)
- In clinical studies of adolescents 12 through 15 years of age, the most commonly reported adverse reactions (≥8%) were pain at the injection site (90.5%), fatigue (77.5%), headache (75.5%), chills (49.2%), muscle pain (42.2%), fever (24.3%), joint pain (20.2%), injection site swelling (9.2%), and injection site redness (8.6%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer Inc. at 1-800-438-1985 or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 8/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

COMIRNATY is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 12 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

The storage, preparation, and administration information in this Prescribing Information apply to COMIRNATY supplied in:

- single dose vials with gray caps and labels with gray borders, and
- multiple dose vials with gray caps and labels with gray borders.

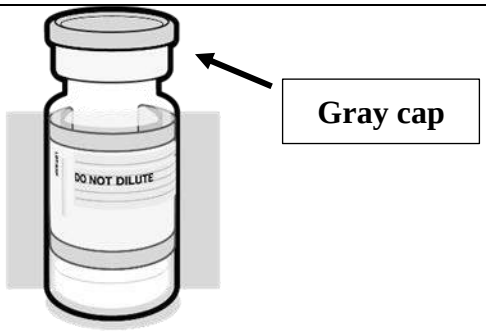
COMIRNATY supplied in vials with gray caps and labels with gray borders MUST NOT be diluted prior to use.

2.1 Preparation for Administration

- COMIRNATY vials with gray caps and labels with gray borders contain a frozen suspension without preservative. Each vial must be thawed prior to administration. **DO NOT DILUTE** prior to use.
- Vials may be thawed in the refrigerator [2ºC to 8ºC (35ºF to 46ºF)] or at room temperature [up to 25ºC (77ºF)].
- Refer to thawing and preparation instructions in the panels below.

Preparation Instructions

COMIRNATY Vial with Gray Cap and Label with Gray Border – Vial Verification



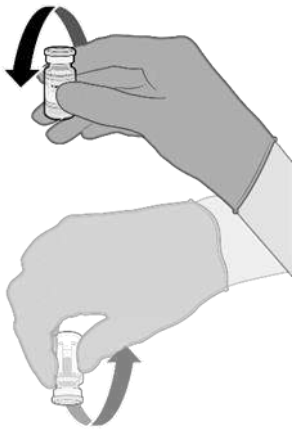
✓ **Gray cap and label with gray border.**

- Verify that the vial of COMIRNATY has a gray cap and a label with a gray border.

Thawing Prior to Use

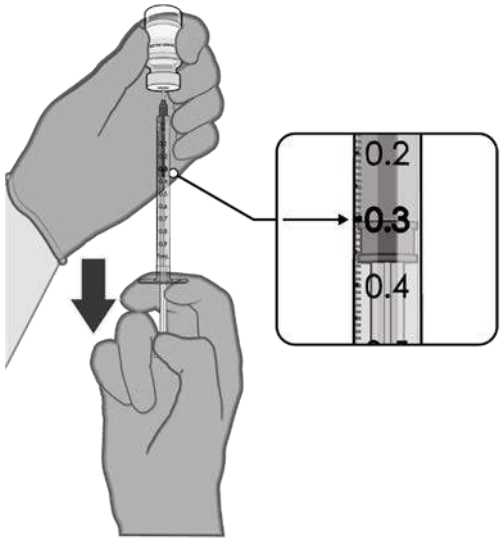
Store in the refrigerator for up to 10 weeks prior to use.

- Thaw vial(s) of COMIRNATY before use either by:
  - Allowing vial(s) to thaw in the refrigerator [2ºC to 8ºC (35ºF to 46ºF)].
    - A carton of 10 single dose vials may take up to 2 hours to thaw
    - A carton of 10 multiple dose vials may take up to 6 hours to thaw.
  - Allowing vial(s) to sit at room temperature [up to 25ºC (77ºF)] for 30 minutes.
- Thawed vials can be stored in the refrigerator [2ºC to 8ºC (35ºF to 46ºF)] for up to 10 weeks prior to use.
- Thawed vials may be stored at room temperature [up to 25ºC (77ºF)] for up to 12 hours prior to use.



Gently × 10

- Before use, mix by inverting vaccine vial gently 10 times.
- Do not shake.
- Prior to mixing, the thawed vaccine may contain white to off-white opaque amorphous particles.
- After mixing, the vaccine should appear as a white to off-white suspension with no visible particles.
- Do not use if liquid is discolored or if particles are observed after mixing.

<table>
<tr><th colspan="2">Preparation of Individual 0.3 mL Doses</th></tr>
<tr><td> 

Withdraw 0.3 mL dose of vaccine.</td><td>Single Dose Vial
Withdraw a single 0.3 mL dose of COMIRNATY vaccine.
Administer immediately.
Discard vial and any excess volume.
Multiple Dose Vial
Multiple dose vials contain 6 doses of 0.3 mL each.
Withdraw 0.3 mL of COMIRNATY preferentially using low dead-volume syringes and/or needles. If standard syringes and needles are used, there may not be sufficient volume to extract 6 doses from a single vial.
Administer immediately.
If the amount of vaccine remaining in a multiple dose vial cannot provide a full dose of 0.3 mL, discard the vial and any excess volume.</td></tr>
<tr><th colspan="2">Multiple Dose Vial – Record Date and Time of First Puncture</th></tr>
<tr><td> 

Record the date and time of first puncture.
Use within 12 hours after first puncture.</td><td>Record the date and time of first vial puncture on the COMIRNATY multiple dose vial label.
Store between 2°C to 25°C (35°F to 77°F).
Discard any unused vaccine 12 hours after first puncture.</td></tr>
</table>

2.2 Administration Information

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. The vaccine will be a white to off-white suspension. Do not administer if vaccine is discolored or contains particulate matter.

After withdrawing a single 0.3 mL dose of COMIRNATY, administer immediately.

2.3 Vaccination Schedule

COMIRNATY is administered intramuscularly as a series of 2 doses (0.3 mL each) 3 weeks apart.

There are no data available on the interchangeability of COMIRNATY with COVID-19 vaccines from other manufacturers to complete the vaccination series. Individuals who have received 1 dose of COMIRNATY should receive a second dose of COMIRNATY to complete the vaccination series.

3 DOSAGE FORMS AND STRENGTHS

COMIRNATY is a suspension for injection. Each dose of COMIRNATY supplied in vials with gray caps and labels with gray borders is 0.3 mL.

4 CONTRAINDICATIONS

Do not administer COMIRNATY to individuals with known history of a severe allergic reaction (e.g., anaphylaxis) to any component of COMIRNATY *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate medical treatment used to manage immediate allergic reactions must be immediately available in the event an acute anaphylactic reaction occurs following administration of COMIRNATY.

5.2 Myocarditis and Pericarditis

Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. The observed risk is higher among males under 40 years of age than among females and older males. The observed risk is highest in males 12 through 17 years of age. Although some cases required intensive care support, available data from short-term follow-up suggest that most individuals have had resolution of symptoms with conservative management. Information is not yet available about potential long-term sequelae. The CDC has published considerations related to myocarditis and pericarditis after vaccination, including for vaccination of individuals with a history of myocarditis or pericarditis (https://www.cdc.gov/vaccines/covid-19/clinical-considerations/myocarditis.html).

5.3 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines, including COMIRNATY. Procedures should be in place to avoid injury from fainting.

5.4 Altered Immunocompetence

Immunocompromised persons, including individuals receiving immunosuppressant therapy, may have a diminished immune response to COMIRNATY.

5.5 Limitation of Effectiveness

COMIRNATY may not protect all vaccine recipients.

6 ADVERSE REACTIONS

In clinical studies, the most commonly reported (≥10%) adverse reactions in participants 16 through 55 years of age following any dose were pain at the injection site (88.6%), fatigue (70.1%), headache (64.9%), muscle pain (45.5%), chills (41.5%), joint pain (27.5%), fever (17.8%), and injection site swelling (10.6%).

In clinical studies, the most commonly reported (≥10%) adverse reactions in participants 56 years of age and older following any dose were pain at the injection site (78.2%), fatigue (56.9%), headache, (45.9%), muscle

pain (32.5%), chills (24.8%), joint pain (21.5%), injection site swelling (11.8%), fever (11.5%), and injection site redness (10.4%).

In a clinical study, the most commonly reported (≥8%) adverse reactions in adolescents 12 through 15 years of age following any dose were pain at the injection site (90.5%), fatigue (77.5%), headache (75.5%), chills (49.2%), muscle pain (42.2%), fever (24.3%), joint pain (20.2%), injection site swelling (9.2%), and injection site redness (8.6%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of COMIRNATY was evaluated in participants 12 years of age and older in 2 clinical studies conducted in Germany (Study 1), United States, Argentina, Brazil, Turkey, South Africa, and Germany (Study 2). Study BNT162-01 (Study 1) was a Phase 1/2, 2-part, dose-escalation trial that enrolled 60 participants, 18 through 55 years of age and 36 participants, 56 through 85 years of age. Study C4591001 (Study 2) is a Phase 1/2/3 multicenter, multinational, randomized, saline placebo-controlled, double-blinded (Phase 2/3), dose-finding, vaccine candidate-selection and efficacy study that has enrolled approximately 46,000 participants 12 years of age or older. Of these, approximately 44,047 participants (22,026 COMIRNATY; 22,021 placebo) in Phase 2/3 are 16 years of age or older (including 378 and 376 participants 16 through 17 years of age in the COMIRNATY and placebo groups, respectively) and 2,260 adolescents are 12 through 15 years of age (1,131 and 1,129 in the COMIRNATY and placebo groups, respectively). Upon issuance of the Emergency Use Authorization for COMIRNATY, participants were unblinded to offer placebo participants COMIRNATY. Participants were unblinded in a phased manner over a period of months to offer placebo participants COMIRNATY. Study 2 also included 200 participants with confirmed stable human immunodeficiency virus (HIV) infection; HIV-positive participants are included in safety population disposition but are summarized separately in safety analyses. Confirmed stable HIV infection was defined as documented viral load <50 copies/mL and CD4 count >200 cells/mm^3 within 6 months before enrollment, and on stable antiretroviral therapy for at least 6 months.

In Study 2, all participants 12 through 15 years of age, and 16 years and older in the reactogenicity subset were monitored for solicited local and systemic reactions and use of antipyretic medication after each vaccination in an electronic diary. Participants are being monitored for unsolicited adverse events, including serious adverse events, throughout the study [from Dose 1 through 1 month (all unsolicited adverse events) or 6 months (serious adverse events) after the last vaccination]. Tables 1 through 6 present the frequency and severity of solicited local and systemic reactions, respectively, within 7 days following each dose of COMIRNATY and placebo.

Participants 16 Years of Age and Older

At the time of the analysis of the ongoing Study 2 with a data cutoff of March 13, 2021, there were 25,651 (58.2%) participants (13,031 COMIRNATY and 12,620 placebo) 16 years of age and older followed for ≥4 months after the second dose.

Demographic characteristics in Study 2 were generally similar with regard to age, gender, race, and ethnicity among participants who received COMIRNATY and those who received placebo. Overall, among the total participants who received either COMIRNATY or placebo, 50.9% were male, 49.1% were female, 79.3% were 16 through 64 years of age, 20.7% were 65 years of age and older, 82.0% were White, 9.6% were Black or

African American, 25.9% were Hispanic/Latino, 4.3% were Asian, and 1.0% were American Indian or Alaska Native.

Local and Systemic Adverse Reactions Solicited in the Study 2

In participants 16 through 55 years of age after receiving Dose 2, the mean duration of pain at the injection site was 2.5 days (range 1 to 70 days), for redness 2.2 days (range 1 to 9 days), and for swelling 2.1 days (range 1 to 8 days) for participants in the COMIRNATY group. In participants 56 years of age and older after receiving Dose 2, the mean duration of pain at the injection site was 2.4 days (range 1 to 36 days), for redness 3.0 days (range 1 to 34 days), and for swelling 2.6 days (range 1 to 34 days) for participants in the COMIRNATY group.

Table 1: Study 2 – Frequency and Percentages of Participants with Solicited Local Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 16 Through 55 Years of Age – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2899 n[b] (%)	**Placebo Dose 1 N[a]=2908 n[b] (%)**	**COMIRNATY Dose 2 N[a]=2682 n[b] (%)**	**Placebo Dose 2 N[a]=2684 n[b] (%)**
Redness[c]				
Any (>2.0 cm)	156 (5.4)	28 (1.0)	151 (5.6)	18 (0.7)
Mild	113 (3.9)	19 (0.7)	90 (3.4)	12 (0.4)
Moderate	36 (1.2)	6 (0.2)	50 (1.9)	6 (0.2)
Severe	7 (0.2)	3 (0.1)	11 (0.4)	0
Swelling[c]				
Any (>2.0 cm)	184 (6.3)	16 (0.6)	183 (6.8)	5 (0.2)
Mild	124 (4.3)	6 (0.2)	110 (4.1)	3 (0.1)
Moderate	54 (1.9)	8 (0.3)	66 (2.5)	2 (0.1)
Severe	6 (0.2)	2 (0.1)	7 (0.3)	0
Pain at the injection site[d]				
Any	2426 (83.7)	414 (14.2)	2101 (78.3)	312 (11.6)
Mild	1464 (50.5)	391 (13.4)	1274 (47.5)	284 (10.6)
Moderate	923 (31.8)	20 (0.7)	788 (29.4)	28 (1.0)
Severe	39 (1.3)	3 (0.1)	39 (1.5)	0

Notes: Reactions were collected in the electronic diary (e-diary) from Day 1 to Day 7 after vaccination.
No Grade 4 solicited local reactions were reported in participants 16 through 55 years of age.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. The N for each reaction was the same, therefore, this information was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: >2.0 to ≤5.0 cm; Moderate: >5.0 to ≤10.0 cm; Severe: >10.0 cm.

d. Mild: does not interfere with activity; Moderate: interferes with activity; Severe: prevents daily activity.

Table 2: Study 2 – Frequency and Percentages of Participants with Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 16 Through 55 Years of Age – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2899 n[b] (%)	Placebo Dose 1 N[a]=2908 n[b] (%)	COMIRNATY Dose 2 N[a]=2682 n[b] (%)	Placebo Dose 2 N[a]=2684 n[b] (%)
Fever				
≥38.0°C	119 (4.1)	25 (0.9)	440 (16.4)	11 (0.4)
≥38.0°C to 38.4°C	86 (3.0)	16 (0.6)	254 (9.5)	5 (0.2)
>38.4°C to 38.9°C	25 (0.9)	5 (0.2)	146 (5.4)	4 (0.1)
>38.9°C to 40.0°C	8 (0.3)	4 (0.1)	39 (1.5)	2 (0.1)
>40.0°C	0	0	1 (0.0)	0
Fatigue[c]				
Any	1431 (49.4)	960 (33.0)	1649 (61.5)	614 (22.9)
Mild	760 (26.2)	570 (19.6)	558 (20.8)	317 (11.8)
Moderate	630 (21.7)	372 (12.8)	949 (35.4)	283 (10.5)
Severe	41 (1.4)	18 (0.6)	142 (5.3)	14 (0.5)
Headache[c]				
Any	1262 (43.5)	975 (33.5)	1448 (54.0)	652 (24.3)
Mild	785 (27.1)	633 (21.8)	699 (26.1)	404 (15.1)
Moderate	444 (15.3)	318 (10.9)	658 (24.5)	230 (8.6)
Severe	33 (1.1)	24 (0.8)	91 (3.4)	18 (0.7)
Chills[c]				
Any	479 (16.5)	199 (6.8)	1015 (37.8)	114 (4.2)
Mild	338 (11.7)	148 (5.1)	477 (17.8)	89 (3.3)
Moderate	126 (4.3)	49 (1.7)	469 (17.5)	23 (0.9)
Severe	15 (0.5)	2 (0.1)	69 (2.6)	2 (0.1)
Vomiting[d]				
Any	34 (1.2)	36 (1.2)	58 (2.2)	30 (1.1)
Mild	29 (1.0)	30 (1.0)	42 (1.6)	20 (0.7)
Moderate	5 (0.2)	5 (0.2)	12 (0.4)	10 (0.4)
Severe	0	1 (0.0)	4 (0.1)	0
Diarrhea[e]				
Any	309 (10.7)	323 (11.1)	269 (10.0)	205 (7.6)
Mild	251 (8.7)	264 (9.1)	219 (8.2)	169 (6.3)
Moderate	55 (1.9)	58 (2.0)	44 (1.6)	35 (1.3)
Severe	3 (0.1)	1 (0.0)	6 (0.2)	1 (0.0)
New or worsened muscle pain[c]				
Any	664 (22.9)	329 (11.3)	1055 (39.3)	237 (8.8)
Mild	353 (12.2)	231 (7.9)	441 (16.4)	150 (5.6)
Moderate	296 (10.2)	96 (3.3)	552 (20.6)	84 (3.1)
Severe	15 (0.5)	2 (0.1)	62 (2.3)	3 (0.1)
New or worsened joint pain[c]				
Any	342 (11.8)	168 (5.8)	638 (23.8)	147 (5.5)
Mild	200 (6.9)	112 (3.9)	291 (10.9)	82 (3.1)
Moderate	137 (4.7)	55 (1.9)	320 (11.9)	61 (2.3)
Severe	5 (0.2)	1 (0.0)	27 (1.0)	4 (0.1)

	COMIRNATY Dose 1 N[a]=2899 n[b] (%)	Placebo Dose 1 N[a]=2908 n[b] (%)	COMIRNATY Dose 2 N[a]=2682 n[b] (%)	Placebo Dose 2 N[a]=2684 n[b] (%)
Use of antipyretic or pain medication[f]	805 (27.8)	398 (13.7)	1213 (45.2)	320 (11.9)

Notes: Reactions and use of antipyretic or pain medication were collected in the electronic diary (e-diary) from Day 1 to Day 7 after each dose.

No Grade 4 solicited systemic reactions were reported in participants 16 through 55 years of age.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. The N for each reaction or use of antipyretic or pain medication was the same, therefore, this information was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: does not interfere with activity; Moderate: some interference with activity; Severe: prevents daily activity.

d. Mild: 1 to 2 times in 24 hours; Moderate: >2 times in 24 hours; Severe: requires intravenous hydration.

e. Mild: 2 to 3 loose stools in 24 hours; Moderate: 4 to 5 loose stools in 24 hours; Severe: 6 or more loose stools in 24 hours.

f. Severity was not collected for use of antipyretic or pain medication.

Table 3: Study 2 – Frequency and Percentages of Participants with Solicited Local Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 56 Years of Age and Older – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)
Redness[c]				
Any (>2.0 cm)	106 (5.3)	20 (1.0)	133 (7.2)	14 (0.8)
Mild	71 (3.5)	13 (0.7)	65 (3.5)	10 (0.5)
Moderate	30 (1.5)	5 (0.3)	58 (3.1)	3 (0.2)
Severe	5 (0.2)	2 (0.1)	10 (0.5)	1 (0.1)
Swelling[c]				
Any (>2.0 cm)	141 (7.0)	23 (1.2)	145 (7.8)	13 (0.7)
Mild	87 (4.3)	11 (0.6)	80 (4.3)	5 (0.3)
Moderate	52 (2.6)	12 (0.6)	61 (3.3)	7 (0.4)
Severe	2 (0.1)	0	4 (0.2)	1 (0.1)
Pain at the injection site[d]				
Any (>2.0 cm)	1408 (70.1)	185 (9.3)	1230 (66.1)	143 (7.8)
Mild	1108 (55.2)	177 (8.9)	873 (46.9)	138 (7.5)
Moderate	296 (14.7)	8 (0.4)	347 (18.7)	5 (0.3)
Severe	4 (0.2)	0	10 (0.5)	0

Notes: Reactions were collected in the electronic diary (e-diary) from Day 1 to Day 7 after vaccination.

No Grade 4 solicited local reactions were reported in participants 56 years of age and older.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. The N for each reaction was the same, therefore, the information was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: >2.0 to ≤5.0 cm; Moderate: >5.0 to ≤10.0 cm; Severe: >10.0 cm.

d. Mild: does not interfere with activity; Moderate: interferes with activity; Severe: prevents daily activity.

Table 4: Study 2 – Frequency and Percentages of Participants with Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Each Dose – Participants 56 Years of Age and Older – Reactogenicity Subset of the Safety Population*

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)
Fever				
≥38.0°C	26 (1.3)	8 (0.4)	219 (11.8)	4 (0.2)
≥38.0°C to 38.4°C	23 (1.1)	3 (0.2)	158 (8.5)	2 (0.1)
>38.4°C to 38.9°C	2 (0.1)	3 (0.2)	54 (2.9)	1 (0.1)
>38.9°C to 40.0°C	1 (0.0)	2 (0.1)	7 (0.4)	1 (0.1)
>40.0°C	0	0	0	0
Fatigue[c]				
Any	677 (33.7)	447 (22.5)	949 (51.0)	306 (16.7)
Mild	415 (20.7)	281 (14.1)	391 (21.0)	183 (10.0)
Moderate	259 (12.9)	163 (8.2)	497 (26.7)	121 (6.6)
Severe	3 (0.1)	3 (0.2)	60 (3.2)	2 (0.1)
Grade 4	0	0	1 (0.1)	0
Headache[c]				
Any	503 (25.0)	363 (18.3)	733 (39.4)	259 (14.1)
Mild	381 (19.0)	267 (13.4)	464 (24.9)	189 (10.3)
Moderate	120 (6.0)	93 (4.7)	256 (13.8)	65 (3.5)
Severe	2 (0.1)	3 (0.2)	13 (0.7)	5 (0.3)
Chills[c]				
Any	130 (6.5)	69 (3.5)	435 (23.4)	57 (3.1)
Mild	102 (5.1)	49 (2.5)	229 (12.3)	45 (2.5)
Moderate	28 (1.4)	19 (1.0)	185 (9.9)	12 (0.7)
Severe	0	1 (0.1)	21 (1.1)	0
Vomiting[d]				
Any	10 (0.5)	9 (0.5)	13 (0.7)	5 (0.3)
Mild	9 (0.4)	9 (0.5)	10 (0.5)	5 (0.3)
Moderate	1 (0.0)	0	1 (0.1)	0
Severe	0	0	2 (0.1)	0
Diarrhea[e]				
Any	168 (8.4)	130 (6.5)	152 (8.2)	102 (5.6)
Mild	137 (6.8)	109 (5.5)	125 (6.7)	76 (4.1)
Moderate	27 (1.3)	20 (1.0)	25 (1.3)	22 (1.2)
Severe	4 (0.2)	1 (0.1)	2 (0.1)	4 (0.2)
New or worsened muscle pain[c]				
Any	274 (13.6)	165 (8.3)	537 (28.9)	99 (5.4)
Mild	183 (9.1)	111 (5.6)	229 (12.3)	65 (3.5)
Moderate	90 (4.5)	51 (2.6)	288 (15.5)	33 (1.8)
Severe	1 (0.0)	3 (0.2)	20 (1.1)	1 (0.1)

	COMIRNATY Dose 1 N[a]=2008 n[b] (%)	Placebo Dose 1 N[a]=1989 n[b] (%)	COMIRNATY Dose 2 N[a]=1860 n[b] (%)	Placebo Dose 2 N[a]=1833 n[b] (%)
New or worsened joint pain[c]				
Any	175 (8.7)	124 (6.2)	353 (19.0)	72 (3.9)
Mild	119 (5.9)	78 (3.9)	183 (9.8)	44 (2.4)
Moderate	53 (2.6)	45 (2.3)	161 (8.7)	27 (1.5)
Severe	3 (0.1)	1 (0.1)	9 (0.5)	1 (0.1)
Use of antipyretic or pain medication[f]	382 (19.0)	224 (11.3)	688 (37.0)	170 (9.3)

Notes: Reactions and use of antipyretic or pain medication were collected in the electronic diary (e-diary) from Day 1 to Day 7 after each dose.

The only Grade 4 solicited systemic reaction reported in participants 56 years of age and older was fatigue.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention. Participants with chronic, stable HIV infection were excluded.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose. N for each reaction or use of antipyretic or pain medication was the same, therefore was included in the column header.

b. n = Number of participants with the specified reaction.

c. Mild: does not interfere with activity; Moderate: some interference with activity; Severe: prevents daily activity; Grade 4 reactions were defined in the clinical study protocol as emergency room visit or hospitalization for severe fatigue, severe headache, severe chills, severe muscle pain, or severe joint pain.

d. Mild: 1 to 2 times in 24 hours; Moderate: >2 times in 24 hours; Severe: requires intravenous hydration; Grade 4 emergency visit or hospitalization for severe vomiting.

e. Mild: 2 to 3 loose stools in 24 hours; Moderate: 4 to 5 loose stools in 24 hours; Severe: 6 or more loose stools in 24 hours; Grade 4: emergency room or hospitalization for severe diarrhea.

f. Severity was not collected for use of antipyretic or pain medication.

In participants with chronic, stable HIV infection the frequencies of solicited local and systemic adverse reactions were similar to or lower than those observed for all participants 16 years of age and older.

Unsolicited Adverse Events

Overall, 11,253 (51.1%) participants in the COMIRNATY group and 11,316 (51.4%) participants in the placebo group had follow-up time between ≥4 months to <6 months after Dose 2 in the blinded placebo-controlled follow-up period with an additional 1,778 (8.1%) and 1,304 (5.9%) with ≥6 months of blinded follow-up time in the COMIRNATY and placebo groups, respectively.

A total of 12,006 (54.5%) participants originally randomized to COMIRNATY had ≥6 months total (blinded and unblinded) follow-up after Dose 2.

In an analysis of all unsolicited adverse events reported following any dose, through 1 month after Dose 2, in participants 16 years of age and older (N=43,847; 21,926 COMIRNATY group vs. 21,921 placebo group), those assessed as adverse reactions not already captured by solicited local and systemic reactions were nausea (274 vs. 87), malaise (130 vs. 22), lymphadenopathy (83 vs. 7), asthenia (76 vs. 25), decreased appetite (39 vs. 9), hyperhidrosis (31 vs. 9), lethargy (25 vs. 6), and night sweats (17 vs. 3).

In analyses of all unsolicited adverse events in Study 2 from Dose 1 up to the participant unblinding date, 58.2% of study participants had at least 4 months of follow-up after Dose 2. Among participants 16 through 55 years of age who received at least 1 dose of study vaccine, 12,995 of whom received COMIRNATY and 13,026 of whom received placebo, unsolicited adverse events were reported by 4,396 (33.8%) participants in the COMIRNATY group and 2,136 (16.4%) participants in the placebo group. In a similar analysis in

participants 56 years of age and older that included 8,931 COMIRNATY recipients and 8,895 placebo recipients, unsolicited adverse events were reported by 2,551 (28.6%) participants in the COMIRNATY group and 1,432 (16.1%) participants in the placebo group. Among participants with confirmed stable HIV infection that included 100 COMIRNATY recipients and 100 placebo recipients, unsolicited adverse events were reported by 29 (29%) participants in the COMIRNATY group and 15 (15%) participants in the placebo group. The higher frequency of reported unsolicited adverse events among COMIRNATY recipients compared to placebo recipients was primarily attributed to events that are consistent with adverse reactions solicited among participants in the reactogenicity subset (Table 3 and Table 4).

Throughout the placebo-controlled safety follow-up period, Bell's palsy (facial paralysis) was reported by 4 participants in the COMIRNATY group and 2 participants in the placebo group. Onset of facial paralysis was Day 37 after Dose 1 (participant did not receive Dose 2) and Days 3, 9, and 48 after Dose 2. In the placebo group the onset of facial paralysis was Day 32 and Day 102. Currently available information is insufficient to determine a causal relationship with the vaccine. In the analysis of blinded, placebo-controlled follow-up, there were no other notable patterns or numerical imbalances between treatment groups for specific categories of non-serious adverse events (including other neurologic or neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to COMIRNATY. In the analysis of unblinded follow-up, there were no notable patterns of specific categories of non-serious adverse events that would suggest a causal relationship to COMIRNATY.

Serious Adverse Events

In Study 2, among participants 16 through 55 years of age who had received at least 1 dose of vaccine or placebo (COMIRNATY =12,995; placebo = 13,026), serious adverse events from Dose 1 up to the participant unblinding date in ongoing follow-up were reported by 103 (0.8%) COMIRNATY recipients and 117 (0.9%) placebo recipients. In a similar analysis, in participants 56 years of age and older (COMIRNATY = 8,931; placebo = 8,895), serious adverse events were reported by 165 (1.8%) COMIRNATY recipients and 151 (1.7%) placebo recipients who received at least 1 dose of COMIRNATY or placebo, respectively. In these analyses, 58.2% of study participants had at least 4 months of follow-up after Dose 2. Among participants with confirmed stable HIV infection serious adverse events from Dose 1 up to the participant unblinding date in ongoing follow-up were reported by 2 (2%) COMIRNATY recipients and 2 (2%) placebo recipients.

In the analysis of blinded, placebo-controlled follow-up, there were no notable patterns between treatment groups for specific categories of serious adverse events (including neurologic, neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to COMIRNATY. In the analysis of unblinded follow-up, there were no notable patterns of specific categories of serious adverse events that would suggest a causal relationship to COMIRNATY.

Adolescents 12 Through 15 Years of Age

In Study 2, 2,260 adolescents (1,131 COMIRNATY; 1,129 placebo) were 12 through 15 years of age. At the time of the analysis of the ongoing Study 2 with a data cutoff of September 2, 2021, there were 1,559 (69.0%) adolescents (786 COMIRNATY and 773 placebo) 12 through 15 years of age followed for ≥4 months after the second dose. The safety evaluation in Study 2 is ongoing.

Demographic characteristics in Study 2 were generally similar with regard to age, gender, race, and ethnicity among adolescents who received COMIRNATY and those who received placebo. Overall, among the adolescents who received COMIRNATY, 50.1% were male and 49.9% were female, 85.8% were White, 4.6% were Black or African American, 11.7% were Hispanic/Latino, 6.4% were Asian, and 0.4% were American Indian/Alaska Native.

Local and Systemic Adverse Reactions Solicited in Study 2

In adolescents 12 through 15 years of age after receiving Dose 2, the mean duration of pain at the injection site was 2.5 days (range 1 to 11 days), for redness 1.8 days (range 1 to 5 days), and for swelling 1.6 days (range 1 to 5 days) in the COMIRNATY group.

Table 5: Study 2 – Frequency and Percentages of Adolescents With Solicited Local Reactions, by Maximum Severity, Within 7 Days After Each Dose – Adolescents 12 Through 15 Years of Age – Safety Population*

	COMIRNATY Dose 1 N[a]=1127 n[b] (%)	Placebo Dose 1 N[a]=1127 n[b] (%)	COMIRNATY Dose 2 N[a]=1097 n[b] (%)	Placebo Dose 2 N[a]=1078 n[b] (%)
Redness[c]				
Any (>2 cm)	65 (5.8)	12 (1.1)	55 (5.0)	10 (0.9)
Mild	44 (3.9)	11 (1.0)	29 (2.6)	8 (0.7)
Moderate	20 (1.8)	1 (0.1)	26 (2.4)	2 (0.2)
Severe	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Swelling[c]				
Any (>2 cm)	78 (6.9)	11 (1.0)	54 (4.9)	6 (0.6)
Mild	55 (4.9)	9 (0.8)	36 (3.3)	4 (0.4)
Moderate	23 (2.0)	2 (0.2)	18 (1.6)	2 (0.2)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pain at the injection site[d]				
Any	971 (86.2)	263 (23.3)	866 (78.9)	193 (17.9)
Mild	467 (41.4)	227 (20.1)	466 (42.5)	164 (15.2)
Moderate	493 (43.7)	36 (3.2)	393 (35.8)	29 (2.7)
Severe	11 (1.0)	0 (0.0)	7 (0.6)	0 (0.0)

Note: Reactions were collected in the electronic diary (e-diary) from Day 1 to Day 7 after vaccination.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention.

a. N = Number of participants reporting at least 1 yes or no response for the specified reaction after the specified dose.

b. n = Number of participants with the specified reaction.

c. Mild: >2.0 to ≤5.0 cm; Moderate: >5.0 to ≤10.0 cm; Severe: >10.0 cm.

d. Mild: does not interfere with activity; Moderate: interferes with activity; Severe: prevents daily activity.

Table 6: Study 2 – Frequency and Percentages of Adolescents with Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Each Dose – Adolescents 12 Through 15 Years of Age – Safety Population*

	COMIRNATY Dose 1 N[a]=1127 n[b] (%)	Placebo Dose 1 N[a]=1127 n[b] (%)	COMIRNATY Dose 2 N[a]=1097 n[b] (%)	Placebo Dose 2 N[a]=1078 n[b] (%)
Fever				
≥38.0°C	114 (10.1)	12 (1.1)	215 (19.6)	7 (0.6)
≥38.0°C to 38.4°C	74 (6.6)	8 (0.7)	107 (9.8)	5 (0.5)
>38.4°C to 38.9°C	29 (2.6)	2 (0.2)	83 (7.6)	1 (0.1)
>38.9°C to 40.0°C	10 (0.9)	2 (0.2)	25 (2.3)	1 (0.1)
>40.0°C	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Fatigue[c]				
Any	677 (60.1)	457 (40.6)	726 (66.2)	264 (24.5)
Mild	278 (24.7)	250 (22.2)	232 (21.1)	133 (12.3)

	COMIRNATY Dose 1 N[a]=1127 n[b] (%)	Placebo Dose 1 N[a]=1127 n[b] (%)	COMIRNATY Dose 2 N[a]=1097 n[b] (%)	Placebo Dose 2 N[a]=1078 n[b] (%)
Moderate	384 (34.1)	199 (17.7)	468 (42.7)	127 (11.8)
Severe	15 (1.3)	8 (0.7)	26 (2.4)	4 (0.4)
Headache[c]				
Any	623 (55.3)	396 (35.1)	708 (64.5)	264 (24.5)
Mild	361 (32.0)	256 (22.7)	302 (27.5)	170 (15.8)
Moderate	251 (22.3)	131 (11.6)	384 (35.0)	93 (8.6)
Severe	11 (1.0)	9 (0.8)	22 (2.0)	1 (0.1)
Chills[c]				
Any	311 (27.6)	109 (9.7)	455 (41.5)	74 (6.9)
Mild	195 (17.3)	82 (7.3)	221 (20.1)	53 (4.9)
Moderate	111 (9.8)	25 (2.2)	214 (19.5)	21 (1.9)
Severe	5 (0.4)	2 (0.2)	20 (1.8)	0 (0.0)
Vomiting[d]				
Any	31 (2.8)	10 (0.9)	29 (2.6)	12 (1.1)
Mild	30 (2.7)	8 (0.7)	25 (2.3)	11 (1.0)
Moderate	0 (0.0)	2 (0.2)	4 (0.4)	1 (0.1)
Severe	1 (0.1)	0 (0.0)	0 (0.0)	0 (0.0)
Diarrhea[e]				
Any	90 (8.0)	82 (7.3)	65 (5.9)	44 (4.1)
Mild	77 (6.8)	72 (6.4)	59 (5.4)	39 (3.6)
Moderate	13 (1.2)	10 (0.9)	6 (0.5)	5 (0.5)
Severe	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
New or worsened muscle pain[c]				
Any	272 (24.1)	148 (13.1)	355 (32.4)	90 (8.3)
Mild	125 (11.1)	88 (7.8)	152 (13.9)	51 (4.7)
Moderate	145 (12.9)	60 (5.3)	197 (18.0)	37 (3.4)
Severe	2 (0.2)	0 (0.0)	6 (0.5)	2 (0.2)
New or worsened joint pain[c]				
Any	109 (9.7)	77 (6.8)	173 (15.8)	51 (4.7)
Mild	66 (5.9)	50 (4.4)	91 (8.3)	30 (2.8)
Moderate	42 (3.7)	27 (2.4)	78 (7.1)	21 (1.9)
Severe	1 (0.1)	0 (0.0)	4 (0.4)	0 (0.0)
Use of antipyretic or pain medication[f]	413 (36.6)	111 (9.8)	557 (50.8)	95 (8.8)

Note: Events and use of antipyretic or pain medication were collected in the electronic diary (e-diary) from Day 1 to Day 7 after each dose.

* Randomized participants in the safety analysis population who received at least 1 dose of the study intervention.

a. N = Number of participants reporting at least 1 yes or no response for the specified event after the specified dose.

b. n = Number of participants with the specified reaction.

c. Mild: does not interfere with activity; Moderate: some interference with activity; Severe: prevents daily activity.

d. Mild: 1 to 2 times in 24 hours; Moderate: >2 times in 24 hours; Severe: requires intravenous hydration.

e. Mild: 2 to 3 loose stools in 24 hours; Moderate: 4 to 5 loose stools in 24 hours; Severe: 6 or more loose stools in 24 hours.

f. Severity was not collected for use of antipyretic or pain medication.

Unsolicited Adverse Events
In Study 2, 2,260 adolescents (1,131 COMIRNATY; 1,129 placebo) were 12 through 15 years of age. Of these, 634 (56.1%) participants in the COMIRNATY group and 629 (55.7%) participants in the placebo group had follow-up time between ≥4 months to <6 months after Dose 2 in the blinded placebo-controlled follow-up period with an additional 152 (13.4%) and 144 (12.8%) with ≥6 months of blinded follow-up time in the COMIRNATY and placebo groups, respectively.

A total of 1,113 (98.4%) participants 12 through 15 years of age originally randomized to COMIRNATY had ≥6 months total (blinded and unblinded) follow-up after Dose 2.

An analysis of all unsolicited adverse events in Study 2 from Dose 1 up to the participant unblinding date was conducted. Among participants 12 through 15 years of age who received at least one dose of study vaccine, unsolicited adverse events were reported by 95 (8.4%) participants in the COMIRNATY group and 113 (10.0%) participants in the placebo group.

In an analysis of all unsolicited adverse events reported during blinded follow-up from Dose 1 through 1 month after Dose 2, in adolescents 12 to 15 years of age, those assessed as adverse reactions not already captured by solicited local and systemic reactions were lymphadenopathy (9 vs. 2), and nausea (5 vs. 2).

In the analysis of blinded, placebo-controlled follow-up, there were no other notable patterns or numerical imbalances between treatment groups for specific categories of unsolicited adverse events (including other neurologic or neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to COMIRNATY. In the analysis of unblinded follow-up, there were no notable patterns of specific categories of non-serious adverse events that would suggest a causal relationship to COMIRNATY.

Serious Adverse Events
In Study 2, among participants 12 through 15 years of age who had received at least 1 dose of vaccine or placebo (COMIRNATY = 1,131; placebo = 1,129), serious adverse events from Dose 1 up to the participant unblinding date in ongoing follow-up were reported by 10 (0.9%) COMIRNATY recipients and 2 (0.2%) placebo recipients. In these analyses, 69.0% of study participants had at least 4 months of follow-up after Dose 2. In the analysis of blinded, placebo-controlled follow-up, there were no notable patterns between treatment groups for specific categories of serious adverse events (including neurologic, neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to COMIRNATY. In the analysis of unblinded follow-up, there were no notable patterns of specific categories of serious adverse events that would suggest a causal relationship to COMIRNATY.

6.2 Postmarketing Experience

The following adverse reactions have been identified during postmarketing use of COMIRNATY, including under Emergency Use Authorization. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Cardiac Disorders: myocarditis, pericarditis
Gastrointestinal Disorders: diarrhea, vomiting
Immune System Disorders: severe allergic reactions, including anaphylaxis, and other hypersensitivity reactions (e.g., rash, pruritus, urticaria, angioedema)
Musculoskeletal and Connective Tissue Disorders: pain in extremity (arm)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to COMIRNATY during pregnancy. Women who are vaccinated with COMIRNATY during pregnancy are encouraged to enroll in the registry by visiting https://mothertobaby.org/ongoing-study/covid19-vaccines/.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. Available data on COMIRNATY administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study has been performed in female rats administered the equivalent of a single human dose of COMIRNATY on 4 occasions, twice prior to mating and twice during gestation. These studies revealed no evidence of harm to the fetus due to the vaccine *(see Animal Data)*.

Data

Animal Data

In a developmental toxicity study, 0.06 mL of a vaccine formulation containing the same quantity of nucleoside-modified messenger ribonucleic acid (mRNA) (30 mcg) and other ingredients included in a single human dose of COMIRNATY was administered to female rats by the intramuscular route on 4 occasions: 21 and 14 days prior to mating, and on gestation days 9 and 20. No vaccine-related adverse effects on female fertility, fetal development, or postnatal development were reported in the study.

8.2 Lactation

Risk Summary

It is not known whether COMIRNATY is excreted in human milk. Data are not available to assess the effects of COMIRNATY on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for COMIRNATY and any potential adverse effects on the breastfed child from COMIRNATY or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of COMIRNATY in individuals 12 through 17 years of age is based on safety and effectiveness data in this age group and in adults *[see Adverse Reactions (6) and Clinical Studies (14.1)]*.

The safety and effectiveness of COMIRNATY in individuals younger than 12 years of age have not been established.

8.5 Geriatric Use

Of the total number of COMIRNATY recipients in Study 2 as of March 13, 2021 (N = 22,026), 20.7% (n = 4,552) were 65 years of age and older and 4.2% (n = 925) were 75 years of age and older *[see Clinical Studies (14.1)]*. No overall differences in safety or effectiveness were observed between these recipients and younger recipients.

11 DESCRIPTION

COMIRNATY (COVID-19 Vaccine, mRNA) is a sterile suspension for injection for intramuscular use. Each 0.3 mL dose of COMIRNATY contains 30 mcg of a nucleoside-modified messenger RNA (mRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2.

Each 0.3 mL dose of COMIRNATY supplied in vials with gray caps and labels with gray borders also includes the following ingredients:
lipids (0.43 mg ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate),
0.05 mg 2-(polyethylene glycol 2000)-N,N-ditetradecylacetamide,
0.09 mg 1,2-distearoyl-sn-glycero-3-phosphocholine, and 0.19 mg cholesterol), 0.06 mg tromethamine,
0.4 mg tromethamine hydrochloride, and 31 mg sucrose.

COMIRNATY does not contain preservative.

The vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The nucleoside-modified mRNA in COMIRNATY is formulated in lipid particles, which enable delivery of the mRNA into host cells to allow expression of the SARS-CoV-2 S antigen. The vaccine elicits an immune response to the S antigen, which protects against COVID-19.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

COMIRNATY has not been evaluated for the potential to cause carcinogenicity, genotoxicity, or impairment of male fertility. In a developmental toxicity study in rats with COMIRNATY there were no vaccine-related effects on female fertility *[see Use in Specific Populations (8.1)]*.

14 CLINICAL STUDIES

14.1 Efficacy in Participants 16 Years of Age and Older

Study 2 is an ongoing, multicenter, multinational, randomized, placebo-controlled, observer-blind, dose-finding, vaccine candidate–selection, and efficacy study in participants 12 years of age and older. Randomization was stratified by age: 12 through 15 years of age, 16 through 55 years of age, or 56 years of age and older, with a minimum of 40% of participants in the ≥56-year stratum. The study excluded participants who were immunocompromised and those who had previous clinical or microbiological diagnosis of COVID-19. Participants with preexisting stable disease, defined as disease not requiring significant change in therapy or

hospitalization for worsening disease during the 6 weeks before enrollment, were included as were participants with known stable infection with HIV, hepatitis C virus (HCV), or hepatitis B virus (HBV).

In Study 2, based on data accrued through March 13, 2021, approximately 44,000 participants 12 years of age and older were randomized equally and received 2 doses of COMIRNATY or placebo. Participants are planned to be followed for up to 24 months, for assessments of safety and efficacy against COVID-19.

Overall, among the total participants who received COMIRNATY or placebo, 51.4% or 50.3% were male and 48.6% or 49.7% were female, 79.1% or 79.2% were 16 through 64 years of age, 20.9% or 20.8% were 65 years of age and older, 81.9% or 82.1% were White, 9.5% or 9.6% were Black or African American, 1.0% or 0.9% were American Indian or Alaska Native, 4.4% or 4.3% were Asian, 0.3% or 0.2% Native Hawaiian or other Pacific Islander, 25.6% or 25.4% were Hispanic/Latino, 73.9% or 74.1% were non-Hispanic/Latino, 0.5% or 0.5% did not report ethnicity, 46.0% or 45.7% had comorbidities [participants who have 1 or more comorbidities that increase the risk of severe COVID-19 disease: defined as subjects who had at least 1 of the Charlson comorbidity index category or body mass index (BMI) $\geq$30 kg/m^2], respectively. The mean age at vaccination was 49.8 or 49.7 years and median age was 51.0 or 51.0 in participants who received COMIRNATY or placebo, respectively.

Efficacy Against COVID-19

The population for the analysis of the protocol pre-specified primary efficacy endpoint included 36,621 participants 12 years of age and older (18,242 in the COMIRNATY group and 18,379 in the placebo group) who did not have evidence of prior infection with SARS-CoV-2 through 7 days after the second dose. The population in the protocol pre-specified primary efficacy analysis included all participants 12 years of age and older who had been enrolled from July 27, 2020, and followed for the development of COVID-19 through November 14, 2020. Participants 18 through 55 years of age and 56 years of age and older began enrollment from July 27, 2020, 16 through 17 years of age began enrollment from September 16, 2020, and 12 through 15 years of age began enrollment from October 15, 2020.

For participants without evidence of SARS-CoV-2 infection prior to 7 days after Dose 2, vaccine efficacy against confirmed COVID-19 occurring at least 7 days after Dose 2 was 95.0% (95% credible interval: 90.3, 97.6), which met the pre-specified success criterion. The case split was 8 COVID-19 cases in the COMIRNATY group compared to 162 COVID-19 cases in the placebo group.

The population for the updated vaccine efficacy analysis included participants 16 years of age and older who had been enrolled from July 27, 2020, and followed for the development of COVID-19 during blinded placebo-controlled follow-up through March 13, 2021, representing up to 6 months of follow-up after Dose 2. There were 12,796 (60.8%) participants in the COMIRNATY group and 12,449 (58.7%) in the placebo group followed for $\geq$4 months after Dose 2 in the blinded placebo-controlled follow-up period.

SARS-CoV-2 variants of concern identified from COVID-19 cases for this age group from this data cutoff include B.1.1.7 (Alpha) and B.1.351 (Beta). Representation of identified variants among cases in vaccine versus placebo recipients did not suggest decreased vaccine effectiveness against these variants.

The updated vaccine efficacy information is presented in Table 7.

Table 7: Vaccine Efficacy – First COVID-19 Occurrence From 7 Days After Dose 2, by Age Subgroup – Participants 16 Years of Age and Older Without Evidence of Infection and Participants With or Without Evidence of Infection Prior to 7 Days After Dose 2 – Evaluable Efficacy (7 Days) Population During the Placebo-Controlled Follow-up Period

First COVID-19 occurrence from 7 days after Dose 2 in participants without evidence of prior SARS-CoV-2 infection*			
Subgroup	**COMIRNATY Na=19,993 Cases n1b Surveillance Timec (n2d)**	**Placebo Na=20,118 Cases n1b Surveillance Timec (n2d)**	**Vaccine Efficacy % (95% CIe)**
All participants	77 6.092 (19,711)	833 5.857 (19,741)	91.1 (88.8, 93.1)
16 through 64 years	70 4.859 (15,519)	709 4.654 (15,515)	90.5 (87.9, 92.7)
65 years and older	7 1.233 (4192)	124 1.202 (4226)	94.5 (88.3, 97.8)
First COVID-19 occurrence from 7 days after Dose 2 in participants with or without* evidence of prior SARS-CoV-2 infection			
Subgroup	**COMIRNATY Na=21,047 Cases n1b Surveillance Timec (n2d)**	**Placebo Na=21,210 Cases n1b Surveillance Timec (n2d)**	**Vaccine Efficacy % (95% CIe)**
All participants	81 6.340 (20,533)	854 6.110 (20,595)	90.9 (88.5, 92.8)
16 through 64 years	74 5.073 (16,218)	726 4.879 (16,269)	90.2 (87.5, 92.4)
65 years and older	7 1.267 (4315)	128 1.232 (4326)	94.7 (88.7, 97.9)

Note: Confirmed cases were determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and at least 1 symptom consistent with COVID-19 (symptoms included: fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; vomiting).

* Participants who had no evidence of past SARS-CoV-2 infection (i.e., N-binding antibody [serum] negative at Visit 1 and SARS-CoV-2 not detected by NAAT [nasal swab] at Visits 1 and 2), and had negative NAAT (nasal swab) at any unscheduled visit prior to 7 days after Dose 2 were included in the analysis.

a. N = Number of participants in the specified group.

b. n1 = Number of participants meeting the endpoint definition.

c. Total surveillance time in 1000 person-years for the given endpoint across all participants within each group at risk for the endpoint. Time period for COVID-19 case accrual is from 7 days after Dose 2 to the end of the surveillance period.

d. n2 = Number of participants at risk for the endpoint.

e. Two-sided confidence interval (CI) for vaccine efficacy is derived based on the Clopper and Pearson method adjusted to the surveillance time.

Subgroup analyses of vaccine efficacy (although limited by small numbers of cases in some subgroups) did not suggest meaningful differences in efficacy across genders, ethnic groups, geographies, or for participants with obesity or medical comorbidities associated with high risk of severe COVID-19.

Efficacy Against Severe COVID-19

Efficacy analyses of secondary efficacy endpoints supported benefit of COMIRNATY in preventing severe COVID-19. Vaccine efficacy against severe COVID-19 is presented only for participants with or without prior

SARS-CoV-2 infection (Table 8) as the COVID-19 case counts in participants without prior SARS-CoV-2 infection were the same as those in participants with or without prior SARS-CoV-2 infection in both the COMIRNATY and placebo groups.

Table 8: Vaccine Efficacy – First Severe COVID-19 Occurrence in Participants 16 Years of Age and Older With or Without* Prior SARS-CoV-2 Infection Based on Protocol† or Centers for Disease Control and Prevention (CDC)‡ Definition From 7 Days After Dose 2 – Evaluable Efficacy (7 Days) Population During the Placebo-Controlled Follow-up

Vaccine Efficacy – First Severe COVID-19 Occurrence			
	COMIRNATY Cases n1[a] Surveillance Time[b] (n2[c])	**Placebo Cases n1[a] Surveillance Time[b] (n2[c])**	**Vaccine Efficacy % (95% CI[d])**
7 days after Dose 2[d]	1 6.353 (20,540)	21 6.237 (20,629)	95.3 (70.9, 99.9)
Vaccine Efficacy – First Severe COVID-19 Occurrence Based on CDC Definition			
	COMIRNATY Cases n1[a] Surveillance Time[b] (n2[c])	**Placebo Cases n1[a] Surveillance Time[b] (n2[c])**	**Vaccine Efficacy % (95% CI[d])**
7 days after Dose 2[d]	0 6.345 (20,513)	31 6.225 (20,593)	100 (87.6, 100.0)

Note: Confirmed cases were determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and at least 1 symptom consistent with COVID-19 (symptoms included: fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; vomiting).

* Participants who had no evidence of past SARS-CoV-2 infection (i.e., N-binding antibody [serum] negative at Visit 1 and SARS-CoV-2 not detected by NAAT [nasal swab] at Visits 1 and 2), and had negative NAAT (nasal swab) at any unscheduled visit prior to 7 days after Dose 2 were included in the analysis.

† Severe illness from COVID-19 is defined in the protocol as confirmed COVID-19 and presence of at least 1 of the following:

- Clinical signs at rest indicative of severe systemic illness (respiratory rate ≥30 breaths per minute, heart rate ≥125 beats per minute, saturation of oxygen ≤93% on room air at sea level, or ratio of arterial oxygen partial pressure to fractional inspired oxygen <300 mm Hg);
- Respiratory failure [defined as needing high-flow oxygen, noninvasive ventilation, mechanical ventilation or extracorporeal membrane oxygenation (ECMO)];
- Evidence of shock (systolic blood pressure <90 mm Hg, diastolic blood pressure <60 mm Hg, or requiring vasopressors);
- Significant acute renal, hepatic, or neurologic dysfunction;
- Admission to an Intensive Care Unit;
- Death.

‡ Severe illness from COVID-19 as defined by CDC is confirmed COVID-19 and presence of at least 1 of the following:

- Hospitalization;
- Admission to the Intensive Care Unit;
- Intubation or mechanical ventilation;
- Death.

a. n1 = Number of participants meeting the endpoint definition.
b. Total surveillance time in 1000 person-years for the given endpoint across all participants within each group at risk for the endpoint. Time period for COVID-19 case accrual is from 7 days after Dose 2 to the end of the surveillance period.
c. n2 = Number of participants at risk for the endpoint.
d. Two-side confidence interval (CI) for vaccine efficacy is derived based on the Clopper and Pearson method adjusted to the surveillance time.

14.2 Efficacy in Adolescents 12 Through 15 Years of Age

A descriptive efficacy analysis of Study 2 has been performed in 2,260 adolescents 12 through 15 years of age evaluating confirmed COVID-19 cases accrued up to a data cutoff date of September 2, 2021.

The vaccine efficacy information in adolescents 12 through 15 years of age is presented in Table 9.

Table 9: Vaccine Efficacy – First COVID-19 Occurrence From 7 Days After Dose 2: Without Evidence of Infection and With or Without Evidence of Infection Prior to 7 Days After Dose 2 – Blinded Placebo-Controlled Follow-up Period, Adolescents 12 Through 15 Years of Age Evaluable Efficacy (7 Days) Population

First COVID-19 occurrence from 7 days after Dose 2 in adolescents 12 through 15 years of age without evidence of prior SARS-CoV-2 infection*			
	COMIRNATY N[a]=1057 Cases n1[b] Surveillance Time[c] (n2[d])	**Placebo N[a]=1030 Cases n1[b] Surveillance Time[c] (n2[d])**	**Vaccine Efficacy % (95% CI[e])**
Adolescents 12 through 15 years of age	0 0.343 (1043)	28 0.322 (1019)	100.0 (86.8, 100.0)
First COVID-19 occurrence from 7 days after Dose 2 in adolescents 12 through 15 years of age with or without evidence of prior SARS-CoV-2 infection			
	COMIRNATY N[a]=1119 Cases n1[b] Surveillance Time[c] (n2[d])	**Placebo N[a]=1109 Cases n1[b] Surveillance Time[c] (n2[d])**	**Vaccine Efficacy % (95% CI[e])**
Adolescents 12 through 15 years of age	0 0.362 (1098)	30[f] 0.345 (1088)	100.0 (87.5, 100.0)

Note: Confirmed cases were determined by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) and at least 1 symptom consistent with COVID-19 (symptoms included: fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; vomiting).

* Participants who had no evidence of past SARS-CoV-2 infection (i.e., N-binding antibody [serum] negative at Visit 1 and SARS-CoV-2 not detected by NAAT [nasal swab] at Visits 1 and 2), and had negative NAAT (nasal swab) at any unscheduled visit prior to 7 days after Dose 2 were included in the analysis.

a. N = Number of participants in the specified group.

b. n1 = Number of participants meeting the endpoint definition.

c. Total surveillance time in 1000 person-years for the given endpoint across all participants within each group at risk for the endpoint. Time period for COVID-19 case accrual is from 7 days after Dose 2 to the end of the surveillance period.

d. n2 = Number of participants at risk for the endpoint.

e. Two-side confidence interval (CI) for vaccine efficacy is derived based on the Clopper and Pearson method adjusted for surveillance time.

f. The only SARS-CoV-2 variant of concern identified from COVID-19 cases in this age group from this data cutoff was B.1.1.7 (Alpha).

14.3 Immunogenicity in Adolescents 12 Through 15 Years of Age

In Study 2, an analysis of SARS-CoV-2 50% neutralizing titers (NT50) 1 month after Dose 2 in a randomly selected subset of participants demonstrated non-inferior immune responses (within 1.5-fold) comparing adolescents 12 through 15 years of age to participants 16 through 25 years of age who had no serological or virological evidence of past SARS-CoV-2 infection up to 1 month after Dose 2 (Table 10).

Table 10: Summary of Geometric Mean Ratio for 50% Neutralizing Titer – Comparison of Adolescents 12 Through 15 Years of Age to Participants 16 Through 25 Years of Age (Immunogenicity Subset) – Participants Without Evidence of Infection up to 1 Month After Dose 2 – Dose 2 Evaluable Immunogenicity Population

		COMIRNATY		12 Through 15 Years/ 16 Through 25 Years	
		12 Through 15 Years n[a]=190	16 Through 25 Years n[a]=170		
Assay	Time Point[b]	GMT[c] (95% CI[c])	GMT[c] (95% CI[c])	GMR[d] (95% CI[d])	Met Noninferiority Objective[e] (Y/N)
SARS-CoV-2 neutralization assay - NT50 (titer)[f]	1 month after Dose 2	1253.6 (1117.7, 1406.1)	708.1 (625.9, 801.1)	1.77 (1.50, 2.09)	Y

Abbreviations: CI = confidence interval; GMR = geometric mean ratio; GMT = geometric mean titer; LLOQ = lower limit of quantitation; NAAT = nucleic-acid amplification test; NT50 = 50% neutralizing titer; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Note: Participants who had no serological or virological evidence (up to 1 month after receipt of the last dose) of past SARS-CoV-2 infection (i.e., N-binding antibody [serum] negative at Visit 1 and SARS-CoV-2 not detected by NAAT [nasal swab] at Visits 1 and 2), and had negative NAAT (nasal swab) at any unscheduled visit up to 1 month after Dose 2 were included in the analysis.

a. n = Number of participants with valid and determinate assay results for the specified assay at the given dose/sampling time point.
b. Protocol-specified timing for blood sample collection.
c. GMTs and 2-sided 95% CIs were calculated by exponentiating the mean logarithm of the titers and the corresponding CIs (based on the Student t distribution). Assay results below the LLOQ were set to 0.5 × LLOQ.
d. GMRs and 2-sided 95% CIs were calculated by exponentiating the mean difference of the logarithms of the titers (Group 1 [12 through 15 years of age] – Group 2 [16 through 25 years of age]) and the corresponding CI (based on the Student t distribution).
e. Noninferiority is declared if the lower bound of the 2-sided 95% CI for the GMR is greater than 0.67.
f. SARS-CoV-2 NT50 were determined using the SARS-CoV-2 mNeonGreen Virus Microneutralization Assay. The assay uses a fluorescent reporter virus derived from the USA_WA1/2020 strain and virus neutralization is read on Vero cell monolayers. The sample NT50 is defined as the reciprocal serum dilution at which 50% of the virus is neutralized.

16 HOW SUPPLIED/STORAGE AND HANDLING

Single Dose Vials: COMIRNATY is a suspension for intramuscular injection. Single dose vials with gray caps and labels with gray borders are supplied in a carton containing 10 single dose vials. One vial contains 1 dose of 0.3 mL.

- Carton of 10 single dose vials: NDC 0069-3125-10
- Single dose vial: NDC 0069-3125-01

Multiple Dose Vials: COMIRNATY is a suspension for intramuscular injection. Multiple dose vials with gray caps and labels with gray borders are supplied in a carton containing 10 multiple dose vials or 25 multiple dose vials. One vial contains 6 doses of 0.3 mL.

- Carton of 10 multiple dose vials: NDC 0069-2025-10
- Carton of 25 multiple dose vials: NDC 0069-2025-25
- Multiple dose vial: NDC 0069-2025-01

During storage, minimize exposure to room light, and avoid exposure to direct sunlight and ultraviolet light.

Do not refreeze thawed vials.

Vial Storage Prior to Use

Cartons of COMIRNATY single dose vials and multiple dose vials with gray caps and labels with gray borders will arrive frozen at ultra-cold conditions in thermal containers with dry ice.

Once received, frozen vials may be immediately transferred to the refrigerator [2ºC to 8ºC (35ºF to 46ºF)], thawed and stored for up to 10 weeks. The 10-week refrigerated expiry date should be recorded on the carton at the time of transfer. A carton of 10 single dose vials may take up to 2 hours to thaw at this temperature. A carton of 10 multiple dose vials may take up to 6 hours to thaw at this temperature.

Alternatively, frozen vials may be stored in an ultra-low temperature freezer at -90ºC to -60ºC (-130ºF to -76ºF). Do not store vials at -25°C to -15°C (-13°F to 5°F). Once vials are thawed, they should not be refrozen.

If cartons of COMIRNATY single dose vials or multiple dose vials with gray caps and labels with gray borders are received at 2°C to 8°C, they should be stored at 2°C to 8°C. Check that the carton has been updated to reflect the 10-week refrigerated expiry date.

Regardless of storage condition, the vaccine should not be used after the expiration date printed on the vial and cartons.

Vial Storage During Use

If not previously thawed at 2ºC to 8ºC (35ºF to 46ºF), allow COMIRNATY single dose vials and multiple dose vials to thaw at room temperature [up to 25ºC (77ºF)] for 30 minutes.

DO NOT DILUTE SINGLE DOSE VIALS OR MULTIPLE DOSE VIALS PRIOR TO USE.

COMIRNATY single dose vials and multiple dose vials with gray caps and labels with gray borders may be stored at room temperature [8°C to 25°C (46°F to 77°F)] for a total of 12 hours prior to the first puncture. After first puncture, multiple dose vials should be held between 2ºC to 25°C (35°F to 77°F). Multiple dose vials should be discarded 12 hours after first puncture.

Transportation of Vials

If local redistribution is needed, single dose vials and multiple dose vials may be transported at -90°C to -60°C (-130°F to -76°F), or at 2°C to 8°C (35°F to 46°F).

17 PATIENT COUNSELING INFORMATION

Inform vaccine recipient of the potential benefits and risks of vaccination with COMIRNATY.

Inform vaccine recipient of the importance of completing the 2 dose vaccination series.

There is a pregnancy exposure registry for COMIRNATY. Encourage individuals exposed to COMIRNATY around the time of conception or during pregnancy to register by visiting https://mothertobaby.org/ongoing-study/covid19-vaccines/.

Advise vaccine recipient to report any adverse events to their healthcare provider or to the Vaccine Adverse Event Reporting System at 1-800-822-7967 and www.vaers.hhs.gov.

Prior to administering the vaccine, give the vaccine recipient the Vaccine Information Fact Sheet for Recipients and Caregivers about COMIRNATY (COVID-19 Vaccine, mRNA) and the Pfizer-BioNTech COVID-19 Vaccine to Prevent Coronavirus Disease 2019 (COVID-19) for Use in Individuals 12 Years of Age and Older. The Vaccine Information Fact Sheet for Recipients and Caregivers is available at www.cvdvaccine-us.com.

This product's labeling may have been updated. For the most recent prescribing information, please visit https://dailymed.nlm.nih.gov/dailymed/.

BIONTECH
Manufactured for
BioNTech Manufacturing GmbH
An der Goldgrube 12
55131 Mainz, Germany

Pfizer
Manufactured by
Pfizer Inc., New York, NY 10017

LAB-1490-2.4c

US Govt. License No. 2229

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use SPIKEVAX safely and effectively. See full prescribing information for SPIKEVAX.

SPIKEVAX (COVID-19 Vaccine, mRNA)
Suspension for injection, for intramuscular use
Initial U.S. Approval: 2022

----------INDICATIONS AND USAGE----------
SPIKEVAX is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 18 years of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------
- For intramuscular injection only.
- SPIKEVAX is administered intramuscularly as a series of two doses (0.5 mL each) one month apart. (2.3)

----------DOSAGE FORMS AND STRENGTHS----------
Suspension for injection. A single dose is 0.5 mL.

----------CONTRAINDICATIONS----------
Severe allergic reaction (e.g., anaphylaxis) to any component of SPIKEVAX. (4)

----------WARNINGS AND PRECAUTIONS----------
Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. (5.2)

----------ADVERSE REACTIONS----------
- In study participants 18 through 64 years, the most commonly reported (≥10%) adverse reactions were pain at injection site (93.3%), fatigue (71.9%), headache (68.7%), myalgia (64.8%), chills (49.7%), arthralgia (48.6%), nausea/vomiting (25.7%), axillary swelling/tenderness (22.2%), fever (17.3%), swelling at the injection site (15.4%), and erythema at the injection site (10.5%). (6.1)
- In study participants 65 years of age and older, the most commonly reported (≥10%) adverse reactions were pain at injection site (88.3%), fatigue (64.8%), headache (53.3%), myalgia (51.8%), arthralgia (40.2%), chills (32.7%), nausea/vomiting (15.0%), swelling at the injection site (13.0%), and axillary swelling/tenderness (12.7%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact ModernaTX, Inc. at 1-866-663-3762 or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION

Revised: 1/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

SPIKEVAX is a vaccine indicated for active immunization to prevent coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in individuals 18 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Preparation for Administration

- SPIKEVAX is supplied in two presentations:
  - multiple-dose vial containing 5.5 mL
  - multiple-dose vial containing 7.5 mL
- SPIKEVAX multiple-dose vials contain a frozen suspension that does not contain a preservative and must be thawed prior to administration.
- Thaw each vial before use following the instructions below.

Multiple-Dose Vial Containing	Thaw in Refrigerator	Thaw at Room Temperature
5.5 mL	Thaw between 2°C to 8°C (36°F to 46°F) for 2 hours and 30 minutes. Let each vial stand at room temperature for 15 minutes before administering.	Alternatively, thaw between 15°C to 25°C (59°F to 77°F) for 1 hour.
7.5 mL	Thaw between 2°C to 8°C (36°F to 46°F) for 3 hours. Let each vial stand at room temperature for 15 minutes before administering.	Alternatively, thaw between 15°C to 25°C (59°F to 77°F) for 1 hour and 30 minutes.

- After thawing, do not refreeze.
- Swirl vial gently after thawing and between each withdrawal. **Do not shake.** Do not dilute the vaccine.
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.
- SPIKEVAX is a white to off-white suspension. It may contain white or translucent product-related particulates. Do not administer if vaccine is discolored or contains other particulate matter.
- Each dose is 0.5 mL.

- If the amount of vaccine remaining in the vial cannot provide a full dose of 0.5 mL, discard the vial and contents. Do not pool excess vaccine from multiple vials.
- After the first dose has been withdrawn, the vial should be held between 2°C to 25°C (36°F to 77°F). Record the date and time of first use on the SPIKEVAX vial label. Discard vial after 12 hours. Do not refreeze.

2.2 Administration

Administer a single 0.5 mL dose.

2.3 Dosing and Schedule

SPIKEVAX is administered intramuscularly as a series of two doses (0.5 mL each) 1 month apart.

There are no data available on the interchangeability of SPIKEVAX with COVID-19 vaccines from other manufacturers to complete the vaccination series. Individuals who have received one dose of SPIKEVAX should receive a second dose of SPIKEVAX to complete the vaccination series.

3 DOSAGE FORMS AND STRENGTHS

SPIKEVAX is a suspension for injection. A single dose is 0.5 mL.

4 CONTRAINDICATIONS

Do not administer SPIKEVAX to individuals with a known history of severe allergic reaction (e.g., anaphylaxis) to any component of SPIKEVAX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate medical treatment to manage immediate allergic reactions must be immediately available in the event an acute anaphylactic reaction occurs following administration of SPIKEVAX.

5.2 Myocarditis and Pericarditis

Postmarketing data demonstrate increased risks of myocarditis and pericarditis, particularly within 7 days following the second dose. The observed risk is higher among males under 40 years of age than among females and older males. The observed risk is highest in males 18 through 24 years of age. Although some cases required intensive care support, available data from short-term follow-up suggest that most individuals have had resolution of symptoms with conservative management. Information is not yet available about potential long-term sequelae. The CDC has published considerations related to myocarditis and pericarditis after vaccination,

including for vaccination of individuals with a history of myocarditis or pericarditis (https://www.cdc.gov/vaccines/covid-19/clinical-considerations/myocarditis.html).

5.3 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines including SPIKEVAX. Procedures should be in place to avoid injury from fainting.

5.4 Altered Immunocompetence

Immunocompromised persons, including individuals receiving immunosuppressive therapy, may have a diminished immune response to SPIKEVAX.

5.5 Limitations of Vaccine Effectiveness

SPIKEVAX may not protect all vaccine recipients.

6 ADVERSE REACTIONS

In study participants 18 through 64 years of age, the most commonly reported (≥10%) adverse reactions following any dose were pain at injection site (93.3%), fatigue (71.9%), headache (68.7%), myalgia (64.8%), chills (49.7%), arthralgia (48.6%), nausea/vomiting (25.7%), axillary swelling/tenderness (22.2%), fever (17.3%), swelling at the injection site (15.4%), and erythema at the injection site (10.5%).

In study participants 65 years of age and older, the most commonly reported (≥10%) adverse reactions following any dose were pain at injection site (88.3%), fatigue (64.8%), headache (53.3%), myalgia (51.8%), arthralgia (40.2%), chills (32.7%), nausea/vomiting (15.0%), swelling at the injection site (13.0%), and axillary swelling/tenderness (12.7%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of SPIKEVAX was evaluated in an ongoing Phase 3 randomized, placebo-controlled, observer-blind clinical trial conducted in the United States involving 30,346 participants 18 years of age and older who received at least one dose of SPIKEVAX (n=15,184) or placebo (n=15,162) (Study 1, NCT04470427). Upon issuance of the Emergency Use Authorization (December 18, 2020) for Moderna COVID-19 Vaccine (SPIKEVAX), participants were unblinded in a phased manner over a period of months to offer placebo participants SPIKEVAX. The median duration of follow up for safety after the second injection during the blinded phase was 4 months. The median duration of follow up for safety after the second injection including both the blinded phase and the open-label phase was 6 months.

In Study 1, the median age of the population was 52 years (range 18-95); 22,826 (75.2%) participants were 18 to 64 years of age and 7,520 (24.8%) participants were 65 years of age and older. Overall, 52.6% of the participants were male, 47.4% were female, 20.5% were Hispanic or Latino, 79.2% were White, 10.2% were African American, 4.6% were Asian, 0.8% were American Indian or Alaska Native, 0.2% were Native Hawaiian or Pacific Islander, 2.0% were other races, and 2.1% were Multiracial. Demographic characteristics were similar between participants who received SPIKEVAX and those who received placebo.

Solicited Adverse Reactions

Local and systemic adverse reactions and use of antipyretic medication were solicited in an electronic diary for 7 days following each injection (i.e., day of vaccination and the next 6 days) among participants receiving SPIKEVAX (n=15,179) and participants receiving placebo (n=15,159) with at least 1 documented dose. Events that persisted for more than 7 days were followed until resolution. Solicited adverse reactions were reported more frequently among vaccine participants than placebo participants.

The reported number and percentage of the solicited local and systemic adverse reactions by age group and dose are presented in Table 1 and Table 2, respectively.

Table 1: Number and Percentage of Participants With Solicited Local and Systemic Adverse Reactions Starting Within 7 Days* After Each Dose in Participants 18-64 Years (Solicited Safety Set, Dose 1 and Dose 2)

	SPIKEVAX		Placebo[a]	
	Dose 1 (N=11,406) n (%)	**Dose 2** (N=11,000) n (%)	**Dose 1** (N=11,402) n (%)	**Dose 2** (N=10,929) n (%)
Local Adverse Reactions				
Pain	9,908 (86.9)	9,893 (89.9)	2,183 (19.1)	2,048 (18.7)
Pain, Grade 3[b]	366 (3.2)	506 (4.6)	23 (0.2)	22 (0.2)
Axillary swelling/tenderness	1,322 (11.6)	1,777 (16.2)	567 (5.0)	474 (4.3)
Axillary swelling/tenderness, Grade 3[b]	37 (0.3)	47 (0.4)	13 (0.1)	12 (0.1)
Swelling (hardness) ≥25 mm	766 (6.7)	1,399 (12.7)	42 (0.4)	46 (0.4)
Swelling (hardness), Grade 3[c]	62 (0.5)	183 (1.7)	3 (<0.1)	5 (<0.1)
Erythema (redness) ≥25 mm	354 (3.1)	989 (9.0)	54 (0.5)	53 (0.5)
Erythema (redness), Grade 3[c]	34 (0.3)	210 (1.9)	11 (<0.1)	12 (0.1)

	SPIKEVAX		Placebo[a]	
	Dose 1 (N=11,406) n (%)	**Dose 2** (N=11,000) n (%)	**Dose 1** (N=11,402) n (%)	**Dose 2** (N=10,929) n (%)
Systemic Adverse Reactions				
Fatigue	4,385 (38.5)	7,453 (67.8)	3,281 (28.8)	2,701 (24.7)
Fatigue, Grade 3[d]	121 (1.1)	1,178 (10.7)	83 (0.7)	88 (0.8)
Fatigue, Grade 4[e]	1 (<0.1)	0 (0)	0 (0)	0 (0)
Headache	4,028 (35.3)	6,929 (63.0)	3,303 (29.0)	2,775 (25.4)
Headache, Grade 3[f]	220 (1.9)	559 (5.1)	163 (1.4)	132 (1.2)
Myalgia	2,700 (23.7)	6,789 (61.7)	1,625 (14.3)	1,425 (13.0)
Myalgia, Grade 3[d]	74 (0.6)	1,116 (10.1)	38 (0.3)	42 (0.4)
Arthralgia	1,892 (16.6)	5,010 (45.6)	1,327 (11.6)	1,180 (10.8)
Arthralgia, Grade 3[d]	47 (0.4)	650 (5.9)	30 (0.3)	37 (0.3)
Arthralgia, Grade 4[e]	1 (<0.1)	0 (0)	0 (0)	0 (0)
Chills	1,050 (9.2)	5,357 (48.7)	730 (6.4)	662 (6.1)
Chills, Grade 3[g]	17 (0.1)	164 (1.5)	8 (<0.1)	15 (0.1)
Nausea/vomiting	1,068 (9.4)	2,355 (21.4)	908 (8.0)	807 (7.4)
Nausea/vomiting, Grade 3[h]	6 (<0.1)	11 (0.1)	8 (<0.1)	8 (<0.1)
Fever	102 (0.9)	1,909 (17.4)	37 (0.3)	38 (0.3)
Fever, Grade 3[i]	10 (<0.1)	185 (1.7)	1 (<0.1)	2 (<0.1)
Fever, Grade 4[j]	4 (<0.1)	12 (0.1)	4 (<0.1)	2 (<0.1)
Use of antipyretic or pain medication	2,656 (23.3)	6,307 (57.3)	1,523 (13.4)	1,254 (11.5)

* 7 days included day of vaccination and the subsequent 6 days. Events and use of antipyretic or pain medication were collected in the electronic diary (e-diary).

[a] Placebo was a saline solution.

[b] Grade 3 pain and axillary swelling/tenderness: Defined as any use of prescription pain reliever; prevents daily activity.

[c] Grade 3 swelling and erythema: Defined as >100 mm / >10 cm.

[d] Grade 3 fatigue, myalgia, arthralgia: Defined as significant; prevents daily activity.

[e] Grade 4 fatigue, arthralgia: Defined as requires emergency room visit or hospitalization.

[f] Grade 3 headache: Defined as significant; any use of prescription pain reliever or prevents daily activity.

[g] Grade 3 chills: Defined as prevents daily activity and requires medical intervention.

[h] Grade 3 nausea/vomiting: Defined as prevents daily activity; requires outpatient intravenous hydration.
[i] Grade 3 fever: Defined as ≥39.0° – ≤40.0°C / ≥102.1° – ≤104.0°F.
[j] Grade 4 fever: Defined as >40.0°C / >104.0°F.

Table 2: Number and Percentage of Participants With Solicited Local and Systemic Adverse Reactions Starting Within 7 Days* After Each Dose in Participants 65 Years and Older (Solicited Safety Set, Dose 1 and Dose 2)

	SPIKEVAX		Placebo[a]	
	Dose 1 (N=3,760) n (%)	**Dose 2** (N=3,691) n (%)	**Dose 1** (N=3,749) n (%)	**Dose 2** (N=3,649) n (%)
Local Adverse Reactions				
Pain	2,780 (73.9)	3,071 (83.2)	482 (12.9)	438 (12.0)
Pain, Grade 3[b]	50 (1.3)	100 (2.7)	32 (0.9)	19 (0.5)
Axillary swelling/tenderness	231 (6.1)	315 (8.5)	155 (4.1)	97 (2.7)
Axillary swelling/tenderness, Grade 3[b]	12 (0.3)	21 (0.6)	14 (0.4)	8 (0.2)
Swelling (hardness) ≥25 mm	169 (4.5)	408 (11.1)	23 (0.6)	14 (0.4)
Swelling (hardness), Grade 3[c]	20 (0.5)	72 (2.0)	3 (<0.1)	7 (0.2)
Erythema (redness) ≥25 mm	91 (2.4)	285 (7.7)	23 (0.6)	15 (0.4)
Erythema (redness), Grade 3[c]	8 (0.2)	77 (2.1)	2 (<0.1)	3 (<0.1)
Systemic Adverse Reactions				
Fatigue	1,251 (33.3)	2,154 (58.4)	852 (22.7)	717 (19.6)
Fatigue, Grade 3[d]	30 (0.8)	255 (6.9)	22 (0.6)	20 (0.5)
Headache	922 (24.5)	1,708 (46.3)	723 (19.3)	652 (17.9)
Headache, Grade 3[e]	53 (1.4)	107 (2.9)	34 (0.9)	33 (0.9)
Myalgia	742 (19.7)	1,740 (47.2)	444 (11.9)	399 (10.9)
Myalgia, Grade 3[d]	17 (0.5)	205 (5.6)	9 (0.2)	10 (0.3)
Arthralgia	618 (16.4)	1,293 (35.1)	457 (12.2)	399 (10.9)
Arthralgia, Grade 3[d]	13 (0.3)	125 (3.4)	8 (0.2)	7 (0.2)
Chills	201 (5.3)	1,143 (31.0)	148 (4.0)	151 (4.1)

	SPIKEVAX		Placebo[a]	
	Dose 1 (N=3,760) n (%)	**Dose 2** (N=3,691) n (%)	**Dose 1** (N=3,749) n (%)	**Dose 2** (N=3,649) n (%)
Chills, Grade 3[f]	7 (0.2)	27 (0.7)	6 (0.2)	2 (<0.1)
Nausea/vomiting	194 (5.2)	439 (11.9)	167 (4.5)	134 (3.7)
Nausea/vomiting, Grade 3[g]	4 (0.1)	10 (0.3)	5 (0.1)	3 (<0.1)
Nausea/vomiting, Grade 4[h]	0 (0)	1 (<0.1)	0 (0)	0 (0)
Fever	10 (0.3)	367 (9.9)	7 (0.2)	5 (0.1)
Fever, Grade 3[i]	1 (<0.1)	18 (0.5)	1 (<0.1)	0 (0)
Fever, Grade 4[j]	0 (0)	1 (<0.1)	2 (<0.1)	1 (<0.1)
Use of antipyretic or pain medication	673 (17.9)	1,548 (41.9)	477 (12.7)	331 (9.1)

* 7 days included day of vaccination and the subsequent 6 days. Events and use of antipyretic or pain medication were collected in the electronic diary (e-diary).

[a] Placebo was a saline solution.

[b] Grade 3 pain and axillary swelling/tenderness: Defined as any use of prescription pain reliever; prevents daily activity.

[c] Grade 3 swelling and erythema: Defined as >100 mm / >10 cm.

[d] Grade 3 fatigue, myalgia, arthralgia: Defined as significant; prevents daily activity.

[e] Grade 3 headache: Defined as significant; any use of prescription pain reliever or prevents daily activity.

[f] Grade 3 chills: Defined as prevents daily activity and requires medical intervention.

[g] Grade 3 nausea/vomiting: Defined as prevents daily activity; requires outpatient intravenous hydration.

[h] Grade 4 nausea/vomiting: Defined as requires emergency room visit or hospitalization for hypotensive shock.

[i] Grade 3 fever: Defined as ≥39.0° – ≤40.0°C / ≥102.1° – ≤104.0°F.

[j] Grade 4 fever: Defined as >40.0°C / >104.0°F.

Solicited local and systemic adverse reactions reported following administration of SPIKEVAX had a median duration of 1 to 3 days.

Grade 3 solicited local adverse reactions were more frequently reported after Dose 2 than after Dose 1. Solicited systemic adverse reactions were more frequently reported by vaccine recipients after Dose 2 than after Dose 1.

In Study 1, 2.3% of participants (vaccine=347, placebo=337) had evidence of prior SARS-CoV-2 infection at baseline (immunologic or virologic evidence of prior SARS-CoV-2 infection [defined as positive RT-PCR test and/or positive Elecsys immunoassay result at Day 1]). Overall, among the 347 vaccine participants, there were no notable differences in reactogenicity compared to the 14,750 vaccine participants who had no evidence of prior SARS-CoV-2 infection at baseline (negative RT-PCR test and negative Elecsys immunoassay result at Day 1).

Unsolicited Adverse Events

Participants were monitored for unsolicited adverse events for 28 days following each dose. Serious adverse events and medically attended adverse events will be recorded for the entire study duration (2 years). Among the 30,346 participants who had received at least 1 dose of vaccine (N=15,184) or placebo (N=15,162), unsolicited adverse events that occurred within 28 days following any vaccination were reported by 31.3% of participants (n=4,752) who received SPIKEVAX and 28.6% of participants (n=4,338) who received placebo.

During the 28-day follow-up period following any dose, lymphadenopathy-related events were reported by 1.7% of vaccine recipients and 0.8% of placebo recipients. These events included lymphadenopathy, lymphadenitis, lymph node pain, vaccination-site lymphadenopathy, injection-site lymphadenopathy, and axillary mass. This imbalance is consistent with the imbalance observed for solicited axillary swelling/tenderness at the injected arm.

During the 7-day follow-up period of any vaccination, hypersensitivity events of injection site rash or injection site urticaria, likely related to vaccination, were reported by 6 participants in the SPIKEVAX group and none in the placebo group. Delayed injection site reactions that began >7 days after vaccination were reported in 1.4% of vaccine recipients and 0.7% of placebo recipients. Delayed injection site reactions included pain, erythema, and swelling and are likely related to vaccination.

In the blinded portion of the study, there were 8 reports of facial paralysis (including Bell's palsy) in the SPIKEVAX group, and 3 in the placebo group. In the 28-day follow-up period there were two cases of facial paralysis in the SPIKEVAX group, which occurred on 8 and 22 days, respectively, after vaccination, and one in the placebo group, which occurred 17 days after vaccination. Currently available information on facial paralysis is insufficient to determine a causal relationship with the vaccine.

In the blinded portion of the study, there were 50 reports of herpes zoster in the SPIKEVAX group, and 23 in the placebo group. In the 28-day period after any vaccination, there were 22 cases of herpes zoster in the SPIKEVAX group, and 15 in the placebo group. Currently available information on herpes zoster infection is insufficient to determine a causal relationship with the vaccine.

There were no other notable patterns or numerical imbalances between treatment groups for specific categories of adverse events (including other neurologic, neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to SPIKEVAX.

Serious Adverse Events

During the blinded phase of the study, serious adverse events were reported by 1.8% (n=268) of participants who received SPIKEVAX and 1.9% (n=292) of participants who received placebo.

There were three serious adverse events of angioedema/facial swelling in the vaccine group in recipients with a history of injection of dermatological fillers. The onset of swelling was reported

1-2 days after the second dose and was likely related to vaccination.

There were no other notable patterns or imbalances between treatment groups for specific categories of serious adverse events (including neurologic, neuro-inflammatory, and thrombotic events) that would suggest a causal relationship to SPIKEVAX.

6.2 Emergency Use Authorization Experience

The following adverse reactions have been identified during emergency use authorization of SPIKEVAX (Moderna COVID-19 Vaccine). Because these reactions are reported voluntarily, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Cardiac Disorders: myocarditis, pericarditis
Immune System Disorders: anaphylaxis
Nervous System Disorders: syncope

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to SPIKEVAX during pregnancy. Women who are vaccinated with SPIKEVAX during pregnancy are encouraged to enroll in the registry by calling 1-866-MODERNA (1-866-663-3762).

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. Available data on SPIKEVAX administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study was performed in female rats administered the equivalent of a single human dose of SPIKEVAX twice prior to mating and twice during gestation. The study revealed no evidence of harm to the fetus due to the vaccine (*see Animal Data*).

Data

Animal Data

In a developmental toxicity study, 0.2 mL of a vaccine formulation containing nucleoside-modified messenger ribonucleic acid (mRNA) (100 mcg) and other ingredients that are included in a 0.5 mL single human dose of SPIKEVAX was administered to female rats by the

intramuscular route on four occasions: 28 and 14 days prior to mating, and on gestation days 1 and 13. No vaccine-related fetal malformations or variations and no adverse effect on postnatal development were observed in the study.

8.2 Lactation

Risk Summary

It is not known whether SPIKEVAX is excreted in human milk. Data are not available to assess the effects of SPIKEVAX on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for SPIKEVAX and any potential adverse effects on the breastfed infant from SPIKEVAX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness have not been established in persons less than 18 years of age.

8.5 Geriatric Use

Clinical studies of SPIKEVAX included participants 65 years of age and older receiving vaccine or placebo, and their data contribute to the overall assessment of safety and efficacy. In a Phase 3 clinical study, 24.8% (n=7,520) of participants were 65 years of age and older and 4.6% (n=1,398) of participants were 75 years of age and older. Vaccine efficacy in participants 65 years of age and older was 91.5% (95% CI 83.2, 95.7) compared to 93.4% (95% CI 91.1, 95.1) in participants 18 to <65 years of age *[see Clinical Studies (14)]*. A lower proportion of participants 65 years of age and older reported solicited local and systemic adverse reactions compared to participants 18-64 years of age *[*see *Adverse Reactions (6.1)]*.

11 DESCRIPTION

SPIKEVAX (COVID-19 Vaccine, mRNA) is a sterile white to off-white suspension for intramuscular injection. Each 0.5 mL dose of SPIKEVAX contains 100 mcg of nucleoside-modified messenger RNA (mRNA) encoding the pre-fusion stabilized Spike glycoprotein (S) of SARS-CoV-2 virus.

Each 0.5 mL dose of SPIKEVAX also contains the following ingredients: a total lipid content of 1.93 mg (SM-102, polyethylene glycol [PEG] 2000 dimyristoyl glycerol [DMG], cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphocholine [DSPC]), 0.31 mg tromethamine, 1.18 mg tromethamine hydrochloride, 0.043 mg acetic acid, 0.20 mg sodium acetate trihydrate, and 43.5 mg sucrose.

SPIKEVAX does not contain a preservative.

The vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The nucleoside-modified mRNA in SPIKEVAX is encapsulated in lipid particles, which enable delivery of the nucleoside-modified mRNA into host cells to allow expression of the SARS-CoV-2 S antigen. The vaccine elicits an immune response to the S antigen, which protects against COVID-19.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

SPIKEVAX has not been evaluated for carcinogenic, mutagenic potential, or impairment of male fertility in animals. A developmental toxicity study was conducted in female rats that received a vaccine formulation containing nucleoside-modified messenger ribonucleic acid (mRNA) (100 mcg) and other ingredients included in a single human dose of SPIKEVAX. No impact on female fertility was reported *(see Use in Specific Populations [8.1])*.

14 CLINICAL STUDIES

Study 1 is an ongoing Phase 3 randomized, placebo-controlled, observer-blind clinical trial to evaluate the efficacy, safety, and immunogenicity of SPIKEVAX in participants 18 years of age and older in the United States. Randomization was stratified by age and health risk: 18 to <65 years of age without comorbidities (not at risk for progression to severe COVID-19), 18 to <65 years of age with comorbidities (at risk for progression to severe COVID-19), and 65 years of age and older with or without comorbidities. Participants who were immunocompromised and those with a known history of SARS-CoV-2 infection were excluded from the study. Participants with no known history of SARS-CoV-2 infection but with positive laboratory results indicative of infection at study entry were included. The study allowed for the inclusion of participants with stable pre-existing medical conditions, defined as disease not requiring significant change in therapy or hospitalization for worsening disease during the 3 months before enrollment, as well as participants with stable human immunodeficiency virus (HIV) infection. A total of 30,415 participants were randomized equally to receive 2 doses of SPIKEVAX or saline placebo 1 month apart. Participants will be followed for efficacy and safety until 2 years after the second dose.

The primary efficacy analysis population (referred to as the Per-Protocol Set) included 28,451 participants who received two doses (at 0 and 1 month) of either SPIKEVAX (n=14,287) or placebo (n=14,164), and had a negative baseline SARS-CoV-2 status. In the Per-Protocol Set, 47.5% of participants were female, 19.7% were Hispanic or Latino; 79.7% were White, 9.7% were African American, 4.7% were Asian, and 2.0% other races. The median age of participants was 53 years (range 18-95) and 25.4% of participants were 65 years of age and older. Of the study participants in the Per-Protocol Set, 22.8% were at increased risk of severe COVID-19 due to at least one pre-existing medical condition (chronic lung disease, significant cardiac disease,

severe obesity, diabetes, liver disease, or HIV infection) regardless of age. There were no notable differences in demographics or pre-existing medical conditions between participants who received SPIKEVAX and those who received placebo.

The population for the vaccine efficacy analysis included participants 18 years of age and older who were enrolled from July 27, 2020, and followed for the development of COVID-19 through the data cutoff of March 26, 2021, or the Participant Decision Visit for treatment unblinding, whichever was earlier. The median length of follow-up for participants in the blinded placebo-controlled phase of the study was 4 months following Dose 2.

Efficacy Against COVID-19

COVID-19 was defined based on the following criteria: The participant must have experienced at least two of the following systemic symptoms: fever (≥38ºC / ≥100.4ºF), chills, myalgia, headache, sore throat, new olfactory and taste disorder(s); or the participant must have experienced at least one of the following respiratory signs/symptoms: cough, shortness of breath or difficulty breathing, or clinical or radiographical evidence of pneumonia; and the participant must have at least one NP swab, nasal swab, or saliva sample (or respiratory sample, if hospitalized) positive for SARS-CoV-2 by RT-PCR. COVID-19 cases were adjudicated by a Clinical Adjudication Committee.

There were 55 COVID-19 cases in the SPIKEVAX group and 744 cases in the placebo group, with a vaccine efficacy of 93.2% (95% confidence interval of 91.0% to 94.8%) (Table 3).

SARS-CoV-2 identified in the majority of COVID-19 cases in this study were sequenced to be the B.1.2 variant. Additional SARS-CoV-2 variants identified in this study included B.1.427/B.1.429 (Epsilon), P.1 (Gamma), and P.2 (Zeta). Representation of identified variants among cases in the vaccine versus placebo recipients did not suggest decreased vaccine effectiveness against these variants.

Table 3: Vaccine Efficacy Against COVID-19* in Participants 18 Years of Age and Older Starting 14 Days After Dose 2 per Adjudication Committee Assessments – Per-Protocol Set

Age Subgroup (Years)	SPIKEVAX			Placebo			% Vaccine Efficacy (95% CI)†
	Participants(N)	COVID-19 Cases (n)	Incidence Rate of COVID-19 per 1,000 Person-Years	Participants (N)	COVID-19 Cases (n)	Incidence Rate of COVID-19 per 1,000 Person-Years	
All participants	14,287	55	9.6	14,164	744	136.6	93.2 (91.0, 94.8)
18 to <65	10,661	46	10.7	10,569	644	159.0	93.4 (91.1, 95.1)
≥65	3,626	9	6.2	3,595	100	71.7	91.5 (83.2, 95.7)

* COVID-19: symptomatic COVID-19 requiring positive RT-PCR result and at least two systemic symptoms (fever [≥38ºC / ≥100.4ºF], chills, myalgia, headache, sore throat, new olfactory and taste disorder[s]) or one respiratory symptom (cough, shortness of breath or difficulty breathing, or clinical or radiographical evidence of pneumonia). Cases starting 14 days after Dose 2.
† VE and 95% CI from the stratified Cox proportional hazard model.

Severe COVID-19 was defined based on confirmed COVID-19 as per the primary efficacy endpoint case definition, plus any of the following: Clinical signs indicative of severe systemic illness, respiratory rate ≥30 per minute, heart rate ≥125 beats per minute, SpO2 ≤93% on room air at sea level or PaO2/FIO2 <300 mm Hg; or respiratory failure or ARDS (defined as needing high-flow oxygen, non-invasive or mechanical ventilation, or ECMO), evidence of shock (systolic blood pressure <90 mmHg, diastolic BP <60 mmHg or requiring vasopressors); or significant acute renal, hepatic, or neurologic dysfunction; or admission to an intensive care unit or death.

Among all participants in the Per-Protocol Set analysis, which included COVID-19 cases confirmed by an adjudication committee, 2 cases of severe COVID-19 were reported in the SPIKEVAX group compared with 106 cases reported in the placebo group, with a vaccine efficacy of 98.2% (95% confidence interval of 92.8% to 99.6%) (Table 4).

Table 4: Vaccine Efficacy Against Severe COVID-19* in Participants 18 Years of Age and Older Starting 14 Days After Dose 2 per Adjudication Committee Assessments – Per-Protocol Set

SPIKEVAX			Placebo			
Participants (N)	Severe COVID-19 Cases (n)	Incidence Rate of COVID-19 per 1,000 Person-Years	Participants (N)	Severe COVID-19 Cases (n)	Incidence Rate of COVID-19 per 1,000 Person-Years	% Vaccine Efficacy (95% CI)†
14,287	2	0.3	14,164	106	19.1	98.2 (92.8, 99.6)

* Severe COVID-19: symptomatic COVID-19 requiring positive RT-PCR result and at least two systemic symptoms or one respiratory symptom, plus any of the following: Clinical signs indicative of severe systemic illness, respiratory rate ≥30 per minute, heart rate ≥125 beats per minute, SpO2 ≤93% on room air at sea level or PaO2/FIO2 <300 mm Hg; or respiratory failure or ARDS (defined as needing high-flow oxygen, non-invasive or mechanical ventilation, or ECMO), evidence of shock (systolic blood pressure <90 mmHg, diastolic BP <60 mmHg or requiring vasopressors); or significant acute renal, hepatic, or neurologic dysfunction; or admission to an intensive care unit or death. Cases starting 14 days after Dose 2.

† VE and 95% CI from the stratified Cox proportional hazard model.

In an exploratory analysis, occurrence of asymptomatic SARS-CoV-2 infection was assessed among participants in the Per-Protocol Set (enrolled from July 27, 2020, and followed maximally through March 26, 2021). Asymptomatic SARS-CoV-2 infection was defined as having a positive scheduled serology test based on binding antibody against SARS-CoV-2 nucleocapsid protein as measured by the Roche Elecsys immunoassay (N-serology) and/or a positive RT-PCR test for SARS-CoV-2, in the absence of any reported COVID-19 symptoms included as part of the primary efficacy endpoint case definition (described above) or symptoms included in the secondary COVID-19 endpoint case definition (fever ≥38°C / ≥100.4°F, chills, cough, shortness of breath or difficulty breathing, fatigue, muscle aches, body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea, vomiting, or diarrhea) at any time during the study. To assess for asymptomatic infection starting 14 days after Dose 2, all participants had scheduled blood draws for N-serology collected at the 1 month post-Dose 2 visit and the 6 months post-Dose 2 visit (if still blinded to treatment arm), and scheduled N-serology and nasopharyngeal swab for RT-PCR collection at the Participant Decision Visit for treatment unblinding.

In the Per-Protocol Set, 14,287 participants in the SPIKEVAX group and 14,164 participants in the placebo group had N-serology and/or RT-PCR results available from one or more of the pre-specified timepoints listed above. Among these participants, there were 180 cases of asymptomatic SARS-CoV-2 infection in the SPIKEVAX group compared with 399 cases in the placebo group. Limitations of this analysis include the infrequently scheduled assessments for serology and PCR testing, which may not have captured all cases of asymptomatic infections which occurred during the study.

16 HOW SUPPLIED/STORAGE AND HANDLING

SPIKEVAX is supplied in multiple-dose vials as follows:

NDC 80777-100-99	Carton of 10 multiple-dose vials, each vial containing 5.5 mL
NDC 80777-100-98	Carton of 10 multiple-dose vials, each vial containing 7.5 mL

During storage, minimize exposure to room light, and avoid exposure to direct sunlight and ultraviolet light.

Frozen Storage
Store frozen between -50°C to -15°C (-58°F to 5°F).

Storage after Thawing

- Storage at 2°C to 8°C (36°F to 46°F):
  - Vials may be stored refrigerated between 2°C to 8°C (36°F to 46°F) for up to 30 days prior to first use.
  - Vials should be discarded 12 hours after the first puncture.
- Storage at 8°C to 25°C (46°F to 77°F):
  - Vials may be stored between 8°C to 25°C (46°F to 77°F) for a total of 24 hours.
  - Vials should be discarded 12 hours after the first puncture.
  - Total storage at 8°C to 25°C (46°F to 77°F) must not exceed 24 hours.

Do not refreeze once thawed.

Thawed vials can be handled in room light conditions.

Transportation of Thawed Vials at 2°C to 8°C (36F° to 46°F)
If transport at -50°C to -15°C (-58°F to 5°F) is not feasible, available data support transportation of one or more thawed vials for up to 12 hours at 2°C to 8°C (36°F to 46°F) when shipped using shipping containers which have been qualified to maintain 2°C to 8°C (36°F to 46°F) and under routine road and air transport conditions with shaking and vibration minimized. Once thawed and transported at 2°C to 8°C (36°C to 46°F), vials should not be refrozen and should be stored at 2°C to 8°C (36°F to 46°F) until use.

17 PATIENT COUNSELING INFORMATION

Advise the vaccine recipient or caregiver to read the FDA-approved patient labeling.

Inform the vaccine recipient or caregiver of the potential benefits and risks of vaccination with SPIKEVAX.

Inform the vaccine recipient or caregiver of the importance of completing the two dose vaccination series.

Instruct the vaccine recipient or caregiver to report any adverse events to their healthcare

provider or to the Vaccine Adverse Event Reporting System at 1-800-822-7967 and www.vaers.hhs.gov.

There is a pregnancy exposure registry for SPIKEVAX. Encourage individuals who receive SPIKEVAX around the time of conception or while pregnant to enroll in the pregnancy exposure registry. Pregnant individuals can enroll in the pregnancy exposure registry by calling 1-866-MODERNA (1-866-663-3762).

Prior to administering the vaccine, provide the vaccine recipient the Vaccine Information Fact Sheet for Recipients and Caregivers about SPIKEVAX (COVID-19 Vaccine, mRNA) and the Moderna COVID-19 Vaccine to Prevent Coronavirus Disease 2019 (COVID-19) for Use in Individuals 18 Years of Age and Older. The Vaccine Information Fact Sheet for Recipients and Caregivers is available at https://www.modernatx.com/covid19vaccine-eua/eua-fact-sheet-recipients.pdf.

This product's labeling may have been updated. For the most recent prescribing information, please visit https://dailymed.nlm.nih.gov/dailymed/.

Manufactured for:
Moderna US, Inc.
Cambridge, MA 02139

US Govt. License No. 2256

Revised: 1/2022

Diptheria / Tetanus / Pertussis (DTaP)

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use DAPTACEL safely and effectively. See full prescribing information for DAPTACEL.

DAPTACEL (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed)
Suspension for Intramuscular Injection
Initial U.S. Approval: 2002

----------RECENT MAJOR CHANGES ----------
Dosage and Administration (2.1) xx/202x

----------INDICATIONS AND USAGE----------
- DAPTACEL is a vaccine indicated for active immunization against diphtheria, tetanus and pertussis as a five-dose series in infants and children 6 weeks through 6 years of age (prior to 7th birthday). (1)

----------DOSAGE AND ADMINISTRATION----------
- The five dose immunization series consists of a 0.5 mL intramuscular injection administered at 2, 4, 6 and 15-20 months of age, and at 4-6 years of age. (2.1, 2.2)

----------DOSAGE FORMS AND STRENGTHS----------
- Suspension for injection, supplied in single-dose (0.5 mL) vials (3)

----------CONTRAINDICATIONS----------
- Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid, tetanus toxoid, or pertussis-containing vaccine, or any component of DAPTACEL. (4.1)
- Encephalopathy within 7 days of a previous pertussis-containing vaccine with no other identifiable cause. (4.2)
- Progressive neurologic disorder until a treatment regimen has been established and the condition has stabilized. (4.3)

----------WARNINGS AND PRECAUTIONS----------
- Carefully consider benefits and risks before administering DAPTACEL to persons with a history of:
  - fever ≥40.5°C (105°F), hypotonic-hyporesponsive episode (HHE) or persistent, inconsolable crying lasting ≥3 hours within 48 hours after a previous pertussis-containing vaccine. (5.2)
  - seizures within 3 days after a previous pertussis-containing vaccine. (5.2)
- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following DAPTACEL. (5.3)
- For infants and children with a history of previous seizures, an antipyretic may be administered (in the dosage recommended in its prescribing information) at the time of vaccination with DAPTACEL and for the next 24 hours. (5.4)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including DAPTACEL, to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.7)

----------ADVERSE REACTIONS----------
- Rates of adverse reactions varied by dose number, with systemic reactions most frequent following doses 1-3 and injection site reactions most frequent following doses 4 and 5. Systemic reactions that occurred in >50% of subjects following any dose included fussiness/irritability, inconsolable crying, and decreased activity/lethargy. Fever ≥38.0°C occurred in 6-16% of US subjects, depending on dose number. Injection site reactions that occurred in >30% of subjects following any dose included tenderness, redness and increase in arm circumference. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc., at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 and http://vaers.hhs.gov.

----------DRUG INTERACTIONS----------
- In cases where DAPTACEL and Menactra are to be administered to children 4 through 6 years of age, the two vaccines should be administered concomitantly or Menactra should be administered prior to DAPTACEL. Administration of Menactra one month after DAPTACEL has been shown to reduce meningococcal antibody responses to Menactra. (7.1)
- Do not mix with any other vaccine in the same syringe or vial. (7.1)
- Immunosuppressive therapies may reduce the immune response to DAPTACEL. (7.2)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: xx/202x

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

DAPTACEL® is a vaccine indicated for active immunization against diphtheria, tetanus and pertussis as a five-dose series in infants and children 6 weeks through 6 years of age (prior to seventh birthday).

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Immunization Series

DAPTACEL is to be administered as a 5 dose series at 2, 4 and 6 months of age (at intervals of 6-8 weeks), at 15-20 months of age and at 4-6 years of age. The first dose may be given as early as 6 weeks of age. Four doses of DAPTACEL constitute a primary immunization course for pertussis. The fifth dose is a booster for pertussis immunization. Three doses of DAPTACEL constitute a primary immunization course for diphtheria and tetanus. The fourth and fifth doses are boosters for diphtheria and tetanus immunization. [See *Clinical Studies (14.1, 14.2, 14.3)*.]

Mixed Sequences of DAPTACEL and other DTaP-containing Vaccines

DAPTACEL contains the same pertussis antigens, manufactured by the same process, as Pentacel® [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) vaccine] and VAXELIS (Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Haemophilus b Conjugate and Hepatitis B Vaccine). Pentacel and VAXELIS contain twice the amount of detoxified pertussis toxin (PT) and four times the amount of filamentous hemagglutinin (FHA) as DAPTACEL.

DAPTACEL may be used as any of the doses in a 5-dose DTaP series initiated with Pentacel or VAXELIS.

Data are not available on the safety and effectiveness of using mixed sequences of DAPTACEL and DTaP-containing vaccines from different manufacturers for successive doses of the DTaP vaccination series.

2.2 Administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exist, the product should not be administered.

Just before use, shake the vial well, until a uniform, white, cloudy suspension results.

Withdraw and administer a single 0.5 mL dose of DAPTACEL intramuscularly. Discard unused portion. Use a separate sterile needle and syringe for each injection. Changing needles between withdrawing the vaccine from the vial and injecting it into a recipient is not necessary unless the needle has been damaged or contaminated. In infants younger than 1 year, the anterolateral aspect of the thigh provides the largest muscle and is the preferred site of injection. In older children, the

deltoid muscle is usually large enough for injection. The vaccine should not be injected into the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously or subcutaneously.

DAPTACEL should not be combined through reconstitution or mixed with any other vaccine.

3 DOSAGE FORMS AND STRENGTHS

DAPTACEL is a suspension for injection in 0.5 mL single-dose vials. See *Description (0)* for a complete listing of ingredients.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A severe allergic reaction (e.g., anaphylaxis) after a previous dose of DAPTACEL or any other tetanus toxoid, diphtheria toxoid, or pertussis-containing vaccine, or any other component of this vaccine is a contraindication to administration of DAPTACEL. [See *Description (0).*] Because of uncertainty as to which component of the vaccine may be responsible, none of the components should be administered. Alternatively, such individuals may be referred to an allergist for evaluation if further immunizations are to be considered.

4.2 Encephalopathy

Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of a previous dose of a pertussis containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including DAPTACEL.

4.3 Progressive Neurologic Disorder

Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy is a contraindication to administration of any pertussis-containing vaccine, including DAPTACEL. Pertussis vaccine should not be administered to individuals with such conditions until a treatment regimen has been established and the condition has stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

5.2 Adverse Reactions Following Prior Pertussis Vaccination

If any of the following events occur within the specified period after administration of a whole-cell pertussis vaccine or a vaccine containing an acellular pertussis component, the decision to administer DAPTACEL should be based on careful consideration of potential benefits and possible risks. [See *Dosage and Administration (2.1).*]

- Temperature of ≥40.5°C (105°F) within 48 hours, not attributable to another identifiable cause.
- Collapse or shock-like state (hypotonic-hyporesponsive episode [HHE]) within 48 hours.
- Persistent, inconsolable crying lasting ≥3 hours within 48 hours.
- Seizures with or without fever within 3 days.

5.3 Guillain-Barré Syndrome and Brachial Neuritis

A review by the Institute of Medicine found evidence for a causal relation between tetanus toxoid and both brachial neuritis and Guillain-Barré syndrome. (1) If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following DAPTACEL.

5.4 Infants and Children with a History of Previous Seizures

For infants or children with a history of previous seizures, an appropriate antipyretic may be administered (in the dosage recommended in its prescribing information) at the time of vaccination with a vaccine containing an acellular pertussis component (including DAPTACEL) and for the following 24 hours, to reduce the possibility of post-vaccination fever.

5.5 Limitations of Vaccine Effectiveness

Vaccination with DAPTACEL may not protect all individuals.

5.6 Altered Immunocompetence

If DAPTACEL is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained. [See *Immunosuppressive Treatments (7.2).*]

5.7 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including DAPTACEL, to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

5.8 Syncope

Syncope (fainting) has been reported following vaccination with DAPTACEL. Procedures should be in place to avoid injury from fainting.

6 ADVERSE REACTIONS

6.1 Data from Clinical Studies

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events.

Approximately 18,000 doses of DAPTACEL have been administered to infants and children in 9 clinical studies. Of these, 3 doses of DAPTACEL were administered to 4,998 children, 4 doses of DAPTACEL were administered to 1,725 children, and 5 doses of DAPTACEL were administered to 485 children. A total of 989 children received 1 dose of DAPTACEL following 4 prior doses of Pentacel.

In a randomized, double-blinded pertussis vaccine efficacy trial, the Sweden I Efficacy Trial, conducted in Sweden during 1992-1995, the safety of DAPTACEL was compared with DT and a whole-cell pertussis DTP vaccine. A standard diary card was kept for 14 days after each dose and follow-up telephone calls were made 1 and 14 days after each injection. Telephone calls were made monthly to monitor the occurrence of severe events and/or hospitalizations for the 2 months after the last injection. There were fewer of the solicited common local and systemic reactions following DAPTACEL than following the whole-cell pertussis DTP vaccine. As shown in Table 1, the 2,587 infants who received DAPTACEL at 2, 4 and 6 months of age had similar rates of reactions within 24 hours as recipients of DT and significantly lower rates than infants receiving whole-cell pertussis DTP.

Table 1: Percentage of Infants from Sweden I Efficacy Trial with Local or Systemic Reactions within 24 Hours Post-Dose 1, 2 and 3 of DAPTACEL compared with DT and Whole-Cell Pertussis DTP Vaccines

Event	Dose 1 (2 MONTHS) DAPTACEL N = 2,587	Dose1 (2 MONTHS) DT N = 2,574	Dose 1 (2 MONTHS) DTP N = 2,102	Dose 2 (4 MONTHS) DAPTACEL N = 2,563	Dose 2 (4 MONTHS) DT N = 2,555	Dose 2 (4 MONTHS) DTP N = 2,040	Dose 3 (6 MONTHS) DAPTACEL N = 2,549	Dose 3 (6 MONTHS) DT N = 2,538	Dose 3 (6 MONTHS) DTP N = 2,001
Local									
Tenderness (Any)	8.0*	8.4	59.5	10.1*	10.3	60.2	10.8*	10.0	50.0
Redness ≥2 cm	0.3*	0.3	6.0	1.0*	0.8	5.1	3.7*	2.4	6.4
Swelling ≥2 cm	0.9*	0.7	10.6	1.6*	2.0	10.0	6.3*†	3.9	10.5
Systemic									
Fever‡ ≥38°C (100.4°F)	7.8*	7.6	72.3	19.1*	18.4	74.3	23.6*	22.1	65.1
Fretfulness§	32.3	33.0	82.1	39.6	39.8	85.4	35.9	37.7	73.0
Anorexia	11.2*	10.3	39.2	9.1*	8.1	25.6	8.4*	7.7	17.5
Drowsiness	32.7*	32.0	56.9	25.9*	25.6	50.6	18.9*	20.6	37.6
Crying ≥1 hour	1.7*	1.6	11.8	2.5*	2.7	9.3	1.2*	1.0	3.3
Vomiting	6.9*	6.3	9.5	5.2¶	5.8	7.4	4.3	5.2	5.5

DT: Swedish National Biologics Laboratories
DTP: whole-cell pertussis DTP, Sanofi Pasteur Inc.
N = Number of evaluable subjects
* p<0.001: DAPTACEL versus whole-cell pertussis DTP
† p<0.0001: DAPTACEL versus DT
‡ Rectal temperature
§ Statistical comparisons were not made for this variable
¶ p<0.003: DAPTACEL versus whole-cell pertussis DTP

The incidence of serious and less common selected systemic events in the Sweden I Efficacy Trial is summarized in table 2.

Table 2: Selected Systemic Events: Rates Per 1,000 Doses after Vaccination at 2, 4 and 6 Months of Age in Sweden I Efficacy Trial

EVENT	Dose 1 (2 MONTHS) DAPTACEL N = 2,587	Dose 1 (2 MONTHS) DT N = 2,574	Dose 1 (2 MONTHS) DTP N = 2,102	Dose 1 (4 MONTHS) DAPTACEL N = 2,565	Dose 1 (4 MONTHS) DT N = 2556	Dose 1 (4 MONTHS) DTP N = 2,040	Dose 3 (6 MONTHS) DAPTACEL N = 2,551	Dose 3 (6 MONTHS) DT N = 2,539	Dose 3 (6 MONTHS) DTP N = 2,002
Rectal temperature ≥40°C (104°F) within 48 hours of vaccination	0.39	0.78	3.33	0	0.78	3.43	0.39	1.18	6.99
Hypotonic-hypo-responsive episode within 24 hours of vaccination	0	0	1.9	0	0	0.49	0.39	0	0
Persistent crying ≥3 hours within 24 hours of vaccination	1.16	0	8.09	0.39	0.39	1.96	0	0	1.0
Seizures within 72 hours of vaccination	0	0.39	0	0	0.39	0.49	0	0.39	0

DT: Swedish National Biologics Laboratories
DTP: whole-cell pertussis DTP, Sanofi Pasteur Inc.
N = Number of evaluable subjects

In the Sweden I Efficacy Trial, one case of whole limb swelling and generalized symptoms, with resolution within 24 hours, was observed following dose 2 of DAPTACEL. No episodes of anaphylaxis or encephalopathy were observed. No seizures were reported within 3 days of vaccination with DAPTACEL. Over the entire study period, 6 seizures were reported in the DAPTACEL group, 9 in the DT group and 3 in the whole-cell pertussis DTP group, for overall rates of 2.3, 3.5 and 1.4 per 1,000 vaccinees, respectively. One case of infantile spasms was reported in the DAPTACEL group. There were no instances of invasive bacterial infection or death.

In a US study, children received 4 doses of DAPTACEL at 2, 4, 6 and 15-17 months of age. A total of 1,454 children received DAPTACEL and were included in the safety analyses. Of these, 51.7% were female, 77.2% Caucasian, 6.3% Black, 6.5% Hispanic, 0.9% Asian and 9.1% other races. The use of DAPTACEL as a fifth dose of DTaP vaccine was evaluated in 2 subsequent US clinical studies. In one study, a total of 485 children received DAPTACEL at 4-6 years of age following 4 prior doses of DAPTACEL in infancy (DAPTACEL-primed). In a separate study, a total of 989 children received DAPTACEL at 4-6 years of age following 4 prior doses of Pentacel in infancy (Pentacel-primed). The children included in these fifth dose studies were non-random subsets of participants from previous DAPTACEL or Pentacel studies. The subsets were representative of all children who received 4 doses of DAPTACEL or Pentacel in the earlier studies with regard to frequencies of solicited local and systemic adverse events following the fourth dose.

In the US 4-dose DAPTACEL study, at 2, 4, and 6 months of age, DAPTACEL was administered concomitantly with *Haemophilus influenzae* type b (Hib) conjugate vaccine (tetanus toxoid conjugate) (Sanofi Pasteur SA), inactivated poliovirus vaccine (IPV) (Sanofi Pasteur SA), and 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.). Infants had received the first dose of hepatitis B vaccine at 0 months of age. At 2 and 6 months of age, hepatitis B vaccine (recombinant) (Merck & Co., Inc.) was also administered concomitantly with DAPTACEL. Based on random assignment, the fourth dose of DAPTACEL was administered either alone; concomitantly with Hib conjugate (tetanus toxoid conjugate) vaccine; or concomitantly with Hib conjugate (tetanus toxoid conjugate) vaccine, 7-valent pneumococcal conjugate vaccine, measles, mumps, rubella (MMR) vaccine (Merck & Co., Inc.), and varicella vaccine (Merck & Co., Inc.). In the fifth dose studies, DAPTACEL was administered concomitantly with IPV (all DAPTACEL-primed subjects and 47% of Pentacel-primed subjects) and MMR vaccine.

In the US studies, the occurrence of solicited local and systemic adverse events listed in Table 3 was recorded daily by parents or guardians for Days 0-7 following vaccination. For Days 0 and 1 following the first three doses of DAPTACEL, signs and symptoms of HHE also were solicited. Periodic telephone calls were made to inquire about adverse events. Serious adverse events were monitored during the three studies, through 6 months following the last dose of DAPTACEL.

The incidence and severity of selected solicited local and systemic adverse events that occurred within 3 days following each dose of DAPTACEL are shown in Table 3. The incidence of redness, tenderness and swelling at the DAPTACEL injection site increased with the fourth and fifth doses, with the highest rates reported after the fifth dose. The incidence of redness, tenderness and swelling at the DAPTACEL injection site was similarly increased when DAPTACEL was given as a fifth dose of DTaP vaccine in Pentacel-primed children.

Table 3: Number (Percentage) of Children from US Studies with Selected Solicited Local and Systemic Adverse Events by Severity Occurring Between 0 to 3 Days after Each Dose of DAPTACEL

	Dose 1* **N = 1390-1406** **%**	**Dose 2*** **N = 1346-1360** **%**	**Dose 3*** **N = 1301-1312** **%**	**Dose 4*** **N = 1118-1144** **%**	**Dose 5 DAPTACEL-primed*** **N = 473-481** **%**	**Dose 5 Pentacel-primed*** **N = 936-981** **%**
Injection Site Reactions (DAPTACEL injection site)						
Redness						
>5 mm	6.2	7.1	9.6	17.3	35.8	20.2
25 - 50 mm	0.6	0.5	1.9	6.3	10.4	6.8
>50 mm	0.4	0.1	0.0	3.1	15.8	6.6
Swelling						
>5 mm	4.0	4.0	6.5	11.7	23.9	12.0
25 - 50 mm	1.2	0.6	1.0	3.2	5.8	4.1
>50 mm	0.4	0.1	0.1	1.6	7.7	2.9
Tenderness[†]						
Any	48.8	38.2	40.9	49.5	61.5	50.0
Moderate	16.5	9.9	10.6	12.3	11.2	7.4
Severe	4.1	2.3	1.7	2.2	1.7	0.3
Increase in Arm Circumference[‡]						
>5 mm	-	-	-	30.1	38.3	28.6
20 - 40 mm	-	-	-	7.0	14.0	7.6
>40 mm	-	-	-	0.4	1.5	1.2
Interference with Normal Activity of the Arm[§]						
Any	-	-	-	-	20.4	8.8
Moderate	-	-	-	-	5.6	1.7
Severe	-	-	-	-	0.4	0.0
Systemic Reactions						
Fever[¶]						
≥38.0°C	9.3	16.1	15.8	10.5	6.1	4.6
>38.5-39.5°C	1.5	3.9	4.8	2.7	2.1	2.0
>39.5°C	0.1	0.4	0.3	0.7	0.2	0.2
Decreased Activity/Lethargy[#]						
Any	51.1	37.4	33.2	25.3	21.0	12.6
Moderate	23.0	14.4	12.1	8.2	5.8	3.6
Severe	1.2	1.4	0.6	1.0	0.8	0.4
Inconsolable Crying[Þ]						
Any	58.5	51.4	47.9	37.1	14.1	7.2
Moderate	14.2	12.6	10.8	7.7	3.5	1.9
Severe	2.2	3.4	1.4	1.5	0.4	0.3

	Dose 1* N = 1390-1406 %	Dose 2* N = 1346-1360 %	Dose 3* N = 1301-1312 %	Dose 4* N = 1118-1144 %	Dose 5 DAPTACEL-primed* N = 473-481 %	Dose 5 Pentacel-primed* N = 936-981 %
Fussiness/Irritability[β]						
Any	75.8	70.7	67.1	54.4	34.9	22.9
Moderate	27.7	25.0	22.0	16.3	7.5	5.3
Severe	5.6	5.5	4.3	3.9	0.4	0.5

* In one US study, children received four doses of DAPTACEL. A non-random subset of these children received a fifth dose of DAPTACEL in a subsequent study. A non-random subset of children previously vaccinated with 4 doses of Pentacel in previous clinical studies received a dose of DAPTACEL at 4-6 years of age as the fifth dose of DTaP vaccine in another clinical study.

† Doses 1-4 - Moderate: subject cries when site is touched; Severe: subject cries when leg or arm is moved. Dose 5 - Moderate: interfered with activities, but did not require medical care or absenteeism; Severe: incapacitating, unable to perform usual activities, may have/or required medical care or absenteeism.

‡ The circumference of the DAPTACEL-injected arm at the level of the axilla was monitored following the fourth and fifth doses only. Increase in arm circumference was calculated by subtracting the baseline circumference pre-vaccination (Day 0) from the circumference post-vaccination.

§ Moderate: decreased use of arm, but did not require medical care or absenteeism; Severe: incapacitating, refusal to move arm, may have/or required medical care or absenteeism.

¶ For Doses 1-3, 53.7% of temperatures were measured rectally, 45.1% were measured axillary, 1.0% were measured orally, and 0.1% were measured by an unspecified route. For Dose 4, 35.7% of temperatures were measured rectally, 62.3% were measured axillary, 1.5% were measured orally, and 0.5% were measured by an unspecified route. For Dose 5 in DAPTACEL-primed children, 0.2% of temperatures were measured rectally, 11.3% were measured axillary, and 88.4% were measured orally. For Dose 5 in Pentacel-primed children, 0.2% of temperatures were measured rectally, 0.5% were measured tympanically, 17% were measured axillary, and 81.7% were measured orally. Fever is based upon actual temperatures recorded with no adjustments to the measurement for route.

Dose 1-4 - Moderate: interferes with and limits daily activity, less interactive; Severe: disabling (not interested in usual daily activity, subject cannot be coaxed to interact with caregiver). Dose 5 - Moderate: interfered with activities, but did not require medical care or absenteeism; Severe: incapacitating, unable to perform usual activities, may have/or required medical care or absenteeism.

Þ Doses 1-4 - Moderate: 1 to 3 hours inconsolable crying; Severe: >3 hours inconsolable crying. Dose 5 - Moderate: interfered with activities, but did not require medical care or absenteeism; Severe: incapacitating, unable to perform usual activities, may have/or required medical care or absenteeism.

β Doses 1-4 - Moderate: Irritability for 1 to 3 hours; Severe: irritability for >3 hours. Dose 5 - Moderate: interfered with activities, but did not require medical care or absenteeism; Severe: incapacitating, unable to perform usual activities, may have/or required medical care or absenteeism.

In the US study in which children received 4 doses of DAPTACEL, of 1,454 subjects who received DAPTACEL, 5 (0.3%) subjects experienced a seizure within 60 days following any dose of DAPTACEL. One seizure occurred within 7 days post-vaccination: an infant who experienced an afebrile seizure with apnea on the day of the first vaccination. Three other cases of seizures occurred between 8 and 30 days post-vaccination. Of the seizures that occurred within 60 days post-vaccination, 3 were associated with fever. In this study, there were no reported cases of HHE following DAPTACEL. There was one death due to aspiration 222 days post-vaccination in a subject with ependymoma. Within 30 days following any dose of DAPTACEL, 57 (3.9%) subjects reported at least one serious adverse event. During this period, the most frequently reported serious adverse event was bronchiolitis, reported in 28 (1.9%)

subjects. Other serious adverse events that occurred within 30 days following DAPTACEL include three cases of pneumonia, two cases of meningitis and one case each of sepsis, pertussis (post-dose 1), irritability and unresponsiveness.

In the US study in which DAPTACEL was administered as a fifth DTaP dose in DAPTACEL-primed subjects, within 30 days following the fifth consecutive dose of DAPTACEL, 1 (0.2%) subject reported 2 serious adverse events (bronchospasm and hypoxia). In the US study in which DAPTACEL was administered as a fifth DTaP dose in Pentacel-primed subjects, within 30 days following DAPTACEL, 4 (0.4%) subjects reported one or more serious adverse events (asthma and pneumonia; idiopathic thrombocytopenic purpura; vomiting; cellulitis not at the injection site). In these two studies, there were no reports of seizures within 30 days following DAPTACEL in either the DAPTACEL-primed subjects or Pentacel-primed subjects.

In another study (Sweden II Efficacy Trial), 3 DTaP vaccines and a whole-cell pertussis DTP vaccine, none of which are licensed in the US, were evaluated to assess relative safety and efficacy. This study included HCPDT, a vaccine made of the same components as DAPTACEL but containing twice the amount of detoxified PT and four times the amount of FHA (20 mcg detoxified PT and 20 mcg FHA). HHE was observed following 29 (0.047%) of 61,220 doses of HCPDT; 16 (0.026%) of 61,219 doses of an acellular pertussis vaccine made by another manufacturer; and 34 (0.056%) of 60,792 doses of a whole-cell pertussis DTP vaccine. There were 4 additional cases of HHE in other studies using HCPDT vaccine for an overall rate of 33 (0.047%) in 69,525 doses.

In a randomized, parallel-group, US multi-center clinical trial conducted in children 4 through 6 years of age, DAPTACEL was administered as follows: concomitantly with IPV (Sanofi Pasteur SA) followed 30 days later by Menactra® [Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate vaccine, Sanofi Pasteur Inc.] [Group A]; concomitantly with Menactra followed 30 days later by IPV [Group B]; or 30 days after concomitant administration of Menactra and IPV [Group C]. Solicited injection site and systemic reactions were recorded in a diary card for 7 consecutive days after each vaccination. For all study groups, the most frequently reported solicited local reaction at the DAPTACEL injection site was pain: 71.7%, 69.4% and 52.1% of subjects in Groups A, B and C, respectively. For all study groups, the most frequently reported systemic reaction after DAPTACEL vaccination was myalgia: 46.2%, 37.3% and 25.8% of subjects in Groups A, B and C, respectively. Fever >39.5ºC occurred at <1.0% in all groups.

6.2 Postmarketing Experience

The following adverse events have been spontaneously reported during the postmarketing use of DAPTACEL in the US and other countries. Because these events are reported voluntarily from a population of uncertain size, it may not be possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

The following adverse events were included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to DAPTACEL.

- **Blood and lymphatic disorders**

  Lymphadenopathy

- **Cardiac disorders**

Cyanosis

- **Gastro-intestinal disorders**

  Nausea, diarrhea

- **General disorders and administration site conditions**

  Local reactions: injection site pain, injection site rash, injection site nodule, injection site mass, extensive swelling of injected limb (including swelling that involves adjacent joints).

- **Infections and infestations**

  Injection site cellulitis, cellulitis, injection site abscess

- **Immune system disorders**

  Hypersensitivity, allergic reaction, anaphylactic reaction (edema, face edema, swelling face, pruritus, rash generalized) and other types of rash (erythematous, macular, maculo-papular)

- **Nervous system disorders**

  Convulsions: febrile convulsion, grand mal convulsion, partial seizures
  HHE, hypotonia, somnolence, syncope

- **Psychiatric disorders**

  Screaming

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

In clinical trials, DAPTACEL was administered concomitantly with one or more of the following US licensed vaccines: Hib conjugate vaccine, IPV, hepatitis B vaccine, pneumococcal conjugate vaccine, Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate vaccine, MMR vaccine, and varicella vaccine. [See *Adverse Reactions (6.1)* and *Clinical Studies (0)*.] When DAPTACEL is given at the same time as another injectable vaccine(s), the vaccines should be administered with different syringes and at different injection sites.

In cases where DAPTACEL and Menactra are to be administered to children 4 through 6 years of age, the two vaccines should be administered concomitantly or Menactra should be administered prior to DAPTACEL. Administration of Menactra one month after DAPTACEL has been shown to reduce meningococcal antibody responses to Menactra. [See *Adverse Reactions (6.1)* and *Clinical Studies (0)*.]

7.2 Immunosuppressive Treatments

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to DAPTACEL.

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

DAPTACEL is not indicated for use in infants below 6 weeks of age or children 7 years of age or older. Safety and effectiveness of DAPTACEL in these age groups have not been established.

11 DESCRIPTION

DAPTACEL is a sterile isotonic suspension of pertussis antigens and diphtheria and tetanus toxoids adsorbed on aluminum phosphate, for intramuscular injection.

Each 0.5 mL dose contains 15 Lf diphtheria toxoid, 5 Lf tetanus toxoid and acellular pertussis antigens [10 mcg detoxified pertussis toxin (PT), 5 mcg filamentous hemagglutinin (FHA), 3 mcg pertactin (PRN), and 5 mcg fimbriae types 2 and 3 (FIM)].

Other ingredients per 0.5 mL dose include 1.5 mg aluminum phosphate (0.33 mg of aluminum) as the adjuvant, ≤5 mcg residual formaldehyde, <50 ng residual glutaraldehyde and 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative).

The acellular pertussis vaccine components are produced from *Bordetella pertussis* cultures grown in Stainer-Scholte medium (2) modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. The FIM components are extracted and co-purified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT is detoxified with glutaraldehyde. FHA is treated with formaldehyde, and the residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate.

Corynebacterium diphtheriae is grown in modified Mueller's growth medium. (3) After purification by ammonium sulfate fractionation, diphtheria toxin is detoxified with formaldehyde and diafiltered. *Clostridium tetani* is grown in modified Mueller-Miller casamino acid medium without beef heart infusion. (4) Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

The adsorbed diphtheria, tetanus and acellular pertussis components are combined with aluminum phosphate (as adjuvant), 2-phenoxyethanol (not as a preservative) and water for injection.

Both diphtheria and tetanus toxoids induce at least 2 units of antitoxin per mL in the guinea pig potency test. The potency of the acellular pertussis vaccine components is determined by the antibody response of immunized mice to detoxified PT, FHA, PRN and FIM as measured by enzyme-linked immunosorbent assay (ELISA).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of

protection. Antitoxin levels of at least 0.1 IU/mL are generally regarded as protective. (5) Levels of 1.0 IU/mL have been associated with long-term protection. (6)

Tetanus

Tetanus is an acute disease caused by an extremely potent neurotoxin produced by C tetani. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assay is considered the minimum protective level. (5) (7) A tetanus antitoxin level ≥0.1 IU/mL as measured by the ELISA used in clinical studies of DAPTACEL is considered protective.

Pertussis

Pertussis (whooping cough) is a respiratory disease caused by B pertussis. This Gram-negative coccobacillus produces a variety of biologically active components, though their role in either the pathogenesis of, or immunity to, pertussis has not been clearly defined.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

DAPTACEL has not been evaluated for carcinogenic or mutagenic potential or impairment of fertility.

14 CLINICAL STUDIES

14.1 Diphtheria

In a US study in which children received 4 doses of DAPTACEL at 2, 4, 6 and 15-17 months of age, after the third dose, 100% (N = 1,099) achieved diphtheria antitoxin levels of ≥0.01 IU/mL and 98.5% achieved diphtheria antitoxin levels of ≥0.10 IU/mL. Among a random subset of children who received the fourth dose of DAPTACEL at 15-16 months of age, 96.5% (N = 659) achieved diphtheria antitoxin levels of ≥1.0 IU/mL after the fourth dose.

14.2 Tetanus

In a US study in which children received 4 doses of DAPTACEL at 2, 4, 6 and 15-17 months of age, after the third dose, 100% (N = 1,037) achieved tetanus antitoxin levels of ≥0.10 IU/mL. Among a random subset of children who received the fourth dose of DAPTACEL at 15-16 months of age, 98.8% (N = 681) achieved tetanus antitoxin levels of ≥1.0 IU/mL after the fourth dose.

14.3 Pertussis

A randomized, double-blinded, placebo-controlled efficacy and safety study was conducted in Sweden during 1992-1995 (Sweden I Efficacy Trial) under the sponsorship of the National Institute of Allergy and Infectious Diseases. A total of 9,829 infants received 1 of 4 vaccines: DAPTACEL (N = 2,587); another investigational acellular pertussis vaccine (N = 2,566); whole-cell pertussis DTP vaccine (N = 2,102); or DT vaccine as placebo (Swedish National Bacteriological Laboratory, N = 2,574). Infants were immunized at 2, 4 and 6 months of age. The mean length of follow-up was 2 years after the third dose of vaccine. The protective efficacy of DAPTACEL against pertussis after 3 doses using the World Health Organization (WHO) case definition (≥21 consecutive days of paroxysmal cough with culture or serologic confirmation or

epidemiologic link to a confirmed case) was 84.9% (95% confidence interval [CI] 80.1 to 88.6). The protective efficacy of DAPTACEL against mild pertussis (≥1 day of cough with laboratory confirmation) was 77.9% (95% CI 72.6 to 82.2). Protection against pertussis by DAPTACEL was sustained for the 2-year follow-up period.

In order to assess the antibody response to the pertussis antigens of DAPTACEL in the US population, 2 lots of DAPTACEL, including the lot used in the Sweden I Efficacy Trial, were administered to US infants in the US Bridging Study. In this study, antibody responses following 3 doses of DAPTACEL given to US children at 2, 4 and 6 months of age were compared to those from a subset of the infants enrolled in the Sweden I Efficacy Trial. Assays were performed in parallel on the available sera from the US and Swedish infants. Antibody responses to all the antigens were similar except for those to the PRN component. For both lots of DAPTACEL, the geometric mean concentration (GMC) and percent response to PRN in US infants (Lot 006, N = 107; Lot 009, N = 108) were significantly lower after 3 doses of vaccine than in Swedish infants (N = 83). In separate US and Canadian studies in which children received DAPTACEL at 2, 4 and 6 months of age, with a fourth dose at either 17-20 months (Canadian study) or 15-16 months (random subset from US study) of age, antibody responses to each pertussis antigen following the fourth dose (Canadian study N = 275; US study N = 237-347) were at least as high as those seen in the Swedish infants after 3 doses. While a serologic correlate of protection for pertussis has not been established, the antibody response to all antigens in North American infants after 4 doses of DAPTACEL at 2, 4, 6 and 15-20 months of age was comparable to that achieved in Swedish infants in whom efficacy was demonstrated after 3 doses of DAPTACEL at 2, 4 and 6 months of age.

In the US Study 005, infants were randomized to receive 3 doses of VAXELIS at 2, 4, and 6 months of age and DAPTACEL and PedvaxHIB [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)] at 15 months of age, or Control group vaccines (3 doses of Pentacel vaccine at 2, 4, and 6 months of age + RECOMBIVAX HB [Hepatitis B Vaccine (Recombinant)] at 2 and 6 months of age and DAPTACEL and ActHIB [Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)] at 15 months of age). All subjects received concomitant Prevnar 13 (Pneumococcal 13-valent Conjugate Vaccine [Diphtheria CRM197 Protein]) at 2, 4, 6, and 15 months of age. To complete the 4-dose pertussis primary vaccination series, participants in both groups received DAPTACEL at 15 months of age and were evaluated for immune responses to pertussis antigens one month later.

The non-inferiority criteria for vaccine response rates and GMCs for all pertussis antigens were met following the fourth dose. (8)

14.4 Concomitantly Administered Vaccines

In the US Bridging study, DAPTACEL was given concomitantly with Hib conjugate vaccine (Sanofi Pasteur SA) according to local practices. Anti-PRP immune response was evaluated in 261 infants who received 3 doses of Hib conjugate vaccine. One month after the third dose, 96.9% achieved anti-PRP antibody levels of at least 0.15 mcg/mL and 82.7% achieved antibody levels of at least 1.0 mcg/mL.

In the US study in which infants received DAPTACEL concomitantly with Hib conjugate (tetanus toxoid conjugate) vaccine, IPV, 7-valent pneumococcal conjugate vaccine, and hepatitis B vaccine [see *Adverse Reactions (6.1)*], at 7 months of age, 100.0% of subjects (N = 1,050-1,097) had protective neutralizing antibody levels (≥1:8 1/dil) for poliovirus types 1, 2 and 3; and

92.4% (N = 998) achieved anti-hepatitis B surface antigen levels ≥10.0 mIU/mL. Although there is no established serologic correlate of protection for any of the pneumococcal serotypes, at 7 months of age 91.3%-98.9% (N = 1,027-1,029) achieved anti-pneumococcal polysaccharide levels ≥0.5 mcg/mL for serotypes 4, 9V, 14, 18C, 19F and 23F and 80.7% (N = 1,027) achieved an anti-pneumococcal polysaccharide level ≥0.5 mcg/mL for serotype 6B. The mumps seroresponse rate was lower when DAPTACEL was administered concomitantly (86.6%; N = 307) vs. non-concomitantly (90.1%; N = 312) with the first dose of MMR vaccine [upper limit of 90% confidence interval for difference in rates (non-concomitant minus concomitant) >5%]. There was no evidence for interference in the immune response to the measles, rubella, and varicella antigens or to the fourth dose of the 7-valent pneumococcal conjugate vaccine with concomitant administration of DAPTACEL.

In a randomized, parallel-group, US multi-center clinical trial conducted in children 4 through 6 years of age, DAPTACEL was administered as follows: concomitantly with IPV (Sanofi Pasteur SA) followed 30 days later by Menactra [Group A]; concomitantly with Menactra followed 30 days later by IPV [Group B]; or 30 days after concomitant administration of Menactra and IPV [Group C]. Sera were obtained approximately 30 days after each respective vaccination. When DAPTACEL was administered concomitantly with Menactra [Group B], antibody responses to PT, FHA and PRN (GMC), tetanus (% participants with antibody concentrations ≥1.0 IU/mL), and diphtheria (%participants with antibody concentrations ≥1.0 IU/mL) were non-inferior to those observed when DAPTACEL (and IPV) were administered [Group A]. The anti-FIM GMCs were marginally lower when DAPTACEL and Menactra were administered concomitantly but the clinical significance is unknown because there are no established serological correlates of protection for pertussis. When DAPTACEL (and IPV) were administered 30 days prior to Menactra [Group A], significantly lower serum-bactericidal assay-human complement (SBA-H) GMTs to all 4 meningococcal serogroups were observed compared to when Menactra (and IPV) were administered 30 days prior to DAPTACEL [Group C]. When DAPTACEL was administered concomitantly with Menactra [Group B], SBA-H GMTs to meningococcal serogroups A, C, and W-135 were non-inferior to those observed when Menactra (and IPV) were administered [Group C]. The non-inferiority criterion was marginally missed for meningococcal serogroup Y. [See *Drug Interactions (7.1)*.]

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5 Department of Health and Human Services, Food and Drug Administration. Biological products; bacterial vaccines and toxoids; implementation of efficacy review; proposed rule. Federal Register 1985;50(240):51002-117.

6 Wharton M, et al. Diphtheria Toxoid. In: Plotkin SA, Orenstein WA, editors. Vaccines. 4th ed. Philadelphia, PA: W. B. Saunders 2004 p. 211-28.

7 Wassilak SGF, et al. Tetanus Toxoid. In: Plotkin SA, Orenstein WA, editors. Vaccines. 4th ed. Philadelphia, PA: W. B. Saunders 2004 p. 745-81.

8 VAXELIS® [full prescribing information]. Toronto, ON: MSP Vaccine Company.

16 HOW SUPPLIED/STORAGE AND HANDLING

The vial stopper for this product is not made with natural rubber latex.

DAPTACEL is supplied in a single-dose vial (NDC No. 49281-286-58):

in packages of 1 vial: NDC No. 49281-286-01;

in packages of 5 vials: NDC No. 49281-286-05;

in packages of 10 vials: NDC No. 49281-286-10.

DAPTACEL should be stored at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Product which has been exposed to freezing should not be used. Do not use after expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

Inform the parent or guardian of the following:

- The potential benefits and risks of immunization with DAPTACEL.
- The common adverse reactions that have occurred following administration of DAPTACEL or other vaccines containing similar components.
- Other adverse reactions can occur. Call healthcare provider with any adverse reactions of concern.

Provide the Vaccine Information Statements (VIS), which are required by the National Childhood Vaccine Injury Act of 1986.

Manufactured by:

Sanofi Pasteur Limited
Toronto Ontario Canada

Distributed by:

Sanofi Pasteur Inc.

Swiftwater PA 18370 USA

US Patents: 4500639, 4687738, 4784589, 4997915, 5444159, 5667787, 5877298.

DAPTACEL® is a registered trademark of Sanofi, its affiliates and subsidiaries..

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use INFANRIX safely and effectively. See full prescribing information for INFANRIX.

INFANRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) Suspension for Intramuscular Injection
Initial U.S. Approval: 1997

---------- INDICATIONS AND USAGE----------
INFANRIX is a vaccine indicated for active immunization against diphtheria, tetanus, and pertussis as a 5-dose series in infants and children aged 6 weeks through 6 years (prior to the 7th birthday). (1)

----------DOSAGE AND ADMINISTRATION----------
A 0.5-mL intramuscular injection given as a 5-dose series: (2.2)
- One dose each at 2, 4, and 6 months of age.
- One booster dose at 15 to 20 months of age and another booster dose at 4 to 6 years of age.

---------- DOSAGE FORMS AND STRENGTHS----------
Single-dose vials and single-dose, prefilled syringes containing a 0.5-mL suspension for injection. (3)

---------- CONTRAINDICATIONS ----------
- Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid-, tetanus toxoid-, or pertussis-containing vaccine, or to any component of INFANRIX. (4.1)
- Encephalopathy within 7 days of administration of a previous pertussis-containing vaccine. (4.2)
- Progressive neurologic disorders. (4.3)

---------- WARNINGS AND PRECAUTIONS----------
- If Guillain-Barré syndrome occurs within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give INFANRIX should be based on potential benefits and risks. (5.1)
- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.2)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including INFANRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.3)
- If temperature ≥105°F, collapse or shock-like state, or persistent, inconsolable crying lasting ≥3 hours have occurred within 48 hours after receipt of a pertussis-containing vaccine, or if seizures have occurred within 3 days after receipt of a pertussis-containing vaccine, the decision to give INFANRIX should be based on potential benefits and risks. (5.4)
- For children at higher risk for seizures, an antipyretic may be administered at the time of vaccination with INFANRIX. (5.5)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including INFANRIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination. (5.6)

---------- ADVERSE REACTIONS ----------
Rates of injection site reactions (pain, redness, swelling) ranged from 10% to 53%, depending on reaction and dose number, and were highest following Doses 4 and 5. Fever was common (20% to 30%) following Doses 1-3. Other common solicited adverse reactions were drowsiness, irritability/fussiness, and loss of appetite, reported in approximately 15% to 60% of subjects, depending on reaction and dose number. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- DRUG INTERACTIONS----------
Do not mix INFANRIX with any other vaccine in the same syringe or vial. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

***Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

INFANRIX is indicated for active immunization against diphtheria, tetanus, and pertussis as a 5-dose series in infants and children aged 6 weeks through 6 years (prior to the 7th birthday).

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

Shake vigorously to obtain a homogeneous, turbid, white suspension. Do not use if resuspension does not occur with vigorous shaking. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

For the prefilled syringes, attach a sterile needle and administer intramuscularly.

For the vials, use a sterile needle and sterile syringe to withdraw the 0.5-mL dose and administer intramuscularly. Changing needles between drawing vaccine from a vial and injecting it into a recipient is not necessary unless the needle has been damaged or contaminated. Use a separate sterile needle and syringe for each individual.

Do not administer this product intravenously, intradermally, or subcutaneously.

2.2 Dose and Schedule

A 0.5-mL dose of INFANRIX is approved for intramuscular administration in infants and children aged 6 weeks through 6 years (prior to the 7th birthday) as a 5-dose series. The series consists of a primary immunization course of 3 doses administered at 2, 4, and 6 months of age (at intervals of 4 to 8 weeks), followed by 2 booster doses, administered at 15 to 20 months of age and at 4 to 6 years of age. The first dose may be given as early as 6 weeks of age.

The preferred administration site is the anterolateral aspect of the thigh for most infants aged younger than 12 months and the deltoid muscle of the upper arm for most children aged 12 months through 6 years.

2.3 Use of INFANRIX with Other DTaP Vaccines

Sufficient data are not available on the safety and effectiveness of interchanging INFANRIX and Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) vaccines from different manufacturers for successive doses of the DTaP vaccination series. Because the pertussis antigen components of INFANRIX and PEDIARIX [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine] are the same, INFANRIX may be used to complete a DTaP vaccination series initiated with PEDIARIX.

2.4 Additional Dosing Information

If any recommended dose of pertussis vaccine cannot be given *[see Contraindications (4.2, 4.3), Warnings and Precautions (5.5)]*, Diphtheria and Tetanus Toxoids Adsorbed (DT) For Pediatric Use should be given according to its prescribing information.

3 DOSAGE FORMS AND STRENGTHS

INFANRIX is a suspension for injection available in 0.5-mL single-dose vials and 0.5-mL

single-dose, prefilled TIP-LOK syringes.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid-, tetanus toxoid-, or pertussis-containing vaccine, or to any component of INFANRIX is a contraindication *[see Description (11)]*. Because of the uncertainty as to which component of the vaccine might be responsible, no further vaccination with any of these components should be given. Alternatively, such individuals may be referred to an allergist for evaluation if immunization with any of these components is being considered.

4.2 Encephalopathy

Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of administration of a previous dose of a pertussis-containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including INFANRIX.

4.3 Progressive Neurologic Disorder

Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy, is a contraindication to administration of any pertussis-containing vaccine, including INFANRIX. Pertussis vaccine should not be administered to individuals with these conditions until a treatment regimen has been established and the condition has stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome occurs within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including INFANRIX, should be based on careful consideration of the potential benefits and possible risks. When a decision is made to withhold tetanus toxoid, other available vaccines should be given, as indicated.

5.2 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.3 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including INFANRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.4 Adverse Reactions following Prior Pertussis Vaccination

If any of the following reactions occur in temporal relation to receipt of a pertussis-containing vaccine, the decision to give any pertussis-containing vaccine, including INFANRIX, should be based on careful consideration of the potential benefits and possible risks:

- Temperature of ≥40.5°C (105°F) within 48 hours not due to another identifiable cause;
- Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours;
- Persistent, inconsolable crying lasting ≥3 hours, occurring within 48 hours;
- Seizures with or without fever occurring within 3 days.

5.5 Children at Risk for Seizures

For children at higher risk for seizures than the general population, an appropriate antipyretic may be administered at the time of vaccination with a pertussis-containing vaccine, including INFANRIX, and for the ensuing 24 hours to reduce the possibility of post-vaccination fever.

5.6 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including INFANRIX, to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

5.7 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the patient's immunization history for possible vaccine hypersensitivity. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

Approximately 95,000 doses of INFANRIX have been administered in clinical studies. In these studies, 29,243 infants have received INFANRIX in primary series studies: 6,081 children have received a fourth consecutive dose of INFANRIX, 1,764 children have received a fifth consecutive dose of INFANRIX, and 559 children have received a dose of INFANRIX following 3 doses of PEDIARIX.

Solicited Adverse Reactions

In a U.S. study, 335 infants received INFANRIX, ENGERIX-B [Hepatitis B Vaccine (Recombinant)], inactivated poliovirus vaccine (IPV, Sanofi Pasteur SA), Haemophilus b (Hib)

conjugate vaccine (Wyeth Pharmaceuticals Inc.), and pneumococcal 7-valent conjugate (PCV7) vaccine (Wyeth Pharmaceuticals Inc.) concomitantly at separate sites. All vaccines were administered at 2, 4, and 6 months of age. Data on solicited local reactions and general adverse reactions were collected by parents using standardized diary cards for 4 consecutive days following each vaccine dose (i.e., day of vaccination and the next 3 days) (Table 1). Among subjects, 69% were white, 16% were Hispanic, 8% were black, 4% were Asian, and 2% were of other racial/ethnic groups.

Table 1. Solicited Local and General Adverse Reactions (%) Occurring within 4 Days of Vaccination[a] with Separate Concomitant Administration of INFANRIX, ENGERIX-B, IPV, Haemophilus b (Hib) Conjugate Vaccine, and Pneumococcal Conjugate Vaccine (PCV7) (Modified Intent-to-Treat Cohort)

Adverse Reaction	INFANRIX, ENGERIX-B, IPV, Hib Vaccine, & PCV7		
	Dose 1	Dose 2	Dose 3
Local[b]			
n	**335**	**323**	**315**
Pain, any	32	30	30
Pain, Grade 2 or 3	9	9	9
Pain, Grade 3	3	2	1
Redness, any	18	33	39
Redness, >20 mm	0	0	2
Swelling, any	10	20	25
Swelling, >20 mm	1	0	1
General			
n	**333**	**321**	**311**
Fever[c] (≥100.4°F)	20	30	24
Fever[c] (>101.3°F)	5	8	6
Fever[c] (>102.2°F)	0	3	2
Fever[c] (>103.1°F)	0	0	0
n	**335**	**323**	**315**
Drowsiness, any	54	48	38
Drowsiness, Grade 2 or 3	18	12	11
Drowsiness, Grade 3	4	1	2
Irritability/Fussiness, any	62	62	57
Irritability/Fussiness, Grade 2 or 3	19	21	19
Irritability/Fussiness, Grade 3	4	3	3
Loss of appetite, any	28	27	24
Loss of appetite, Grade 2 or 3	5	3	5
Loss of appetite, Grade 3	1	0	0

Hib conjugate vaccine and PCV7 manufactured by Wyeth Pharmaceuticals Inc. IPV manufactured

by Sanofi Pasteur SA.
Modified intent-to-treat cohort = All vaccinated subjects for whom safety data were available.
n = Number of infants for whom at least one symptom sheet was completed; for fever; numbers exclude missing temperature recordings or tympanic measurements.
Grade 2: Pain defined as cried/protested on touch; drowsiness defined as interfered with normal daily activities; irritability/fussiness defined as crying more than usual/interfered with normal daily activities; loss of appetite defined as eating less than usual/interfered with normal daily activities.
Grade 3: Pain defined as cried when limb was moved/spontaneously painful; drowsiness defined as prevented normal daily activities; irritability/fussiness defined as crying that could not be comforted/prevented normal daily activities; loss of appetite defined as no eating at all.
[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.
[b] Local reactions at the injection site for INFANRIX.
[c] Axillary temperatures increased by 1°C and oral temperatures increased by 0.5°C to derive equivalent rectal temperature.

In a U.S. study, the safety of a booster dose of INFANRIX was evaluated in children aged 15 to 18 months whose previous 3 DTaP doses were with INFANRIX (n = 251) or PEDIARIX (n = 559). Vaccines administered concurrently with the fourth dose of INFANRIX included measles, mumps, and rubella (MMR) vaccine (Merck & Co., Inc.), varicella vaccine (Merck & Co., Inc.), pneumococcal 7-valent conjugate (PCV7) vaccine (Wyeth Pharmaceuticals Inc.), and any U.S.-licensed Hib conjugate vaccine; these were given concomitantly in 13.2%, 6.3%, 37.4%, and 41.2% of subjects, respectively. Data on solicited adverse reactions were collected by parents using standardized diary cards for 4 consecutive days following each vaccine dose (i.e., day of vaccination and the next 3 days) (Table 2). Among subjects, 85% were white, 6% were Hispanic, 6% were black, 1% were Asian, and 2% were of other racial/ethnic groups.

Table 2. Solicited Local and General Adverse Reactions (%) Occurring within 4 Days of Vaccination[a] with INFANRIX Administered as the Fourth Dose following 3 Previous Doses of INFANRIX or PEDIARIX (Total Vaccinated Cohort)

Adverse Reaction	Group Primed with INFANRIX[b] n = 247	Group Primed with PEDIARIX[c] n = 553
Local[d]		
Pain, any	45	48
Pain, Grade 2 or 3	19	19
Pain, Grade 3	4	3
Redness, any	48	50
Redness, >20 mm	6	6
Swelling, any	33	33
Swelling, >20 mm	4	5
Increase in mid-thigh circumference, any	33	26
Increase in mid-thigh circumference, >40 mm	0	1
General		
Fever[e] (>99.5°F)	9	15
Fever[e] (>100.4°F)	5	7
Fever[e] (>101.3°F)	2	2
Drowsiness, any	36	31
Drowsiness, Grade 2 or 3	9	7
Drowsiness, Grade 3	2	1
Irritability, any	52	54
Irritability, Grade 2 or 3	18	20
Irritability, Grade 3	3	1
Loss of appetite, any	25	23
Loss of appetite, Grade 2 or 3	5	5
Loss of appetite, Grade 3	2	0

Total Vaccinated Cohort = All subjects who received a dose of study vaccine.
n = Number of subjects for whom at least one symptom sheet was completed.
Grade 2: Pain defined as cried/protested on touch; drowsiness defined as interfered with normal daily activities; irritability defined as crying more than usual/interfered with normal daily activities; loss of appetite defined as eating less than usual/no effect on normal daily activities.
Grade 3: Pain defined as cried when limb was moved/spontaneously painful; drowsiness defined as prevented normal daily activities; irritability defined as crying that could not be comforted/prevented normal daily activities; loss of appetite defined as eating less than usual/interfered with normal daily activities.
[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.
[b] Received INFANRIX, ENGERIX-B, IPV (Sanofi Pasteur SA), PCV7 vaccine (Wyeth

Pharmaceuticals Inc.), and Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.) at 2, 4, and 6 months of age.

[c] Received PEDIARIX, PCV7 vaccine (Wyeth Pharmaceuticals Inc.), and Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.) at 2, 4, and 6 months of age or PCV7 vaccine 2 weeks later.

[d] Local reactions at the injection site for INFANRIX.

[e] Axillary temperatures.

In a U.S. study, the safety of a fifth consecutive dose of INFANRIX coadministered at separate sites with a fourth dose of IPV (Sanofi Pasteur SA) and a second dose of MMR vaccine (Merck & Co., Inc.) was evaluated in 1,053 children aged 4 to 6 years. Data on solicited adverse reactions were collected by parents using standardized diary cards for 4 consecutive days following each vaccine dose (i.e., day of vaccination and the next 3 days) (Table 3). Among subjects, 43% were white, 18% Hispanic, 15% Asian, 7% black, and 17% were of other racial/ethnic groups.

Table 3. Solicited Local and General Adverse Reactions (%) Occurring within 4 Days of Vaccination[a] with a Fifth Consecutive Dose of INFANRIX when Coadministered with IPV and MMR Vaccine (Total Vaccinated Cohort)

Local[b]	**n = 1,039-1,043**
Pain, any	53
Pain, Grade 2 or 3[c]	12
Pain, Grade 3[c]	1
Redness, any	37
Redness, ≥50 mm	20
Redness, ≥110 mm	4
Arm circumference increase, any	38
Arm circumference increase, >20 mm	7
Arm circumference increase, >30 mm	3
Swelling, any	27
Swelling, ≥50 mm	12
Swelling, ≥110 mm	2
General	**n = 993-1,036**
Drowsiness, any	18
Drowsiness, Grade 3[d]	1
Fever, ≥99.5°F	15
Fever, >100.4°F	4
Fever, >102.2°F	1
Fever, >104°F	0
Loss of appetite, any	16
Loss of appetite, Grade 3[e]	1

IPV manufactured by Sanofi Pasteur SA. MMR vaccine manufactured by Merck & Co., Inc.
Total Vaccinated Cohort = All vaccinated subjects for whom safety data were available.
n = Number of children with evaluable data for the reactions listed.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] Local reactions at the injection site for INFANRIX.

[c] Grade 2 defined as painful when the limb was moved; Grade 3 defined as preventing normal daily activities.

[d] Grade 3 defined as preventing normal daily activities.

[e] Grade 3 defined as not eating at all.

In the U.S. booster immunization studies in which INFANRIX was administered as the fourth or fifth dose in the DTaP series following previous doses with INFANRIX or PEDIARIX, large swelling reactions of the limb injected with INFANRIX were assessed.

In the fourth-dose study, a large swelling reaction was defined as injection site swelling with a diameter of >50 mm, a >50 mm increase in the mid-thigh circumference compared with the

pre-vaccination measurement, and/or any diffuse swelling that interfered with or prevented daily activities. The overall incidence of large swelling reactions occurring within 4 days (Day 0-Day 3) following INFANRIX was 2.3%.

In the fifth-dose study, a large swelling reaction was defined as swelling that involved >50% of the injected upper arm length and that was associated with a >30 mm increase in mid-upper arm circumference within 4 days following vaccination. The incidence of large swelling reactions following the fifth consecutive dose of INFANRIX was 1.0%.

Less Common and Serious General Adverse Reactions

Selected adverse reactions reported from a double-blind, randomized Italian clinical efficacy trial involving 4,696 children administered INFANRIX or 4,678 children administered whole-cell DTP vaccine (DTwP) (manufactured by Connaught Laboratories, Inc.) as a 3-dose primary series are shown in Table 4. The incidence of rectal temperature ≥104°F, hypotonic-hyporesponsive episodes, and persistent crying ≥3 hours following administration of INFANRIX was significantly less than that following administration of whole-cell DTP vaccine.

Table 4. Selected Adverse Reactions Occurring within 48 Hours following Vaccination with INFANRIX or Whole-Cell DTP in Italian Infants at 2, 4, or 6 Months of Age

	INFANRIX (n = 13,761 Doses)		**Whole-Cell DTP Vaccine (n = 13,520 Doses)**	
Reaction	**Number**	**Rate/1,000 Doses**	**Number**	**Rate/1,000 Doses**
Fever (≥104°F)[a,b]	5	0.36	32	2.4
Hypotonic-hyporesponsive episode[c]	0	0	9	0.67
Persistent crying ≥3 hours[a]	6	0.44	54	4.0
Seizures[d]	1[e]	0.07	3[f]	0.22

[a] P <0.001.

[b] Rectal temperatures.

[c] P = 0.002.

[d] Not statistically significant at P <0.05.

[e] Maximum rectal temperature within 72 hours of vaccination = 103.1°F.

[f] Maximum rectal temperature within 72 hours of vaccination = 99.5°F, 101.3°F, and 102.2°F.

In a German safety study that enrolled 22,505 infants (66,867 doses of INFANRIX administered as a 3-dose primary series at 3, 4, and 5 months of age), all subjects were monitored for unsolicited adverse events that occurred within 28 days following vaccination using report cards. In a subset of subjects (n = 2,457), these cards were standardized diaries which solicited specific adverse reactions that occurred within 8 days of each vaccination in addition to unsolicited adverse events which occurred from enrollment until approximately 30 days following the third vaccination. Cards from the whole cohort were returned at subsequent visits and were supplemented by spontaneous reporting by parents and a medical history after the first and

second doses of vaccine. In the subset of 2,457, adverse events following the third dose of vaccine were reported via standardized diaries and spontaneous reporting at a follow-up visit. Adverse events in the remainder of the cohort were reported via report cards which were returned by mail approximately 28 days after the third dose of vaccine. Adverse reactions (rates per 1,000 doses) occurring within 7 days following any of the first 3 doses included: unusual crying (0.09), febrile seizure (0.0), afebrile seizure (0.13), and hypotonic-hyporesponsive episodes (0.01).

6.2 Postmarketing Experience

In addition to reports in clinical trials for INFANRIX, the following adverse reactions have been identified during postapproval use of INFANRIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccination.

Infections and Infestations

Bronchitis, cellulitis, respiratory tract infection.

Blood and Lymphatic System Disorders

Lymphadenopathy, thrombocytopenia.

Immune System Disorders

Anaphylactic reaction, hypersensitivity.

Nervous System Disorders

Encephalopathy, headache, hypotonia, syncope.

Ear and Labyrinth Disorders

Ear pain.

Cardiac Disorders

Cyanosis.

Respiratory, Thoracic, and Mediastinal Disorders

Apnea, cough.

Skin and Subcutaneous Tissue Disorders

Angioedema, erythema, pruritus, rash, urticaria.

General Disorders and Administration Site Conditions

Fatigue, injection site induration, injection site reaction, Sudden Infant Death Syndrome.

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

In clinical trials, INFANRIX was given concomitantly with Hib conjugate vaccine, pneumococcal 7-valent conjugate vaccine, hepatitis B vaccine, IPV, and the second dose of MMR vaccine *[see Adverse Reactions (6.1), Clinical Studies (14.3)]*.

When INFANRIX is administered concomitantly with other injectable vaccines, they should be given with separate syringes. INFANRIX should not be mixed with any other vaccine in the same syringe or vial.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to INFANRIX.

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

Safety and effectiveness of INFANRIX in infants aged younger than 6 weeks and children aged 7 to 16 years have not been established. INFANRIX is not approved for use in these age groups.

11 DESCRIPTION

INFANRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) is a noninfectious, sterile vaccine for intramuscular administration. Each 0.5-mL dose is formulated to contain 25 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid, 25 mcg of inactivated pertussis toxin (PT), 25 mcg of filamentous hemagglutinin (FHA), and 8 mcg of pertactin (69 kiloDalton outer membrane protein).

The diphtheria toxin is produced by growing *Corynebacterium diphtheriae* (*C. diphtheriae)* in Fenton medium containing a bovine extract. Tetanus toxin is produced by growing *Clostridium tetani* (*C. tetani)* in a modified Latham medium derived from bovine casein. The bovine materials used in these extracts are sourced from countries which the United States Department of Agriculture (USDA) has determined neither have nor present an undue risk for bovine spongiform encephalopathy (BSE). Both toxins are detoxified with formaldehyde, concentrated by ultrafiltration, and purified by precipitation, dialysis, and sterile filtration.

The acellular pertussis antigens (PT, FHA, and pertactin) are isolated from *Bordetella pertussis* (*B. pertussis)* culture grown in modified Stainer-Scholte liquid medium. PT and FHA are isolated from the fermentation broth; pertactin is extracted from the cells by heat treatment and flocculation. The antigens are purified in successive chromatographic and precipitation steps. PT is detoxified using glutaraldehyde and formaldehyde. FHA and pertactin are treated with formaldehyde.

Diphtheria and tetanus toxoids and pertussis antigens (PT, FHA, and pertactin) are individually adsorbed onto aluminum hydroxide.

Diphtheria and tetanus toxoid potency is determined by measuring the amount of neutralizing antitoxin in previously immunized guinea pigs. The potency of the acellular pertussis components (PT, FHA, and pertactin) is determined by enzyme-linked immunosorbent assay (ELISA) on sera from previously immunized mice.

Each 0.5-mL dose contains aluminum hydroxide as adjuvant (not more than 0.625 mg aluminum by assay) and 4.5 mg of sodium chloride. Each dose also contains ≤100 mcg of residual formaldehyde and ≤100 mcg of polysorbate 80 (Tween 80).

INFANRIX is available in vials and prefilled syringes. The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex. The vial stoppers are not made with natural rubber latex.

INFANRIX is formulated without preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated infectious disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to the diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection; a level of 0.1 IU/mL is regarded as protective.[1]

Tetanus

Tetanus is an acute toxin-mediated infectious disease caused by a potent exotoxin released by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to the tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assays, is considered the minimum protective level.[2,3] A level of 0.1 IU/mL is considered protective.[4]

Pertussis

Pertussis (whooping cough) is a disease of the respiratory tract caused by *B. pertussis*. The role of the different components produced by *B. pertussis* in either the pathogenesis of, or the immunity to, pertussis is not well understood. There is no well-established serological correlate of protection for pertussis.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

INFANRIX has not been evaluated for carcinogenic or mutagenic potential or for impairment of

fertility.

14 CLINICAL STUDIES

14.1 Diphtheria and Tetanus

Efficacy of diphtheria toxoid used in INFANRIX was determined on the basis of immunogenicity studies. A VERO cell toxin-neutralizing test confirmed the ability of infant sera (N = 45), obtained one month after a 3-dose primary series, to neutralize diphtheria toxin. Levels of diphtheria antitoxin ≥0.01 IU/mL were achieved in 100% of the sera tested.

Efficacy of tetanus toxoid used in INFANRIX was determined on the basis of immunogenicity studies. An in vivo mouse neutralization assay confirmed the ability of infant sera (N = 45), obtained1 month after a 3-dose primary series, to neutralize tetanus toxin. Levels of tetanus antitoxin ≥0.01 IU/mL were achieved in 100% of the sera tested.

14.2 Pertussis

Efficacy of a 3-dose primary series of INFANRIX has been assessed in 2 clinical studies.

A double-blind, randomized, active Diphtheria and Tetanus Toxoids (DT)-controlled trial conducted in Italy assessed the absolute protective efficacy of INFANRIX when administered at 2, 4, and 6 months of age. The population used in the primary analysis of the efficacy of INFANRIX included 4,481 infants vaccinated with INFANRIX and 1,470 DT vaccinees. The mean length of follow-up was 17 months, beginning 30 days after the third dose of vaccine. After 3 doses, the absolute protective efficacy of INFANRIX against WHO-defined typical pertussis (21 days or more of paroxysmal cough with infection confirmed by culture and/or serologic testing) was 84% (95% CI: 76, 89). When the definition of pertussis was expanded to include clinically milder disease with respect to type and duration of cough, with infection confirmed by culture and/or serologic testing, the efficacy of INFANRIX was calculated to be 71% (95% CI: 60, 78) against >7 days of any cough and 73% (95% CI: 63, 80) against ≥14 days of any cough. Vaccine efficacy after 3 doses and with no booster dose in the second year of life was assessed in 2 subsequent follow-up periods. A follow-up period from 24 months to a mean age of 33 months was conducted in a partially unblinded cohort (children who received DT were offered pertussis vaccine and those who declined were retained in the study cohort). During this period, the efficacy of INFANRIX against WHO-defined pertussis was 78% (95% CI: 62, 87). During the third follow-up period, which was conducted in an unblinded manner among children aged 3 to 6 years, the efficacy of INFANRIX against WHO-defined pertussis was 86% (95% CI: 79, 91). Thus, protection against pertussis in children administered 3 doses of INFANRIX in infancy was sustained to 6 years of age.

A prospective efficacy trial was also conducted in Germany employing a household contact study design. In preparation for this study, 3 doses of INFANRIX were administered at 3, 4, and 5 months of age to more than 22,000 children living in 6 areas of Germany in a safety and immunogenicity study. Infants who did not participate in the safety and immunogenicity study

could have received a DTwP vaccine or DT vaccine. Index cases were identified by spontaneous presentation to a physician. Households with at least one other member (i.e., besides index case) aged 6 through 47 months were enrolled. Household contacts of index cases were monitored for incidence of pertussis by a physician who was blinded to the vaccination status of the household. Calculation of vaccine efficacy was based on attack rates of pertussis in household contacts classified by vaccination status. Of the 173 household contacts who had not received a pertussis vaccine, 96 developed WHO-defined pertussis, as compared with 7 of 112 contacts vaccinated with INFANRIX. The protective efficacy of INFANRIX was calculated to be 89% (95% CI: 77, 95), with no indication of waning of protection up until the time of the booster vaccination. The average age of infants vaccinated with INFANRIX at the end of follow-up in this trial was 13 months (range: 6 to 25 months). When the definition of pertussis was expanded to include clinically milder disease, with infection confirmed by culture and/or serologic testing, the efficacy of INFANRIX against ≥7 days of any cough was 67% (95% CI: 52, 78) and against ≥7 days of paroxysmal cough was 81% (95% CI: 68, 89). The corresponding efficacy of INFANRIX against ≥14 days of any cough or paroxysmal cough were 73% (95% CI: 59, 82) and 84% (95% CI: 71, 91), respectively.

Pertussis Immune Response to INFANRIX Administered as a 3-Dose Primary Series

The immune responses to each of the 3 pertussis antigens contained in INFANRIX were evaluated in sera obtained 1 month after the third dose of vaccine in each of 3 studies (schedule of administration: 2, 4, and 6 months of age in the Italian efficacy study and one U.S. study; 3, 4, and 5 months of age in the German efficacy study). One month after the third dose of INFANRIX, the response rates to each pertussis antigen were similar in all 3 studies. Thus, although a serologic correlate of protection for pertussis has not been established, the antibody responses to these 3 pertussis antigens (PT, FHA, and pertactin) in a U.S. population were similar to those achieved in 2 populations in which efficacy of INFANRIX was demonstrated.

14.3 Immune Response to Concomitantly Administered Vaccines

In a U.S. study, INFANRIX was given concomitantly, at separate sites, with Hib conjugate vaccine (Sanofi Pasteur SA) at 2, 4, and 6 months of age. Subjects also received ENGERIX-B and oral poliovirus vaccine (OPV). One month after the third dose of Hib conjugate vaccine, 90% of 72 infants had anti-PRP (polyribosyl-ribitol-phosphate) ≥1.0 mcg/mL.

In a U.S. study, INFANRIX was given concomitantly, at separate sites, with ENGERIX-B, IPV (Sanofi Pasteur SA), pneumococcal 7-valent conjugate (PCV7), and Hib conjugate vaccines (Wyeth Pharmaceuticals Inc.) at 2, 4, and 6 months of age. Immune responses were measured in sera obtained approximately 1 month after the third dose of vaccines. Among 121 subjects who had not received a birth dose of hepatitis B vaccine, 99.2% had anti-HBsAg (hepatitis B surface antigen) ≥10 mIU/mL following the third dose of ENGERIX-B. Among 153 subjects, 100% had anti-poliovirus 1, 2, and 3, ≥1:8 following the third dose of IPV. Although serological correlates for protection have not been established for the pneumococcal serotypes, a threshold level of

≥0.3 mcg/mL was evaluated. Following the third dose of PCV7 vaccine, 91.8% to 99.4% of subjects (n = 146-156) had anti-pneumococcal polysaccharide ≥0.3 mcg/mL for serotypes 4, 9V, 14, 18C, 19F, and 23F, and 73.0% had a level ≥0.3 mcg/mL for serotype 6B.

15 REFERENCES

1. Vitek CR and Wharton M. Diphtheria Toxoid. In: Plotkin SA, Orenstein WA, and Offit PA, eds. *Vaccines.* 5th ed. Saunders; 2008:139-156.
2. Wassilak SGF, Roper MH, Kretsinger K, and Orenstein WA. Tetanus Toxoid. In: Plotkin SA, Orenstein WA, and Offit PA, eds. *Vaccines.* 5th ed. Saunders; 2008:805-839.
3. Department of Health and Human Services, Food and Drug Administration. Biological products; Bacterial vaccines and toxoids; Implementation of efficacy review; Proposed rule. *Federal Register.* December 13, 1985;50(240):51002-51117.
4. Centers for Disease Control and Prevention. General Recommendations on Immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR.* 2006;55(RR-15):1-48.

16 HOW SUPPLIED/STORAGE AND HANDLING

INFANRIX is available in 0.5-mL single-dose vials and 0.5-mL single-dose, disposable, prefilled TIP-LOK syringes (packaged without needles):

NDC 58160-810-01 Vial in Package of 10: NDC 58160-810-11

NDC 58160-810-43 Syringe in Package of 10: NDC 58160-810-52

Store refrigerated between 2° and 8°C (36° and 46°F). Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

Provide the following information to the parent or guardian:

- Inform of the potential benefits and risks of immunization with INFANRIX, and of the importance of completing the immunization series.
- Inform about the potential for adverse reactions that have been temporally associated with administration of INFANRIX or other vaccines containing similar components.
- Instruct to report any adverse events to their healthcare provider.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

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Research Triangle Park, NC 27709

INF:29PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use KINRIX safely and effectively. See full prescribing information for KINRIX.

KINRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine) Suspension for Intramuscular Injection
Initial U.S. Approval: 2008

---------- **INDICATIONS AND USAGE** ----------

A single dose of KINRIX is indicated for active immunization against diphtheria, tetanus, pertussis, and poliomyelitis as the fifth dose in the diphtheria, tetanus, and acellular pertussis (DTaP) vaccine series and the fourth dose in the inactivated poliovirus vaccine (IPV) series in children aged 4 through 6 years (prior to the 7th birthday) whose previous DTaP vaccine doses have been with INFANRIX and/or PEDIARIX for the first 3 doses and INFANRIX for the fourth dose. (1)

---------- **DOSAGE AND ADMINISTRATION** ----------

A single intramuscular injection (0.5 mL). (2.2)

---------- **DOSAGE FORMS AND STRENGTHS** ----------

Single-dose vials and single-dose, prefilled syringes containing a 0.5-mL suspension for injection. (3)

---------- **CONTRAINDICATIONS** ----------

- Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid-, tetanus toxoid-, pertussis- or poliovirus-containing vaccine, or to any component of KINRIX, including neomycin and polymyxin B. (4.1)
- Encephalopathy within 7 days of administration of a previous pertussis-containing vaccine. (4.2)
- Progressive neurologic disorders. (4.3)

---------- **WARNINGS AND PRECAUTIONS** ----------

- If Guillain-Barré syndrome occurs within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give KINRIX should be based on potential benefits and risks. (5.1)
- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.2)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including KINRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.3)
- If temperature ≥105°F, collapse or shock-like state, or persistent, inconsolable crying lasting ≥3 hours have occurred within 48 hours after receipt of a pertussis-containing vaccine, or if seizures have occurred within 3 days after receipt of a pertussis-containing vaccine, the decision to give KINRIX should be based on potential benefits and risks. (5.4)
- For children at higher risk for seizures, an antipyretic may be administered at the time of vaccination with KINRIX. (5.5)

---------- **ADVERSE REACTIONS** ----------

- The most frequently reported solicited local reaction (>50%) was injection site pain. Other common solicited local reactions (≥25%) were redness, increase in arm circumference, and swelling. (6.1)
- Common solicited general adverse reactions (≥15%) were drowsiness, fever (≥99.5°F), and loss of appetite. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- **DRUG INTERACTIONS** ----------

Do not mix KINRIX with any other vaccine in the same syringe or vial. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

***Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

A single dose of KINRIX is indicated for active immunization against diphtheria, tetanus, pertussis, and poliomyelitis as the fifth dose in the diphtheria, tetanus, and acellular pertussis (DTaP) vaccine series and the fourth dose in the inactivated poliovirus vaccine (IPV) series in children aged 4 through 6 years (prior to the 7th birthday) whose previous DTaP vaccine doses have been with INFANRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) and/or PEDIARIX [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine] for the first 3 doses

and INFANRIX for the fourth dose.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

Shake vigorously to obtain a homogeneous, turbid, white suspension. Do not use if resuspension does not occur with vigorous shaking. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

For the prefilled syringes, attach a sterile needle and administer intramuscularly.

For the vials, use a sterile needle and sterile syringe to withdraw the 0.5-mL dose and administer intramuscularly. Changing needles between drawing vaccine from a vial and injecting it into a recipient is not necessary unless the needle has been damaged or contaminated. Use a separate sterile needle and syringe for each individual.

Do not administer this product intravenously, intradermally, or subcutaneously.

2.2 Recommended Dose and Schedule

KINRIX is to be administered as a 0.5-mL dose by intramuscular injection. The preferred site of administration is the deltoid muscle of the upper arm.

KINRIX may be used for the fifth dose in the DTaP immunization series and the fourth dose in the IPV immunization series in children aged 4 through 6 years (prior to the 7th birthday) whose previous DTaP vaccine doses have been with INFANRIX and/or PEDIARIX for the first 3 doses and INFANRIX for the fourth dose *[see Indications and Usage (1)]*.

3 DOSAGE FORMS AND STRENGTHS

KINRIX is a suspension for injection available in 0.5-mL single-dose vials and 0.5-mL single-dose prefilled TIP-LOK syringes.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid-, tetanus toxoid-, pertussis- or poliovirus-containing vaccine, or to any component of KINRIX, including neomycin and polymyxin B, is a contraindication to administration of KINRIX *[see Description (11)]*. Because of the uncertainty as to which component of the vaccine might be responsible, no further vaccination with any of these components should be given. Alternatively, such individuals may be referred to an allergist for evaluation if immunization with any of these components is considered.

4.2 Encephalopathy

Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of administration of a previous dose of a pertussis-containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including KINRIX.

4.3 Progressive Neurologic Disorder

Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy, is a contraindication to administration of any pertussis-containing vaccine, including KINRIX. Pertussis vaccine should not be administered to individuals with such conditions until a treatment regimen has been established and the condition has stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome occurs within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including KINRIX, should be based on careful consideration of the potential benefits and possible risks. When a decision is made to withhold tetanus toxoid, other available vaccines should be given, as indicated.

5.2 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.3 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including KINRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.4 Adverse Reactions following Prior Pertussis Vaccination

If any of the following reactions occur in temporal relation to receipt of a pertussis-containing vaccine, the decision to give any pertussis-containing vaccine, including KINRIX, should be based on careful consideration of the potential benefits and possible risks:

- Temperature of ≥40.5°C (105°F) within 48 hours not due to another identifiable cause;
- Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours;
- Persistent, inconsolable crying lasting ≥3 hours, occurring within 48 hours;
- Seizures with or without fever occurring within 3 days.

When a decision is made to withhold pertussis vaccination, other available vaccines should be given, as indicated.

5.5 Children at Risk for Seizures

For children at higher risk for seizures than the general population, an appropriate antipyretic may be administered at the time of vaccination with a pertussis-containing vaccine, including KINRIX, and for the ensuing 24 hours to reduce the possibility of post-vaccination fever.

5.6 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the patient's immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions to allow an assessment of benefits and risks. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

A total of 4,013 children were vaccinated with a single dose of KINRIX in 4 clinical trials. Of these, 381 children received a non-U.S. formulation of KINRIX (containing ≤2.5 mg 2-phenoxyethanol per dose as preservative).

The primary study (Study 048), conducted in the United States, was a randomized, controlled clinical trial in which children aged 4 to 6 years were vaccinated with KINRIX (n = 3,156) or control vaccines (INFANRIX and IPOL vaccine [IPV, Sanofi Pasteur SA]; n = 1,053) as a fifth DTaP vaccine dose following 4 doses of INFANRIX and as a fourth IPV dose following 3 doses of IPOL. Subjects also received the second dose of U.S.-licensed measles, mumps, and rubella (MMR) vaccine (Merck & Co., Inc.) administered concomitantly, at separate sites.

Data on adverse events were collected by parents/guardians using standardized forms for 4 consecutive days following vaccination with KINRIX or control vaccines (i.e., day of vaccination and the next 3 days). The reported frequencies of solicited local reactions and general adverse reactions in Study 048 are presented in Table 1.

In 3 studies (Studies 046, 047, and 048), children were monitored for unsolicited adverse events, including serious adverse events that occurred in the 31-day period following vaccination, and in 2 studies (Studies 047 and 048), parents/guardians were actively queried about changes in the child's health status, including the occurrence of serious adverse events, through 6 months post-vaccination.

Table 1. Percentage of Children Aged 4 to 6 Years Reporting Solicited Local or General Adverse Reactions within 4 Days of Vaccination[a] with KINRIX or Separate Concomitant Administration of INFANRIX and IPV when Coadministered with MMR Vaccine (Study 048) (Total Vaccinated Cohort)

Adverse Reaction	KINRIX	INFANRIX + IPV
Local[b]	**n = 3,121-3,128**	**n = 1,039-1,043**
Pain, any	57[c]	53
Pain, Grade 2 or 3[d]	14	12
Pain, Grade 3[d]	2[c]	1
Redness, any	37	37
Redness, ≥50 mm	18	20
Redness, ≥110 mm	3	4
Arm circumference increase, any	36	38
Arm circumference increase, >20 mm	7	7
Arm circumference increase, >30 mm	2	3
Swelling, any	26	27
Swelling, ≥50 mm	10	12
Swelling, ≥110 mm	1	2
General	**n = 3,037-3,120**	**n = 993-1,036**
Drowsiness, any	19	18
Drowsiness, Grade 3[e]	1	1
Fever, ≥99.5°F	16	15
Fever, >100.4°F	7[c]	4
Fever, >102.2°F	1	1
Fever, >104°F	0	0
Loss of appetite, any	16	16
Loss of appetite, Grade 3[f]	1	1

IPV = Inactivated poliovirus vaccine (Sanofi Pasteur SA); MMR = Measles, mumps, and rubella vaccine (Merck & Co., Inc.).

Total Vaccinated Cohort = All vaccinated subjects for whom safety data were available.

n = Number of children with evaluable data for the reactions listed.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] Local reactions at the injection site for KINRIX or INFANRIX.

[c] Statistically higher than comparator group (P <0.05).

[d] Grade 2 defined as painful when the limb was moved; Grade 3 defined as preventing normal daily activities.

[e] Grade 3 defined as preventing normal daily activities.

[f] Grade 3 defined as not eating at all.

In Study 048, KINRIX was non-inferior to INFANRIX with regard to swelling that involved

>50% of the injected upper arm length and that was associated with a >30 mm increase in mid-upper arm circumference within 4 days following vaccination (upper limit of 2-sided 95% Confidence Interval for difference in percentage of KINRIX [0.6%, n = 20] minus INFANRIX [1.0%, n = 11] ≤2%).

Serious Adverse Events

Within the 31-day period following study vaccination in 3 studies (Studies 046, 047, and 048) in which all subjects received concomitant MMR vaccine (U.S.-licensed MMR vaccine [Merck & Co., Inc.] in Studies 047 and 048, non—U.S.-licensed MMR vaccine in Study 046), 3 subjects (0.1% [3/3,537]) who received KINRIX reported serious adverse events (dehydration and hypernatremia; cerebrovascular accident; dehydration and gastroenteritis) and 4 subjects (0.3% [4/1,434]) who received INFANRIX and inactivated poliovirus vaccine (Sanofi Pasteur SA) reported serious adverse events (cellulitis, constipation, foreign body trauma, fever without identified etiology).

6.2 Postmarketing Experience

In addition to reports in clinical trials for KINRIX, the following adverse reactions have been identified during postapproval use of KINRIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccination.

General Disorders and Administration Site Conditions

Injection site vesicles.

Nervous System Disorders

Syncope.

Skin and Subcutaneous Tissue Disorders

Pruritus.

Additional adverse reactions reported following postmarketing use of INFANRIX, for which a causal relationship to vaccination is plausible, are: Allergic reactions, including anaphylactoid reactions, anaphylaxis, angioedema, and urticaria; apnea; collapse or shock-like state (hypotonic-hyporesponsive episode); convulsions (with or without fever); lymphadenopathy; and thrombocytopenia.

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

In U.S. clinical trials, KINRIX was administered concomitantly with the second dose of MMR vaccine (Merck & Co., Inc.); in one of these trials (Study 055), KINRIX was also administered concomitantly with varicella vaccine (Merck & Co., Inc.) *[see Clinical Studies (14.2)]*.

When KINRIX is administered concomitantly with other injectable vaccines, they should be given with separate syringes. KINRIX should not be mixed with any other vaccine in the same syringe or vial.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to KINRIX.

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

Safety and effectiveness of KINRIX in children younger than 4 years and children aged 7 to 16 years have not been evaluated. KINRIX is not approved for use in persons in these age groups.

11 DESCRIPTION

KINRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine) is a noninfectious, sterile vaccine for intramuscular administration. Each 0.5-mL dose is formulated to contain 25 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid, 25 mcg of inactivated pertussis toxin (PT), 25 mcg of filamentous hemagglutinin (FHA), 8 mcg of pertactin (69 kiloDalton outer membrane protein), 40 D-antigen Units (DU) of Type 1 poliovirus (Mahoney), 8 DU of Type 2 poliovirus (MEF-1), and 32 DU of Type 3 poliovirus (Saukett). The diphtheria, tetanus, and pertussis components of KINRIX are the same as those in INFANRIX and PEDIARIX and the poliovirus component is the same as that in PEDIARIX.

The diphtheria toxin is produced by growing *Corynebacterium diphtheriae (C. diphtheriae)* in Fenton medium containing a bovine extract. Tetanus toxin is produced by growing *Clostridium tetani (C. tetani)* in a modified Latham medium derived from bovine casein. The bovine materials used in these extracts are sourced from countries which the United States Department of Agriculture (USDA) has determined neither have nor are at risk of bovine spongiform encephalopathy (BSE). Both toxins are detoxified with formaldehyde, concentrated by ultrafiltration, and purified by precipitation, dialysis, and sterile filtration.

The acellular pertussis antigens (PT, FHA, and pertactin) are isolated from *Bordetella pertussis* (*B. pertussis)* culture grown in modified Stainer-Scholte liquid medium. PT and FHA are isolated from the fermentation broth; pertactin is extracted from the cells by heat treatment and flocculation. The antigens are purified in successive chromatographic and precipitation steps. PT is detoxified using glutaraldehyde and formaldehyde. FHA and pertactin are treated with formaldehyde.

Diphtheria and tetanus toxoids and pertussis antigens (inactivated PT, FHA, and pertactin) are individually adsorbed onto aluminum hydroxide.

The inactivated poliovirus component of KINRIX is an enhanced potency component. Each of the 3 strains of poliovirus is individually grown in VERO cells, a continuous line of monkey kidney cells, cultivated on microcarriers. Calf serum and lactalbumin hydrolysate are used during VERO cell culture and/or virus culture. Calf serum is sourced from countries the USDA has determined neither have nor are at risk of BSE. After clarification, each viral suspension is purified by ultrafiltration, diafiltration, and successive chromatographic steps, and inactivated with formaldehyde. The 3 purified viral strains are then pooled to form a trivalent concentrate.

Diphtheria and tetanus toxoid potency is determined by measuring the amount of neutralizing antitoxin in previously immunized guinea pigs. The potency of the acellular pertussis components (inactivated PT, FHA, and pertactin) is determined by enzyme-linked immunosorbent assay (ELISA) on sera from previously immunized mice. The potency of the inactivated poliovirus component is determined by using the D-antigen ELISA and by a poliovirus-neutralizing cell culture assay on sera from previously immunized rats.

Each 0.5-mL dose contains aluminum hydroxide as adjuvant (not more than 0.6 mg aluminum by assay) and 4.5 mg of sodium chloride. Each dose also contains ≤100 mcg of residual formaldehyde and ≤100 mcg of polysorbate 80 (Tween 80). Neomycin sulfate and polymyxin B are used in the poliovirus vaccine manufacturing process and may be present in the final vaccine at ≤0.05 ng neomycin and ≤0.01 ng polymyxin B per dose.

The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex. The vial stoppers are not made with natural rubber latex.

KINRIX does not contain a preservative.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated infectious disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to the diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection; a level of 0.1 IU/mL is regarded as protective.[1]

Tetanus

Tetanus is an acute toxin-mediated disease caused by a potent exotoxin released by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to the tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assays, is considered the minimum protective level.[2,3] A level of ≥0.1 IU/mL is considered protective.[4]

Pertussis

Pertussis (whooping cough) is a disease of the respiratory tract caused by *B. pertussis*. The role

of the different components produced by *B. pertussis* in either the pathogenesis of, or the immunity to, pertussis is not well understood. There is no well-established serological correlate of protection for pertussis. The efficacy of the pertussis component of KINRIX was determined in clinical trials of INFANRIX administered as a 3-dose series in infants (see INFANRIX prescribing information).

Poliomyelitis

Poliovirus is an enterovirus that belongs to the picornavirus family. Three serotypes of poliovirus have been identified (Types 1, 2, and 3). Neutralizing antibodies against the 3 poliovirus serotypes are recognized as conferring protection against poliomyelitis disease.[5]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

KINRIX has not been evaluated for carcinogenic or mutagenic potential or for impairment of fertility.

14 CLINICAL STUDIES

14.1 Immunological Evaluation

In a U.S. multicenter study (Study 048), 4,209 children were randomized in a 3:1 ratio to receive either KINRIX or INFANRIX and IPV (Sanofi Pasteur SA) administered concomitantly at separate sites. Subjects also received MMR vaccine (Merck & Co., Inc.) administered concomitantly at a separate site. Subjects were children aged 4 through 6 years who previously received 4 doses of INFANRIX, 3 doses of IPV, and 1 dose of MMR vaccine. Among subjects in both vaccine groups combined, 49.6% were female; 45.6% of subjects were white, 18.8% Hispanic, 13.6% Asian, 7.0% black, and 15.0% were of other racial/ethnic groups.

Levels of antibodies to the diphtheria, tetanus, pertussis (PT, FHA, and pertactin), and poliovirus antigens were measured in sera obtained immediately prior to vaccination and 1 month (range: 31 to 48 days) after vaccination (Table 2). The co-primary immunogenicity endpoints were anti-diphtheria toxoid, anti-tetanus toxoid, anti-PT, anti-FHA, and anti-pertactin booster responses, and anti-poliovirus Type 1, Type 2, and Type 3 geometric mean antibody titers (GMTs) 1 month after vaccination. KINRIX was shown to be non-inferior to INFANRIX and IPV administered separately, in terms of booster responses to DTaP antigens and post-vaccination GMTs for anti-poliovirus antibodies (Table 2).

Table 2. Pre-Vaccination Antibody Levels and Post-Vaccination[a] Antibody Responses following KINRIX Compared with Separate Concomitant Administration of INFANRIX and IPV in Children Aged 4 to 6 Years when Coadministered with MMR Vaccine (Study 048) (ATP Cohort for Immunogenicity)

	KINRIX n = 787-851	INFANRIX + IPV n = 237-262
Anti-diphtheria Toxoid		
Pre-vaccination % ≥0.1 IU/mL (95% CI)[b]	87.7 (85.3, 89.9)	85.5 (80.6, 89.5)
Post-vaccination % ≥0.1 IU/mL (95% CI)[b]	100 (99.6, 100)	100 (98.6, 100)
% Booster Response (95% CI)[c]	99.5 (98.8, 99.9)[d]	100 (98.6, 100)
Anti-tetanus Toxoid		
Pre-vaccination % ≥0.1 IU/mL (95% CI)[b]	87.8 (85.4, 90.0)	88.2 (83.6, 91.8)
Post-vaccination % ≥0.1 IU/mL (95% CI)[b]	100 (99.6, 100)	100 (98.6, 100)
% Booster Response (95% CI)[c]	96.7 (95.2, 97.8)[d]	93.9 (90.2, 96.5)
Anti-PT		
% Booster Response (95% CI)[e]	92.2 (90.2, 94.0)[d]	92.6 (88.7, 95.5)
Anti-FHA		
% Booster Response (95% CI)[e]	95.4 (93.7, 96.7)[d]	96.2 (93.1, 98.1)
Anti-pertactin		
% Booster Response (95% CI)[e]	97.8 (96.5, 98.6)[d]	96.9 (94.1, 98.7)
Anti-poliovirus 1		
Pre-vaccination % ≥1:8 (95% CI)[b]	88.3 (85.9, 90.4)	85.1 (80.1, 89.2)
Post-vaccination % ≥1:8 (95% CI)[b]	99.9 (99.3, 100)	100 (98.5, 100)
Post-vaccination GMT (95% CI)	2,127 (1,976, 2,290)[f]	1,685 (1,475, 1,925)
Anti-poliovirus 2		
Pre-vaccination % ≥1:8 (95% CI)[b]	91.8 (89.7, 93.6)	87.0 (82.3, 90.8)
Post-vaccination % ≥1:8 (95% CI)[b]	100 (99.6, 100)	100 (98.5, 100)
Post-vaccination GMT (95% CI)	2,265 (2,114, 2,427)[f]	1,818 (1,606, 2,057)
Anti-poliovirus 3		
Pre-vaccination % ≥1:8 (95% CI)[b]	84.7 (82.0, 87.0)	85.0 (80.1, 89.1)
Post-vaccination % ≥1:8 (95% CI)[b]	100 (99.5, 100)	100 (98.5, 100)
Post-vaccination GMT (95% CI)	3,588 (3,345, 3,849)[f]	3,365 (2,961, 3,824)

ATP = According-to-protocol; CI = Confidence Interval; GMT = Geometric mean antibody titer; IPV = Inactivated poliovirus vaccine (Sanofi Pasteur SA); MMR = Measles, mumps, and rubella vaccine (Merck & Co., Inc.).

n = Number of subjects with available results.

[a] One-month blood sampling, range 31 to 48 days.

[b] Seroprotection defined as anti-diphtheria toxoid and anti-tetanus toxoid antibody concentrations ≥0.1 IU/mL by ELISA and as anti-poliovirus Type 1, Type 2, and Type 3

antibody titer ≥1:8 by micro-neutralization assay for poliovirus.

[c] Booster response: In subjects with pre-vaccination <0.1 IU/mL, post-vaccination concentration ≥0.4 IU/mL. In subjects with pre-vaccination concentration ≥0.1 IU/mL, an increase of at least 4 times the pre-vaccination concentration.

[d] KINRIX was non-inferior to INFANRIX + IPV based on booster response rates (upper limit of 2-sided 95% CI on the difference of INFANRIX + IPV minus KINRIX ≤10%).

[e] Booster response: In subjects with pre-vaccination <5 EL.U./mL, post-vaccination concentration ≥20 EL.U./mL. In subjects with pre-vaccination ≥5 EL.U./mL and <20 EL.U./mL, an increase of at least 4 times the pre-vaccination concentration. In subjects with pre-vaccination ≥20 EL.U./mL, an increase of at least 2 times the pre-vaccination concentration.

[f] KINRIX was non-inferior to INFANRIX + IPV based on post-vaccination anti-poliovirus antibody GMTs adjusted for baseline titer (upper limit of 2-sided 95% CI for the GMT ratio [INFANRIX + IPV:KINRIX] ≤1.5).

14.2 Concomitant Vaccine Administration

In a U.S. study (Study 055) that enrolled children aged 4 to 6 years, KINRIX was administered concomitantly at separate sites with MMR vaccine (Merck & Co., Inc.) (n = 237) or with MMR vaccine and varicella vaccine (Merck & Co., Inc.) (n = 239). Immune responses to the antigens contained in KINRIX were measured approximately 1 month (28 to 48 days) after vaccination. Booster responses to diphtheria, tetanus, and pertussis antigens and GMTs for poliovirus (Type 1, 2, and 3) after the receipt of KINRIX administered concomitantly with MMR vaccine and varicella vaccine were non-inferior to immune responses following concomitant administration of KINRIX administered with MMR vaccine.

15 REFERENCES

1. Vitek CR and Wharton M. Diphtheria Toxoid. In: Plotkin SA, Orenstein WA, and Offit PA, eds. *Vaccines*. 5th ed. Saunders; 2008:139-156.
2. Wassilak SGF, Roper MH, Kretsinger K, and Orenstein WA. Tetanus Toxoid. In: Plotkin SA, Orenstein WA, and Offit PA, eds. *Vaccines*. 5th ed. Saunders; 2008:805-839.
3. Department of Health and Human Services, Food and Drug Administration. Biological products; Bacterial vaccines and toxoids; Implementation of efficacy review; Proposed rule. *Federal Register*. December 13, 1985;50(240):51002-51117.
4. Centers for Disease Control and Prevention. General Recommendations on Immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR*. 2006;55(RR-15):1-48.
5. Sutter RW, Pallansch MA, Sawyer LA, et al. Defining surrogate serologic tests with respect to predicting protective vaccine efficacy: Poliovirus vaccination. In: Williams JC, Goldenthal KL, Burns DL, Lewis Jr BP, eds. Combined vaccines and simultaneous administration.

Current issues and perspectives. New York, NY: The New York Academy of Sciences; 1995:289-299.

16 HOW SUPPLIED/STORAGE AND HANDLING

KINRIX is available in 0.5-mL single-dose vials and 0.5-mL single-dose, disposable, prefilled TIP-LOK syringes (packaged without needles):

NDC 58160-812-01 Vial in Package of 10: NDC 58160-812-11

NDC 58160-812-43 Syringe in Package of 10: NDC 58160-812-52

Store refrigerated between 2° and 8°C (36° and 46°F). Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

Provide the following information to the parent or guardian:

- Inform of the potential benefits and risks of immunization with KINRIX.
- Inform about the potential for adverse reactions that have been temporally associated with administration of KINRIX or other vaccines containing similar components.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by **GlaxoSmithKline Biologicals**

Rixensart, Belgium, U.S. License 1617, and

GSK Vaccines GmbH

Marburg, Germany, U.S. License 1617

Distributed by **GlaxoSmithKline**

Research Triangle Park, NC 27709

KNX:15PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use Quadracel safely and effectively. See full prescribing information for Quadracel.

Quadracel (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine) Suspension for Intramuscular Injection
Initial U.S. Approval: 2015

----------RECENT MAJOR CHANGES ----------

Indications and Usage (1)	xx/202x
Warnings and Precautions (5.6)	xx/202x

----------INDICATIONS AND USAGE----------

Quadracel is a vaccine indicated for active immunization against diphtheria, tetanus, pertussis and poliomyelitis. A single dose of Quadracel is approved as a fifth dose in the diphtheria, tetanus, pertussis (DTaP) vaccination series, and as a fourth or fifth dose in the inactivated poliovirus (IPV) vaccination series in children 4 through 6 years of age whose previous DTaP vaccine doses have been with Pentacel, DAPTACEL and/or VAXELIS. (1)

----------DOSAGE AND ADMINISTRATION----------

A single intramuscular injection of 0.5 mL. (2)

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection, supplied in single dose (0.5 mL) vials. (3)

----------CONTRAINDICATIONS----------

- Severe allergic reaction (e.g., anaphylaxis) to any ingredient of Quadracel, or following any diphtheria toxoid, tetanus toxoid, pertussis-containing vaccine or inactivated poliovirus vaccine. (4.1) (11)
- Encephalopathy within 7 days of a previous pertussis-containing vaccine with no other identifiable cause. (4.2)
- Progressive neurologic disorder until a treatment regimen has been established and the condition has stabilized. (4.3)

----------WARNINGS AND PRECAUTIONS----------

- Carefully consider benefits and risks before administering Quadracel to persons with a history of:
  - fever ≥40.5°C (≥105°F), hypotonic-hyporesponsive episode (HHE) or persistent, inconsolable crying lasting ≥3 hours within 48 hours after a previous pertussis-containing vaccine. (5.2)
  - seizures within 3 days after a previous pertussis-containing vaccine. (5.2)
- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including Quadracel, should be based on careful consideration of the potential benefits and possible risks. (5.3)

----------ADVERSE REACTIONS----------

In a clinical study, the most common solicited injection site reactions were pain (>75%), increase in arm circumference (>65%), erythema (>55%), and swelling (>40%). Common solicited systemic reactions were myalgia (>50%), malaise (>35%), and headache (>15%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pharmacovigilance Department, Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or http://vaers.hhs.gov

See 17 for PATIENT COUNSELING INFORMATION.

Revised: xx/202x

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Quadracel® is a vaccine indicated for active immunization against diphtheria, tetanus, pertussis and poliomyelitis. A single dose of Quadracel is approved for use as a fifth dose in the diphtheria, tetanus, pertussis (DTaP) vaccination series, and as a fourth or fifth dose in the inactivated poliovirus (IPV) vaccination series in children 4 through 6 years of age whose previous DTaP vaccine doses have been with Pentacel® [Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed, Inactivated Poliovirus and Haemophilus b conjugate (Tetanus Toxoid Conjugate) Vaccine], DAPTACEL® (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) and/or VAXELIS (Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Haemophilus b Conjugate and Hepatitis B Vaccine).

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only

Just before use, shake the vial well, until a uniform, white, cloudy suspension results. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exist, the product should not be administered.

Withdraw and administer a 0.5 mL dose of Quadracel vaccine intramuscularly into the deltoid muscle of the upper arm. Discard unused portion.

Quadracel should not be combined through reconstitution or mixed with any other vaccine.

3 DOSAGE FORMS AND STRENGTHS

Quadracel is a suspension for injection in 0.5 mL single-dose vials.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Severe allergic reaction (e.g., anaphylaxis) to any ingredient of Quadracel [see *Description (11)*] or following any diphtheria toxoid, tetanus toxoid, pertussis-containing vaccine, or inactivated poliovirus vaccine, is a contraindication to administration of Quadracel.

4.2 Encephalopathy

Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of a previous dose of a pertussis-containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including Quadracel.

4.3 Progressive Neurologic Disorder

Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy is a contraindication to administration of any pertussis-containing vaccine including Quadracel. Pertussis vaccine should not be administered to individuals with such conditions until a treatment regimen has been established and the condition has stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

5.2 Adverse Reactions Following Prior Pertussis Vaccination

If any of the following events have occurred within the specified period after administration of a pertussis vaccine, the decision to administer Quadracel should be based on careful consideration of benefits and risks.

- Temperature of ≥40.5°C (≥105°F) within 48 hours, not attributable to another identifiable cause.
- Collapse or shock-like state (hypotonic-hyporesponsive episode [HHE]) within 48 hours.
- Persistent, inconsolable crying lasting ≥3 hours within 48 hours.
- Seizures with or without fever within 3 days.

5.3 Guillain-Barré Syndrome

If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any vaccine containing tetanus toxoid, including Quadracel, should be based on careful consideration of the potential benefits and possible risks.

5.4 Limitations of Vaccine Effectiveness

Vaccination with Quadracel may not protect all individuals.

5.5 Altered Immunocompetence

If Quadracel is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained. [See *Drug Interactions (7.2)*.]

5.6 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines including Quadracel. Procedures should be in place to avoid injury from fainting.

6 ADVERSE REACTIONS

In a clinical study, the most common solicited injection site reactions were pain (>75%), increase in arm circumference (>65%), erythema (>55%), and swelling (>40%). Common solicited systemic reactions were myalgia (>50%), malaise (>35%), and headache (>15%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events.

In a randomized, controlled, multicenter study conducted in the US and Puerto Rico (Study M5I02; ClinicalTrials.gov Identifier: NCT01346293), 3,372 children, 4 to 6 years of age, who had received 4 doses of DAPTACEL and/or Pentacel vaccine(s) received Quadracel, or DAPTACEL + IPOL (Poliovirus Vaccine Inactivated) vaccines administered concomitantly but at separate sites. Subjects also received Measles, Mumps, and Rubella Virus Vaccine Live (MMR) (Merck & Co., Inc.) and Varicella Virus Vaccine Live (Varicella vaccine) (Merck & Co., Inc.) administered concomitantly at separate sites. Safety was evaluated in 2,733 subjects who received Quadracel and 621 subjects who received DAPTACEL + IPOL vaccines.

Among these subjects, 51.5% were male, 48.5% were female, 75.7% were Caucasian, 8.6% were Black, 7.9% were Hispanic, 0.9% were Asian, and 7.8% were of other racial/ethnic groups. The mean age for both groups was 4.4 years and the ratio of male to female subjects and ethnicity were balanced between both groups.

Solicited injection site reactions and systemic reactions were collected daily for 7 days following vaccination, via diary cards. Participants were monitored for unsolicited adverse events for 28 days and serious adverse events (SAEs) for 6 months after vaccination.

Solicited Adverse Reactions

The incidence and severity of solicited injection site and systemic adverse reactions that occurred within 7 days after vaccination in each study group are shown in Table 1.

Table 1: Percentage of Children 4 through 6 years of Age with Solicited Adverse Reactions by Intensity Within 7 Days of Vaccination with Quadracel or Concomitant but Separate DAPTACEL and IPOL vaccines Co-Administered with MMR and Varicella Vaccines*

	Quadracel (N†= 2,500-2,689)	**DAPTACEL + IPOL (N† = 598-603)**
Injection Site Reactions	Quadracel site	DAPTACEL or IPOL site
Pain,Any	77.4	76.5
Pain‡, Grade 1	56.4	54.9
Pain‡, Grade 2	19.0	18.6
Pain‡, Grade 3	2.0	3.0
Change in limb circumference§, Any	68.1	65.1
Change in limb circumference§, Grade 1	59.8	58.6
Change in limb circumference§, Grade 2	8.2	6.5
Change in limb circumference§, Grade 3	0.2	0.0
Erythema, Any	59.1	53.4
Erythema, >0 to <25 mm	31.6	31.8
Erythema, ≥25 to <50 mm	9.5	9.6
Erythema, ≥50 mm	18.0	11.9
Swelling, Any	40.2	36.4
Swelling, >0 to <25 mm	23.5	23.1
Swelling, ≥25 to <50 mm	8.1	6.1
Swelling, ≥50 mm	8.6	7.1
Extensive limb swelling, Any	1.5	1.3
Systemic Reactions		
Myalgia#, Any	53.8	52.6
Myalgia#, Grade 1	36.0	33.5
Myalgia#, Grade 2	15.8	16.3
Myalgia#, Grade 3	1.9	2.8
Malaise#, Any	35.0	33.2
Malaise#, Grade 1	21.7	18.7
Malaise#, Grade 2	10.6	11.1
Malaise#, Grade 3	2.6	3.3
Headache#, Any	15.6	16.6
Headache#, Grade 1	11.9	11.9
Headache#, Grade 2	3.1	4.0
Headache#, Grade 3	0.6	0.7
Fever, Any	6.0	6.9

	Quadracel (N†= 2,500-2,689)	DAPTACEL + IPOL (N† = 598-603)
Fever ≥38.0°C to ≤38.4°C	2.6	3.0
Fever, ≥38.5°C to ≤38.9°C	2.1	1.8
Fever, ≥39.0°C	1.3	2.0

***** ClinicalTrials.gov Identifier: NCT01346293.
† N = The number of subjects with available data.
‡ Grade 1: Easily tolerated, Grade 2: Sufficiently discomforting to interfere with normal behavior or activities, Grade 3: Incapacitating, unable to perform usual activities.
§ Grade 1: >0 to <25 mm increase over pre-vaccination measurement, Grade 2: ≥25 to ≤50 mm increase over pre-vaccination measurement, Grade 3: >50 mm increase over pre-vaccination measurement.
¶ Swelling of the injected limb including the adjacent joint (i.e., elbow and/or shoulder) as compared to baseline.
# Grade 1: No interference with activity, Grade 2: Some interference with activity, Grade 3: Significant; prevents daily activity.

Serious Adverse Events

In Study M5I02, within 28 days following vaccination with Quadracel, or DAPTACEL + IPOL vaccines, and concomitant MMR and varicella vaccines, 0.1% of subjects (3/2,733) in the Quadracel group experienced a serious adverse event. During the same time period, 0.2% subjects (1/621) in the DAPTACEL + IPOL group experienced a SAE. Within the 6-month follow-up period after vaccination, SAEs were reported in 0.8% of subjects (21/2,733) who received Quadracel and 0.5% of subjects (3/621) who received DAPTACEL + IPOL vaccines, none of which were assessed as related to vaccination.

6.2 Postmarketing Experience

The following adverse events have been spontaneously reported, during the post-marketing use of Quadracel outside the US, in infants and children from 2 months through 6 years of age. Because these events are reported voluntarily from a population of uncertain size, it is not possible to estimate their frequency reliably or establish a causal relationship to vaccine exposure. This list includes adverse events based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Quadracel.

- ***Immune system disorders***
  Anaphylactic reaction, hypersensitivity and allergic reactions (such as rash, urticaria, dyspnea)
- ***Psychiatric disorders***
  Screaming
- ***Nervous system disorders***
  Somnolence, convulsion, febrile convulsion, HHE, hypotonia
- ***Cardiac disorders***
  Cyanosis
- ***Vascular disorders***
  Pallor

- ***General disorders and administration site conditions***
  Listlessness
  Injection site reactions (including inflammation, mass, sterile abscess, and edema)
  Large injection site reactions (>50 mm), including limb swelling which may extend from the injection site beyond one or both joints
- ***Infections and Infestations***
  Injection site cellulitis, injection site abscess

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

In the US clinical trial, Study M5I02, Quadracel was administered concomitantly with one or more of the following US-licensed vaccines: MMR vaccine and varicella vaccine. [See *Adverse Reactions (6.1)*.]

When Quadracel is given at the same time as another injectable vaccine(s), the vaccines should be administered with different syringes and at different injection sites.

7.2 Immunosuppressive Treatments

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to Quadracel. [See *Warnings and Precautions (5.5)*.]

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

The safety and effectiveness of Quadracel has not been established in children less than 4 years of age or children 7 through 16 years of age and is not approved for use in these age groups.

11 DESCRIPTION

Quadracel (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine) is a sterile suspension for intramuscular injection.

Each 0.5 mL dose is formulated to contain 15 Lf diphtheria toxoid, 5 Lf tetanus toxoid, acellular pertussis antigens [20 mcg detoxified pertussis toxin (PT), 20 mcg filamentous hemagglutinin (FHA), 3 mcg pertactin (PRN), 5 mcg fimbriae types 2 and 3 (FIM)], and inactivated polioviruses [40 D-antigen units (DU) Type 1 (Mahoney), 8 DU Type 2 (MEF-1), 32 DU Type 3 (Saukett)].

Corynebacterium diphtheriae is grown in modified Mueller's growth medium. (1) After purification by ammonium sulfate fractionation, the diphtheria toxin is detoxified with formaldehyde and diafiltered.

Clostridium tetani is grown in modified Mueller-Miller casamino acid medium without beef heart infusion. (2) Tetanus toxin is detoxified with formaldehyde and purified by

ammonium sulfate fractionation and diafiltration. Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

The acellular pertussis vaccine antigens are produced from Bordetella pertussis cultures grown in Stainer-Scholte medium (3) modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. FIM are extracted and copurified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT is detoxified with glutaraldehyde. FHA is treated with formaldehyde and the residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate.

Poliovirus Type 1, Type 2 and Type 3 are each grown in separate cultures of MRC-5 cells, a line of normal human diploid cells, by the microcarrier method. (4) (5) The cells are grown in CMRL (Connaught Medical Research Laboratories) 1969 medium, supplemented with calf serum. For viral growth, the culture medium is replaced by Medium 199, without calf serum. After clarification and filtration, the viral suspensions are concentrated by ultrafiltration, and purified by liquid chromatography steps. The monovalent viral suspensions are inactivated with formaldehyde. Monovalent concentrates of each inactivated poliovirus are combined to produce a trivalent poliovirus concentrate.

The adsorbed diphtheria, tetanus and acellular pertussis antigens are combined with aluminum phosphate, 2-phenoxyethanol (not as a preservative) and water for injection, into an intermediate concentrate. The trivalent poliovirus concentrate is added and the vaccine is diluted to its final concentration.

Each 0.5 mL dose contains 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, polysorbate 80 (approximately 10 ppm by calculation), <2 mcg residual formaldehyde, <50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative), <4 pg of neomycin and <4 pg polymyxin B sulfate.

Quadracel does not contain a preservative.

Both diphtheria and tetanus toxoids induce at least 2 neutralizing units per mL in the guinea pig potency test. The potency of the acellular pertussis antigens is evaluated by the antibody response of immunized mice to detoxified PT, FHA, PRN and FIM as measured by enzyme-linked immunosorbent assay (ELISA). The potency of the inactivated poliovirus antigens is determined by measuring antibody-mediated neutralization of poliovirus in sera from immunized rats.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the

lowest level giving some degree of protection. Antitoxin levels of at least 0.1 IU/mL are generally regarded as protective. (6) Levels of 1.0 IU/mL have been associated with long-term protection. (7)

Tetanus

Tetanus is an acute disease caused by an extremely potent neurotoxin produced by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assay, is considered the minimum protective level. (6) (8). A tetanus antitoxoid level ≥0.1 IU/mL as measured by the ELISA used in clinical studies of Quadracel is considered protective.

Pertussis

Pertussis (whooping cough) is a respiratory disease caused by *B. pertussis*. This Gram-negative coccobacillus produces a variety of biologically active components, though their role in either the pathogenesis of, or immunity to, pertussis has not been clearly defined.

There is no well-established serological correlate of protection for pertussis. Because DAPTACEL contains the same pertussis antigens manufactured by the same process as those in Quadracel, the effectiveness of Quadracel against pertussis was based on a comparison of pertussis immune responses following Quadracel to those following DAPTACEL (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed). [See *Clinical Studies (14)*]. The efficacy of the pertussis component of DAPTACEL was determined in clinical trials of DAPTACEL administered to infants (see DAPTACEL prescribing information). Quadracel contains twice as much detoxified PT and four times as much FHA as DAPTACEL. Quadracel contains the same quantity of the same pertussis antigens manufactured by the same process as those in Pentacel and VAXELIS.

Poliomyelitis

Polioviruses, of which there are three serotypes (Types 1, 2, and 3), are enteroviruses. The presence of poliovirus type-specific neutralizing antibodies has been correlated with protection against poliomyelitis. (9)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Quadracel has not been evaluated for carcinogenic or mutagenic potential or impairment of fertility.

14 CLINICAL STUDIES

14.1 Immunogenicity

In Study M5I02, children 4 through 6 years of age received Quadracel or DAPTACEL + IPOL as the fifth dose in the diphtheria, tetanus, and pertussis vaccination series and the fourth or fifth dose in the inactivated poliovirus vaccination series. Subjects also

received their second dose of MMR and Varicella vaccines, concomitantly. The immunogenicity subset comprised 263 subjects in the Quadracel group and 253 subjects in the DAPTACEL + IPOL vaccines group. [See study description in *Adverse Reactions (6.1)*].

Antibody levels to diphtheria, tetanus, pertussis (PT, FHA, PRN and FIM) and poliovirus antigens were measured in sera obtained immediately prior to vaccination and 28 days after vaccination. The co-primary endpoints were booster response rates and antibody geometric mean concentrations/titers (GMCs/GMTs) to diphtheria, tetanus, pertussis and poliovirus antigens elicited after vaccination. Booster response rates and antibody GMCs/GMTs following Quadracel vaccination were compared to those after DAPTACEL + IPOL vaccination.

Quadracel was non-inferior to DAPTACEL + IPOL vaccines administered concomitantly at separate sites, as demonstrated by comparison of the post-vaccination antibody booster response rates and GMCs/GMTs to diphtheria and tetanus (Table 2), to all pertussis antigens (Table 3) and to poliovirus 1, 2 and 3 (Table 4).

Table 2: Booster Response Rates, Pre- and Post-Vaccination Seroprotection Rates and Post-Vaccination Antibody Levels to Diphtheria and Tetanus Antigens Following Quadracel or Concomitant but Separate DAPTACEL and IPOL Vaccines Co-Administered with MMR and Varicella Vaccines*

	Quadracel (N† =253-262)	**DAPTACEL + IPOL (N† =248-253)**
Anti-Diphtheria		
% Booster Response‡	97.3§	99.2
Pre-vaccination % ≥0.1 IU/mL¶	90.7	83.1
Post-vaccination % ≥0.1 IU/mL¶	100.0	99.6
Post-vaccination % ≥1.0 IU/mL¶	99.6	99.6
Post-vaccination GMC (IU/mL)	18.6#	15.5
Anti-Tetanus		
% Booster Response‡	84.2§	84.3
Pre-vaccination % ≥0.1 IU/mL¶	91.7	89.1
Post-vaccination % ≥0.1 IU/mL¶	100.0	99.2
Post-vaccination % ≥1.0 IU/mL¶	98.9	96.8
Post-vaccination GMC (IU/mL)	6.4#	5.5

* ClinicalTrials.gov Identifier: NCT01346293.

† N = The number of subjects with available data.

‡ Booster response: In subjects with pre-vaccination antibody concentrations <0.1 IU/mL, a post-vaccination level ≥0.4 IU/mL; in subjects with pre-vaccination antibody concentrations ≥0.1 IU/mL but <2.0 IU/mL, a 4-fold rise in post-vaccination level; in subjects with pre-vaccination antibody level ≥2.0 IU/mL, a 2-fold rise in post-vaccination level.

§ Quadracel was non-inferior to DAPTACEL + IPOL based on the post-vaccination booster response rates for diphtheria and tetanus (lower limits of the 2-sided 95% CIs of the difference [Quadracel minus DAPTACEL + IPOL] were >-10%).

¶ Seroprotection: anti-diphtheria and anti-tetanus antibody concentrations ≥0.1 IU/mL and ≥1.0 IU/mL.

[#] Quadracel was non-inferior to DAPTACEL + IPOL based on the post-vaccination GMCs for diphtheria and tetanus (lower limits of the 2-sided 95% CIs of the ratio [Quadracel / DAPTACEL + IPOL] were >2/3).

Table 3: Booster Response Rates and Post-vaccination Antibody Levels to Pertussis Antigens Following Quadracel or Concomitant but Separate DAPTACEL and IPOL Vaccines Co-Administered with MMR and Varicella Vaccines*

	Quadracel (N† =250-255)	DAPTACEL + IPOL (N† =247-249)
Anti-PT		
% Booster Response‡	95.2§	89.9
Post-vaccination GMC (EU/mL)	120.7¶	61.3
Anti-FHA		
% Booster Response‡	94.9§	87.5
Post-vaccination GMC (EU/mL)	123.5¶	79.0
Anti-PRN		
% Booster Response‡	96.9§	93.1
Post-vaccination GMC (EU/mL)	282.6¶	187.5
Anti-FIM		
% Booster Response‡	97.2§	92.4
Post-vaccination GMC (EU/mL)	505.8¶	378.9

* ClinicalTrials.gov Identifier: NCT01346293.
† N = The number of subjects with available data.
‡ Booster response: In subjects with pre-vaccination antibody concentrations <LLOQ, a post-vaccination level ≥4xLLOQ; in subjects with pre-vaccination antibody concentrations ≥LLOQ but <4xLLOQ, a 4-fold rise in post-vaccination level; in subjects with pre-vaccination antibody level ≥4xLLOQ, a 2-fold rise in post-vaccination level.
§ Quadracel was non-inferior to DAPTACEL + IPOL based on the post-vaccination booster response rates for all pertussis antigens (lower limits of the 2-sided 95% CIs of the difference [Quadracel minus DAPTACEL + IPOL] were > -10%).
¶ Quadracel was non-inferior to DAPTACEL + IPOL based on the post-vaccination GMCs for all pertussis antigens (lower limits of the 2-sided 95% CIs of the ratio [DTaP-IPV / DAPTACEL + IPOL] were >2/3).

Table 4: Booster Response Rates, Pre- and Post-Vaccination Seroprotection Rates and Post-vaccination Antibody Levels to Poliovirus Antigens Following Quadracel or Concomitant but Separate DAPTACEL and IPOL Vaccines Co-Administered with MMR and Varicella Vaccines*

	Quadracel (N† =247-258)	DAPTACEL + IPOL (N† =248-253)
Anti-Poliovirus 1		
% Booster Response‡	85.9§	82.3
Pre-vaccination % ≥1:8 dilution	98.4	98.8
Post-vaccination % ≥1:8 dilution	100.0	99.6
Post-vaccination GMT	3,477¶	2,731
Anti-Poliovirus 2		
% Booster Response‡	78.3§	79.0
Pre-vaccination % ≥1:8 dilution	99.6	99.6
Post-vaccination % ≥1:8 dilution	100.0	100.0
Post-vaccination GMT	3,491¶	3,894
Anti-Poliovirus 3		
% Booster Response‡	85.0§	84.7
Pre-vaccination % ≥1:8 dilution	96.8	93.1
Post-vaccination % ≥1:8 dilution	100.0	100.0
Post-vaccination GMT	4,591¶	3,419

* ClinicalTrials.gov Identifier: NCT01346293.
† N = The number of subjects with available data.
‡ Booster response: In subjects with pre-vaccination antibody concentrations <1:8 dilution, post-vaccination levels ≥1:8 dil; in subjects with pre-vaccination antibody concentrations ≥1:8 dilution, a 4-fold rise in post-vaccination antibody levels.
§ Quadracel was non-inferior to DAPTACEL + IPOL based on the post-vaccination booster response rates for polio types 1, 2 and 3 (lower limits of the 2-sided 95% CIs of the difference [Quadracel minus DAPTACEL + IPOL] were > -10%).
¶ Quadracel was non-inferior to DAPTACEL + IPOL based on the post-vaccination GMTs for polio types 1, 2 and 3 (lower limits of the 2-sided 95% CIs of the ratio [Quadracel / DAPTACEL + IPOL] were >2/3).

15 REFERENCES

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4 van Wezel AL, et al. Inactivated poliovirus vaccine: current production methods and new developments. Rev Infect Dis 1984;6 (Suppl 2):S335-40.

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16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

The vial stopper for this product is not made with natural latex rubber.

Quadracel is supplied in a single-dose vial (NDC No. 49281-562-58) in packages of 10 vials (NDC No. 49281-562-10).

16.2 Storage and Handling

Quadracel should be stored at 2° to 8°C (35° to 46°F). **Do not freeze**. Product which has been exposed to freezing should not be used. Do not use after expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

Inform the parent or guardian of the following:

- The potential benefits and risks of immunization with Quadracel.
- The common adverse reactions that have occurred following administration of Quadracel or other vaccines containing similar components.
- Other adverse reactions can occur. Call healthcare provider with any adverse reactions of concern.

Provide the Vaccine Information Statements (VIS), which are required by the National Childhood Vaccine Injury Act of 1986.

Manufactured by:

Sanofi Pasteur Limited
Toronto Ontario Canada

Distributed by:

Sanofi Pasteur Inc.

Swiftwater PA 18370 USA

R6-xx22 USA

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use Pentacel safely and effectively. See full prescribing information for Pentacel.

Pentacel (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine
Suspension for Intramuscular Injection
Initial U.S. Approval: 2008

----------RECENT MAJOR CHANGES ----------

Dosage and Administration (2.1)	xx/202x
Warnings and Precautions (5.8)	xx/202x

----------INDICATIONS AND USAGE----------

- Pentacel is a vaccine indicated for active immunization against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease due to *Haemophilus influenzae* type b. Pentacel is approved for use as a four dose series in children 6 weeks through 4 years of age (prior to 5th birthday). (1)

----------DOSAGE AND ADMINISTRATION----------

- The four dose immunization series consists of a 0.5 mL intramuscular injection, after reconstitution, administered at 2, 4, 6 and 15-18 months of age. (2.1)
- Pentacel consists of a liquid vaccine component (DTaP-IPV component) and a lyophilized vaccine component (ActHIB vaccine). Reconstitute the ActHIB vaccine component with the DTaP-IPV component immediately before administration. (2.2)

----------DOSAGE FORMS AND STRENGTHS----------

- Suspension for injection (0.5 mL dose) supplied as a liquid vaccine component that is combined through reconstitution with a lyophilized vaccine component, both in single-dose vials. (3)

----------CONTRAINDICATIONS----------

- Severe allergic reaction (eg, anaphylaxis) after a previous dose of Pentacel, any ingredient of Pentacel, or any other diphtheria toxoid, tetanus toxoid, pertussis-containing vaccine, inactivated poliovirus vaccine or *H. influenzae* type b vaccine. (4.1)
- Encephalopathy within 7 days of a previous pertussis-containing vaccine with no other identifiable cause. (4.2)
- Progressive neurologic disorder until a treatment regimen has been established and the condition has stabilized. (4.3)

----------WARNINGS AND PRECAUTIONS----------

- Carefully consider benefits and risks before administering Pentacel to persons with a history of:
  - fever ≥40.5°C (≥105°F), hypotonic-hyporesponsive episode (HHE) or persistent, inconsolable crying lasting ≥3 hours within 48 hours after a previous pertussis-containing vaccine. (5.2)
  - seizures within 3 days after a previous pertussis-containing vaccine. (5.2)
- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following Pentacel. (5.3)
- For infants and children with a history of previous seizures, an antipyretic may be administered (in the dosage recommended in its prescribing information) at the time of vaccination with Pentacel and for the next 24 hours. (5.4)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including Pentacel, to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.7)

----------ADVERSE REACTIONS----------

- Rates of adverse reactions varied by dose number. Systemic reactions that occurred in >50% of participants following any dose included fussiness/irritability and inconsolable crying. Fever ≥38.0°C occurred in 6-16% of participants, depending on dose number. Injection site reactions that occurred in >30% of participants following any dose included tenderness and increase in arm circumference. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pharmacovigilance Department, Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 and http://vaers.hhs.gov.

----------DRUG INTERACTIONS----------

- Do not mix Pentacel or any of its components with any other vaccine or diluent. (7.1)
- Immunosuppressive therapies may reduce the immune response to Pentacel. (7.2)
- Urine antigen detection may not have definitive diagnostic value in suspected *H. influenzae* type b disease within one week following Pentacel. (7.3)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: xx/202x

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Pentacel® is a vaccine indicated for active immunization against diphtheria, tetanus, pertussis, poliomyelitis and invasive disease due to *Haemophilus influenzae* type b. Pentacel is approved for use as a four dose series in children 6 weeks through 4 years of age (prior to fifth birthday).

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only

2.1 Immunization Series

Pentacel is to be administered as a 4-dose series at 2, 4, 6 and 15-18 months of age. The first dose may be given as early as 6 weeks of age. Four doses of Pentacel constitute a primary immunization course against pertussis. Three doses of Pentacel constitute a primary immunization course against diphtheria, tetanus, *H. influenzae* type b invasive disease, and poliomyelitis; the fourth dose is a booster for diphtheria, tetanus, *H. influenzae* type b invasive disease, and poliomyelitis immunizations. [See *14 Clinical Studies (14.1, 14.2, 14.3, 14.4, 14.5).]*

Mixed Sequences of Pentacel and other DTaP-containing Vaccines

Pentacel, DAPTACEL (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed), Quadracel (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine), and VAXELIS (Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Haemophilus b Conjugate and Hepatitis B Vaccine) contain the same pertussis antigens manufactured by the same process. The amount of each of the pertussis antigens is the same in Pentacel, Quadracel, and VAXELIS. Pentacel contains twice the amount of detoxified pertussis toxin (PT) and four times the amount of filamentous hemagglutinin (FHA) as DAPTACEL.

Pentacel may be used as the fourth dose in the 5-dose DTaP series in children who have received a 3-dose series of VAXELIS [See *CLINICAL STUDIES (14)].*

Pentacel may be used to complete the first 4 doses of the 5-dose DTaP series in infants and children who have received 1 or more doses of DAPTACEL and are also scheduled to receive the other antigens of Pentacel.

Children who have completed a 4-dose series with Pentacel should receive a fifth dose of DTaP vaccine using DAPTACEL or Quadracel at 4-6 years of age. (1) (2)

Data are not available on the safety and effectiveness of using mixed sequences of Pentacel and DTaP vaccine from different manufacturers.

Mixed Sequences of Pentacel and IPV Vaccine

Pentacel may be used in infants and children who have received 1 or more doses of another licensed IPV vaccine and are scheduled to receive the antigens of Pentacel. However, data are not available on the safety and immunogenicity of Pentacel in such infants and children.

The Advisory Committee on Immunization Practices (ACIP) recommends that the final dose in the 4-dose IPV series be administered at age ≥4 years. (3) When Pentacel is administered at ages 2, 4, 6, and 15-18 months, an additional booster dose of IPV vaccine should be administered at age 4-6 years, resulting in a 5-dose IPV series. (3)

Mixed Sequences of Pentacel and Haemophilus b Conjugate Vaccine

Pentacel may be used to complete the vaccination series in infants and children previously vaccinated with one or more doses of Haemophilus b Conjugate Vaccine (either separately administered or as part of another combination vaccine), who are also scheduled to receive the other antigens of Pentacel. However, data are not available on the safety and immunogenicity of Pentacel in such infants and children. If different brands of Haemophilus b Conjugate Vaccines are administered to complete the series, three primary immunizing doses are needed, followed by a booster dose.

2.2 Administration

The package contains a vial of the DTaP-IPV component and a vial of lyophilized ActHIB vaccine component.

Before use, thoroughly but gently shake the vial of DTaP-IPV component, withdraw the entire liquid content and inject into the vial of the lyophilized ActHIB vaccine component. Gently swirl the vial now containing Pentacel until a cloudy, uniform, white to off-white (yellow tinge) suspension results.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If these conditions exist, Pentacel should not be administered.

Withdraw and administer a single 0.5 mL dose of Pentacel intramuscularly. Pentacel should be used immediately after reconstitution. Discard unused portion. Refer to Figures 1, 2, 3, 4 and 5.

Pentacel: Instructions for Reconstitution of ActHIB Vaccine Component with DTaP-IPV Component

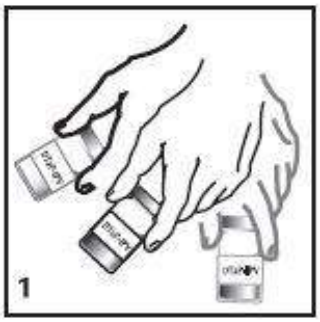

Figure 1
Gently shake the vial of DTaP-IPV component.

Figure 2
Withdraw the entire liquid content.

Figure 3
Insert the syringe needle through the stopper of the vial of lyophilized ActHIB vaccine component and inject the liquid into the vial.

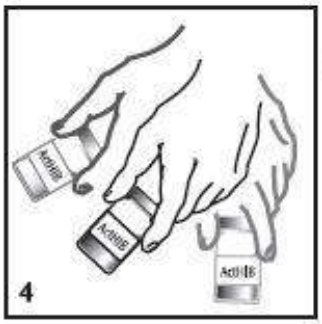

Figure 4
Swirl vial gently.

Figure 5
After reconstitution, immediately withdraw 0.5 mL of Pentacel vaccine and administer intramuscularly. Pentacel vaccine should be used immediately after reconstitution.

In infants younger than 1 year, the anterolateral aspect of the thigh provides the largest muscle and is the preferred site of injection. In older children, the deltoid muscle is usually large enough for injection. The vaccine should not be injected into the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously or subcutaneously.

Pentacel should not be mixed in the same syringe with other parenteral products.

3 DOSAGE FORMS AND STRENGTHS

Pentacel is a suspension for injection (0.5 mL dose) supplied as a liquid vaccine component that is combined through reconstitution with a lyophilized vaccine component,

both in single-dose vials. [See *Dosage and Administration (2.2)* and *How Supplied/Storage and Handling (16)*.]

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A severe allergic reaction (eg, anaphylaxis) after a previous dose of Pentacel or any other diphtheria toxoid, tetanus toxoid, or pertussis-containing vaccine, inactivated poliovirus vaccine or *H. influenzae* type b vaccine, or any ingredient of this vaccine is a contraindication to administration of Pentacel. [See *Description (11).*]

4.2 Encephalopathy

Encephalopathy (eg, coma, decreased level of consciousness, prolonged seizures) within 7 days of a previous dose of a pertussis containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including Pentacel.

4.3 Progressive Neurologic Disorder

Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy is a contraindication to administration of any pertussis-containing vaccine including Pentacel. Pertussis vaccine should not be administered to individuals with such conditions until a treatment regimen has been established and the condition has stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

5.2 Adverse Reactions Following Prior Pertussis Vaccination

If any of the following events occur within the specified period after administration of a pertussis vaccine, the decision to administer Pentacel should be based on careful consideration of potential benefits and possible risks.

- Temperature of ≥40.5°C (≥105°F) within 48 hours, not attributable to another identifiable cause.
- Collapse or shock-like state (hypotonic-hyporesponsive episode (HHE)) within 48 hours.
- Persistent, inconsolable crying lasting ≥3 hours within 48 hours.
- Seizures with or without fever within 3 days.

5.3 Guillain-Barré Syndrome and Brachial Neuritis

A review by the Institute of Medicine (IOM) found evidence for a causal relation between tetanus toxoid and both brachial neuritis and Guillain-Barré syndrome. (4) If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following Pentacel.

5.4 Infants and Children with a History of Previous Seizures

For infants or children with a history of previous seizures, an appropriate antipyretic may be administered (in the dosage recommended in its prescribing information) at the time of vaccination with a vaccine containing acellular pertussis antigens (including Pentacel) and for the following 24 hours, to reduce the possibility of post-vaccination fever.

5.5 Limitations of Vaccine Effectiveness

Vaccination with Pentacel may not protect all individuals.

5.6 Altered Immunocompetence

If Pentacel is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained. [See *Drug Interactions (7.2)*.]

5.7 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including Pentacel, to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

5.8 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines including Pentacel. Procedures should be in place to avoid injury from fainting.

6 ADVERSE REACTIONS

Rates of adverse reactions varied by dose number. The most frequent (>50% of participants) systemic reactions following any dose were fussiness/irritability and inconsolable crying. The most frequent (>30% of participants) injection site reactions following any dose were tenderness and increased circumference of the injected arm.

6.1 Data from Clinical Studies

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events.

The safety of Pentacel was evaluated in four clinical studies in which a total of 5,980 participants received at least one dose of Pentacel. In three of the studies, conducted in the US, a total of 4,198 participants were enrolled to receive four consecutive doses of Pentacel. In the fourth study, conducted in Canada, 1,782 participants previously vaccinated with three doses of Pentacel received a fourth dose. The vaccination schedules of Pentacel, Control vaccines, and concomitantly administered vaccines used in these studies are provided in Table 1.

Across the four studies, 50.8% of participants were female. Among participants in the three US studies, 64.5% were Caucasian, 9.2% were Black, 12.9% were Hispanic, 3.9% were Asian, and 9.5% were of other racial/ethnic groups. In the two controlled studies, the racial/ethnic distribution of participants who received Pentacel and Control vaccines was similar. In the Canadian fourth dose study, 86.0% of participants were Caucasian, 1.9% were Black, 0.8% were Hispanic, 4.3% were Asian, 2.0% were East Indian, 0.5% were Native Indian, and 4.5% were of other racial/ethnic groups.

Table 1: Clinical Safety Studies of Pentacel: Vaccination Schedules

Study	Pentacel	Control Vaccines	Concomitantly Administered Vaccines
494-01	2, 4, 6 and 15 months	HCPDT + POLIOVAX + ActHIB at 2, 4, 6, and 15 months	7-valent pneumococcal conjugate vaccine* (PCV7) at 2, 4, and 6 months in a subset of participants† Hepatitis B vaccine at 2 and 6 months‡
P3T06	2, 4, 6, and 15-16 months	DAPTACEL + IPOL + ActHIB at 2, 4, and 6 months; and DAPTACEL + ActHIB at 15-16 months	PCV7* at 2, 4, and 6 months Hepatitis B vaccine at 2 and 6 months‡
494-03	2, 4, 6, and 15-16 months	None	PCV7* at 2, 4, and 6 months in all participants; and at 15 months in a random subset of participants Hepatitis B vaccine at 2 and 6 months (if a dose was previously administered)‡ or at 2, 4, and 6 months (if no previous dose) Measles, mumps, rubella vaccine§ (MMR) and varicella§ vaccine at 12 or 15 months in random subsets of participants
5A9908	15-18 months¶	None	None

HCPDT: non-US licensed DTaP vaccine that is identical to the DTaP component of Pentacel.
POLIOVAX: US licensed Poliovirus Vaccine Inactivated, Sanofi Pasteur Limited.

IPOL: US licensed Poliovirus Vaccine Inactivated, Sanofi Pasteur SA.

* PCV7 manufactured by Wyeth Laboratories.

† PCV7 was introduced after the study was initiated, and thus, administered concomitantly with Pentacel vaccine in a subset of participants.

‡ The first dose of hepatitis B vaccine (manufacturer not specified) was administered prior to study initiation, from birth to 21 days of age. Subsequent doses were with hepatitis B vaccine manufactured by Merck and Co.

§ MMR and varicella vaccines were both manufactured by Merck and Co.

¶ Study participants previously had received three doses of Pentacel vaccine by 8 months of age.

Solicited Adverse Reactions

The incidence and severity of selected solicited injection site and systemic adverse reactions that occurred within 3 days following each dose of Pentacel or Control vaccines in Study P3T06 is shown in Table 2. Information on these reactions was recorded daily by parents or guardians on diary cards. In Table 2, injection site reactions are reported for the Pentacel and DAPTACEL injection sites.

Table 2: Number (Percentage) of Children with Selected Solicited Adverse Reactions by Severity Occurring within 0-3 days of Pentacel or Control Vaccines in Study P3T06

Injection Site Reactions	Pentacel **Dose 1** N=465-467%	Pentacel **Dose 2** N = 451 %	Pentacel **Dose 3** N = 438-440 %	Pentacel **Dose 4** N = 387-396 %	DAPTACEL **Dose 1** N = 1,400-1,404 %	DAPTACEL **Dose 2** N = 1,358-1,359 %	DAPTACEL **Dose 3** N = 1,311-1,312 %	DAPTACEL **Dose 4** N = 376-380 %
Redness >5 mm	7.1	8.4	8.7	17.3	6.2	7.1	9.6	16.4
Redness >25 mm	2.8	1.8	1.8	9.2	1.0	0.6	1.9	7.9
Redness >50 mm	0.6	0.2	0.0	2.3	0.4	0.1	0.0	2.4
Swelling >5 mm	7.5	7.3	5.0	9.7	4.0	4.0	6.5	10.3
Swelling >25 mm	3.0	2.0	1.6	3.8	1.6	0.7	1.1	4.0
Swelling >50 mm	0.9	0.0	0.0	0.8	0.4	0.1	0.1	1.3
Tenderness* Any	47.5	39.2	42.7	56.1	48.8	38.2	40.9	51.1
Tenderness* Moderate or Severe	19.6	10.6	11.6	16.7	20.7	12.2	12.3	15.8
Tenderness* Severe	5.4	1.6	1.4	3.3	4.1	2.3	1.7	2.4
Increase in Arm Circumference >5 mm	-	-	-	33.6	-	-	-	30.6
Increase in Arm Circumference **>**20 mm	-	-	-	4.7	-	-	-	6.9
Increase in Arm Circumference >40 mm	-	-	-	0.5	-	-	-	0.8

Systemic Reactions	**Pentacel Dose 1** **N =** 466-467 %	**Penatcel Dose 2** **N =** 451-452 %	**Pentacel Dose 3** **N =** 435-440 %	**Pentacel Dose 4** **N =** 389-398 %	**DAPTACEL + IPOL + ActHIB Dose 1** **N =** 1,390-1,406 %	**DAPTACEL + IPOL + ActHIB Dose 2** **N =** 1,346-1,360 %	**DAPTACEL + IPOL + ActHIB Dose 3** **N =** 1,301-1,312 %	**DAPTACEL + ActHIB Dose 4** **N =** 379-381 %
Fever†‡ ≥38.0°C	5.8	10.9	16.3	13.4	9.3	16.1	15.8	8.7
Fever†‡ >38.5°C	1.3	2.4	4.4	5.1	1.6	4.3	5.1	3.2
Fever†‡ >39.5°C	0.4	0.0	0.7	0.3	0.1	0.4	0.3	0.8
Decreased Activity/Lethargy§ Any	45.8	32.7	32.5	24.1	51.1	37.4	33.2	24.1
Decreased Activity/Lethargy§ Moderate or Severe	22.9	12.4	12.7	9.8	24.3	15.8	12.7	9.2
Decreased Activity/Lethargy§ Severe	2.1	0.7	0.2	2.5	1.2	1.4	0.6	0.3
Inconsolable Crying Any	59.3	49.8	47.3	35.9	58.5	51.4	47.9	36.2
Inconsolable Crying ≥1 hour	19.7	10.6	13.6	11.8	16.4	16.0	12.2	10.5
Inconsolable Crying >3 hours	1.9	0.9	1.1	2.3	2.2	3.4	1.4	1.8
Fussiness/Irritability Any	76.9	71.2	68.0	53.5	75.8	70.7	67.1	53.8
Fussiness/Irritability ≥1 hour	34.5	27.0	26.4	23.6	33.3	30.5	26.2	19.4
Fussiness/Irritability >3 hours	4.3	4.0	5.0	5.3	5.6	5.5	4.3	4.5

* Any: Mild, Moderate or Severe; Mild: subject whimpers when site is touched; Moderate: subject cries when site is touched; Severe: subject cries when leg or arm is moved.

† Fever is based upon actual temperatures recorded with no adjustments to the measurement route.

‡ Following Doses 1-3 combined, the proportion of temperature measurements that were taken by axillary, rectal or other routes, or not recorded were 46.0%, 53.0%, 1.0%, and 0% respectively, for Pentacel vaccine and 44.8%, 54.0%, 1.0%, and 0.1%, respectively, for DAPTACEL + IPOL + ActHIB. Following Dose 4, the proportion of temperature measurements that were taken by axillary, rectal or other routes, or not recorded were 62.7%, 34.4%, 2.4% and 0.5%, respectively, for Pentacel vaccine, and 61.1%, 36.6%, 1.7% and 0.5%, respectively, for DAPTACEL + ActHIB.

§ Moderate: interferes with or limits usual daily activity; Severe: disabling, not interested in usual daily activity.

Hypotonic Hyporesponsive Episodes

In Study P3T06, the diary cards included questions pertaining to HHEs. In Studies 494-01, 494-03, and 5A9908, a question about the occurrence of fainting or change in mental status was asked during post-vaccination phone calls. Across these 4 studies, no HHEs, as defined in a report of a US Public Health Service workshop (6) were reported among participants who received Pentacel (N = 5,979), separately administered HCPDT + POLIOVAX + ActHIB (N = 1,032) or separately administered DAPTACEL + IPOL + ActHIB (N = 1,455). Hypotonia not fulfilling HHE criteria within 7 days following vaccination was reported in 4 participants after the administration of Pentacel (1 on the same day as the 1st dose; 3 on the same day as the 3rd dose) and in 1 participant after the administration of DAPTACEL + IPOL + ActHIB (4 days following the 1st dose).

Seizures

Across Studies 494-01, 494-03, 5A9908 and P3T06, a total of 8 participants experienced a seizure within 7 days following either Pentacel (4 participants; N = 4,197 for at least one of Doses 1-3; N = 5,033 for Dose 4), separately administered HCPDT + POLIOVAX + ActHIB (3 participants; N = 1,032 for at least one of Doses 1-3, N = 739 for Dose 4), separately administered DAPTACEL + IPOL + ActHIB (1 participant; N = 1,455 for at least one of Doses 1-3), or separately administered DAPTACEL + ActHIB (0 participants; N = 418 for Dose 4). Among the four participants who experienced a seizure within 7 days following Pentacel, one participant in Study 494-01 had an afebrile seizure 6 days after the first dose, one participant in Study 494-01 had a possible seizure the same day as the third dose, and two participants in Study 5A9908 had a febrile seizure 2 and 4 days, respectively, after the fourth dose. Among the four participants who experienced a seizure within 7 days following Control vaccines, one participant had an afebrile seizure the same day as the first dose of DAPTACEL + IPOL + ActHIB, one participant had an afebrile seizure the same day as the second dose of HCPDT + POLIOVAX + ActHIB, and two participants had a febrile seizure 6 and 7 days, respectively, after the fourth dose of HCPDT + POLIOVAX + ActHIB.

Serious Adverse Events

In Study P3T06, within 30 days following any of Doses 1-3 of Pentacel or Control vaccines, 19 of 484 (3.9%) participants who received Pentacel and 50 of 1,455 (3.4%) participants who received DAPTACEL + IPOL + ActHIB experienced a serious adverse event. Within 30 days following Dose 4 of Pentacel or Control vaccines, 5 of 431 (1.2%) participants who received Pentacel and 4 of 418 (1.0%) participants who received DAPTACEL + ActHIB experienced a serious adverse event. In Study 494-01, within 30 days following any of Doses 1-3 of Pentacel or Control vaccines, 23 of 2,506 (0.9%) participants who received Pentacel and 11 of 1,032 (1.1%) participants who received HCPDT + POLIOVAX + ActHIB experienced a serious adverse event. Within 30 days following Dose 4 of Pentacel or Control vaccines, 6 of 1,862 (0.3%) participants who received Pentacel and 2 of 739 (0.3%) participants who received HCPDT + POLIOVAX + ActHIB experienced a serious adverse event.

Across Studies 494-01, 494-03 and P3T06, within 30 days following any of Doses 1-3 of Pentacel or Control vaccines, overall, the most frequently reported serious adverse

events were bronchiolitis, dehydration, pneumonia and gastroenteritis. Across Studies 494-01, 494-03, 5A9908 and P3T06, within 30 days following Dose 4 of Pentacel or Control vaccines, overall, the most frequently reported serious adverse events were dehydration, gastroenteritis, asthma, and pneumonia.

Across Studies 494-01, 494-03, 5A9908 and P3T06, two cases of encephalopathy were reported, both in participants who had received Pentacel (N = 5,979). One case occurred 30 days post-vaccination and was secondary to cardiac arrest following cardiac surgery. One infant who had onset of neurologic symptoms 8 days post-vaccination was subsequently found to have structural cerebral abnormalities and was diagnosed with congenital encephalopathy.

A total of 5 deaths occurred during Studies 494-01, 494-03, 5A9908 and P3T06: 4 in children who had received Pentacel (N = 5,979) and one in a participant who had received DAPTACEL + IPOL + ActHIB (N = 1,455). There were no deaths reported in children who received HCPDT + POLIOVAX + ActHIB (N = 1,032). Causes of death among children who received Pentacel were asphyxia due to suffocation, head trauma, Sudden Infant Death syndrome, and neuroblastoma (8, 23, 52 and 256 days post-vaccination, respectively). One participant with ependymoma died secondary to aspiration 222 days following DAPTACEL + IPOL + ActHIB.

6.2 Postmarketing Experience

The following additional adverse events have been spontaneously reported during the post-marketing use of Pentacel worldwide, since 1997. Between 1997 and 2007, Pentacel was primarily used in Canada. Because these events are reported voluntarily from a population of uncertain size, it may not be possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

The following adverse events were included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Pentacel.

- ***Cardiac disorders***
  Cyanosis
- ***Gastrointestinal disorders***
  Vomiting, diarrhea
- ***General disorders and administration site conditions***
  Injection site reactions (including inflammation, mass, abscess and sterile abscess), extensive swelling of the injected limb (including swelling that involved adjacent joints), vaccination failure/therapeutic response decreased (invasive *H. influenzae* type b disease)
- ***Immune system disorders***
  Anaphylaxis/anaphylactic reaction, hypersensitivity (such as rash and urticaria)
- ***Infections and infestations***
  Meningitis, rhinitis, viral infection
- ***Metabolism and nutrition disorders***

Decreased appetite

- ***Nervous system disorders***
  Somnolence, HHE, depressed level of consciousness
- ***Psychiatric disorders***
  Screaming
- ***Respiratory, thoracic and mediastinal disorders***
  Apnea, cough
- ***Skin and subcutaneous tissue disorders***
  Erythema, skin discoloration
- ***Vascular disorders***
  Pallor

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

In clinical trials, Pentacel was administered concomitantly with one or more of the following US licensed vaccines: hepatitis B vaccine, 7-valent pneumococcal conjugate vaccine, MMR and varicella vaccines. [See *Adverse Reactions (5)* and *Clinical Studies (14)*.] When Pentacel is given at the same time as another injectable vaccine(s), the vaccine(s) should be administered with different syringes and at different injection sites.

7.2 Immunosuppressive Treatments

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to Pentacel. [See *Warnings and Precautions (5.6)*.]

7.3 Drug/Laboratory Test Interactions

Antigenuria has been detected in some instances following receipt of ActHIB. Urine antigen detection may not have definite diagnostic value in suspected *H. influenzae* type b disease within one week following receipt of Pentacel. (6)

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

The safety and effectiveness of Pentacel was established in the age group 6 weeks through 18 months on the basis of clinical studies. [See *Adverse Reactions (6.1)* and *Clinical Studies (14)*.] The safety and effectiveness of Pentacel in the age group 19 months through 4 years is supported by evidence in children 6 weeks through 18 months. The safety and effectiveness of Pentacel in infants less than 6 weeks of age and in children 5 to 16 years of age have not been established.

11 DESCRIPTION

Pentacel consists of a Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus (DTaP-IPV) component and an ActHIB® component combined through reconstitution for intramuscular injection. ActHIB (Haemophilus b Conjugate Vaccine [Tetanus Toxoid Conjugate]), consists of *H. influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate [PRP]) covalently bound to tetanus toxoid (PRP-T). The DTaP-IPV component is supplied as a sterile liquid used to reconstitute the lyophilized ActHIB component to form Pentacel. Pentacel is a uniform, cloudy, white to off-white (yellow tinge) suspension.

Each 0.5 mL dose contains 15 Lf diphtheria toxoid, 5 Lf tetanus toxoid, acellular pertussis antigens [20 mcg detoxified pertussis toxin (PT), 20 mcg filamentous hemagglutinin (FHA), 3 mcg pertactin (PRN), 5 mcg fimbriae types 2 and 3 (FIM)], inactivated polioviruses
[40 D-antigen units (DU) Type 1 (Mahoney), 8 DU Type 2 (MEF-1), 32 DU Type 3 (Saukett)] and 10 mcg PRP of *H. influenzae* type b covalently bound to 24 mcg of tetanus toxoid (PRP-T).

Other ingredients per 0.5 mL dose include 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, polysorbate 80 (approximately 10 ppm by calculation), 42.5 mg sucrose, <2 mcg residual formaldehyde, <50 ng residual glutaraldehyde, ≤50 ng residual bovine serum albumin, 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative), <4 pg of neomycin and <4 pg polymyxin B sulfate.

Corynebacterium diphtheriae is grown in modified Mueller's growth medium. (7) After purification by ammonium sulfate fractionation, the diphtheria toxin is detoxified with formaldehyde and diafiltered.

Clostridium tetani is grown in modified Mueller-Miller casamino acid medium without beef heart infusion. (8) Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

The acellular pertussis vaccine antigens are produced from *Bordetella pertussis* cultures grown in Stainer-Scholte medium (9) modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. FIM are extracted and copurified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT is detoxified with glutaraldehyde. FHA is treated with formaldehyde and the residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate.

Poliovirus Type 1, Type 2 and Type 3 are each grown in separate cultures of MRC-5 cells, a line of normal human diploid cells, by the microcarrier method. (10) (11) The cells are grown in CMRL (Connaught Medical Research Laboratories) 1969 medium, supplemented with calf serum. For viral growth, the culture medium is replaced by Medium 199, without calf serum. After clarification and filtration, the viral suspensions

are concentrated by ultrafiltration, and purified by liquid chromatography steps. The monovalent viral suspensions are inactivated with formaldehyde. Monovalent concentrates of each inactivated poliovirus are combined to produce a trivalent poliovirus concentrate.

The adsorbed diphtheria, tetanus and acellular pertussis antigens are combined with aluminum phosphate (as adjuvant), 2-phenoxyethanol (not as a preservative) and water for injection, into an intermediate concentrate. The trivalent poliovirus concentrate is added and the DTaP-IPV component is diluted to its final concentration. The DTaP-IPV component does not contain a preservative.

Both diphtheria and tetanus toxoids induce at least 2 neutralizing units per mL in the guinea pig potency test. The potency of the acellular pertussis antigens is evaluated by the antibody response of immunized mice to detoxified PT, FHA, PRN and FIM as measured by enzyme-linked immunosorbent assay (ELISA). The potency of inactivated poliovirus antigens is determined by measuring antibody-mediated neutralization of poliovirus in sera from immunized rats.

PRP, a high molecular weight polymer, is prepared from the *Haemophilus influenzae* type b strain 1482 grown in a semi-synthetic medium. (12) The tetanus toxoid for conjugation to PRP is prepared by ammonium sulfate purification, and formalin inactivation of the toxin from cultures of *Clostridium tetani* (Harvard strain) grown in a modified Mueller and Miller medium. (13) The toxoid is filter sterilized prior to the conjugation process. The ActHIB component does not contain a preservative. Potency of the ActHIB component is specified on each lot by limits on the content of PRP polysaccharide and protein per dose and the proportion of polysaccharide and protein that is characterized as high molecular weight conjugate.

The vial stoppers for the DTaP-IPV and ActHIB components of Pentacel are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection. Antitoxin levels of at least 0.1 IU/mL are generally regarded as protective. (14) Levels of 1.0 IU/mL have been associated with long-term protection. (15)

Tetanus

Tetanus is an acute disease caused by an extremely potent neurotoxin produced by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assay is considered the minimum protective level. (14) (16) A tetanus

antitoxoid level ≥0.1 IU/mL as measured by the ELISA used in clinical studies of Pentacel is considered protective.

Pertussis

Pertussis (whooping cough) is a respiratory disease caused by *B. pertussis*. This Gram-negative coccobacillus produces a variety of biologically active components, though their role in either the pathogenesis of, or immunity to, pertussis has not been clearly defined.

Poliomyelitis

Polioviruses, of which there are three serotypes (Types 1, 2, and 3) are enteroviruses. The presence of poliovirus type-specific neutralizing antibodies has been correlated with protection against poliomyelitis. (17)

Invasive Disease Due to *H. influenzae* Type b

H. influenzae type b can cause invasive disease such as meningitis and sepsis. Anti-PRP antibody has been shown to correlate with protection against invasive disease due to *H. influenzae* type b.

Based on data from passive antibody studies (18) and an efficacy study with *H. influenzae* type b polysaccharide vaccine in Finland, (19) a post-vaccination anti-PRP level of 0.15 mcg/mL has been accepted as a minimal protective level. Data from an efficacy study with *H. influenzae* type b polysaccharide vaccine in Finland indicate that a level >1.0 mcg/mL 3 weeks after vaccination predicts protection through a subsequent one-year period. (20) (21) These levels have been used to evaluate the effectiveness of Haemophilus b Conjugate Vaccines, including the ActHIB component of Pentacel.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Pentacel has not been evaluated for carcinogenic or mutagenic potential or impairment of fertility.

14 CLINICAL STUDIES

The efficacy of Pentacel is based on the immunogenicity of the individual antigens compared to separately administered vaccines. Serological correlates of protection exist for diphtheria, tetanus, poliomyelitis, and invasive disease due to *H. influenzae* type b. [See *Clinical Pharmacology (12.1).]* The efficacy against pertussis, for which there is no well established serological correlate of protection, was based, in part, on a comparison of pertussis immune responses following Pentacel in US children to responses following DAPTACEL (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) manufactured by Sanofi Pasteur Limited) in an efficacy study conducted in Sweden (Sweden I Efficacy Trial). While Pentacel and DAPTACEL contain the same pertussis antigens, manufactured by the same process, Pentacel contains twice as much detoxified PT and four times as much FHA as DAPTACEL.

Immune responses to Pentacel were evaluated in four US studies: Studies 494-01, P3T06, 494-03, and M5A10. The vaccination schedules of Pentacel, Control vaccines, and concomitantly administered vaccines used in Studies 494-01, P3T06, and 494-03 are provided in Table 1. [See *Adverse Reactions (6.1)*.] In Study M5A10, participants were randomized to receive Pentacel or separately administered DAPTACEL, IPOL, and ActHIB at 2, 4, and 6 months of age. 7-valent pneumococcal conjugate (PCV7, Wyeth Pharmaceuticals Inc.) at 2, 4, and 6 months of age, and Hepatitis B vaccine (Merck and Co. or GlaxoSmithKline Biologicals) at 2 and 6 months of age, were administered concomitantly with Pentacel or Control vaccines. (22)

14.1 Diphtheria

The proportions of participants achieving diphtheria antitoxin seroprotective levels one month following three and four doses of Pentacel or DAPTACEL in Study P3T06 are provided in Table 3.

14.2 Tetanus

The proportions of participants achieving tetanus antitoxoid seroprotective levels one month following three and four doses of Pentacel or DAPTACEL in Study P3T06 are provided in Table 3.

Table 3: Study P3T06 Diphtheria Antitoxin and Tetanus Antitoxoid Responses One Month Following Dose 3 and Dose 4 of Pentacel or DAPTACEL + IPOL + ActHIB in US Children Vaccinated at 2, 4, 6, and 15-16 Months of Age

	Pentacel	**DAPTACEL + IPOL + ActHIB**
Post-Dose 3	**N = 331-345**	**N = 1,037-1,099**
Diphtheria Antitoxin		
% ≥0.01 IU/mL*	100.0%	100.0%
% ≥0.10 IU/mL†	98.8%	98.5%
Tetanus Antitoxoid		
% ≥0.10 IU/mL†	99.7%	100.0%
Post-Dose 4	**N = 341-352**	**N = 328-334**
Diphtheria Antitoxin		
% ≥0.10 IU/mL*	100.0%	100.0%
% ≥1.0 IU/mL†	96.5%	95.7%
Tetanus Antitoxoid		
% ≥0.10 IU/mL*	100.0%	100.0%
% ≥1.0 IU/mL†‡	92.9%	99.4%

Per Protocol Immunogenicity population.

* Seroprotection rate following Pentacel vaccine is not inferior to DAPTACEL vaccine (upper limit of 90% CI of the difference DAPTACEL – Pentacel is <10%).

† Non-inferiority criteria were not pre-specified.

‡ With the ELISA used in this study, a tetanus antitoxoid level of 1.0 IU/mL is 10 times the protective level.

14.3 Pertussis

In a clinical pertussis vaccine efficacy study conducted in Sweden during 1992-1995 (Sweden I Efficacy Trial), 2,587 infants received DAPTACEL and 2,574 infants received a non-US licensed DT vaccine as placebo at 2, 4, and 6 months of age. (1) The mean length of follow-up was 2 years after the third dose of vaccine. The protective efficacy of DAPTACEL against pertussis after 3 doses of vaccine using the World Health Organization (WHO) case definition (≥21 consecutive days of paroxysmal cough with culture or serologic confirmation or epidemiologic link to a confirmed case) was 84.9% (95% confidence interval [CI] 80.1%, 88.6%). The protective efficacy of DAPTACEL against mild pertussis (≥1 day of cough with laboratory confirmation) was 77.9% (95% CI 72.6%, 82.2%). Protection against pertussis by DAPTACEL was sustained for the 2-year follow-up period.

Based on comparisons of the immune responses to DAPTACEL in US infants (Post-Dose 3) and Canadian children (Post-Dose 4) relative to infants who participated in the Sweden I Efficacy Trial, it was concluded that 4 doses of DAPTACEL were needed for primary immunization against pertussis in US children. (1)

In a serology bridging analysis, immune responses to FHA, PRN and FIM in a subset of infants who received three doses of DAPTACEL in the Sweden I Efficacy Trial were compared to the Post-Dose 3 and Post-Dose 4 responses in a subset of US children from Study 494-01 who received Pentacel (Table 4). Available stored sera from infants who received DAPTACEL in the Sweden I Efficacy Trial and sera from children who received PCV7 concomitantly with the first three doses of Pentacel in Study 494-01 (Table 1) were assayed in parallel. Data on levels of antibody to PT using an adequately specific assay were not available for this serology bridging analysis.

Geometric mean antibody concentrations (GMCs) and seroconversion rates for antibodies to FHA, PRN and FIM one month following Dose 3 of DAPTACEL in the subset of infants from the Sweden I Efficacy Trial and one month following Dose 3 and Dose 4 of Pentacel in a subset of infants from US Study 494-01 are presented in Table 4. Seroconversion was defined as 4-fold rise in antibody level (Post-Dose 3/Pre-Dose 1 or Post-Dose 4/Pre-Dose 1). For anti-FHA and anti-FIM, the non-inferiority criteria were met for seroconversion rates, and for anti-FHA, anti-PRN, and anti-FIM, the non-inferiority criteria were met for GMCs, following Dose 4 of Pentacel relative to Dose 3 of DAPTACEL. The non-inferiority criterion for anti-PRN seroconversion following Dose 4 of Pentacel relative to Dose 3 of DAPTACEL was not met [upper limit of 95% CI for difference in rate (DAPTACEL minus Pentacel) = 13.24%]. Whether the lower anti-PRN seroconversion rate following Dose 4 of Pentacel in US children relative to Dose 3 of DAPTACEL in Swedish infants correlates with diminished efficacy of Pentacel against pertussis is unknown.

Table 4: FHA, PRN and FIM Antibody Responses One Month Following Dose 3 of DAPTACEL in a Subset of Infants Vaccinated at 2, 4, and 6 Months of Age in the Sweden I Efficacy Trial and One Month Following Dose 3 and Dose 4 of Pentacel in a Subset of Infants Vaccinated at 2, 4, 6, and 15-16 Months of Age in US Study 494-01

	Post-Dose 3 DAPTACEL Sweden I Efficacy Trial N = 80	Post-Dose 3 Pentacel* US Study 494-01 N = 730-995	Post-Dose 4 Pentacel† US Study 494-01 N = 507-554
Anti-FHA			
% achieving 4-fold rise‡	68.8	79.8	91.7§
GMC (EU/mL)	40.70	71.46	129.85§
Anti-PRN			
% achieving 4-fold rise‡	98.8	74.4	89.2¶
GMC (EU/mL)	111.26	38.11	90.82§
Anti-FIM			
% achieving 4-fold rise‡	86.3	86.5	91.5§
GMC (EU/mL)	339.31	265.02	506.57§

Analyzed sera were from subsets of the Per Protocol Immunogenicity populations in each study.

Data on anti-PT levels using an adequately specific assay were not available.

* Non-inferiority criteria were not pre-specified for the comparisons of immune responses to Pentacel vaccine Post-Dose 3 vs. DAPTACEL vaccine Post-Dose 3.

† Pre-specified non-inferiority analyses compared immune responses to Pentacel vaccine Post-Dose 4 vs. DAPTACEL vaccine Post-Dose 3.

‡ Fold rise was calculated as Post-Dose 3/Pre-Dose 1 antibody level or Post-Dose 4/Pre-Dose 1 antibody level.

§ Percent achieving 4-fold rise or GMC Post-Dose 4 Pentacel vaccine is not inferior to Post-Dose 3 DAPTACEL vaccine [upper limit of 95% CI for difference in rates (DAPTACEL minus Pentacel) <10% and upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) <1.5].

¶ Non-inferiority criterion is not met for percent achieving 4-fold rise in anti-PRN Post-Dose 4 Pentacel vaccine relative to Post-Dose 3 DAPTACEL vaccine [upper limit of 95% CI for difference in rates (DAPTACEL minus Pentacel) = 13.24%, exceeds the non-inferiority criterion of <10%].

In a separate study, Study P3T06, US infants were randomized to receive either Pentacel or DAPTACEL + IPOL + ActHIB at 2, 4, 6, and 15-16 months of age (Table 1). The pertussis immune responses (GMCs and seroconversion rates) one month following the third and fourth doses were compared between the two groups (Table 5). Seroconversion was defined as a 4-fold rise in antibody level (Post-Dose 3/Pre-Dose 1 or Post-Dose 4/Pre-Dose 1). Data on anti-PT responses obtained from an adequately specific assay were available on only a non-random subset of study participants. The subset of study participants was representative of all study participants with regard to Pre-Dose 1, Post-Dose 3 and Post-Dose 4 GMCs of antibodies to FHA, PRN and FIM.

For each of the pertussis antigens, non-inferiority criteria were met for seroconversion rates and GMCs following Dose 3 of Pentacel relative to Dose 3 of DAPTACEL. Following Dose 4 of Pentacel relative to Dose 4 of DAPTACEL, non-inferiority criteria were met for all comparisons except for anti-PRN GMCs [upper limit of 90% CI for ratio of GMCs (DAPTACEL/Pentacel) = 2.25]. Whether the lower anti-PRN GMC following Dose 4 of Pentacel relative to Dose 4 of DAPTACEL in US children correlates with diminished efficacy of Pentacel against pertussis is unknown.

Table 5: Pertussis Antibody Responses One Month Following Doses 3 and 4 of Pentacel or DAPTACEL + IPOL + ActHIB in US Infants Vaccinated at 2, 4, 6, and 15-16 Months of Age in Study P3T06

	Post-Dose 3 **Pentacel** **N = 143**	**Post-Dose 3** **DAPTACEL + IPOL + ActHIB** **N = 481-485**	**Post-Dose 4** **Pentacel** **N = 113**	**Post-Dose 4** **DAPTACEL + ActHIB** **N = 127-128**
Anti-PT % achieving 4-fold rise*	95.8[†]	87.3	93.8[‡]	91.3
Anti-PT GMC (EU/mL)	102.62[†]	61.88	107.89[‡]	100.29
	Post-Dose 3 **Pentacel** **N = 218-318**	**Post-Dose 3** **DAPTACEL + IPOL + ActHIB** **N = 714-1,016**	**Post-Dose 4** **Pentacel** **N = 230-367**	**Post-Dose 4** **DAPTACEL + ActHIB** **N = 237-347**
Anti-FHA % achieving 4-fold rise*	81.9[§]	60.9	88.4¶	79.3
Anti-FHA GMC (EU/mL)	73.68[§]	29.22	107.89¶	64.02
Anti-PRN % achieving 4-fold rise*	74.2[§]	75.4	92.7¶	98.3
Anti-PRN GMC (EU/mL)	36.05[§]	43.25	93.59[#]	186.07
Anti-FIM % achieving 4-fold rise*	91.7[§]	86.3	93.5¶	91.6
Anti-FIM GMC (EU/mL)	268.15[§]	267.18	553.39¶	513.54

Per Protocol Immunogenicity population for anti-FHA, anti-PRN, and anti-FIM.

Non-random subset of per Protocol Immunogenicity population for anti-PT. See text for further information on the subset evaluated.

* Fold rise was calculated as Post-Dose 3/Pre-Dose 1 antibody level or Post-Dose 4/Pre-Dose 1 antibody level.

† Percent achieving 4-fold rise or GMC Post-Dose 3 Pentacel vaccine not inferior to Post-Dose 3 DAPTACEL vaccine [upper limit of 95% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 95% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

‡ Percent achieving 4-fold rise or GMC Post-Dose 4 Pentacel vaccine not inferior to Post-Dose 4 DAPTACEL vaccine [upper limit of 95% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 95% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

§ Percent achieving 4-fold rise or GMC Post-Dose 3 Pentacel vaccine not inferior to Post-Dose 3 DAPTACEL vaccine [upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 90% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

¶ Percent achieving 4-fold rise or GMC Post-Dose 4 Pentacel vaccine not inferior to Post-Dose 4 DAPTACEL vaccine [upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) <1.5 and upper limit of 90% CI for differences in rates (DAPTACEL minus Pentacel) <10%].

\# Non-inferiority criterion is not met for GMC Post-Dose 4 Pentacel vaccine relative to Post-Dose 4 DAPTACEL vaccine [upper limit of 90% CI for GMC ratio (DAPTACEL/Pentacel) = 2.25, which exceeds the non-inferiority criterion of <1.5].

Study 006 was a study conducted in the US, where infants were randomized to receive 3 doses of VAXELIS at 2, 4, and 6 months of age and Pentacel at 15 months of age (N=2,406), or control group vaccines (4 doses of Pentacel at 2, 4, 6, and 15 months of age + RECOMBIVAX HB [Hepatitis B Vaccine (Recombinant)] at 2 and 6 months of age; N=402). All subjects received concomitant Prevnar 13 (Pneumococcal 13-valent Conjugate Vaccine [Diphtheria CRM197 Protein]) at 2, 4, 6, and 15 months of age.

Participants were evaluated for immune responses to pertussis antigens one month following the dose of Pentacel administered at 15 months of age. The non-inferiority criteria for antibody vaccine response rates and GMCs for all pertussis antigens were met following the fourth dose except for GMCs for PRN (lower bound of 2-sided 95% CI for GMC ratio [VAXELIS group/Control group vaccines] was 0.66, which was below the non-inferiority criterion >0.67). (22)

14.4 Poliomyelitis

In Study P3T06 (Table 1), in which infants were randomized to receive the first three doses of Pentacel or DAPTACEL + IPOL + ActHIB at 2, 4, and 6 months of age, one month following the third dose of study vaccines, ≥99.4% of participants in both groups (Pentacel: N = 338-350), (DAPTACEL + IPOL + ActHIB: N = 1,050-1,097) achieved neutralizing antibody levels of ≥1:8 for Poliovirus types 1, 2, and 3.

In Study 494-01 (Table 1), in which infants were randomized to receive Pentacel or HCPDT + POLIOVAX + ActHIB, GMTs (1/dil) of antibodies to Poliovirus types 1, 2, and 3 one month following Dose 4 of Pentacel (N = 851-857) were 2,304, 4,178, and 4,415, respectively, and one month following Dose 4 of POLIOVAX (N = 284-287) were 2,330, 2,840, and 3,300, respectively.

14.5 Invasive Disease due to *H. Influenzae* Type b

Anti-PRP seroprotection rates and GMCs one month following Dose 3 of Pentacel or separately administered ActHIB in studies 494-01, P3T06, and M5A10 are presented in Table 6. In Study 494-01, non-inferiority criteria were not met for the proportion of participants who achieved an anti-PRP level ≥1.0 mcg/mL and for anti-PRP GMCs following Pentacel compared with separately administered ActHIB. In each of Studies P3T06 and M5A10, the non-inferiority criterion was met for the proportion of participants who achieved an anti-PRP level ≥1.0 mcg/mL following Pentacel compared with separately administered ActHIB. In Study M5A10, the non-inferiority criterion was met for anti-PRP GMCs following Pentacel compared with separately administered ActHIB.

Table 6: Anti-PRP Seroprotection Rates and GMCs One Month Following Three Doses of Pentacel or Separate DTaP + IPV + ActHIB Administered at 2, 4, and 6 Months of Age in Studies 494-01, P3T06, and M5A10

	Study 494-01 **Pentacel** **N = 1,127**	**Study 494-01** **HCPDT + POLIOVAX + ActHIB** **N = 401**
% achieving anti-PRP ≥0.15 mcg/mL	95.4*	98.3
% achieving anti-PRP ≥1.0 mcg/mL	79.1†	88.8
Anti-PRP GMC (mcg/mL)	3.19‡	6.23
	Study P3T06 **Pentacel** **N = 365**	**Study P3T06** **DAPTACEL + IPOL + ActHIB** **N = 1,128**
% achieving anti-PRP ≥0.15 mcg/mL	92.3*	93.3
% achieving anti-PRP ≥1.0 mcg/mL	72.1*	70.8
Anti-PRP GMC (mcg/mL)	2.31§	2.29
	Study M5A10 **Pentacel** **N = 826**	**Study M5A10** **DAPTACEL + IPOL + ActHIB** **N = 421**
% achieving anti-PRP ≥0.15 mcg/mL	93.8¶	90.3
% achieving anti-PRP ≥1.0 mcg/mL	75.1¶	74.8
Anti-PRP GMC (mcg/mL)	2.52#	2.38

Per Protocol Immunogenicity population for all studies.

IPV indicates Poliovirus Vaccine Inactivated.

* Percent achieving specified level following Pentacel vaccine not inferior to ActHIB vaccine [upper limit of 90% CI for difference in rates (ActHIB minus Pentacel) <10%].

† Non-inferiority criterion not met for percent achieving anti-PRP ≥1.0 mcg/mL following Pentacel vaccine relative to ActHIB vaccine [upper limit of 90% CI for difference in rates (ActHIB minus Pentacel), 12.9%, exceeds the non-inferiority criterion <10%].

‡ Non-inferiority criterion not met for GMC following Pentacel vaccine relative to ActHIB vaccine [upper limit of 90% CI of GMC ratio (ActHIB/Pentacel), 2.26, exceeds the non-inferiority criterion <1.5].

§ Non-inferiority criterion not pre-specified.

¶ Percent achieving specified level following Pentacel vaccine not inferior to ActHIB vaccine [upper limit of 95% CI for difference in rates (ActHIB minus Pentacel) <10%].

GMC following Pentacel vaccine not inferior to ActHIB vaccine [upper limit of 90% CI of GMC ratio (ActHIB/Pentacel) <1.5].

In Study 494-01, at 15 months of age prior to receipt of Dose 4 of study vaccines, 68.6% of Pentacel recipients (N = 829) and 80.8% of separately administered ActHIB recipients (N = 276) had an anti-PRP level ≥0.15 mcg/mL. Following Dose 4 of study vaccines, 98.2% of Pentacel recipients (N = 874) and 99.0% of separately administered ActHIB recipients (N = 291) had an anti-PRP level ≥1.0 mcg/mL.

In Study P3T06, at 15 months of age prior to receipt of Dose 4 of study vaccines, 65.4% of Pentacel recipients (N = 335) and 60.7% of separately administered ActHIB recipients (N = 323) had an anti-PRP level ≥0.15 mcg/mL. Following Dose 4 of study vaccines, 97.8% of Pentacel recipients (N = 361) and 95.9% of separately administered ActHIB recipients (N = 340) had an anti-PRP level ≥1.0 mcg/mL.

14.6 Concomitantly Administered Vaccines

In Study P3T06, (Table 1) there was no evidence for reduced antibody responses to hepatitis B vaccine (percent of participants with anti-HBsAg ≥10 mIU/mL and GMCs) or PCV7 (percent of participants with antibody levels ≥0.15 mcg/mL and ≥0.5 mcg/mL and GMCs to each serotype) administered concomitantly with Pentacel (N = 321-325) relative to these vaccines administered concomitantly with DAPTACEL + IPOL + ActHIB (N = 998-1,029). The immune responses to hepatitis B vaccine and PCV7 were evaluated one month following the third dose.

In Study 494-03, (Table 1) there was no evidence for interference in the immune response to the fourth dose of PCV7 (percent of participants with antibody levels ≥0.15 mcg/mL and ≥0.5 mcg/mL and GMCs to each serotype) administered at 15 months of age concomitantly with Pentacel (N = 155) relative to this vaccine administered concomitantly with MMR and varicella vaccines (N = 158). There was no evidence for interference in the immune response to MMR and varicella vaccines (percent of participants with pre-specified seroresponse level) administered at 15 months of age concomitantly with Pentacel (N = 154) relative to these vaccines administered concomitantly with PCV7 (N = 144). The immune responses to MMR, varicella vaccine and the fourth dose of PCV7 were evaluated one month post-vaccination.

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16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

The vial stoppers for the DTaP-IPV and ActHIB vaccine components of Pentacel are not made with natural rubber latex.

5 Dose Package (NDC No. 49281-510-05) containing 5 vials of DTaP-IPV component (NDC No. 49281-560-05) to be used to reconstitute 5 single-dose vials of lyophilized ActHIB vaccine component (NDC No. 49281-548-58).

16.2 Storage and Handling

Pentacel should be stored at 2° to 8°C (35° to 46°F). Do not freeze. Product which has been exposed to freezing should not be used. Do not use after expiration date shown on the label.

Pentacel should be used immediately after reconstitution.

17 PATIENT COUNSELING INFORMATION

Before administration of Pentacel, health-care personnel should inform the parent or guardian of the benefits and risks of the vaccine and the importance of completing the immunization series unless a contraindication to further immunization exists.

The health-care provider should inform the parent or guardian about the potential for adverse reactions that have been temporally associated with Pentacel or other vaccines containing similar ingredients. The health-care provider should provide the Vaccine Information Statements (VIS) which are required by the National Childhood Vaccine Injury Act of 1986 to be given with each immunization. The parent or guardian should be instructed to report adverse reactions to their health-care provider.

Manufactured by:
Sanofi Pasteur Limited
Toronto Ontario Canada

and **Sanofi Pasteur SA**
Marcy L'Etoile France

Distributed by:
Sanofi Pasteur Inc.
Swiftwater PA 18370 USA

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PEDIARIX safely and effectively. See full prescribing information for PEDIARIX.

PEDIARIX [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine], Suspension for Intramuscular Injection
Initial U.S. Approval: 2002

---------- **INDICATIONS AND USAGE**----------
PEDIARIX is a vaccine indicated for active immunization against diphtheria, tetanus, pertussis, infection caused by all known subtypes of hepatitis B virus, and poliomyelitis. PEDIARIX is approved for use as a 3-dose series in infants born of hepatitis B surface antigen (HBsAg)-negative mothers. PEDIARIX may be given as early as 6 weeks of age through 6 years of age (prior to the 7th birthday). (1)

----------**DOSAGE AND ADMINISTRATION**----------
Three doses (0.5-mL each) by intramuscular injection at 2, 4, and 6 months of age. (2.2)

---------- **DOSAGE FORMS AND STRENGTHS**----------
Single-dose, prefilled syringes containing a 0.5-mL suspension for injection. (3)

---------- **CONTRAINDICATIONS** ----------
- Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid-, tetanus toxoid-, pertussis-, hepatitis B-, or poliovirus-containing vaccine, or to any component of PEDIARIX. (4.1)
- Encephalopathy within 7 days of administration of a previous pertussis-containing vaccine. (4.2)
- Progressive neurologic disorders. (4.3)

---------- **WARNINGS AND PRECAUTIONS**----------
- In clinical trials, PEDIARIX was associated with higher rates of fever, relative to separately administered vaccines. (5.1)
- If Guillain-Barré syndrome occurs within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give PEDIARIX should be based on potential benefits and risks. (5.2)
- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.3)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including PEDIARIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.4)
- If temperature ≥105°F, collapse or shock-like state, or persistent, inconsolable crying lasting ≥3 hours have occurred within 48 hours after receipt of a pertussis-containing vaccine, or if seizures have occurred within 3 days after receipt of a pertussis-containing vaccine, the decision to give PEDIARIX should be based on potential benefits and risks. (5.5)
- For children at higher risk for seizures, an antipyretic may be administered at the time of vaccination with PEDIARIX. (5.6)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including PEDIARIX, to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.7)

---------- **ADVERSE REACTIONS** ----------
Common solicited adverse reactions following any dose (≥25%) included local injection site reactions (pain, redness, and swelling), fever (≥100.4°F), drowsiness, irritability/fussiness, and loss of appetite. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- **DRUG INTERACTIONS**----------
Do not mix PEDIARIX with any other vaccine in the same syringe. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

***Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

PEDIARIX is indicated for active immunization against diphtheria, tetanus, pertussis, infection caused by all known subtypes of hepatitis B virus, and poliomyelitis. PEDIARIX is approved for

use as a 3-dose series in infants born of hepatitis B surface antigen (HBsAg)-negative mothers. PEDIARIX may be given as early as 6 weeks of age through 6 years of age (prior to the 7th birthday).

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

Shake vigorously to obtain a homogeneous, turbid, white suspension. Do not use if resuspension does not occur with vigorous shaking. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Attach a sterile needle and administer intramuscularly.

The preferred administration site is the anterolateral aspect of the thigh for children younger than 1 year. In older children, the deltoid muscle is usually large enough for an intramuscular injection. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk. Gluteal injections may result in suboptimal hepatitis B immune response.

Do not administer this product intravenously, intradermally, or subcutaneously.

2.2 Recommended Dose and Schedule

Immunization with PEDIARIX consists of 3 doses of 0.5 mL each by intramuscular injection at 2, 4, and 6 months of age (at intervals of 6 to 8 weeks, preferably 8 weeks). The first dose may be given as early as 6 weeks of age. Three doses of PEDIARIX constitute a primary immunization course for diphtheria, tetanus, pertussis, and poliomyelitis and the complete vaccination course for hepatitis B.

2.3 Modified Schedules in Previously Vaccinated Children

Children Previously Vaccinated with Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP)

PEDIARIX may be used to complete the first 3 doses of the DTaP series in children who have received 1 or 2 doses of INFANRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed), manufactured by GlaxoSmithKline, identical to the DTaP component of PEDIARIX *[see Description (11)]* and are also scheduled to receive the other vaccine components of PEDIARIX. Data are not available on the safety and effectiveness of using PEDIARIX following 1 or more doses of a DTaP vaccine from a different manufacturer.

Children Previously Vaccinated with Hepatitis B Vaccine

PEDIARIX may be used to complete the hepatitis B vaccination series following 1 or 2 doses of another hepatitis B vaccine (monovalent or as part of a combination vaccine), including vaccines from other manufacturers, in children born of HBsAg-negative mothers who are also scheduled to receive the other vaccine components of PEDIARIX.

A 3-dose series of PEDIARIX may be administered to infants born of HBsAg-negative mothers and who received a dose of hepatitis B vaccine at or shortly after birth. However, data are limited regarding the safety of PEDIARIX in such infants *[see Adverse Reactions (6.1)]*. There are no data to support the use of a 3-dose series of PEDIARIX in infants who have previously received more than 1 dose of hepatitis B vaccine.

Children Previously Vaccinated with Inactivated Poliovirus Vaccine (IPV)

PEDIARIX may be used to complete the first 3 doses of the IPV series in children who have received 1 or 2 doses of IPV from a different manufacturer and are also scheduled to receive the other vaccine components of PEDIARIX.

2.4 Booster Immunization following PEDIARIX

Children who have received a 3-dose series with PEDIARIX should complete the DTaP and IPV series according to the recommended schedule.[1] Because the pertussis antigens contained in INFANRIX and KINRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine), manufactured by GlaxoSmithKline, are the same as those in PEDIARIX, these children should receive INFANRIX as their fourth dose of DTaP and either INFANRIX or KINRIX as their fifth dose of DTaP, according to the respective prescribing information for these vaccines. KINRIX or another manufacturer's IPV may be used to complete the 4-dose IPV series according to the respective prescribing information.

3 DOSAGE FORMS AND STRENGTHS

PEDIARIX is a suspension for injection available in 0.5-mL single-dose prefilled TIP-LOK syringes.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A severe allergic reaction (e.g., anaphylaxis) after a previous dose of any diphtheria toxoid-, tetanus toxoid-, pertussis antigen-, hepatitis B-, or poliovirus-containing vaccine or any component of this vaccine, including yeast, neomycin, and polymyxin B, is a contraindication to administration of PEDIARIX *[see Description (11)]*.

4.2 Encephalopathy

Encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of administration of a previous dose of a pertussis-containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis-containing vaccine, including PEDIARIX.

4.3 Progressive Neurologic Disorder

Progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or

progressive encephalopathy, is a contraindication to administration of any pertussis-containing vaccine, including PEDIARIX. PEDIARIX should not be administered to individuals with such conditions until the neurologic status is clarified and stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Fever

In clinical trials, administration of PEDIARIX in infants was associated with higher rates of fever relative to separately administered vaccines *[see Adverse Reactions (6.1)]*.

5.2 Guillain-Barré Syndrome

If Guillain-Barré syndrome occurs within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give PEDIARIX or any vaccine containing tetanus toxoid should be based on careful consideration of the potential benefits and possible risks.

5.3 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.4 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including PEDIARIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.5 Adverse Reactions following Prior Pertussis Vaccination

If any of the following reactions occur in temporal relation to receipt of a vaccine containing a pertussis component, the decision to give any pertussis-containing vaccine, including PEDIARIX, should be based on careful consideration of the potential benefits and possible risks:

- Temperature of ≥40.5ºC (105ºF) within 48 hours not due to another identifiable cause;
- Collapse or shock-like state (hypotonic-hyporesponsive episode) within 48 hours;
- Persistent, inconsolable crying lasting ≥3 hours, occurring within 48 hours;
- Seizures with or without fever occurring within 3 days.

5.6 Children at Risk for Seizures

For children at higher risk for seizures than the general population, an appropriate antipyretic may be administered at the time of vaccination with a vaccine containing a pertussis component, including PEDIARIX, and for the ensuing 24 hours to reduce the possibility of post-vaccination fever.

5.7 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely.

Decisions about when to administer an intramuscular vaccine, including PEDIARIX, to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

5.8 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions to allow an assessment of benefits and risks. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

A total of 23,849 doses of PEDIARIX have been administered to 8,088 infants who received 1 or more doses as part of the 3-dose series during 14 clinical studies. Common adverse reactions that occurred in ≥25% of subjects following any dose of PEDIARIX included local injection site reactions (pain, redness, and swelling), fever, drowsiness, irritability/fussiness, and loss of appetite. In comparative studies (including the German and U.S. studies described below), administration of PEDIARIX was associated with higher rates of fever relative to separately administered vaccines *[see Warnings and Precautions (5.1)]*. The prevalence of fever was highest on the day of vaccination and the day following vaccination. More than 96% of episodes of fever resolved within the 4-day period following vaccination (i.e., the period including the day of vaccination and the next 3 days).

In the largest of the 14 studies conducted in Germany, safety data were available for 4,666 infants who received PEDIARIX administered concomitantly at separate sites with 1 of 4 *Haemophilus influenzae* type b (Hib) conjugate vaccines (GlaxoSmithKline [licensed in the United States only for booster immunization], Wyeth Pharmaceuticals Inc. [no longer licensed in the United States], Sanofi Pasteur SA [U.S.-licensed], or Merck & Co, Inc. [U.S.-licensed]) at 3, 4, and 5 months of age and for 768 infants in the control group that received separate U.S.-licensed vaccines (INFANRIX, Hib conjugate vaccine [Sanofi Pasteur SA], and oral poliovirus vaccine [OPV] [Wyeth Pharmaceuticals, Inc.; no longer licensed in the United States]). In this study, information on adverse events that occurred within 30 days following vaccination was collected. More than 95% of study participants were white.

In a U.S. study, the safety of PEDIARIX administered to 673 infants was compared with the safety of separately administered INFANRIX, ENGERIX-B [Hepatitis B Vaccine (Recombinant)], and IPV (Sanofi Pasteur SA) in 335 infants. In both groups, infants received

Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.; no longer licensed in the United States) and 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.) concomitantly at separate sites. All vaccines were administered at 2, 4, and 6 months of age. Data on solicited local reactions and general adverse reactions were collected by parents using standardized diary cards for 4 consecutive days following each vaccine dose (i.e., day of vaccination and the next 3 days). Telephone follow-up was conducted 1 month and 6 months after the third vaccination to inquire about serious adverse events. At the 6-month follow-up, information also was collected on new onset of chronic illnesses. A total of 638 subjects who received PEDIARIX and 313 subjects who received INFANRIX, ENGERIX-B, and IPV completed the 6-month follow-up. Among subjects in both study groups combined, 69% were white, 18% were Hispanic, 7% were black, 3% were Oriental, and 3% were of other racial/ethnic groups.

Solicited Adverse Reactions

Data on solicited local reactions and general adverse reactions from the U.S. safety study are presented in Table 1. This study was powered to evaluate fever >101.3°F following Dose 1. The rate of fever ≥100.4°F following each dose was significantly higher in the group that received PEDIARIX compared with separately administered vaccines. Other statistically significant differences between groups in rates of fever, as well as other solicited adverse reactions, are noted in Table 1. Medical attention (a visit to or from medical personnel) for fever within 4 days following vaccination was sought in the group who received PEDIARIX for 8 infants after the first dose (1.2%), 1 infant following the second dose (0.2%), and 5 infants following the third dose (0.8%) (Table 1). Following Dose 2, medical attention for fever was sought for 2 infants (0.6%) who received separately administered vaccines (Table 1). Among infants who had a medical visit for fever within 4 days following vaccination, 9 of 14 who received PEDIARIX and 1 of 2 who received separately administered vaccines, had 1 or more diagnostic studies performed to evaluate the cause of fever.

Table 1. Percentage of Infants with Solicited Local and General Adverse Reactions within 4 Days of Vaccination[a] at 2, 4, and 6 Months of Age with PEDIARIX Administered Concomitantly with Hib Conjugate Vaccine and 7-Valent Pneumococcal Conjugate Vaccine (PCV7) or with Separate Concomitant Administration of INFANRIX, ENGERIX-B, IPV, Hib Conjugate Vaccine, and PCV7 (Modified Intent-to-Treat Cohort)

Adverse Reaction	PEDIARIX, Hib Vaccine, & PCV7			INFANRIX, ENGERIX-B, IPV, Hib Vaccine, & PCV7		
	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3
Local[b]						
n	**671**	**653**	**648**	**335**	**323**	**315**
Pain, any	36	36	31	32	30	30
Pain, Grade 2 or 3	12	11	11	9	9	9
Pain, Grade 3	2	3	2	3	2	1
Redness, any	25[c]	37	40	18	33	39
Redness, >5 mm	6[c]	10[c]	13[c]	2	6	7
Redness, >20 mm	1	1[c]	3	0	0	2
Swelling, any	17[c]	27[c]	29	10	20	25
Swelling, >5 mm	6[c]	10[c]	9[c]	2	5	4
Swelling, >20 mm	2	3[c]	3	1	0	1
General						
n	**667**	**644**	**645**	**333**	**321**	**311**
Fever[d], ≥100.4°F	28[c]	39[c]	34[c]	20	30	24
Fever[d], >101.3°F	7	14[c]	9	5	10	6
Fever[d], >102.2°F	2[c]	4	3	0	3	2
Fever[d], >103.1°F	1	1	1	0	0	0
Fever[d], M.A.	1[c]	0	1	0	1	0
n	**671**	**653**	**648**	**335**	**323**	**315**
Drowsiness, any	57	52	41	54	48	38
Drowsiness, Grade 2 or 3	16	14	11	18	12	11
Drowsiness, Grade 3	3	1	1	4	1	2
Irritability/Fussiness, any	61	65	61	62	62	57
Irritability/Fussiness, Grade 2 or 3	20	28[c]	25[c]	19	21	19
Irritability/Fussiness, Grade 3	3	4	4	4	3	3
Loss of appetite, any	30	31	26	28	27	24
Loss of appetite, Grade 2 or 3	7	8[c]	6	5	3	5
Loss of appetite, Grade 3	1	0	0	1	0	0

Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.; no longer licensed in the United States); PCV7 (Wyeth Pharmaceuticals Inc.); IPV (Sanofi Pasteur SA).

Modified intent-to-treat cohort = All vaccinated subjects for whom safety data were available.

n = Number of infants for whom at least 1 symptom sheet was completed; for fever, numbers

exclude missing temperature recordings or tympanic measurements.
M.A. = Medically attended (a visit to or from medical personnel).
Grade 2 defined as sufficiently discomforting to interfere with daily activities.
Grade 3 defined as preventing normal daily activities.
[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.
[b] Local reactions at the injection site for PEDIARIX or INFANRIX.
[c] Rate significantly higher in the group that received PEDIARIX compared with separately administered vaccines (*P* value <0.05 [2-sided Fisher Exact test] or the 95% CI on the difference between groups [Separate minus PEDIARIX] does not include 0).
[d] Axillary temperatures increased by 1°C and oral temperatures increased by 0.5°C to derive equivalent rectal temperature.

Serious Adverse Events

Within 30 days following any dose of vaccine in the U.S. safety study in which all subjects received concomitant Hib and pneumococcal conjugate vaccines, 7 serious adverse events were reported in 7 subjects (1% [7/673]) who received PEDIARIX (1 case each of pyrexia, gastroenteritis, and culture-negative clinical sepsis and 4 cases of bronchiolitis) and 5 serious adverse events were reported in 4 subjects (1% [4/335]) who received INFANRIX, ENGERIX-B, and IPV (uteropelvic junction obstruction and testicular atrophy in 1 subject and 3 cases of bronchiolitis).

Deaths

In 14 clinical trials, 5 deaths were reported among 8,088 (0.06%) recipients of PEDIARIX and 1 death was reported among 2,287 (0.04%) recipients of comparator vaccines. Causes of death in the group that received PEDIARIX included 2 cases of Sudden Infant Death Syndrome (SIDS) and 1 case of each of the following: convulsive disorder, congenital immunodeficiency with sepsis, and neuroblastoma. One case of SIDS was reported in the comparator group. The rate of SIDS among all recipients of PEDIARIX across the 14 trials was 0.25/1,000. The rate of SIDS observed for recipients of PEDIARIX in the German safety study was 0.2/1,000 infants (reported rate of SIDS in Germany in the latter part of the 1990s was 0.7/1,000 newborns). The reported rate of SIDS in the United States from 1990 to 1994 was 1.2/1,000 live births. By chance alone, some cases of SIDS can be expected to follow receipt of pertussis-containing vaccines.

Onset of Chronic Illnesses

In the U.S. safety study in which all subjects received concomitant Hib and pneumococcal conjugate vaccines, 21 subjects (3%) who received PEDIARIX and 14 subjects (4%) who received INFANRIX, ENGERIX-B, and IPV reported new onset of a chronic illness during the period from 1 to 6 months following the last dose of study vaccines. Among the chronic illnesses reported in the subjects who received PEDIARIX, there were 4 cases of asthma and 1 case each of diabetes mellitus and chronic neutropenia. There were 4 cases of asthma in subjects who received INFANRIX, ENGERIX-B, and IPV.

Seizures

In the German safety study over the entire study period, 6 subjects in the group that received PEDIARIX (n = 4,666) reported seizures. Two of these subjects had a febrile seizure, 1 of whom also developed afebrile seizures. The remaining 4 subjects had afebrile seizures, including 2 with infantile spasms. Two subjects reported seizures within 7 days following vaccination (1 subject had both febrile and afebrile seizures, and 1 subject had afebrile seizures), corresponding to a rate of 0.22 seizures per 1,000 doses (febrile seizures 0.07 per 1,000 doses, afebrile seizures 0.14 per 1,000 doses). No subject who received concomitant INFANRIX, Hib vaccine, and OPV (n = 768) reported seizures. In a separate German study that evaluated the safety of INFANRIX in 22,505 infants who received 66,867 doses of INFANRIX administered as a 3-dose primary series, the rate of seizures within 7 days of vaccination with INFANRIX was 0.13 per 1,000 doses (febrile seizures 0.0 per 1,000 doses, afebrile seizures 0.13 per 1,000 doses).

Over the entire study period in the U.S. safety study in which all subjects received concomitant Hib and pneumococcal conjugate vaccines, 4 subjects in the group that received PEDIARIX (n = 673) reported seizures. Three of these subjects had a febrile seizure and 1 had an afebrile seizure. Over the entire study period, 2 subjects in the group that received INFANRIX, ENGERIX-B, and IPV (n = 335) reported febrile seizures. There were no afebrile seizures in this group. No subject in either study group had seizures within 7 days following vaccination.

Other Neurological Events of Interest

No cases of hypotonic-hyporesponsiveness or encephalopathy were reported in either the German or U.S. safety studies.

Safety of PEDIARIX after a Previous Dose of Hepatitis B Vaccine

Limited data are available on the safety of administering PEDIARIX after a previous dose of hepatitis B vaccine. In 2 separate studies, 160 Moldovan infants and 96 U.S. infants, respectively, received 3 doses of PEDIARIX following 1 previous dose of hepatitis B vaccine. Neither study was designed to detect significant differences in rates of adverse events associated with PEDIARIX administered after a previous dose of hepatitis B vaccine compared with PEDIARIX administered without a previous dose of hepatitis B vaccine.

6.2 Postmarketing Safety Surveillance Study

In a safety surveillance study conducted at a health maintenance organization in the United States, infants who received 1 or more doses of PEDIARIX from approximately mid-2003 through mid-2005 were compared with age-, gender-, and area-matched historical controls who received 1 or more doses of separately administered U.S.-licensed DTaP vaccine from 2002 through approximately mid-2003. Only infants who received 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.) concomitantly with PEDIARIX or DTaP vaccine were included in the cohorts. Other U.S.-licensed vaccines were administered according to routine practices at the study sites, but concomitant administration with PEDIARIX or DTaP was not a

criterion for inclusion in the cohorts. A birth dose of hepatitis B vaccine had been administered routinely to infants in the historical DTaP control cohort, but not to infants who received PEDIARIX. For each of Doses 1-3, a random sample of 40,000 infants who received PEDIARIX was compared with the historical DTaP control cohort for the incidence of seizures (with or without fever) during the 8-day period following vaccination. For each dose, random samples of 7,500 infants in each cohort were also compared for the incidence of medically-attended fever (fever ≥100.4°F that resulted in hospitalization, an emergency department visit, or an outpatient visit) during the 4-day period following vaccination. Possible seizures and medical visits plausibly related to fever were identified by searching automated inpatient and outpatient data files. Medical record reviews of identified events were conducted to verify the occurrence of seizures or medically-attended fever. The incidence of verified seizures and medically-attended fever from this study are presented in Table 2.

Table 2. Percentage of Infants with Seizures (with or without Fever) within 8 Days of Vaccination and Medically-Attended Fever within 4 Days of Vaccination with PEDIARIX Compared with Historical Controls

	PEDIARIX			Historical DTaP Controls			Difference (PEDIARIX–DTaP Controls)
Adverse Reaction	**N**	**n**	**% (95% CI)**	**N**	**n**	**% (95% CI)**	**% (95% CI)**
All Seizures (with or without fever)							
Dose 1, Days 0-7	40,000	7	0.02 (0.01, 0.04)	39,232	6	0.02 (0.01, 0.03)	0.00 (-0.02, 0.02)
Dose 2, Days 0-7	40,000	3	0.01 (0.00, 0.02)	37,405	4	0.01 (0.00, 0.03)	0.00 (-0.02, 0.01)
Dose 3, Days 0-7	40,000	6	0.02 (0.01, 0.03)	40,000	5	0.01 (0.00, 0.03)	0.00 (-0.01, 0.02)
Total doses	120,000	16	0.01 (0.01, 0.02)	116,637	15	0.01 (0.01, 0.02)	0.00 (-0.01, 0.01)
Medically-Attended Fever[a]							
Dose 1, Days 0-3	7,500	14	0.19 (0.11, 0.30)	7,500	14	0.19 (0.11, 0.30)	0.00 (-0.14, 0.14)
Dose 2, Days 0-3	7,500	25	0.33 (0.22, 0.48)	7,500	15	0.20 (0.11, 0.33)	0.13 (-0.03, 0.30)
Dose 3, Days 0-3	7,500	21	0.28 (0.17, 0.43)	7,500	19	0.25 (0.15, 0.39)	0.03 (-0.14, 0.19)
Total doses	22,500	60	0.27 (0.20, 0.34)	22,500	48	0.21 (0.16, 0.28)	0.05 (-0.01, 0.14)

DTaP – any U.S.-licensed DTaP vaccine. Infants received 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.) concomitantly with each dose of PEDIARIX or DTaP. Other U.S.-licensed vaccines were administered according to routine practices at the study sites.
N = Number of subjects in the given cohort.
n = Number of subjects with reactions reported in the given cohort.
[a] Medically-attended fever defined as fever ≥100.4°F that resulted in hospitalization, an emergency department visit, or an outpatient visit.

6.3 Postmarketing Spontaneous Reports for PEDIARIX

In addition to reports in clinical trials for PEDIARIX, the following adverse reactions have been identified during postapproval use of PEDIARIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Cardiac Disorders

Cyanosis.

Gastrointestinal Disorders

Diarrhea, vomiting.

General Disorders and Administration Site Conditions

Fatigue, injection site cellulitis, injection site induration, injection site itching, injection site nodule/lump, injection site reaction, injection site vesicles, injection site warmth, limb pain, limb swelling.

Immune System Disorders

Anaphylactic reaction, anaphylactoid reaction, hypersensitivity.

Infections and Infestations

Upper respiratory tract infection.

Investigations

Abnormal liver function tests.

Nervous System Disorders

Bulging fontanelle, depressed level of consciousness, encephalitis, hypotonia, hypotonic-hyporesponsive episode, lethargy, somnolence, syncope.

Psychiatric Disorders

Crying, insomnia, nervousness, restlessness, screaming, unusual crying.

Respiratory, Thoracic, and Mediastinal Disorders

Apnea, cough, dyspnea.

Skin and Subcutaneous Tissue Disorders

Angioedema, erythema, rash, urticaria.

Vascular Disorders

Pallor, petechiae.

6.4 Postmarketing Spontaneous Reports for INFANRIX and/or ENGERIX-B

The following adverse reactions have been identified during postapproval use of INFANRIX and/or ENGERIX-B in children younger than 7 years but not already reported for PEDIARIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Blood and Lymphatic System Disorders

Idiopathic thrombocytopenic purpura,[a,b] lymphadenopathy,[a] thrombocytopenia.[a,b]

Gastrointestinal Disorders

Abdominal pain,[b] intussusception,[a,b] nausea.[b]

General Disorders and Administration Site Conditions

Asthenia,[b] malaise.[b]

Hepatobiliary Disorders

Jaundice.[b]

Immune System Disorders

Anaphylactic shock,[a] serum sickness–like disease.[b]

Musculoskeletal and Connective Tissue Disorders

Arthralgia,[b] arthritis,[b] muscular weakness,[b] myalgia.[b]

Nervous System Disorders

Encephalopathy,[a] headache,[a] meningitis,[b] neuritis,[b] neuropathy,[b] paralysis.[b]

Skin and Subcutaneous Tissue Disorders

Alopecia,[b] erythema multiforme,[b] lichen planus,[b] pruritus,[a,b] Stevens Johnson syndrome.[a]

Vascular Disorders

Vasculitis.[b]

[a] Following INFANRIX (licensed in the United States in 1997).

[b] Following ENGERIX-B (licensed in the United States in 1989).

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

Immune responses following concomitant administration of PEDIARIX, Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.; no longer licensed in the U.S.), and 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.) were evaluated in a clinical trial *[see Clinical Studies (14.3)]*.

When PEDIARIX is administered concomitantly with other injectable vaccines, they should be given with separate syringes and at different injection sites. PEDIARIX should not be mixed with any other vaccine in the same syringe.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to PEDIARIX.

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

Safety and effectiveness of PEDIARIX were established in the age group 6 weeks through 6 months on the basis of clinical studies *[see Adverse Reactions (6.1), Clinical Studies (14.1, 14.2)]*. Safety and effectiveness of PEDIARIX in the age group 7 months through 6 years are supported by evidence in infants aged 6 weeks through 6 months. Safety and effectiveness of PEDIARIX in infants younger than 6 weeks and children aged 7 to 16 years have not been evaluated.

11 DESCRIPTION

PEDIARIX [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine] is a noninfectious, sterile vaccine for intramuscular administration. Each 0.5-mL dose is formulated to contain 25 Lf of diphtheria toxoid, 10 Lf of tetanus toxoid, 25 mcg of inactivated pertussis toxin (PT), 25 mcg of filamentous hemagglutinin (FHA), 8 mcg of pertactin (69 kiloDalton outer membrane protein), 10 mcg of HBsAg, 40 D-antigen Units (DU) of Type 1 poliovirus (Mahoney), 8 DU of Type 2 poliovirus (MEF-1), and 32 DU of Type 3 poliovirus (Saukett). The diphtheria, tetanus, and pertussis components are the same as those in INFANRIX and KINRIX. The hepatitis B surface antigen is the same as that in ENGERIX-B.

The diphtheria toxin is produced by growing *Corynebacterium diphtheriae (C. diphtheriae)* in Fenton medium containing a bovine extract. Tetanus toxin is produced by growing *Clostridium tetani (C. tetani)* in a modified Latham medium derived from bovine casein. The bovine materials used in these extracts are sourced from countries which the United States Department

of Agriculture (USDA) has determined neither have nor present an undue risk for bovine spongiform encephalopathy (BSE). Both toxins are detoxified with formaldehyde, concentrated by ultrafiltration, and purified by precipitation, dialysis, and sterile filtration.

The acellular pertussis antigens (PT, FHA, and pertactin) are isolated from *Bordetella pertussis* (*B. pertussis)* culture grown in modified Stainer-Scholte liquid medium. PT and FHA are isolated from the fermentation broth; pertactin is extracted from the cells by heat treatment and flocculation. The antigens are purified in successive chromatographic and precipitation steps. PT is detoxified using glutaraldehyde and formaldehyde. FHA and pertactin are treated with formaldehyde.

The hepatitis B surface antigen is obtained by culturing genetically engineered *Saccharomyces cerevisiae (S. cerevisiae)* cells, which carry the surface antigen gene of the hepatitis B virus, in synthetic medium. The surface antigen expressed in the *S. cerevisiae* cells is purified by several physiochemical steps, which include precipitation, ion exchange chromatography, and ultrafiltration.

The inactivated poliovirus component is an enhanced potency component. Each of the 3 strains of poliovirus is individually grown in VERO cells, a continuous line of monkey kidney cells, cultivated on microcarriers. Calf serum and lactalbumin hydrolysate are used during VERO cell culture and/or virus culture. Calf serum is sourced from countries the USDA has determined neither have nor present an undue risk for BSE. After clarification, each viral suspension is purified by ultrafiltration, diafiltration, and successive chromatographic steps, and inactivated with formaldehyde. The 3 purified viral strains are then pooled to form a trivalent concentrate.

Diphtheria and tetanus toxoids and pertussis antigens (inactivated PT, FHA, and pertactin) are individually adsorbed onto aluminum hydroxide. The hepatitis B component is adsorbed onto aluminum phosphate.

Diphtheria and tetanus toxoid potency is determined by measuring the amount of neutralizing antitoxin in previously immunized guinea pigs. The potency of the acellular pertussis component (inactivated PT, FHA, and pertactin) is determined by enzyme-linked immunosorbent assay (ELISA) on sera from previously immunized mice. Potency of the hepatitis B component is established by HBsAg ELISA. The potency of the inactivated poliovirus component is determined by using the D-antigen ELISA and by a poliovirus-neutralizing cell culture assay on sera from previously immunized rats.

Each 0.5-mL dose contains aluminum salts as adjuvant (not more than 0.85 mg aluminum by assay) and 4.5 mg of sodium chloride. Each dose also contains ≤100 mcg of residual formaldehyde and ≤100 mcg of polysorbate 80 (Tween 80). Neomycin sulfate and polymyxin B are used in the poliovirus vaccine manufacturing process and may be present in the final vaccine at ≤0.05 ng neomycin and ≤0.01 ng polymyxin B per dose. The procedures used to manufacture the HBsAg antigen result in a product that contains ≤5% yeast protein.

The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex.

PEDIARIX is formulated without preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated infectious disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to the diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection; a level of 0.1 IU/mL is regarded as protective.[2]

Tetanus

Tetanus is an acute toxin-mediated disease caused by a potent exotoxin released by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to the tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assays, is considered the minimum protective level.[3,4] A level ≥0.1 IU/mL is considered protective.[5]

Pertussis

Pertussis (whooping cough) is a disease of the respiratory tract caused by *B. pertussis*. The role of the different components produced by *B. pertussis* in either the pathogenesis of, or the immunity to, pertussis is not well understood. There is no established serological correlate of protection for pertussis.

Hepatitis B

Infection with hepatitis B virus can have serious consequences including acute massive hepatic necrosis and chronic active hepatitis. Chronically infected persons are at increased risk for cirrhosis and hepatocellular carcinoma.

Antibody concentrations ≥10 mIU/mL against HBsAg are recognized as conferring protection against hepatitis B virus infection.[6]

Poliomyelitis

Poliovirus is an enterovirus that belongs to the picornavirus family. Three serotypes of poliovirus have been identified (Types 1, 2, and 3). Poliovirus-neutralizing antibodies confer protection against poliomyelitis disease.[7]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

PEDIARIX has not been evaluated for carcinogenic or mutagenic potential or for impairment of

fertility.

14 CLINICAL STUDIES

The efficacy of PEDIARIX is based on the immunogenicity of the individual antigens compared with licensed vaccines. Serological correlates of protection exist for the diphtheria, tetanus, hepatitis B, and poliovirus components. The efficacy of the pertussis component, which does not have a well-established correlate of protection, was determined in clinical trials of INFANRIX.

14.1 Efficacy of INFANRIX

Efficacy of a 3-dose primary series of INFANRIX has been assessed in 2 clinical studies.

A double-blind, randomized, active Diphtheria and Tetanus Toxoids (DT)-controlled trial conducted in Italy, sponsored by the National Institutes of Health (NIH), assessed the absolute protective efficacy of INFANRIX when administered at 2, 4, and 6 months of age. The population used in the primary analysis of the efficacy of INFANRIX included 4,481 infants vaccinated with INFANRIX and 1,470 DT vaccinees. After 3 doses, the absolute protective efficacy of INFANRIX against WHO-defined typical pertussis (21 days or more of paroxysmal cough with infection confirmed by culture and/or serologic testing) was 84% (95% CI: 76%, 89%). When the definition of pertussis was expanded to include clinically milder disease, with infection confirmed by culture and/or serologic testing, the efficacy of INFANRIX was 71% (95% CI: 60%, 78%) against >7 days of any cough and 73% (95% CI: 63%, 80%) against ≥14 days of any cough. A longer unblinded follow-up period showed that after 3 doses and with no booster dose in the second year of life, the efficacy of INFANRIX against WHO-defined pertussis was 86% (95% CI: 79%, 91%) among children followed to 6 years of age. For details see INFANRIX prescribing information.

A prospective efficacy trial was also conducted in Germany employing a household contact study design. In this study, the protective efficacy of INFANRIX administered to infants at 3, 4, and 5 months of age against WHO-defined pertussis was 89% (95% CI: 77%, 95%). When the definition of pertussis was expanded to include clinically milder disease, with infection confirmed by culture and/or serologic testing, the efficacy of INFANRIX against ≥7 days of any cough was 67% (95% CI: 52%, 78%) and against ≥7 days of paroxysmal cough was 81% (95% CI: 68%, 89%). For details see INFANRIX prescribing information.

14.2 Immunological Evaluation of PEDIARIX

In a U.S. multicenter study, infants were randomized to 1 of 3 groups: (1) a combination vaccine group that received PEDIARIX concomitantly with Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.; no longer licensed in the United States) and U.S.-licensed 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.); (2) a separate vaccine group that received U.S.-licensed INFANRIX, ENGERIX-B, and IPV (Sanofi Pasteur SA) concomitantly with the same Hib and pneumococcal conjugate vaccines; and (3) a staggered vaccine group that received PEDIARIX concomitantly with the same Hib conjugate vaccine but with the same

pneumococcal conjugate vaccine administered 2 weeks later. The schedule of administration was 2, 4, and 6 months of age. Infants either did not receive a dose of hepatitis B vaccine prior to enrollment or were permitted to receive 1 dose of hepatitis B vaccine administered at least 30 days prior to enrollment. For the separate vaccine group, ENGERIX-B was not administered at 4 months of age to subjects who received a dose of hepatitis B vaccine prior to enrollment. Among subjects in all 3 vaccine groups combined, 84% were white, 7% were Hispanic, 6% were black, 0.7% were Oriental, and 2.4% were of other racial/ethnic groups.

The immune responses to the pertussis (PT, FHA, and pertactin), diphtheria, tetanus, poliovirus, and hepatitis B antigens were evaluated in sera obtained 1 month (range: 20 to 60 days) after the third dose of PEDIARIX or INFANRIX. Geometric mean antibody concentrations (GMCs) adjusted for pre-vaccination values for PT, FHA, and pertactin and the seroprotection rates for diphtheria, tetanus, and the polioviruses among subjects who received PEDIARIX in the combination vaccine group were shown to be non-inferior to those achieved following separately administered vaccines (Table 3).

Because of differences in the hepatitis B vaccination schedule among subjects in the study, no clinical limit for non-inferiority was pre-defined for the hepatitis B immune response. However, in a previous U.S. study, non-inferiority of PEDIARIX relative to separately administered INFANRIX, ENGERIX-B, and an oral poliovirus vaccine, with respect to the hepatitis B immune response was demonstrated.

Table 3. Antibody Responses following PEDIARIX as Compared with Separate Concomitant Administration of INFANRIX, ENGERIX-B, and IPV (1 Month[a] after Administration of Dose 3) in Infants Vaccinated at 2, 4, and 6 Months of Age when Administered Concomitantly with Hib Conjugate Vaccine and Pneumococcal Conjugate Vaccine (PCV7)

Antibody	PEDIARIX, Hib Vaccine, & PCV7 (n = 154-168)	INFANRIX, ENGERIX-B, IPV, Hib Vaccine, & PCV7 (n = 141-155)
Anti-diphtheria Toxoid		
% ≥0.1 IU/mL[b]	99.4	98.7
Anti-tetanus Toxoid		
% ≥0.1 IU/mL[b]	100	98.1
Anti-PT		
% VR[c]	98.7	95.1
GMC[b]	48.1	28.6
Anti-FHA		
% VR[c]	98.7	96.5
GMC[b]	111.9	97.6
Anti-pertactin		
% VR[c]	91.7	95.1
GMC[b]	95.3	80.6
Anti-polio 1		
% ≥1:8[b,d]	100	100
Anti-polio 2		
% ≥1:8[b,d]	100	100
Anti-polio 3		
% ≥1:8[b,d]	100	100
	(n = 114-128)	**(n = 111-121)**
Anti-HBsAg[e]		
% ≥10 mIU/mL[f]	97.7	99.2
GMC (mIU/mL)[f]	1032.1	614.5

Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.; no longer licensed in the United States); PCV7 (Wyeth Pharmaceuticals Inc.); IPV (Sanofi Pasteur SA).

Assay methods used: ELISA for anti-diphtheria, anti-tetanus, anti-PT, anti-FHA, anti-pertactin, and anti-HBsAg; micro-neutralization for anti-polio (1, 2, and 3).

VR = Vaccine response: In initially seronegative infants, appearance of antibodies (concentration ≥5 EL.U./mL); in initially seropositive infants, at least maintenance of pre-vaccination concentration.

GMC = Geometric mean antibody concentration. GMCs are adjusted for pre-vaccination levels.

[a] One-month blood sampling, range: 20 to 60 days.

[b] Seroprotection rate or GMC for PEDIARIX not inferior to separately administered vaccines (upper limit of 90% CI on GMC ratio [separate vaccine group/combination vaccine group] <1.5 for anti-PT, anti-FHA, and anti-pertactin, and upper limit of 95% CI for the difference in seroprotection rates [separate vaccine group minus combination vaccine group] <10% for diphtheria and tetanus and <5% for the 3 polioviruses). GMCs are adjusted for pre-vaccination levels.

[c] The upper limit of 95% CI for differences in vaccine response rates (separate vaccine group minus combination group) was 0.31, 1.52, and 9.46 for PT, FHA, and pertactin, respectively. No clinical limit defined for non-inferiority.

[d] Poliovirus-neutralizing antibody titer.

[e] Subjects who received a previous dose of hepatitis B vaccine were excluded from the analysis of hepatitis B seroprotection rates and GMCs presented in the table.

[f] No clinical limit defined for non-inferiority.

14.3 Concomitant Vaccine Administration

In a U.S. multicenter study *[see Clinical Studies (14.2)]*, there was no evidence for interference with the immune responses to PEDIARIX when administered concomitantly with 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.) relative to 2 weeks prior.

Anti-PRP (Hib polyribosyl-ribitol-phosphate) seroprotection rates and GMCs of pneumococcal antibodies 1 month (range: 20 to 60 days) after the third dose of vaccines for the combination vaccine group and the separate vaccine group from the U.S. multicenter study *[see Clinical Studies (14.2)]*, are presented in Table 4.

Table 4. Anti-PRP Seroprotection Rates and GMCs (mcg/mL) of Pneumococcal Antibodies 1 Month[a] following the Third Dose of Hib Conjugate Vaccine and Pneumococcal Conjugate Vaccine (PCV7) Administered Concomitantly with PEDIARIX or with INFANRIX, ENGERIX-B, and IPV

	PEDIARIX, Hib Vaccine, & PCV7	**INFANRIX, ENGERIX-B, IPV, Hib Vaccine, & PCV7**
	(n = 161-168)	**(n = 146-156)**
	% (95% CI)	**% (95% CI)**
Anti-PRP ≥0.15 mcg/mL	100 (97.8, 100)	99.4 (96.5, 100)
Anti-PRP ≥1.0 mcg/mL	95.8 (91.6, 98.3)	91.0 (85.3, 95.0)
	GMC (95% CI)	**GMC (95% CI)**
Pneumococcal Serotype		
4	1.7 (1.5, 2.0)	2.1 (1.8, 2.4)
6B	0.8 (0.7, 1.0)	0.7 (0.5, 0.9)
9V	1.6 (1.4, 1.8)	1.6 (1.4, 1.9)
14	4.7 (4.0, 5.4)	6.3 (5.4, 7.4)
18C	2.6 (2.3, 3.0)	3.0 (2.5, 3.5)
19F	1.1 (1.0, 1.3)	1.1 (0.9, 1.2)
23F	1.5 (1.2, 1.8)	1.8 (1.5, 2.3)

Hib conjugate vaccine (Wyeth Pharmaceuticals Inc.; no longer licensed in the United States); PCV7 (Wyeth Pharmaceuticals Inc.); IPV (Sanofi Pasteur SA).
Assay method used: ELISA for anti-PRP and 7 pneumococcal serotypes.
GMC = Geometric mean antibody concentration.
[a] One-month blood sampling, range: 20 to 60 days.

15 REFERENCES

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7. Sutter RW, Pallansch MA, Sawyer LA, et al. Defining surrogate serologic tests with respect to predicting protective vaccine efficacy: Poliovirus vaccination. In: Williams JC, Goldenthal KL, Burns DL, Lewis Jr BP, eds. Combined vaccines and simultaneous administration. Current issues and perspectives. New York, NY: The New York Academy of Sciences; 1995:289-299.

16 HOW SUPPLIED/STORAGE AND HANDLING

PEDIARIX is available in 0.5-mL single-dose, disposable, prefilled TIP-LOK syringes (packaged without needles):

NDC 58160-811-43 Syringe in Package of 10: NDC 58160-811-52

Store refrigerated between 2º and 8ºC (36º and 46ºF). Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

Provide the following information to the parent or guardian:

- Inform of the potential benefits and risks of immunization with PEDIARIX, and of the importance of completing the immunization series.
- Inform about the potential for adverse reactions that have been temporally associated with administration of PEDIARIX or other vaccines containing similar components.
- Instruct to report any adverse events to their healthcare provider.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by **GlaxoSmithKline Biologicals**

Rixensart, Belgium, U.S. License 1617, and

GSK Vaccines GmbH

Marburg, Germany, U.S. License 1617

Distributed by **GlaxoSmithKline**

Research Triangle Park, NC 27709

PDX:26PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use VAXELIS safely and effectively. See full prescribing information for VAXELIS.

VAXELIS® (Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Haemophilus b Conjugate and Hepatitis B Vaccine)

Suspension for Intramuscular Injection

Initial U.S. Approval: 2018

----------INDICATIONS AND USAGE----------

VAXELIS is a vaccine indicated for active immunization to prevent diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and invasive disease due to *Haemophilus influenzae* type b. VAXELIS is approved for use as a 3-dose series in children from 6 weeks through 4 years of age (prior to the 5th birthday). (1)

----------DOSAGE AND ADMINISTRATION----------

The 3-dose immunization series consists of a 0.5 mL intramuscular injection, administered at 2, 4, and 6 months of age. (2.1)

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection (0.5 mL dose) available in single-dose vials and prefilled syringes. (3)

----------CONTRAINDICATIONS----------

- Severe allergic reaction (e.g., anaphylaxis) to a previous dose of VAXELIS, any ingredient of VAXELIS, or any other diphtheria toxoid, tetanus toxoid, pertussis-containing vaccine, inactivated poliovirus vaccine, hepatitis B vaccine, or *Haemophilus influenzae* type b vaccine. (4.1)
- Encephalopathy within 7 days of a previous pertussis-containing vaccine with no other identifiable cause. (4.2)
- Progressive neurologic disorder until a treatment regimen has been established and the condition has stabilized. (4.3)

----------WARNINGS AND PRECAUTIONS----------

- Carefully consider benefits and risks before administering VAXELIS to persons with a history of:
  - fever ≥40.5°C (≥105°F), hypotonic-hyporesponsive episode (HHE) or persistent, inconsolable crying lasting ≥3 hours within 48 hours after a previous pertussis-containing vaccine. (5.2)
  - seizures within 3 days after a previous pertussis-containing vaccine. (5.2)
- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following VAXELIS. (5.3)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including VAXELIS, to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.5)
- Urine antigen detection may not have definitive diagnostic value in suspected *H. influenzae* type b disease following vaccination with VAXELIS. (5.7) (7.1)

----------ADVERSE REACTIONS----------

The solicited adverse reactions following any dose were irritability (≥55%), crying (≥45%), injection site pain (≥44%), somnolence (≥40%), injection site erythema (≥25%), decreased appetite (≥23%), fever ≥38.0°C (≥19%), injection site swelling (≥18%), and vomiting (≥9%). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc., at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 and http://vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 07/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

VAXELIS® is a vaccine indicated for active immunization to prevent diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B, and invasive disease due to *Haemophilus influenzae* (*H. influenzae*) type b. VAXELIS is approved for use as a 3-dose series in children 6 weeks through 4 years of age (prior to the 5th birthday).

2 DOSAGE AND ADMINISTRATION

For intramuscular use only.

2.1 Vaccination Schedule

VAXELIS is to be administered as a 3-dose series at 2, 4, and 6 months of age. The first dose may be given as early as 6 weeks of age. Three doses of VAXELIS constitute a primary immunization course against diphtheria, tetanus, *H. influenzae* type b invasive disease and poliomyelitis.

VAXELIS may be used to complete the hepatitis B immunization series.

A 3-dose series of VAXELIS does not constitute a primary immunization series against pertussis; an additional dose of pertussis-containing vaccine is needed to complete the primary series. [See *Pertussis Vaccination Following VAXELIS.*]

Pertussis Vaccination following VAXELIS

VAXELIS, Pentacel® [(Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate (Tetanus Toxoid Conjugate) Vaccine): DTaP-IPV/Hib], Quadracel® [(Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine): DTaP-IPV] and DAPTACEL® [(Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed): DTaP] contain the same pertussis antigens manufactured by the same process. Children who have received a 3-dose series of VAXELIS should complete the primary and pertussis vaccination series with Pentacel, Quadracel or DAPTACEL according to the respective prescribing information in the approved package inserts. [See *ADVERSE REACTIONS (6.1) AND CLINICAL STUDIES (14).]*

Administration of VAXELIS following previous doses of other DTaP-containing Vaccines

VAXELIS may be used to complete the first 3 doses of the 5-dose DTaP series in infants and children who have received 1 or 2 doses of Pentacel or DAPTACEL and are also scheduled to receive the other antigens in VAXELIS. Data are not available on the safety and immunogenicity of such mixed sequences.

Data are not available on the safety and effectiveness of using VAXELIS following 1 or 2 doses of a DTaP vaccine from a different manufacturer.

Administration of VAXELIS following previous doses of any Hepatitis B Vaccine

A 3-dose series of VAXELIS may be administered to infants born to HBsAg-negative mothers, and who have received a dose of any hepatitis B vaccine, prior to or at 1 month of age. [See *ADVERSE REACTIONS (6.1) AND CLINICAL STUDIES (14)*.]

VAXELIS may be used to complete the hepatitis B vaccination series following 1 or 2 doses of other hepatitis B vaccines, in infants and children born of HBsAg-negative mothers and who are also scheduled to receive the other antigens in VAXELIS. However, data are not available on the safety and effectiveness of VAXELIS in such infants and children.

Administration of VAXELIS following previous doses of Inactivated Polio Vaccine (IPV)

VAXELIS may be administered to infants and children who have received 1 or 2 doses of IPV and are also scheduled to receive the other antigens in VAXELIS. However, data are not available on the safety and effectiveness of VAXELIS in such infants and children.

Administration of VAXELIS following previous doses of Haemophilus b Conjugate Vaccines

VAXELIS may be administered to infants and children who have received 1 or 2 doses of *H. influenzae* type b Conjugate Vaccine and are also scheduled to receive the other antigens in VAXELIS. However, data are not available on the safety and effectiveness of VAXELIS in such infants and children.

2.2 Administration

Just before use, shake the vial or syringe until a uniform, white, cloudy suspension results.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exist, the product should not be administered.

Administer a single 0.5 mL dose of VAXELIS intramuscularly.

In infants younger than 1 year, the anterolateral aspect of the thigh is the preferred site of injection. The vaccine should not be injected into the gluteal area.

VAXELIS should not be combined through reconstitution or mixed with any other vaccine. Discard unused portion.

3 DOSAGE FORMS AND STRENGTHS

VAXELIS is a suspension for injection available in 0.5 mL single-dose vials and prefilled syringes. [See *HOW SUPPLIED/STORAGE AND HANDLING (16)*.]

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Do not administer VAXELIS to anyone with a history of a severe allergic reaction (e.g., anaphylaxis) to a previous dose of VAXELIS, any ingredient of VAXELIS, or any other diphtheria toxoid, tetanus toxoid, pertussis-containing vaccine, inactivated poliovirus vaccine, hepatitis B vaccine, or *H. influenzae* type b vaccine [See *DESCRIPTION (11)*.]

4.2 Encephalopathy

Do not administer VAXELIS to anyone with a history of encephalopathy (e.g., coma, decreased level of consciousness, prolonged seizures) within 7 days of a previous dose of a pertussis-containing vaccine, that is not attributable to another identifiable cause.

4.3 Progressive Neurologic Disorder

Do not administer VAXELIS to anyone with a history of progressive neurologic disorder, including infantile spasms, uncontrolled epilepsy, or progressive encephalopathy until a treatment regimen has been established and the condition has stabilized.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

5.2 Adverse Reactions Following Prior Pertussis Vaccination

If any of the following events occur after administration of a pertussis vaccine, the decision to administer VAXELIS should be based on careful consideration of potential benefits and possible risks.

- Temperature of ≥40.5°C (≥105°F) within 48 hours, not attributable to another identifiable cause.
- Collapse or shock-like state (hypotonic-hyporesponsive episode [HHE]) within 48 hours.
- Persistent, inconsolable crying lasting ≥3 hours within 48 hours.
- Seizures with or without fever within 3 days.

5.3 Guillain-Barré Syndrome and Brachial Neuritis

A review by the Institute of Medicine (IOM) found evidence for a causal relation between tetanus toxoid and both brachial neuritis and Guillain-Barré syndrome. If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following VAXELIS. (1)

5.4 Altered Immunocompetence

If VAXELIS is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained.

5.5 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. The decision about when to administer an intramuscular vaccine, including VAXELIS, to an infant born prematurely should be based on consideration of the infant's medical status and the potential benefits and possible risks of vaccination.

5.6 Limitations of Vaccine Effectiveness

Vaccination with VAXELIS may not protect all individuals.

5.7 Interference with Laboratory Tests

Urine antigen detection may not have definitive diagnostic value in suspected *H. influenzae* type b disease following vaccination with VAXELIS. [See *DRUG INTERACTIONS (7.1).*]

6 ADVERSE REACTIONS

Rates of adverse reactions varied by number of doses of VAXELIS received. The solicited adverse reactions 0-5 days following any dose were irritability (≥55%), crying (≥45%), injection site pain (≥44%), somnolence (≥40%), injection site erythema (≥25%), decreased appetite (≥23%), fever ≥38.0°C (≥19%), injection site swelling (≥18%), and vomiting (≥9%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events.

The safety of VAXELIS was evaluated in 6 clinical studies, in which a total of 5,251 infants 43 to 99 days of age at enrollment received at least 1 dose of VAXELIS. Two of these (study 005 and 006) were controlled clinical studies conducted in the US, in which a total of 3,380 infants 46 to 89 days of age at enrollment received at least 1 dose of VAXELIS. The vaccination schedules of VAXELIS, Control vaccines, and concomitantly administered vaccines used in these studies are provided in Table 1. At 15 months of age, participants in Study 005 received a dose of DAPTACEL and a *H. influenzae* type b conjugate vaccine, whereas participants in Study 006 received a dose of Pentacel. In a non-US study, 294 children received a dose of VAXELIS at 15 months of age.

Across the 2 studies conducted in the US, among all randomized participants (3,392 in the VAXELIS group and 889 in the Control group), 52.6% were male and 47.4% were female. The race distribution was as follows: 71.7% were White, 11.0% were Black, 4.5% were American Indian or Alaska Native, 3.5% were Asian, and 9.3% were of other racial groups. Most participants (81.8%) were of non-Hispanic or Latino ethnicity. The racial/ethnic distribution of participants who received VAXELIS and Control vaccines was similar.

Table 1: Clinical Safety Studies with VAXELIS in the US: Vaccination Schedules

Study	Vaccine	Concomitantly Administered Vaccines
005*	VAXELIS at 2, 4, 6 months and DAPTACEL + PedvaxHIB® at 15 months	RotaTeq® at 2, 4, and 6 months Prevnar 13® at 2, 4, 6, and 15 months
	Control group vaccines: Pentacel at 2, 4, 6 months and RECOMBIVAX HB® at 2 and 6 months DAPTACEL+ ActHIB® at 15 months	RotaTeq at 2, 4, and 6 months Prevnar 13 at 2, 4, 6, and 15 months
006*	VAXELIS at 2, 4, 6 months and Pentacel at 15 months	RotaTeq at 2, 4, and 6 months Prevnar 13 at 2, 4, 6, and 15 months
	Control group vaccines: Pentacel at 2, 4, 6, and 15 months RECOMBIVAX HB at 2 and 6 months	RotaTeq at 2, 4, and 6 months Prevnar 13 at 2, 4, 6, and 15 months

Prevnar 13 (Pneumococcal 13-valent Conjugate Vaccine [Diphtheria CRM197 Protein])
RotaTeq (Rotavirus Vaccine, Live, Oral, Pentavalent)
PedvaxHIB [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)]
RECOMBIVAX HB (Hepatitis B Vaccine [Recombinant])
* The first dose of Hepatitis B vaccine was administered prior to study initiation (prior to or at 1 month of age).

Solicited Adverse Reactions

Information on solicited adverse events was recorded daily by parents or guardians on vaccination report cards. The incidence and severity of solicited injection site and systemic adverse reactions (i.e., vaccine-related adverse events) that occurred within 5 days following each dose of VAXELIS or Control vaccines at 2, 4, and 6 months of age in studies 005 and 006 are shown in Table 2.

Table 2: Percentage of Infants with Solicited Adverse Reactions Occurring within 5 days Following VAXELIS or Control Vaccines Administered Concomitantly at Separate Sites with Prevnar 13 and RotaTeq in Studies 005 and 006

		VAXELIS + Prevnar 13 + RotaTeq			Pentacel + RECOMBIVAX HB + Prevnar 13 + RotaTeq		
		Dose 1 (N=3,370) (%)	Dose 2 (N=3,221) (%)	Dose 3 (N=3,134) (%)	Dose 1 (N=880) (%)	Dose 2 (N=849) (%)	Dose 3 (N=825) (%)
Injection Site Adverse Reactions		**VAXELIS site**			**Pentacel or RECOMBIVAX HB site**		
Injection site erythema	Any	25.8	31.8	31.8	25.0	25.8	30.9
	≥2.5 cm	0.9	1.0	1.3	1.1	1.1	1.2
	>5.0 cm	0.0	0.1	0.2	0.3	0.2	0.1
Injection site pain*	Any	53.3	49.0	44.9	55.8	43.7	44.4
	Moderate or severe	16.3	14.1	12.5	19.1	11.3	10.8
	Severe	2.8	2.5	2.0	3.2	1.9	1.3
Injection site swelling	Any	18.9	22.8	23.4	20.8	20.4	22.9
	≥2.5 cm	2.5	1.6	1.7	2.7	1.3	0.8
	>5.0 cm	0.2	0.2	0.2	0.3	0.1	0.0
Systemic Adverse Reactions							
Fever	≥38°C	19.2	29.0	29.3	14.6	18.0	17.8
	≥38.5°C	5.3	11.5	13.2	3.4	6.5	8.1
	≥39.5°C	0.2	0.7	1.5	0.1	0.2	0.9
Crying	Any	52.0	49.5	45.1	50.6	47.0	40.6
	>1 hour	18.6	19.8	16.7	20.6	16.8	14.1
	>3 hours	3.6	3.8	3.4	4.4	4.0	2.9
Decreased Appetite†	Any	28.9	24.2	23.2	25.8	20.5	20.1
	Moderate or severe	7.0	5.5	4.8	6.8	3.9	5.0
	Severe	0.5	0.5	0.5	0.6	0.2	0.0
Irritability‡	Any	61.8	58.9	55.2	61.7	56.3	51.6
	Moderate or severe	24.6	23.4	20.1	25.7	19.2	16.8
	Severe	2.5	3.8	2.9	2.2	2.7	2.2

		VAXELIS + Prevnar 13 + RotaTeq			Pentacel + RECOMBIVAX HB + Prevnar 13 + RotaTeq		
		Dose 1 (N=3,370) (%)	Dose 2 (N=3,221) (%)	Dose 3 (N=3,134) (%)	Dose 1 (N=880) (%)	Dose 2 (N=849) (%)	Dose 3 (N=825) (%)
Somnolence§	Any	56.3	47.8	40.8	55.2	44.1	38.8
	Moderate or severe	15.0	11.5	8.5	14.5	9.4	8.2
	Severe	1.5	1.1	1.0	1.7	0.6	1.1
Vomiting¶	Any	13.1	11.5	9.5	11.3	9.7	6.9
	Moderate or severe	3.5	2.6	2.1	2.8	3.1	1.0
	Severe	0.4	0.2	0.1	0.5	0.6	0.1

N = Number of vaccinated participants with safety follow-up.

* Moderate: cries and protests when injection site is touched; Severe: cries when injected limb is moved or the movement of the injected limb is reduced.
† Moderate: missed 1 or 2 feeds/meals completely; Severe: refuses ≥3 feeds or refuses most feeds.
‡ Moderate: requiring increased attention; Severe: inconsolable.
§ Moderate: not interested in surroundings or did not wake up for a meal; Severe: Sleeping most of the time or difficult to wake up.
¶ Moderate: 2-5 episodes per 24 hours; Severe: ≥6 episodes per 24 hours or requiring parenteral hydration.
A subject with the same adverse reactions at both the Pentacel and RECOMBIVAX HB injection site, was counted once and was classified according to the highest intensity grading.
Fever is based upon actual temperatures recorded with no adjustments due to the measurement route. Following Doses 1-3 combined, the proportion of temperature measurements that were taken by rectal, axillary, or other routes were 91.7%, 8.1%, and 0% respectively, for VAXELIS group, and 90.3%, 9.7%, and 0%, respectively, for Pentacel + RECOMBIVAX HB vaccines group.

Non-fatal Serious Adverse Events

Across Studies 005 and 006, within 30 days following any infant dose vaccination, 68 participants (2.0%) who received VAXELIS and concomitant vaccines versus 19 participants (2.2%) who received Control and concomitant vaccines experienced a serious adverse event. Of these, a vaccine-related SAE was reported for no participants in the Control vaccines group and for 4 participants (0.1%) in the VAXELIS group:

- 3 of these 4 experienced pyrexia 1 to 2 days following the first study vaccinations; and
- 1 of these 4 experienced an apparent life-threatening event (vomiting followed by pallor and lethargy) on the day of the first study vaccinations, and again 2 days later.

Deaths

In the 2 US studies, death was reported in 6 participants (0.2%) who received VAXELIS and in 1 participant (0.1%) who received Pentacel + RECOMBIVAX HB vaccines; none were assessed as vaccine related. Causes of death among infants who received VAXELIS were asphyxia, hydrocephalus, unknown cause, sepsis and 2 cases of Sudden Infant Death Syndrome (occurring 1, 2, 10, 42, 44 and 49 days post-vaccination, respectively). Across all 6 clinical studies, there were no deaths assessed as related to VAXELIS.

6.2 Postmarketing Experience

The following adverse events have been reported during post-marketing use of VAXELIS or other vaccines containing the antigens of VAXELIS. These adverse events are included based on a suspected causal connection to VAXELIS or the components of DAPTACEL® (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed), IPOL® (Poliovirus Vaccine Inactivated), COMVAX® [Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine].

Because these events are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to vaccination.

- ***Immune System Disorders***
  Hypersensitivity (such as rash, urticaria, dyspnea, erythema multiforme), anaphylactic reaction (such as urticaria, angioedema, edema, face edema, shock).
- ***General Disorders and Administration Site Conditions***
  Extensive swelling of injected limb (including swelling that involves adjacent joints).
- ***Nervous System***
  Seizure, febrile seizure, hypotonic-hyporesponsive episode (HHE).

7 DRUG INTERACTIONS

7.1 Interference with Laboratory Tests

Sensitive tests (e.g., Latex Agglutination kits) have detected vaccine-derived polyribosylribitol phosphate (PRP) in the urine of vaccinees for at least 30 days following vaccination with PedvaxHIB [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)]. (2) Therefore, urine antigen detection may not have definite diagnostic value in suspected *H. influenzae* type b disease following vaccination with VAXELIS. [See *WARNINGS AND PRECAUTIONS (5.7).*]

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

VAXELIS is not approved for use in individuals 5 years of age and older. No human or animal data are available to assess vaccine-associated risks in pregnancy.

8.2 Lactation

VAXELIS is not approved for use in individuals 5 years of age and older. No human or animal data are available to assess the impact of VAXELIS on milk production, its presence in breast milk, or its effects on the breastfed infant.

8.4 Pediatric Use

The safety of VAXELIS has been established in the age group 6 weeks through 15 months, and the effectiveness of VAXELIS was established in the age group 6 weeks through 6 months on the basis of clinical studies. [See *ADVERSE REACTIONS (6.1) AND CLINICAL STUDIES (14).*]

The safety and effectiveness of VAXELIS in older children through 4 years of age are supported by evidence in younger children. The safety and effectiveness of VAXELIS in infants less than 6 weeks of age and in children and adolescents 5 through 17 years of age have not been established.

11 DESCRIPTION

VAXELIS (Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Haemophilus b Conjugate and Hepatitis B Vaccine) is a sterile suspension for intramuscular injection.

Each 0.5 mL dose is formulated to contain 15 Lf diphtheria toxoid, 5 Lf tetanus toxoid, acellular pertussis antigens [20 mcg detoxified pertussis toxin (PT), 20 mcg filamentous hemagglutinin (FHA), 3 mcg pertactin (PRN), 5 mcg fimbriae types 2 and 3 (FIM)], inactivated polioviruses [29 D-antigen units (DU) Type 1 (Mahoney), 7 DU Type 2 (MEF-1), 26 DU Type 3 (Saukett)], 3 mcg polyribosylribitol phosphate (PRP) of *H. influenzae* type b covalently bound to 50 mcg of the outer membrane protein complex (OMPC) of *Neisseria meningitidis* serogroup B, and 10 mcg hepatitis B surface antigen (HBsAg). Each 0.5 mL dose contains 319 mcg aluminum from aluminum salts used as adjuvants.

Other ingredients per 0.5 mL dose include <0.0056% polysorbate 80 and the following residuals from the manufacturing process: ≤14 mcg formaldehyde, ≤50 ng glutaraldehyde, ≤50 ng bovine serum albumin, <5 ng of neomycin, <200 ng streptomycin sulfate, <25 ng polymyxin B sulfate, ≤0.125 µg ammonium thiocyanate and ≤0.1 mcg yeast protein (maximum 1% relative to HBsAg protein).

Corynebacterium diphtheriae is grown in modified Mueller's growth medium. (3) After purification by ammonium sulfate fractionation, the diphtheria toxin is detoxified with formaldehyde and diafiltered.

Clostridium tetani is grown in modified Mueller-Miller casamino acid medium without beef heart infusion. (4) Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

The acellular pertussis vaccine antigens are produced from *Bordetella pertussis* cultures grown in Stainer-Scholte medium (5) modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. FIM are extracted and copurified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT is detoxified with glutaraldehyde. FHA is treated with formaldehyde and the residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed separately onto aluminum phosphate.

The Type 1, Type 2, and Type 3 polioviruses are individually grown in Vero cells. The viral harvests are concentrated and purified, then inactivated with formaldehyde to produce

monovalent suspensions of each serotype. Specified quantities of monovalent suspensions of each serotype are mixed to produce the trivalent poliovirus concentrate.

The HBsAg antigen is harvested and purified from fermentation cultures of a recombinant strain of the yeast *Saccharomyces cerevisiae* containing the gene for the *adw* subtype of HBsAg. The recombinant *Saccharomyces cerevisiae* is grown in a fermentation medium which consists of an extract of yeast, soy peptone, dextrose, amino acids, and mineral salts. The HBsAg protein is released from the yeast cells by cell disruption and purified by a series of physical and chemical methods which includes ion and hydrophobic chromatography, and diafiltration. The purified protein is treated in phosphate buffer with formaldehyde and then co-precipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate.

The purified PRP of *H. influenzae* type b (Haemophilus b, Ross strain) is conjugated to an OMPC of the B11 strain of *N. meningitidis* serogroup B. *H. influenzae* type b is grown in a fermentation medium which includes an extract of yeast, nicotinamide adenine dinucleotide, hemin chloride, soy peptone, dextrose, and mineral salts. The PRP is purified from the culture broth by purification procedures which include ethanol fractionation, enzyme digestion, phenol extraction and diafiltration. *N. meningitidis* serogroup B is grown in a fermentation medium which includes an extract of yeast, amino acids and mineral salts. The OMPC is purified by detergent extraction, ultracentrifugation, diafiltration and sterile filtration. PRP is conjugated to OMPC by chemical coupling and the PRP-OMPC is then adsorbed onto an amorphous aluminum hydroxyphosphate sulfate adjuvant.

The adsorbed diphtheria, tetanus, and acellular pertussis antigens are combined with aluminum phosphate (as adjuvant) and water for injection into an intermediate concentrate. The individual HBsAg and PRP-OMPC adjuvanted bulks are added followed by the trivalent poliovirus concentrate, to produce VAXELIS.

Both diphtheria and tetanus toxoids induce at least 2 neutralizing units per mL of serum in the guinea pig potency test. The potency of the acellular pertussis antigens is evaluated by the antibody response of immunized mice to detoxified PT, FHA, PRN and FIM as measured by enzyme-linked immunosorbent assay (ELISA). The immunogenicity of the inactivated polioviruses is evaluated by the antibody response in rats measured by virus neutralization. The potency of the HBsAg component is measured relative to a standard by an *in vitro* immunoassay. The potency of the PRP-OMPC component is measured by quantitating the polysaccharide concentration using an HPLC method.

VAXELIS does not contain a preservative. The vial stopper, syringe plunger stopper, and syringe tip cap are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of

protection. Antitoxin levels of ≥0.1 IU/mL are generally regarded as protective. (6) Levels of 1.0 IU/mL have been associated with long-term protection. (7)

Tetanus

Tetanus is an acute disease caused by an extremely potent neurotoxin produced by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of ≥0.01 IU/mL, measured by neutralization assay is considered the minimum protective level. (6) (8) A tetanus antitoxoid level ≥0.1 IU/mL as measured by the ELISA used in clinical studies of VAXELIS is considered protective.

Pertussis

Pertussis (whooping cough) is a respiratory disease caused by *B. pertussis*. This Gram-negative coccobacillus produces a variety of biologically active components, though their role in either the pathogenesis of, or immunity to, pertussis has not been clearly defined.

Poliomyelitis

Polioviruses, of which there are 3 serotypes (Types 1, 2, and 3), are enteroviruses. The presence of poliovirus type-specific neutralizing antibodies has been correlated with protection against poliomyelitis. (9)

Hepatitis B

Hepatitis B virus is one of several hepatitis viruses that cause systemic infection, with major pathology in the liver. Antibody concentrations of ≥10 mIU/mL against HBsAg correlate with protection against hepatitis B virus infection.

Haemophilus influenzae type b Invasive Disease

H. influenzae type b can cause invasive disease such as meningitis and sepsis. Anti-PRP antibody has been shown to correlate with protection against invasive disease due to *H. influenzae* type b.

Based on data from passive antibody studies (10) and an efficacy study with *H. influenzae* type b polysaccharide vaccine in Finland, (11) a post-vaccination anti-PRP level of ≥0.15 mcg/mL is considered a minimal protective level. Data from an efficacy study with *H. influenzae* type b polysaccharide vaccine in Finland indicate that an anti-PRP level of ≥1.0 mcg/mL 3 weeks after vaccination predicts protection through a subsequent 1-year period. (11) (12) These levels have been used to evaluate the effectiveness of *H. influenzae* type b conjugate vaccines, including the PRP-OMPC component of VAXELIS.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

VAXELIS has not been evaluated for carcinogenic or mutagenic potential or impairment of fertility.

14 CLINICAL STUDIES

14.1 Effectiveness of VAXELIS

The effectiveness of VAXELIS is based on the immunogenicity of the individual antigens compared to US licensed vaccines. Serological correlates of protection exist for diphtheria,

tetanus, hepatitis B, poliomyelitis, and invasive disease due to *H. influenzae* type b. The effectiveness against pertussis is based upon the pertussis immune responses following 3 doses of VAXELIS compared to 3 doses of Pentacel, as well as the pertussis immune responses following a subsequent dose of DAPTACEL in the same 2 groups of children. VAXELIS, Pentacel and DAPTACEL contain the same pertussis antigens, manufactured by the same processes.

14.2 Immunogenicity

In the US Study 005 (Table 1), infants were randomized to receive 3 doses of VAXELIS at 2, 4, and 6 months of age and DAPTACEL and PedvaxHIB at 15 months of age, or Control group vaccines (3 doses of Pentacel vaccine at 2, 4, and 6 months of age + RECOMBIVAX HB at 2 and 6 months of age and DAPTACEL and ActHIB at 15 months of age). All subjects received concomitant vaccines: RotaTeq at 2, 4 and 6 months and Prevnar 13 at 2, 4, 6, and 15 months of age. [See *ADVERSE REACTIONS (6.1)*.] All infants had received a dose of hepatitis B vaccine prior to study initiation, prior to or at one month of age. Among all randomized participants, 53.0% were male and 47.0% were female. Most (79.2%) participants were White, 14.1% were Black and 5.2% were multi-racial. Most (91.4%) participants were of non-Hispanic or non-Latin ethnicity.

Antibody responses to diphtheria, tetanus, pertussis (PT, FHA, PRN and FIM), poliovirus types 1, 2 and 3, hepatitis B and *H. influenzae* type b antigens were measured in sera obtained one month following the third dose of VAXELIS or Pentacel + RECOMBIVAX HB vaccines. VAXELIS was non-inferior to Pentacel + RECOMBIVAX HB administered concomitantly at separate sites, as demonstrated by the proportions of participants achieving seroprotective levels of antibodies to diphtheria, tetanus, poliovirus, hepatitis B and PRP antigens, and pertussis vaccine response rates and GMCs (except FHA), following 3 doses of the vaccine. See Table 3.

To complete the 4-dose pertussis primary vaccination series, participants in both groups received DAPTACEL at 15 months of age and were evaluated for immune responses to pertussis antigens one month later. The non-inferiority criteria for vaccine response rates and GMCs for all pertussis antigens were met following the fourth dose.

Table 3: Antibody Responses One Month Following Dose 3 of VAXELIS or Control Vaccines Administered Concomitantly with Prevnar 13 and RotaTeq in Study 005

	VAXELIS + Prevnar 13 + RotaTeq (N=688 - 810)	Pentacel + RECOMBIVAX HB + Prevnar 13 + RotaTeq (N=353 - 400)
Anti-Diphtheria Toxoid		
% ≥0.1 IU/mL	82.4*	86.3
Anti-Tetanus Toxoid		
% ≥0.1 IU/mL	99.9†	99.5
Anti-PT		
% vaccine response‡	98.1*	98.5
GMC	109.6§	85.4
Anti-FHA		
% vaccine response‡	87.3*	92.0
GMC	46.6¶	72.3
Anti-PRN		
% vaccine response‡	79.3*	82.0
GMC	55.8§	66.8
Anti-FIM		
% vaccine response‡	90.2*	86.2
GMC	235.9§	184.4
Anti-Poliovirus Type 1		
% ≥1:8 dilution	100.0†	98.2
Anti-Poliovirus Type 2		
% ≥1:8 dilution	100.0†	99.7
Anti-Poliovirus Type 3		
% ≥1:8 dilution	100.0†	99.8
Anti-PRP		
% ≥0.15 μg/mL	97.3†	92.4
% ≥1.0 μg/mL	85.0*	75.3
Anti-HBsAg		
% ≥10 mIU/mL	99.4*	98.6

N = The number of participants with available data.

* Non-inferiority criterion met (lower bound of 2-sided 95% CI for the difference [VAXELIS group minus Control vaccines group] was >-10%).

† Non-inferiority criterion met (lower bound of 2-sided 95% CI for the difference [VAXELIS group minus Control vaccines group] was >-5%).

‡ Vaccine response = if pre-vaccination antibody concentration was <4 x lower limit of quantitation [LLOQ], then the post-vaccination antibody concentration was ≥4 x LLOQ; if pre-vaccination antibody concentration was ≥4 x LLOQ, then the post-vaccination antibody concentration was ≥pre-vaccination levels (pre-Dose 1).

§ Non-inferiority criterion met (lower bound of 2-sided 95% CI for the GMC ratio [VAXELIS group/Control vaccines group] was >0.67).

¶ Non-inferiority criterion not met for anti-FHA GMC (lower bound of 2-sided 95% CI for the GMC ratio [VAXELIS group/Control vaccines group was 0.59 which is below the non-inferiority criterion >0.67).

Study 006 (Table 1) was a lot consistency study conducted in the US, where infants were randomized to receive 3 doses of VAXELIS at 2, 4, and 6 months of age and Pentacel at 15 months of age (N=2,406), or control group vaccines (4 doses of Pentacel at 2, 4, 6, and 15 months of age + RECOMBIVAX HB at 2 and 6 months of age; N=402). All subjects received concomitant vaccines: RotaTeq at 2, 4 and 6 months and Prevnar 13 at 2, 4, 6, and 15 months of age. All infants had received a dose of hepatitis B vaccine prior to study initiation, from birth up to one month of age.

Antibody responses to diphtheria, tetanus, pertussis (PT, FHA, PRN and FIM), poliovirus types 1, 2 and 3, hepatitis B and *H. influenzae* type b antigens were measured in sera obtained one month following the third dose of VAXELIS or Pentacel + RECOMBIVAX HB. VAXELIS was non-inferior to Pentacel + RECOMBIVAX HB administered concomitantly at separate sites, as demonstrated by the proportions of participants achieving seroprotective levels of antibodies to diphtheria, tetanus, poliovirus, hepatitis B and PRP antigens, and pertussis vaccine response rates and GMCs, except for GMCs for FHA (lower bound of 2-sided 95% CI for GMC ratio [VAXELIS group/Control group vaccines] was 0.62, which was below the non-inferiority criterion >0.67).

To complete the 4-dose pertussis primary vaccination series, participants in both groups received Pentacel at 15 months of age and were evaluated for immune responses to pertussis antigens one month later. The non-inferiority criteria for antibody vaccine response rates and GMCs for all pertussis antigens were met following the fourth dose except for GMCs for PRN (lower bound of 2-sided 95% CI for GMC ratio [VAXELIS group/Control group vaccines] was 0.66, which was below the non-inferiority criterion >0.67).

14.3 Concomitantly Administered Vaccines

In Study 006 conducted in the US (Table 1), the immune responses to Prevnar 13 were measured one month after the third dose. Non-inferiority criteria were met for GMCs to 12 of the 13 serotype antigens in Prevnar 13 for participants who received VAXELIS relative to Control vaccines. For serotype 6B, the non-inferiority criterion was not met (lower bound of 2-sided 95% CI for GMC ratio [VAXELIS group/Control vaccines group] is 0.64, which is below the non-inferiority criterion >0.67).

15 REFERENCES

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16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Single-dose vial (NDC 63361-243-58) in packages of 10 vials (NDC 63361-243-10).

Single-dose, prefilled syringe with Luer lock connection and a tip cap, without needle, 0.5 mL (NDC 63361-243-88). Supplied as package of 10 (NDC 63361-243-15).

The vial stopper, syringe plunger stopper, and syringe tip cap are not made with natural rubber latex.

16.2 Storage and Handling

VAXELIS should be stored at 2°C to 8°C (36°F to 46°F). **Do not freeze**. Product which has been exposed to freezing should not be used. Protect from light. Do not use after expiration date shown on the label. Discard unused portion.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Inform the parent or guardian of the following:

- The potential benefits and risks of immunization with VAXELIS.
- The common adverse reactions that have occurred following administration of VAXELIS or other vaccines containing similar ingredients.
- Other adverse reactions can occur. Call healthcare provider with any adverse reactions of concern.

Provide the Vaccine Information Statements (VIS), which are required by the National Childhood Vaccine Injury Act of 1986.

Manufactured by:

Sanofi Pasteur Limited
Toronto Ontario Canada

for:

MSP Vaccine Company

Swiftwater PA 18370 USA

Distributed by:

Merck Sharp & Dohme LLC

A subsidiary of **Merck & Co., Inc.**

Rahway NJ 07065 USA

and **Sanofi Pasteur Inc.**

Swiftwater PA 18370 USA

R8-0722 USA

Patient Information

VAXELIS® (pronounced "vak-sel-lis")

(Diphtheria and Tetanus Toxoids and Acellular Pertussis, Inactivated Poliovirus, Haemophilus b Conjugate and Hepatitis B Vaccine)

Before your child gets VAXELIS, read this document and be sure you understand all of the information. Keep this document, you may need to read it again. If you have questions or side effects, ask your child's healthcare provider. This information does not take the place of talking about VAXELIS with your child's healthcare provider.

What is VAXELIS?

VAXELIS is a vaccine given to protect your child from getting diphtheria, tetanus (lockjaw), pertussis (whooping cough), polio, Hib (*Haemophilus influenzae* type b), and hepatitis B.

Your child cannot get any of these diseases from VAXELIS.

VAXELIS may not completely protect your child from these diseases.

Who should not get VAXELIS?

Your child should not get VAXELIS, if your child:

- is allergic to any of the ingredients.
- had an allergic reaction to any prior shot for diphtheria, tetanus, pertussis, polio, Hib, or hepatitis B.
- had a serious reaction affecting their brain and nervous system after a whooping cough shot.
- has a brain and nerve illness that is getting worse.

Before your child gets VAXELIS, tell your healthcare provider if your child:

- had problems with any shots for these diseases.
- is taking steroids, getting treatment for cancer, or has another problem that weakens the immune system.

How is VAXELIS given?

- VAXELIS is given to children from 6 weeks through 4 years of age (up to 5th birthday).
- Your child will need 3 shots:
    - one shot at 2 months old, **and**
    - one shot at 4 months old, **and**
    - one shot at 6 months old
- If your child misses a shot of VAXELIS, your healthcare provider may suggest a catch-up schedule.
- Your child may get VAXELIS at the same time they get other vaccines.

What are the most common side effects of VAXELIS?

- pain, redness, or swelling where the shot was given
- fever (100.4°F or higher)
- crying more than usual

- eating less than usual
- fussy more than usual
- sleepy more than usual
- throwing up

There may be other side effects that are not listed. If your child has any side effects that worry you or seem to get worse, tell your child's healthcare provider right away.

You may report any side effects directly to the Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967 or *http://vaers.hhs.gov*, or contact Sanofi Pasteur Inc., at 1-800-822-2463 (1-800-VACCINE).

To learn more about VAXELIS, ask your healthcare provider. You can also find the Full Prescribing Information written for doctors at www.fda.gov/media/119465/download.

What is in VAXELIS?

- Active ingredients: inactivated bacteria of diphtheria, tetanus, pertussis, Hib, and inactivated hepatitis B and polio viruses. The bacteria and viruses in VAXELIS are not alive and do not cause disease.
- Other ingredients: aluminum salts, polysorbate 80, glutaraldehyde, formaldehyde, bovine serum albumin, neomycin, streptomycin, polymyxin B, ammonium thiocyanate, yeast protein, and water.
- VAXELIS does not have any preservatives in it.
- VAXELIS vial stopper, syringe plunger stopper, and syringe tip cap do not contain natural rubber latex.

Manufactured by: **Sanofi Pasteur Limited** Toronto Ontario Canada for: MSP Vaccine Company Swiftwater PA 18370 USA.

Distributed by: **Merck Sharp & Dohme LLC,** a subsidiary of **Merck & Co., Inc.** Rahway NJ 07065 USA, and by **Sanofi Pasteur Inc.** Swiftwater PA 18370 USA.

Initial Approval: 23 October 2020

R2-0722

Haemophilus Influenzae Type B (HIB)

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use ActHIB® safely and effectively. See full prescribing information for ActHIB.

ActHIB® [Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)] Solution for Intramuscular Injection

Initial U.S. Approval: 1993

RECENT MAJOR CHANGES

Warnings and Precautions, Syncope (5.7)	7/2022

INDICATIONS AND USAGE

- ActHIB is a vaccine indicated for the prevention of invasive disease caused by *Haemophilus influenzae* type b. ActHIB vaccine is approved for use as a four dose series in infants and children 2 months through 5 years of age (1)

DOSAGE AND ADMINISTRATION

For intramuscular administration only

Four-dose series (0.5 mL each) by intramuscular injection:

- A three-dose primary series administered at 2, 4, and 6 months of age. (2.1)
- A single booster dose administered at 15-18 months of age. (2.1)

DOSAGE FORMS AND STRENGTHS

- Solution for injection: lyophilized powder to be reconstituted in supplied 0.4% Sodium Chloride diluent. A single dose, after reconstitution is 0.5 mL (3)

CONTRAINDICATIONS

- Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any *Haemophilus influenzae* type b or tetanus toxoid-containing vaccine or any component of ActHIB vaccine. (4)

WARNINGS AND PRECAUTIONS

- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the potential benefits and risks of giving ActHIB vaccine must be evaluated. (5.2)

ADVERSE REACTIONS

- Following administration of ActHIB vaccine in children 2-20 months of age, rates of adverse reactions varied by dose number and age of recipients:
  - The most frequent systemic reactions after any dose for children 2 months to 16 months of age were fussiness/irritability (75%), inconsolable crying (58%) and decreased activity/lethargy (51%). (6.1)
  - In children 15-20 months of age tenderness (20%) was the most common local reaction following a single dose. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pharmacovigilance Department, Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION

Revised: 07/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ActHIB® is a vaccine indicated for the prevention of invasive disease caused by *Haemophilus influenzae (H. influenzae)* type b. ActHIB is approved for use in children 2 months through 5 years of age.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only

2.1 Immunization Series

ActHIB vaccine is administered as a four-dose series (0.5 mL per dose) as:

- A primary three-dose series of a single dose at 2, 4, and 6 months of age.
- A single booster dose at 15 through 18 months of age.

2.2 Reconstitution

ActHIB vaccine is a solution for injection supplied as single-dose vials of lyophilized vaccine to be reconstituted only with the accompanying saline diluent (0.4% Sodium Chloride). To reconstitute ActHIB vaccine, withdraw 0.6 mL of saline diluent and inject into the vial of lyophilized ActHIB vaccine. Agitate the vial to ensure complete reconstitution. The reconstituted ActHIB vaccine will appear clear and colorless. Withdraw a 0.5-mL dose of the reconstituted vaccine and inject intramuscularly. After reconstitution, if ActHIB vaccine is not administered promptly store at 2° to 8°C (35° to 46°F) and administer within 24 hours. Stored vaccine should be re-agitated prior to injection. Refer to Figures 1, 2, 3, and 4.

Instructions for Reconstitution of ActHIB Vaccine with Saline Diluent (0.4% Sodium Chloride)

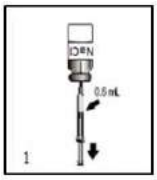

Figure 1. Disinfect the diluent vial stopper, inject the needle and withdraw 0.6 mL of 0.4% Sodium Chloride diluent as indicated.

Figure 2. Cleanse the ActHIB vaccine stopper, insert the syringe needle into the vial, and inject the total volume of diluent.

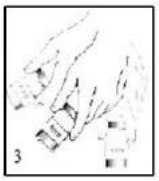

Figure 3. Agitate vial thoroughly.

Figure 4. After reconstitution, withdraw 0.5 mL of reconstituted vaccine and administer **intramuscularly**.

2.3 Administration

Parenteral drug products should be inspected visually for particulate matter and/or discoloration prior to administration, whenever solution and container permit. If either of these conditions exist, the vaccine should not be administered.

ActHIB vaccine is administered as a single dose (0.5 mL) by intramuscular injection into the anterolateral aspect of the thigh or deltoid. Discard unused portion.

Do not administer this product intravenously, intradermally, or subcutaneously.
ActHIB vaccine should not be mixed in the same syringe with other parenteral products.

3 DOSAGE FORMS AND STRENGTHS

ActHIB vaccine is a solution for injection supplied as a single-dose vial of lyophilized powder to be reconstituted with the supplied 0.4% Sodium Chloride diluent. A single dose, after reconstitution is 0.5 mL.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any *H. influenzae* type b or tetanus toxoid-containing vaccine or any component of the vaccine is a contraindication to administration of ActHIB vaccine *[see DESCRIPTION (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine and other appropriate agents must be available should an acute anaphylactic reaction occur.

5.2 Guillain-Barré Syndrome

If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including ActHIB vaccine, should be based on careful consideration of the potential benefits and possible risks.

5.3 Altered Immunocompetence

In immunosuppressed persons, including those receiving immunosuppressive therapy, the expected antibody responses may not be obtained.

5.4 Limitations of Vaccine Effectiveness

Vaccination with ActHIB vaccine may not protect 100% of individuals.

5.5 Tetanus Immunization

Immunization with ActHIB vaccine does not substitute for routine tetanus immunization.

5.6 Interference with Laboratory Tests

Urine antigen detection may not have a diagnostic value in suspected disease due to *H. influenzae* type b within 1 to 2 weeks after receipt of a *H. influenzae* type b-containing vaccine, including ActHIB *[see DRUG INTERACTIONS (7.3)]*.

5.7 Syncope

Syncope (fainting) has been reported following vaccination with ActHIB. Procedures should be in place to avoid injury from fainting.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

More than 7,000 infants and young children (≤2 years of age) have received at least one dose of ActHIB vaccine during US clinical trials. Of these, 1,064 subjects 12 to 24 months of age who received ActHIB vaccine alone reported no serious or life threatening adverse reactions.(1) (2)

Adverse reactions associated with ActHIB vaccine generally subsided after 24 hours and did not persist beyond 48 hours after immunization.

In a US trial, the safety of ActHIB vaccine was evaluated in 110 children 15 to 20 months of age. All children received three doses of *Haemophilus influenzae* type b conjugate vaccine (ActHIB vaccine or a previously licensed Haemophilus b conjugate vaccine) at approximately 2, 4, and 6 months of age. The incidence of selected solicited injection site and systemic adverse reactions which occurred within 48 hours following the dose of ActHIB vaccine is shown in **Table 1**.

Table 1: Local and Systemic Reactions at 6, 24, and 48 Hours Following Immunization with ActHIB Vaccine in Children 15 to 20 months old (2)

Adverse Event	6 Hrs. Post-dose	24 Hrs. Post-dose	48 Hrs. Post-dose
Local (%)	**N=110**	**N=110**	**N=110**
Tenderness	20.0	8.2	0.9
Erythema (>1″)	0.0	0.9	0.0
Induration*	5.5	3.6	0.9
Swelling	3.6	1.8	0.0
Systemic (%)	**N=103-110**	**N=105-110**	**N=104-110**
Fever (>102.2°F) (>39.0°C)	0	1.0	1.9
Irritability	27.3	20.9	12.7
Drowsiness	36.4	17.3	12.7
Anorexia	12.7	10.0	6.4
Vomiting	0.9	0.9	0.9
Persistent cry	0	0	0
Unusual cry	0	0	0

*Induration is defined as hardness with or without swelling.

In a US clinical trial (P3T06), 1,454 children were enrolled and received one dose of ActHIB vaccine at 2 months of age and subsequent doses administered at 4 and 6 months of age (concomitantly with DAPTACEL® [a US-licensed diphtheria, tetanus and pertussis vaccine], IPOL® [a US-licensed inactivated poliovirus vaccine] and PCV7 [Pneumococcal conjugate vaccine, 7-valent]) vaccines at 2, 4, and 6 months of age and hepatitis B vaccine at 2 and 6 months of age). At 15-16 months of age, 418 children received a 4th dose of ActHIB and DAPTACEL vaccines. The most frequent systemic reactions following any dose (>50% of participants) were decreased activity/lethargy, fussiness/irritability, and inconsolable crying.

Table 2: Number (Percentage) of Children with Selected Solicited Systemic Adverse Reactions by Severity Occurring within 0-3 days After Vaccination in Study P3T06

Systemic Reactions	DAPTACEL + IPOL + ActHIB Vaccines			DAPTACEL + ActHIB Vaccines
	Dose 1 N=1,390-1,406 %	Dose 2 N=1,346-1,360 %	Dose 3 N=1,301-1,312 %	Dose 4 N=379-381 %
Fever[*†]				
≥38.0°C	9.3	16.1	15.8	8.7
>38.5°C	1.6	4.3	5.1	3.2
>39.5°C	0.1	0.4	0.3	0.8
Decreased Activity/ Lethargy[‡]				
Any	51.1	37.4	33.2	24.1
Moderate or Severe	24.3	15.8	12.7	9.2
Severe	1.2	1.4	0.6	0.3
Inconsolable Crying				
Any	58.5	51.4	47.9	36.2
≥1 hour	16.4	16.0	12.2	10.5
>3 hours	2.2	3.4	1.4	1.8
Fussiness/ Irritability				
Any	75.8	70.7	67.1	53.8
≥1 hour	33.3	30.5	26.2	19.4
>3 hours	5.6	5.5	4.3	4.5

Note. - Ages of study participants ranged from 1.3 to 19.5 months.

*Fever is based upon actual temperatures recorded with no adjustments to the measurement route.

†Following Doses 1-3 combined, the proportion of temperature measurements that were taken by axillary, rectal or other routes, or not recorded were 44.8%, 54.0%, 1.0%, and 0.1%, respectively. Following Dose 4, the proportion of temperature measurements that were taken by axillary, rectal or other routes, or not recorded were 61.1%, 36.6%, 1.7%, and 0.5%, respectively.

‡Moderate: interferes with or limits usual daily activity; Severe: disabling, not interested in usual daily activity.

In Study P3T06, within 30 days following any of Doses 1-3 of DAPTACEL + IPOL + ActHIB vaccines, 50 of 1,455 (3.4%) participants experienced a serious adverse event (SAE). One SAE of seizure with apnea occurring on the day of vaccination with the first dose of the three vaccines was determined by the investigators as possibly related. Within 30 days following Dose 4, four of 418 (1.0%) participants who received DAPTACEL + ActHIB vaccines experienced a serious adverse event. None was assessed by the investigators as related to the study of vaccines.

6.2 Postmarketing Experience

The following events have been spontaneously reported during the post-approval use of ActHIB vaccine. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

- **Immune system disorders:**
  Anaphylaxis, other allergic/hypersensitivity reactions (including urticaria, angioedema)
- **Nervous system disorders:**
  Convulsions, syncope
- **General disorders and administration site conditions:**
  Extensive limb swelling, peripheral edema, pruritus, rash (including generalized rash)

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

In clinical trials, ActHIB vaccine was administered, at separate sites, concomitantly with one or more of the following vaccines: DTaP; Measles, Mumps and Rubella vaccine (MMR); Hepatitis B vaccine; and Inactivated Poliovirus Vaccine (IPV). No impairment of the antibody response to the individual antigens was demonstrated when ActHIB vaccine was given at the same time but separate sites with these vaccines.(2)

7.2 Immunosuppressive Treatments

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses) may reduce the immune response to ActHIB vaccine *[see WARNINGS AND PRECAUTIONS (5.3)]*.

7.3 Interference with Laboratory Tests

Haemophilus b capsular polysaccharide derived from Haemophilus b Conjugate Vaccines has been detected in the urine of some vaccinees. Urine antigen detection may not have a diagnostic value in suspected disease due to *H. influenzae* type b within 1 to 2 weeks after receipt of a *H. influenzae* type b-containing vaccine, including ActHIB *[see WARNINGS AND PRECAUTIONS (5.6)]*.(3)

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

ActHIB is not approved for use in individuals 6 years of age and older. No human or animal data are available to assess vaccine-associated risks in pregnancy.

8.2 Lactation

ActHIB is not approved for use in individuals 6 years of age and older. Human or animal data are not available to assess the impact of ActHIB on milk production, its presence in breast milk, or its effects on the breastfed infant.

8.4 Pediatric Use

Safety and effectiveness of ActHIB have not been established in infants below the age of 6 weeks and children and adolescents 6 years of age and older *[see DOSAGE AND ADMINISTRATION (2.1)]*.

11 DESCRIPTION

ActHIB vaccine is a sterile, lyophilized powder to be reconstituted with saline diluent (0.4% Sodium Chloride) for intramuscular administration only. The vaccine consists of the *Haemophilus influenzae* type b capsular polysaccharide (polyribosyl-ribitol-phosphate, PRP), a high-molecular-weight polymer prepared from the *H. influenzae* type b strain 1482 grown in a semi-synthetic medium, covalently bound to tetanus toxoid. (4) The lyophilized ActHIB vaccine powder and saline diluent contain no preservative. The tetanus toxoid is prepared by extraction, ammonium sulfate purification, and formalin inactivation of the toxin from cultures of *Clostridium tetani* (Harvard strain) grown in a modified Mueller and Miller medium. (5) The culture medium contains milk-derived raw materials (casein derivatives). Further manufacturing process steps reduce residual formaldehyde to levels below 0.5 micrograms (mcg) per dose by calculation. The toxoid is filtered prior to the conjugation process. In the final formulated vaccine, pH is adjusted using hydrochloric acid. Potency of ActHIB vaccine is specified on each lot by limits on the content of PRP polysaccharide and protein in each dose and the proportion of polysaccharide and protein in the vaccine that is characterized as high molecular weight conjugate.

When ActHIB is reconstituted with saline diluent (0.4% Sodium Chloride), each 0.5-mL dose is formulated to contain 10 mcg of purified capsular polysaccharide conjugated to 24 mcg of inactivated tetanus toxoid and 8.5% of sucrose.

The vial stoppers for ActHIB vaccine and diluent are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Haemophilus influenzae is a gram-negative coccobacillus. Most strains of *H. influenzae* that cause invasive disease (e.g., sepsis and meningitis) are *H. influenzae* type b.

The response to ActHIB vaccine is typical of a T-dependent immune response to antigens. The prominent isotype of anti-capsular PRP antibody induced by ActHIB vaccine is IgG. (6) A booster response for IgG has been demonstrated in children 12 months of age or older who previously received two or three doses of ActHIB vaccine. Bactericidal activity against *H. influenzae* type b was demonstrated in serum after immunization and correlated with the anti-PRP antibody response induced by ActHIB vaccine. (1)

Antibody titers to *H. influenzae* capsular polysaccharide (anti-PRP) of >1.0 mcg/mL following vaccination with unconjugated PRP vaccine correlated with long-term protection against invasive *H. influenzae* type b disease in children older than 24 months of age. (7) Although the relevance of this threshold to clinical protection after immunization with conjugate vaccines is not known, particularly in light of the induced, immunologic memory, this level continues to be considered as indicative of long-term protection. (8) In clinical studies, ActHIB vaccine induced, on average, anti-PRP levels ≥1.0 mcg/mL in 90% of infants after the primary series (2, 4, and 6 months) and in more than 98% of infants following a booster dose given at 15 to 19 months of age. (1)

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

ActHIB vaccine has not been evaluated for its carcinogenic or mutagenic potential or impairment of male fertility.

14 CLINICAL STUDIES

14.1 Immunogenicity of ActHIB Vaccine in Children 2, 4, and 6 Months of Age

Two clinical trials supported by the National Institutes of Health (NIH) have compared the anti-PRP antibody responses to three *Haemophilus influenzae* type b conjugate vaccines in racially mixed populations of children. These studies were done in Tennessee (9) (**Table 3**) and in Minnesota, Missouri, and Texas (10) (**Table 4**) in infants immunized with ActHIB vaccine and other *Haemophilus influenzae* type b conjugate vaccines at 2, 4, and 6 months of age. All *Haemophilus influenzae* type b conjugate vaccines were administered concomitantly with OPV and whole-cell DTP vaccines at separate sites. Neither OPV nor whole-cell DTP vaccines are licensed or distributed in the US currently.

Table 3: Anti-PRP Antibody Responses Following a Two or Three Dose Series of a Haemophilus influenzae type b Vaccine at 2, 4, and 6 Months of Age – Tennessee (9)

Vaccine	N*	Geometric Mean Concentration (GMC) (mcg/mL)			Post Third Immunization % ≥1.0 mcg/mL
		Pre-Immunization at 2 months	Post Second Immunization at 6 months	Post Third Immunization at 7 months	
PRP-T† (ActHIB vaccine)	65	0.10	0.30	3.64	83%
PRP-OMP‡ (PedvaxHIB®)	64	0.11	0.84	N/A	50%§
HbOC¶ (HibTITER®)	61	0.07	0.13	3.08	75%

N/A = Not applicable in this comparison trial although third dose data have been published
*N = Number of children
†*Haemophilus influenzae* type b Conjugate Vaccine (Tetanus Toxoid Conjugate)
‡*Haemophilus influenzae* type b Conjugate Vaccine (Meningococcal Protein Conjugate)
§Seroconversion after the recommended 2-dose primary immunization series is shown
¶*Haemophilus influenzae* type b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate)

Table 4: Anti-PRP Antibody Responses Following a Two or Three Dose Series of a Haemophilus influenzae type b Vaccine at 2, 4, and 6 Months of Age - Minnesota, Missouri, and Texas (10)

Vaccine	N*	Geometric Mean Concentration (GMC) (mcg/mL)			Post Third† Immunization % ≥1.0 mcg/mL
		Pre-Immunization at 2 months	Post Second Immunization at 6 months	Post Third† Immunization at 7 months	
PRP-T‡ (ActHIB vaccine)	142	0.25	1.25	6.37	97%
PRP-OMP§ (PedvaxHIB)	149	0.18	4.00	N/A	85%¶
HbOC# (HibTITER)	167	0.17	0.45	6.31	90%

N/A = Not applicable in this comparison trial although third dose data have been published (10)
*N = Number of children
†Sera were obtained after the third dose from 86 and 110 infants, in PRP-T and HbOC vaccine groups, respectively
‡*Haemophilus influenzae* type b Conjugate Vaccine (Tetanus Toxoid Conjugate)
§*Haemophilus influenzae* type b Conjugate Vaccine (Meningococcal Protein Conjugate)
¶Seroconversion after the recommended 2-dose primary immunization series is shown
#*Haemophilus influenzae* type b Conjugate Vaccine (Diphtheria CRM_{197} Protein Conjugate)

Native American populations have had high rates of *H. influenzae* type b disease and have been observed to have low immune responses to *Haemophilus influenzae* type b conjugate vaccines. In a clinical study enrolling Alaskan Native Americans, following the administration of a three-dose series of ActHIB vaccine at 6 weeks, 4 months, and 6 months of age, 75% of subjects achieved an anti-PRP antibody titer of ≥1.0 mcg/mL at 7 months of age (1 month after the last vaccination). (11)

14.2 Immunogenicity of ActHIB Vaccine in Children 12 to 24 Months of Age

In four separate studies, children 12 to 24 months of age who had not previously received *Haemophilus influenzae* type b conjugate vaccination were immunized with a single dose of ActHIB vaccine (**Table 5**). Geometric Mean Concentration (GMC) of anti-PRP antibody responses were 5.12 mcg/mL (90% responding with ≥1.0 mcg/mL) for children 12 to 15 months of age and 4.4 mcg/mL (82% responding with ≥1.0 mcg/mL) for children 17 to 24 months of age. (2)

Table 5: Anti-PRP Antibody Responses in 12- to 24-month-old Children Immunized with a Single Dose of ActHIB

Age Group	N*	Geometric Mean Concentration (GMC) (mcg/mL)		% Subjects With ≥1.0 mcg/mL	
		Pre-Immunization	Post-Immunization†	Pre-Immunization	Post-Immunization†
12 to 15 months	256	0.06	5.12	1.6	90.2
17 to 24 months	81	0.10	4.40	3.7	81.5

*N = Number of children
†Post immunization responses measured at approximately 1 month after vaccination

ActHIB vaccine has been found to be immunogenic in children with sickle cell anemia, a condition that may cause increased susceptibility to *Haemophilus influenzae* type b disease. Following two doses of ActHIB vaccine given at two-month intervals, 89% of these children (mean age 11 months) had anti-PRP antibody titers of ≥1.0 mcg/mL. This is comparable to anti-PRP antibody levels demonstrated in children without sickle-cell anemia of similar age following two doses of ActHIB vaccine. (12)

15 REFERENCES

1 Data on file, Sanofi Pasteur SA.
2 Data on file, Sanofi Pasteur Inc.
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4 Chu CY, et al. Further studies on the immunogenicity of *Haemophilus influenzae* type b and pneumococcal type 6A polysaccharide-protein conjugate. Infect Immun 40:245-246, 1983.
5 Mueller JH, et al. Production of diphtheria toxin of high potency (100 Lf) on a reproducible medium. J Immunol 40:21-32, 1941.
6 Holmes SJ, et al. Immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in 17- to 19-month-old children. J Pediatr 118:364-371, 1991.
7 Peltola H, et al. Prevention of *Haemophilus influenzae* type b bacteremic infections with the capsular polysaccharide vaccine. N Engl J Med 310:1561-1566, 1984.
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9 Decker MD, et al. Comparative trial in infants of four conjugate *Haemophilus influenzae* type b vaccines. J Pediatr 120:184-189, 1992.
10 Granoff DM, et al. Differences in the immunogenicity of three *Haemophilus influenzae* type b conjugate vaccines in infants. J Pediatr 121:187-194, 1992.
11 Bulkow LR, et al. Comparative immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in Alaska Native infants. Pediatr Infect Dis J 12:484-92, 1993.
12 Kaplan SL, et al. Immunogenicity of *Haemophilus influenzae* type b polysaccharide-tetanus protein conjugate vaccine in children with sickle hemoglobinopathy or malignancies, and after systemic *Haemophilus influenzae* type b infection. J Pediatr 120:367-370, 1992.
13 Vaccine Adverse Event Reporting System United States. MMWR 39:730-733, 1990.

14 CDC. National Childhood Vaccine Injury Act: Requirements for permanent vaccination records and for reporting of selected events after vaccination. MMWR 37:197-200, 1988.
15 National Childhood Vaccine Injury Act of 1986 (Amended 1987).

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Single-dose, lyophilized vaccine vial (NDC 49281-547-58) packaged with single-dose diluent vial (NDC 49281-546-58). Supplied as package of 5 vials each (NDC 49281-545-03).

The vial stoppers for ActHIB vaccine and diluent are not made with natural rubber latex.

16.2 Storage and Handling

Store lyophilized ActHIB vaccine packaged with saline diluent (0.4% Sodium Chloride) at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Discard unused portion.

17 PATIENT COUNSELING INFORMATION

Vaccine Information Statements are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization to the patient, parent, or guardian.

Inform the patients, parents, or guardians about the potential benefits and risks of the vaccine and importance of completing the immunization series unless a contraindication to further immunization exists. In addition to this, parents and guardians must be informed about the potential for adverse reactions that have been temporarily associated with the administration of ActHIB vaccine or other vaccines containing similar ingredients. Prior to administration of ActHIB vaccine, healthcare providers should ask parents or guardians about the recent health status of the infant or child to be immunized. As part of the child's immunization record, the date, lot number, and manufacturer of the vaccine administered should be recorded. (13) (14) (15) Vaccine recipients and guardians must report any adverse reactions upon administration of the vaccine to their healthcare provider and/or to the Vaccine Adverse Event Reporting System (VAERS).

Product information
as of 07/2022.

Manufactured by:
Sanofi Pasteur SA
Marcy L'Etoile France

Distributed by:
Sanofi Pasteur Inc.
Swiftwater PA 18370 USA

HBC-FPLR-SL-JUL22 Rx Only

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use HIBERIX safely and effectively. See full prescribing information for HIBERIX.

HIBERIX [Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)] for injection, for intramuscular use
Initial U.S. Approval: 2009

---------- INDICATIONS AND USAGE----------
HIBERIX is a vaccine indicated for active immunization for the prevention of invasive disease caused by *Haemophilus influenzae* type b. HIBERIX is approved for use in children aged 6 weeks through 4 years (prior to fifth birthday). (1)

----------DOSAGE AND ADMINISTRATION----------
For intramuscular administration only.

A 4-dose series (0.5 mL each) given by intramuscular injection (2.3):
- Primary series: One dose each at 2, 4, and 6 months of age. The first dose may be given as early as 6 weeks of age.
- Booster: One dose at 15 through 18 months of age.

Do not mix HIBERIX with any other vaccine in the same syringe or vial. (2.2)

---------- DOSAGE FORMS AND STRENGTHS----------
Solution for injection supplied as a vial of lyophilized vaccine to be reconstituted with the accompanying vial of saline diluent. A single dose, after reconstitution, is 0.5 mL. (3)

---------- CONTRAINDICATIONS ----------
Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any *H. influenzae* type b- or tetanus toxoid-containing vaccine or any component of HIBERIX. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give HIBERIX should be based on potential benefits and risks. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including HIBERIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including HIBERIX, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination. (5.3)

---------- ADVERSE REACTIONS ----------
Common solicited adverse reactions (≥20%) were pain and redness at the injection site, irritability, drowsiness, fever, loss of appetite, fussiness, and restlessness. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 5/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

HIBERIX is indicated for active immunization for the prevention of invasive disease caused by *Haemophilus influenzae* (*H. influenzae*) type b. HIBERIX is approved for use in children aged 6 weeks through 4 years (prior to fifth birthday).

The evaluation of effectiveness of HIBERIX was based on immune responses in children using serological endpoints that predict protection from invasive disease due to *H. influenzae* type b *[see Clinical Pharmacology (12.1), Clinical Studies (14.1)]*.

2 DOSAGE AND ADMINISTRATION

2.1 Reconstitution

HIBERIX is to be reconstituted only with the accompanying saline diluent. The reconstituted vaccine should be a clear and colorless solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

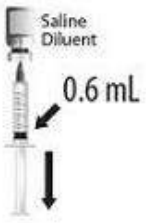

Figure 1. Cleanse both vial stoppers. Withdraw 0.6 mL of saline diluent from accompanying vial.

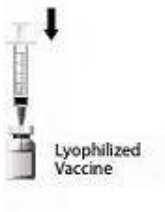

Figure 2. Transfer 0.6 mL saline diluent into lyophilized vaccine vial.

Figure 3. Shake the vial well.

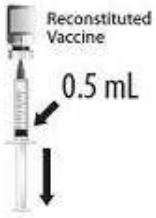

Figure 4. After reconstitution, withdraw 0.5 mL of reconstituted vaccine and administer **intramuscularly**.

Use a separate sterile needle and sterile syringe for each individual.

After reconstitution, administer HIBERIX immediately or store refrigerated between 2° and 8°C (36° and 46°F) and administer within 24 hours. If the vaccine is not administered immediately, shake the solution well again before administration.

2.2 Administration

For intramuscular use only.

HIBERIX is administered as a single dose (0.5 mL) by intramuscular injection into the anterolateral aspect of the thigh or deltoid.

Do not administer this product intravenously, intradermally, or subcutaneously.

If HIBERIX is administered concomitantly with other injectable vaccines, they should be given with separate syringes and at different injection sites. HIBERIX should not be mixed with any other vaccine in the same syringe or vial.

2.3 Dose and Schedule

HIBERIX is administered as a 4-dose series (0.5-mL each dose) given by intramuscular injection. The series consists of a primary immunization course of 3 doses administered at 2, 4, and 6 months of age, followed by a booster dose administered at 15 through 18 months of age. The first dose may be given as early as 6 weeks of age.

3 DOSAGE FORMS AND STRENGTHS

HIBERIX is a solution for injection supplied as a single-dose vial of lyophilized vaccine to be reconstituted with the accompanying vial of saline diluent. A single dose, after reconstitution, is 0.5 mL.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any *H. influenzae* type b- or tetanus toxoid-containing vaccine or any component of the vaccine is a contraindication to administration of HIBERIX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the decision to give any tetanus toxoid-containing vaccine, including HIBERIX, should be based on careful consideration of the potential benefits and possible risks.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including HIBERIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including HIBERIX, to infants born prematurely should be based on consideration of the individual infant’s medical status, and the potential benefits and possible risks of vaccination.

5.4 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the patient's immunization history for possible vaccine hypersensitivity. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

5.5 Altered Immunocompetence

Safety and effectiveness of HIBERIX in immunosuppressed children have not been evaluated. If HIBERIX is administered to immunosuppressed children, including children receiving immunosuppressive therapy, the expected immune response may not be obtained.

5.6 Interference with Laboratory Tests

Urine antigen detection may not have a diagnostic value in suspected disease due to *H. influenzae* type b within 1 to 2 weeks after receipt of a *H. influenzae* type b-containing vaccine, including HIBERIX *[see Drug Interactions (7.1)]*.

5.7 Tetanus Immunization

Immunization with HIBERIX does not substitute for routine tetanus immunization.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. There is the possibility that broad use of HIBERIX could reveal adverse reactions not observed in clinical trials.

Across clinical trials, common solicited adverse reactions (≥20%) were pain and redness at the injection site, irritability, drowsiness, fever, loss of appetite, fussiness, and restlessness.

Study 1: In a randomized, controlled clinical trial conducted in the U.S., children were vaccinated with HIBERIX (n = 2,963), a U.S.-licensed monovalent Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA) (n = 520), or a U.S.-licensed combined Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate Vaccine (DTaP-IPV/Hib) (Sanofi Pasteur Ltd.) (n = 520) at 2, 4, and 6 months of age. HIBERIX and Control PRP-T (Sanofi Pasteur SA) were administered concomitantly with PEDIARIX (DTaP-HBV-IPV) [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine] and Pneumococcal 13-valent Conjugate Vaccine (PCV13) (Wyeth Pharmaceuticals Inc.) with Doses 1, 2, and 3 and ROTARIX [Rotavirus Vaccine, Live, Oral] with Doses 1 and 2. DTaP-IPV/Hib was administered concomitantly with PCV13 and ENGERIX-B [Hepatitis B Vaccine

(Recombinant)] with Doses 1, 2, and 3 and ROTARIX with Doses 1 and 2. If a birth dose of hepatitis B vaccine was received, ENGERIX-B was given with Doses 1 and 3. In the total population, 51.2% were male; 61% were white, 8% were Asian, 9% were black, and 22% were other racial/ethnic groups.

In Study 1, children received a booster dose of either HIBERIX (n = 2,336), a Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA) (n = 435), or a combined Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate Vaccine (DTaP-IPV/Hib) (Sanofi Pasteur Ltd.) (n = 400) at 15 to 18 months of age (mean age: 15.6 months) following primary vaccination at 2, 4, and 6 months of age with the same vaccine. The booster dose of HIBERIX and Control PRP-T (Sanofi Pasteur SA) was administered concomitantly with INFANRIX (DTaP) [Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed].

In 7 additional clinical studies, 1,008 children received HIBERIX as a booster dose following primary vaccination with either HIBERIX (n = 530), Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA) (n = 235), Haemophilus b Conjugate Vaccine (Merck & Co., Inc.) (n = 26), or Haemophilus b Conjugate Vaccine (Wyeth Pharmaceuticals Inc.) (no longer licensed in the U.S., n = 217). None of the studies included a comparator group that received a booster dose with a U.S.-licensed Haemophilus b Conjugate Vaccine. Studies were conducted in Europe, Canada, and Latin America. Across these studies, the mean age of subjects at the time of booster vaccination with HIBERIX ranged from 16 to 19 months. At the time of vaccination, 172 (17.1%) subjects were aged 11 to 14 months, 642 (63.7%) subjects were aged 15 to 18 months, and 194 (19.2%) subjects were aged 19 to 25 months. Approximately half of the subjects were male. Among subjects for whom information on race/ethnicity was available, nearly all subjects were white.

In these 7 studies, HIBERIX was administered concomitantly with non-U.S. formulations (containing 2.5 mg 2-phenoxyethanol per dose as preservative) of one of the following U.S.-licensed vaccines: INFANRIX (DTaP) [Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed], KINRIX (DTaP-IPV) [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine], or PEDIARIX (DTaP-HBV-IPV). In the studies, DTaP-IPV and DTaP-HBV-IPV were administered in dosing regimens not approved in the U.S. Some subjects received DTaP-HBV (GlaxoSmithKline Biologicals, not licensed in U.S.) concomitantly with HIBERIX.

Solicited Adverse Reactions

The reported frequencies of solicited local reactions and general adverse reactions from Study 1 after primary and booster vaccination are presented in Table 1 and Table 2, respectively.

Table 1. Percentage of Children with Solicited Local Reactions and General Adverse Reactions within 4 Days of Primary Series Vaccination[a] (at 2, 4, and 6 Months of Age) with HIBERIX[b], Control PRP-T[b], or DTaP-IPV/Hib[c], Total Vaccinated Cohort[d]

	HIBERIX %			Control PRP-T %			DTaP-IPV/Hib %		
	Dose			Dose			Dose		
Adverse Reactions	**1**	**2**	**3**	**1**	**2**	**3**	**1**	**2**	**3**
Local[e]									
n	**2,828**	**2,668**	**2,553**	**498**	**481**	**463**	**492**	**469**	**443**
Pain	49	45	43	57	53	48	58	50	49
Pain, Grade 3[f]	4	3	2	9	5	4	9	3	3
Redness	19	25	29	24	32	30	26	31	37
Redness, >20 mm	1	1	1	2	1	0	2	2	2
Swelling	13	15	19	19	22	20	20	24	24
Swelling, >20 mm	2	1	1	4	3	1	4	2	2
General									
n	**2,830**	**2,669**	**2,553**	**499**	**480**	**463**	**492**	**469**	**443**
Irritability	69	70	67	76	71	67	73	67	69
Irritability, Grade 3[g]	4	6	5	8	8	5	6	5	3
Drowsiness	60	54	49	66	56	50	61	52	50
Drowsiness, Grade 3[h]	2	3	2	4	2	1	4	3	3
Loss of appetite	29	28	28	33	32	27	34	24	24
Loss of appetite, Grade 3[i]	1	2	2	2	1	0	1	0	1
Fever	14	19	19	16	19	16	12	11	18
Fever, Grade 3[j]	0	1	1	0	0	1	0	0	1

n = All subjects for whom safety data were available.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] Each dose (Doses 1, 2, and 3) of HIBERIX or Control PRP-T (Sanofi Pasteur SA) was concomitantly administered with PEDIARIX (DTaP-HBV-IPV) and PCV13. Doses 1 and 2 were concomitantly administered with ROTARIX.

[c] Each dose (Doses 1, 2, and 3) of DTaP-IPV/Hib was concomitantly administered with PCV13 and ENGERIX-B with Doses 1, 2, and 3 and ROTARIX with Doses 1 and 2. If a birth dose of hepatitis B vaccine was received, ENGERIX-B was given with Doses 1 and 3.

[d] Study 1: NCT01000974.

[e] Local reactions at the injection site for HIBERIX, Control PRP-T, or DTaP-IPV/Hib.

[f] Grade 3 pain defined as cried when limb was moved/spontaneously painful.

[g] Grade 3 irritability defined as crying that could not be comforted/prevented normal activity.

[h] Grade 3 drowsiness defined as prevented normal daily activity.

[i] Grade 3 loss of appetite defined as did not eat at all.

[j] Fever defined as ≥100.4°F (≥38.0°C) rectally; Grade 3 fever defined as >103.1°F (>39.5°C) rectally.

Table 2. Percentage of Children with Solicited Local Reactions and General Adverse Reactions within 4 Days of Booster Vaccination[a] (Dose 4 at 15 through 18 Months of Age) with HIBERIX[b], Control PRP-T[b], or DTaP-IPV/Hib, Total Vaccinated Cohort[c]

	HIBERIX %		Control PRP-T %		DTaP-IPV/Hib%	
Adverse Reactions	**Any**	**Grade 3[d]**	**Any**	**Grade 3[d]**	**Any**	**Grade 3[d]**
Local[e]	**n = 2,224**		**n = 416**		**n = 379**	
Pain	41	1	43	1	43	2
Redness	30	0	31	1	30	3
Swelling	18	1	20	1	20	3
General	**n = 2,225**		**n = 416**		**n = 379**	
Irritability	58	2	60	5	53	2
Drowsiness	39	1	39	3	31	0
Loss of appetite	28	1	34	2	22	1
Fever[f]	15	1	14	1	18	1

n = All subjects for whom safety data were available.

Subjects received primary vaccination at 2, 4, and 6 months of age with the same vaccine as the booster dose.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] The booster dose of HIBERIX and Control PRP-T (Sanofi Pasteur SA) was concomitantly administered with INFANRIX (DTaP).

[c] Study 1: NCT01000974.

[d] Grade 3 pain defined as cried when limb was moved/spontaneously painful.
Grade 3 redness, swelling defined as >20 mm.
Grade 3 irritability defined as crying that could not be comforted/prevented normal activity.
Grade 3 drowsiness defined as prevented normal daily activity.
Grade 3 loss of appetite defined as did not eat at all.
Grade 3 fever defined as >102.2°F (>39.0°C) axillary.

[e] Local reactions at the injection site for HIBERIX, Control PRP-T, or DTaP-IPV/Hib.

[f] Fever defined as ≥99.5°F (≥37.5°C) axillary.

In an open-label, multicenter study conducted in Germany (Study 2), 371 children received a booster dose of HIBERIX administered concomitantly with DTaP-HBV-IPV. The mean age at the time of vaccination was 16 months. Subjects in this study had previously received a primary series with either HIBERIX (n = 92), Control PRP-T (Sanofi Pasteur SA) (n = 96), or Haemophilus b Conjugate Vaccine (Wyeth Pharmaceuticals Inc.) (no longer licensed in the U.S.)

(n = 183). All subjects previously received 3 doses of DTaP-HBV-IPV. The reported frequencies of solicited local reactions and general adverse reactions are presented in Table 3.

Table 3. Percentage of Children with Solicited Local Reactions and General Adverse Reactions within 4 Days of Booster Vaccination[a] (Dose 4) with HIBERIX[b] Coadministered with DTaP-HBV-IPV[c], Intent-to-Treat Cohort (n = 371)

Adverse Reactions	% Any	% Grade 3
Local[d]		
Redness	25	2[e]
Pain	21	1[f]
Swelling	15	2[e]
General		
Fever[g]	35	4
Fussiness	26	1[h]
Loss of appetite	23	1[i]
Restlessness	22	1[i]
Sleepiness	20	1[i]
Diarrhea	15	1[i]
Vomiting	5	1[i]

n = All subjects for whom safety data were available.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] In this study, 92 subjects previously received 3 doses of HIBERIX, 96 subjects previously received 3 doses of a Control PRP-T (Sanofi Pasteur SA), and 183 subjects previously received 3 doses of a Haemophilus b Conjugate Vaccine that is no longer licensed in the U.S.

[c] In this study, DTaP-HBV-IPV was given to subjects who previously received 3 doses of DTaP-HBV-IPV. In the U.S., PEDIARIX is approved for use as a 3-dose primary series; use as a fourth consecutive dose is not approved in the U.S.

[d] Local reactions at the injection site for HIBERIX.

[e] Grade 3 redness or swelling defined as >20 mm.

[f] Grade 3 pain defined as causing crying when limb moved.

[g] Fever defined as ≥100.4°F (≥38.0°C) rectally or ≥99.5°F (≥37.5°C) axillary, oral, or tympanic; Grade 3 fever defined as >103.1°F (>39.5°C) rectally or >102.2°F (>39.0°C) axillary, oral, or tympanic.

[h] Grade 3 fussiness defined as persistent crying and could not be comforted.

[i] Grade 3 for these symptoms defined as preventing normal daily activity.

Serious Adverse Reactions

In Study 1, one of 2,963 subjects who received HIBERIX and coadministered vaccines given at 2, 4, and 6 months of age experienced a serious adverse reaction which was in temporal

association with vaccination and had no alternative plausible causes (convulsion on Day 14 after Dose 1). One of 2,336 subjects who received a booster dose of HIBERIX concomitantly with INFANRIX experienced a serious adverse reaction which was in temporal association with vaccination and had no alternative plausible causes (new onset febrile seizure on Day 1 after Dose 4).

In the 7 additional studies, 2 of 1,008 subjects reported a serious adverse reaction that occurred in the 31-day period following booster immunization with HIBERIX. One subject developed bilateral pneumonia 9 days post-vaccination and one subject experienced asthenia following accidental drug ingestion 18 days post-vaccination.

6.2 Postmarketing Experience

In addition to reports in clinical trials for HIBERIX, the following adverse reactions have been identified during postapproval use of HIBERIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to vaccination.

General Disorders and Administration Site Conditions

Extensive swelling of the vaccinated limb, injection site induration.

Immune System Disorders

Allergic reactions (including anaphylactic and anaphylactoid reactions), angioedema.

Nervous System Disorders

Convulsions (with or without fever), hypotonic-hyporesponsive episode (i.e., sudden onset of hypotonia, hyporesponsiveness, and pallor or cyanosis), somnolence, syncope, or vasovagal responses to injection.

Respiratory, Thoracic, and Mediastinal Disorders

Apnea *[see Warnings and Precautions (5.3)]*.

Skin and Subcutaneous Tissue Disorders

Rash, urticaria.

7 DRUG INTERACTIONS

7.1 Interference with Laboratory Tests

Haemophilus b capsular polysaccharide derived from Haemophilus b Conjugate Vaccines has been detected in the urine of some vaccinees.[1] Urine antigen detection may not have a diagnostic value in suspected disease due to *H. influenzae* type b within 1 to 2 weeks after receipt of a *H. influenzae* type b-containing vaccine, including HIBERIX *[see Warnings and Precautions (5.6)]*.

7.2 Concomitant Vaccine Administration

In clinical studies, HIBERIX was administered concomitantly with routinely recommended pediatric vaccines *[see Clinical Studies (14.2)]*.

7.3 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to HIBERIX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

HIBERIX is not approved for use in individuals aged 5 years and older. No human or animal data with HIBERIX are available to assess vaccine-associated risks in pregnancy.

8.2 Lactation

HIBERIX is not approved for use in individuals aged 5 years and older. No human or animal data are available to assess the impact of HIBERIX on milk production, its presence in breast milk, or its effects on the breastfed infant.

8.4 Pediatric Use

Safety and effectiveness of HIBERIX in children younger than 6 weeks and in children aged 5 to 16 years have not been established.

11 DESCRIPTION

HIBERIX [Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)] is a solution for intramuscular injection, supplied as a sterile, lyophilized powder which is reconstituted at the time of use with the accompanying saline diluent. HIBERIX contains Haemophilus b capsular polysaccharide (polyribosyl-ribitol-phosphate [PRP]), a high molecular weight polymer prepared from the *H. influenzae* type b strain 20,752 grown in a synthetic medium that undergoes heat inactivation and purification. The tetanus toxin, prepared from *Clostridium tetani* grown in a semi-synthetic medium, is detoxified with formaldehyde and purified. The capsular polysaccharide is covalently bound to the tetanus toxoid. After purification, the conjugate is lyophilized in the presence of lactose as a stabilizer. The diluent for HIBERIX is a sterile saline solution (0.9% sodium chloride) supplied in vials.

After reconstitution, each 0.5-mL dose is formulated to contain 10 mcg of purified capsular polysaccharide conjugated to approximately 25 mcg of tetanus toxoid, 12.6 mg of lactose, and ≤0.5 mcg of residual formaldehyde.

HIBERIX does not contain a preservative.

The lyophilized vaccine and saline diluent vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

H. influenzae is a gram-negative coccobacillus. Most strains of *H. influenzae* that cause invasive disease are type b. *H. influenzae* type b can cause invasive disease such as sepsis and meningitis.

Specific levels of antibodies to polyribosyl-ribitol-phosphate (anti-PRP) have been shown to correlate with protection against invasive disease due to *H. influenzae* type b. Based on data from passive antibody studies[2] and a clinical efficacy study with unconjugated *Haemophilus* b polysaccharide vaccine[3], an anti-PRP concentration of 0.15 mcg/mL has been accepted as a minimal protective level. Data from an efficacy study with unconjugated *Haemophilus* b polysaccharide vaccine indicate that an anti-PRP concentration of ≥1.0 mcg/mL predicts protection through at least a 1-year period.[4,5] These antibody levels have been used to evaluate the effectiveness of Haemophilus b Conjugate Vaccines, including HIBERIX.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

HIBERIX has not been evaluated for carcinogenic or mutagenic potential, or for impairment of fertility.

14 CLINICAL STUDIES

14.1 Immunological Evaluation

Primary Series Vaccination (Doses 1, 2, and 3)

The immunogenicity of HIBERIX was evaluated in a randomized, controlled trial (Study 1). HIBERIX or control vaccines were administered concomitantly with U.S.-licensed vaccines *[see Adverse Reactions (6.1)]*.

Anti-PRP geometric mean concentrations (GMCs) and seroprotection rates 1 month following Dose 3 of HIBERIX, Control PRP-T (Sanofi Pasteur SA), or DTaP-IPV/Hib are presented in Table 4.

Table 4. Anti-PRP GMCs and Seroprotection Rates 1 Month following 3 Doses of HIBERIX, Control PRP-T[a], or DTaP-IPV/Hib[b] Administered at 2, 4, and 6 Months of Age, ATP Cohort for Immunogenicity[c]

Vaccine	n	Anti-PRP GMC (mcg/mL) (95% CI)	% Anti-PRP ≥0.15 mcg/mL (95% CI)	% Anti-PRP ≥1.0 mcg/mL (95% CI)
HIBERIX	1,590	5.19 (4.77, 5.66)	96.6 (95.6, 97.4)	81.2 (79.2, 83.1)
Control PRP-T	274	6.74 (5.59, 8.13)	96.7[d] (93.9, 98.5)	89.8[e] (85.6, 93.1)
DTaP-IPV/Hib	253	3.64 (2.89, 4.58)	92.5[f] (88.5, 95.4)	78.3[f] (72.7, 83.2)

[a] U.S.-licensed monovalent Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA).

[b] U.S.-licensed DTaP-IPV/Hib Vaccine (Sanofi Pasteur Ltd.).

[c] Study 1: NCT01000974.

[d] HIBERIX was non-inferior to Control PRP-T for percent of subjects achieving anti-PRP ≥0.15 mcg/mL (lower limit of 95% CI on difference of HIBERIX minus Control PRP-T ≥ predefined limit of -5%).

[e] The non-inferiority criterion was not met (lower limit of 95% CI for the difference in the percentages of subjects with anti-PRP ≥1.0 mcg/mL between two groups [HIBERIX minus Control PRP-T] was -12.28%, which was lower than the predefined limit of -10%).

[f] Analyses of anti-PRP immune responses following DTaP-IPV/Hib vaccination were exploratory.

Booster Vaccination (Dose 4)

The immunogenicity of HIBERIX administered as a booster dose at 15 to 18 months of age was evaluated in a subset of children from Study 1 (n = 336) in comparison with U.S.-licensed vaccines following primary vaccination at 2, 4, and 6 months of age *[see Adverse Reactions (6.1)]*. The booster dose of HIBERIX and Control PRP-T (Sanofi Pasteur SA) was administered concomitantly with INFANRIX.

Antibodies to PRP were measured in sera obtained immediately prior to and 1 month after booster vaccination with HIBERIX or the control vaccines. Anti-PRP GMCs and seroprotection rates are presented in Table 5.

Table 5. Anti-PRP GMCs and Seroprotection Rates prior to and 1 Month following a Booster Dose (Dose 4 at 15 through 18 Months of Age) of HIBERIX, Control PRP-T[a], or DTaP-IPV/Hib[b], ATP Cohort for Immunogenicity[c]

Vaccine	n	Anti-PRP GMC (mcg/mL) (95% CI)		% Anti-PRP ≥0.15 mcg/mL (95% CI)		% Anti-PRP ≥1.0 mcg/mL (95% CI)	
		Pre-	Post-	Pre-	Post-	Pre-	Post-
HIBERIX	329-336	0.50 (0.42, 0.59)	48.78 (42.0, 56.66)	75.1 (70.0, 79.7)	100.0 (98.9, 100.0)	32.2 (27.2, 37.6)	99.1 (97.4, 99.8)
Control PRP-T	226-236	0.47 (0.38, 0.57)	40.29 (33.39, 48.63)	76.1 (70.0, 81.5)	99.6 (97.7, 100.0)	27.0 (21.3, 33.3)	97.9[d] (95.1, 99.3)
DTaP-IPV/Hib	175-186	0.38 (0.30, 0.48)	37.54 (30.53, 46.16)	66.3 (58.8, 73.2)	100.0 (98.0, 100.0)	25.1 (18.9, 32.2)	98.9[e] (96.2, 99.9)

[a] U.S.-licensed monovalent Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA).

[b] U.S.-licensed DTaP-IPV/Hib Vaccine (Sanofi Pasteur Ltd.).

[c] Study 1: NCT01000974.

[d] HIBERIX was non-inferior to Control PRP-T for percent of subjects achieving anti-PRP ≥1.0 mcg/mL (lower limit of 97.5% CI on difference of HIBERIX minus Control PRP-T ≥predefined limit of -10%) at 1 month following the booster dose.

[e] Analyses of anti-PRP immune responses following DTaP-IPV/Hib vaccination were exploratory.

In 6 additional clinical studies, the immune response to HIBERIX administered as a booster dose was evaluated in a total of 415 children aged 12 to 23 months. At the time of vaccination, 30 children were aged 12 to 14 months, 316 children were aged 15 to 18 months, and 69 children were aged 19 to 23 months. Among subjects, 43% to 60% were male. Among subjects for whom information on race/ethnicity was available, nearly all subjects were white. None of the studies included a comparator group that received a booster dose with a U.S.-licensed Haemophilus b Conjugate Vaccine. Characteristics of 3 of these studies are presented in Table 6.

Table 6. Characteristics of 3 Open-Label Booster Immunization Studies of HIBERIX

Study	Country	Per-Protocol Immunogenicity Cohort n	Priming History	Booster Vaccination with HIBERIX	
				Age at Vaccination (months)	Concomitantly Administered Vaccine[a]
3	Canada	42	DTaP-HBV-IPV[b] + Haemophilus b Conjugate Vaccine[c] at 2, 4, and 6 months of age	16-18	DTaP-HBV-IPV[b]
4	Canada	64	DTaP-IPV[d] + HIBERIX at 2, 4, and 6 months of age	16-19	DTaP-IPV[d]
5	Germany	108	DTaP-HBV[e] + HIBERIX at 3, 4, and 5 months of age	16-23	DTaP-HBV[e]

[a] Administered at a separate site.

[b] Non-U.S. formulation equivalent to PEDIARIX with the exception of containing 2.5 mg 2-phenoxyethanol per dose as preservative. In the U.S., PEDIARIX is approved for use as a 3-dose primary series; use as a fourth consecutive dose is not approved in the U.S.

[c] U.S.-licensed Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA).

[d] Non-U.S. formulation equivalent to KINRIX with the exception of containing 2.5 mg 2-phenoxyethanol per dose as preservative. In the U.S., KINRIX is approved for use as the fifth dose of DTaP and the fourth dose of IPV in children aged 4 to 6 years previously primed with approved dosing regimens of INFANRIX and/or PEDIARIX. The DTaP-IPV dosing regimen is not approved in the U.S.

[e] Manufactured by GlaxoSmithKline Biologicals (not licensed in the U.S.).

Antibodies to PRP were measured in sera obtained immediately prior to and 1 month after booster vaccination with HIBERIX. Geometric mean concentrations and anti-PRP seroprotection rates are presented in Table 7.

Table 7. Anti-PRP GMCs and Seroprotection Rates prior to and 1 Month following a Booster Dose of HIBERIX, Per-Protocol Immunogenicity Cohort

Study	n	Anti-PRP GMC (mcg/mL)		% Anti-PRP ≥0.15 mcg/mL		% Anti-PRP ≥1.0 mcg/mL	
		Pre-	Post-	Pre-	Post-	Pre-	Post-
3[a]	42	0.46	59.07	76.2	100	35.7	97.6
4[b]	63-64	0.25	47.78	71.4	100	12.7	100
5[c]	108	0.59	96.12	77.8	100	32.4	100

GMC = Geometric mean antibody concentration.
n = Number of children for whom serological results were available for the pre- and post-dose immunological evaluations.
Studies 3, 4, and 5 correspond to Studies 3, 4, and 5, respectively in Table 6.

[a] Canadian study in children aged 16 to 18 months who previously received 3 doses of DTaP-HBV-IPV and Haemophilus b Conjugate Vaccine (Control PRP-T) (Sanofi Pasteur SA). The booster dose of HIBERIX was coadministered with DTaP-HBV-IPV (a fourth consecutive dose of PEDIARIX is not approved in the U.S.). In this study, pre-vaccination sera may have been obtained up to 1 week prior to booster vaccination with HIBERIX.

[b] Canadian study in children aged 16 to 19 months who previously received 3 doses of DTaP-IPV and HIBERIX. The booster dose of HIBERIX was coadministered with DTaP-IPV. The DTaP-IPV dosing regimen is not approved in the U.S.

[c] German study in children aged 16 to 23 months who previously received 3 doses of DTaP-HBV (GlaxoSmithKline Biologicals, not licensed in the U.S.) and HIBERIX. The booster dose of HIBERIX was coadministered with DTaP-HBV.

14.2 Concomitant Vaccine Administration

Primary Series Vaccination (Doses 1, 2, and 3)

In U.S. Study 1, subjects who received HIBERIX concomitantly with PEDIARIX (DTaP-HBV-IPV) and PCV13 at 2, 4, and 6 months of age had no evidence for reduced antibody responses relative to the response in control subjects administered Control PRP-T (Sanofi Pasteur SA) concomitantly with PEDIARIX (DTaP-HBV-IPV) and PCV13, to pertussis antigens (GMC to pertussis toxin, filamentous hemagglutinin, and pertactin), diphtheria toxoid (antibody levels ≥0.1 IU/mL), tetanus toxoid (antibody levels ≥0.1 IU/mL), poliovirus types 1, 2, and 3 (antibody levels ≥1:8 to each virus), PCV13 (antibody levels ≥0.2 mcg/mL and GMC to each serotype), or hepatitis B (anti-hepatitis B surface antigen ≥10 mIU/mL). The immune responses to PEDIARIX (DTaP-HBV-IPV) and PCV13 were evaluated 1 month following Dose 3. Subjects in both groups received ROTARIX at 2 and 4 months of age.

Booster Vaccination (Dose 4)

In U.S. Study 1, subjects who received a booster dose of HIBERIX concomitantly with INFANRIX at 15 to 18 months of age had no evidence for reduced antibody responses to

pertussis antigens (GMC to pertussis toxin, filamentous hemagglutinin, and pertactin), diphtheria toxoid (antibody levels ≥0.1 IU/mL), and tetanus toxoid (antibody levels ≥0.1 IU/mL), relative to the responses in control subjects administered Control PRP-T (Sanofi Pasteur SA) concomitantly with INFANRIX.

In 7 additional studies, a booster dose of HIBERIX was administered concomitantly with non-U.S. formulations of INFANRIX, KINRIX, and PEDIARIX. Non-U.S. formulations of KINRIX and PEDIARIX were administered in dosing regimens not approved in the U.S.

Sufficient data are not available to confirm lack of interference in immune responses to vaccines other than INFANRIX administered concomitantly with a booster dose of HIBERIX.

15 REFERENCES

1. Rothstein EP, Madore DV, Girone JAC, et al. Comparison of antigenuria after immunization with three Haemophilus influenzae type b conjugate vaccines. *Pediatr Infect Dis J.* 1991;10:311-314.
2. Robbins JB, Parke JC, Schneerson R, et al. Quantitative measurement of “natural” and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. *Pediatr Res.* 1973;7:103-110.
3. Peltola H, Käythy H, Sivonen A, et al. *Haemophilus influenzae* type b capsular polysaccharide vaccine in children: A double-blind field study of 100,000 vaccinees 3 months to 5 years of age in Finland. *Pediatrics.* 1977;60:730-737.
4. Käythy H, Peltola H, Karanko V, et al. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis.* 1983;147:1100.
5. Anderson P. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis.* 1984;149:1034.

16 HOW SUPPLIED/STORAGE AND HANDLING

HIBERIX is available in single-dose vials of lyophilized vaccine, accompanied by vials containing 0.85 mL of saline diluent (packaged without syringes or needles).

Supplied as package of 10 doses (NDC 58160-818-11):

NDC 58160-816-01 Vial of lyophilized vaccine in Package of 10: NDC 58160-816-05

NDC 58160-817-01 Vial of saline diluent in Package of 10: NDC 58160-817-05

16.1 Storage before Reconstitution

Lyophilized vaccine vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light.

Diluent: Store refrigerated or at controlled room temperature between 2° and 25°C (36° and 77°F). Do not freeze. Discard if the diluent has been frozen.

16.2 Storage after Reconstitution

Administer within 24 hours of reconstitution. After reconstitution, store refrigerated between 2° and 8°C (36° and 46°F). Discard the reconstituted vaccine if not used within 24 hours. Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

- Inform parents or guardians of the potential benefits and risks of immunization with HIBERIX.
- Inform parents or guardians about the potential for adverse reactions that have been temporally associated with administration of HIBERIX or other vaccines containing similar components.
- Give parents or guardians the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by GlaxoSmithKline Biologicals

Rixensart, Belgium, U.S. License 1617, and

Distributed by GlaxoSmithKline

Research Triangle Park, NC 27709

HRX:8PI

Liquid PedvaxHIB®
[Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)]

DESCRIPTION

PedvaxHIB® [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)] is a highly purified capsular polysaccharide (polyribosylribitol phosphate or PRP) of *Haemophilus influenzae* type b (Haemophilus b, Ross strain) that is covalently bound to an outer membrane protein complex (OMPC) of the B11 strain of *Neisseria meningitidis* serogroup B. The covalent bonding of the PRP to the OMPC which is necessary for enhanced immunogenicity of the PRP is confirmed by quantitative analysis of the conjugate's components following chemical treatment which yields a unique amino acid. The potency of PedvaxHIB is determined by assay of PRP.

Haemophilus influenzae type b and *Neisseria meningitidis* serogroup B are grown in complex fermentation media. The PRP is purified from the culture broth by purification procedures which include ethanol fractionation, enzyme digestion, phenol extraction and diafiltration. The OMPC from *Neisseria meningitidis* is purified by detergent extraction, ultracentrifugation, diafiltration and sterile filtration.

Liquid PedvaxHIB is ready to use and does not require a diluent. Each 0.5 mL dose of Liquid PedvaxHIB is a sterile product formulated to contain: 7.5 mcg of Haemophilus b PRP, 125 mcg of *Neisseria meningitidis* OMPC and 225 mcg of aluminum as amorphous aluminum hydroxyphosphate sulfate (previously referred to as aluminum hydroxide), in 0.9% sodium chloride, but does not contain lactose or thimerosal. Liquid PedvaxHIB is a slightly opaque white suspension.

This vaccine is for intramuscular administration and not for intravenous injection. (See DOSAGE AND ADMINISTRATION.)

CLINICAL PHARMACOLOGY

Prior to the introduction of Haemophilus b Conjugate Vaccines, *Haemophilus influenzae* type b (Hib) was the most frequent cause of bacterial meningitis and a leading cause of serious, systemic bacterial disease in young children worldwide.[1,2,3,4]

Hib disease occurred primarily in children under 5 years of age in the United States prior to the initiation of a vaccine program and was estimated to account for nearly 20,000 cases of invasive infections annually, approximately 12,000 of which were meningitis. The mortality rate from Hib meningitis is about 5%. In addition, up to 35% of survivors develop neurologic sequelae including seizures, deafness, and mental retardation.[5,6] Other invasive diseases caused by this bacterium include cellulitis, epiglottitis, sepsis, pneumonia, septic arthritis, osteomyelitis and pericarditis.

Prior to the introduction of the vaccine, it was estimated that 17% of all cases of Hib disease occurred in infants less than 6 months of age.[7] The peak incidence of Hib meningitis occurs between 6 to 11 months of age. Forty-seven percent of all cases occur by one year of age with the remaining 53% of cases occurring over the next four years.[2,20]

Among children under 5 years of age, the risk of invasive Hib disease is increased in certain populations including the following:

- Daycare attendees[8,9]
- Lower socio-economic groups[10]
- Blacks[11] (especially those who lack the Km(1) immunoglobulin allotype)[12]
- Caucasians who lack the G2m(n or 23) immunoglobulin allotype[13]
- Native Americans[14,15,16]
- Household contacts of cases[17]
- Individuals with asplenia, sickle cell disease, or antibody deficiency syndromes[18,19]

An important virulence factor of the Hib bacterium is its polysaccharide capsule (PRP). Antibody to PRP (anti-PRP) has been shown to correlate with protection against Hib disease.[3,21] While the anti-PRP level associated with protection using conjugated vaccines has not yet been

determined, the level of anti-PRP associated with protection in studies using bacterial polysaccharide immune globulin or nonconjugated PRP vaccines ranged from >0.15 to >1.0 mcg/mL.[22-28]

Nonconjugated PRP vaccines are capable of stimulating B-lymphocytes to produce antibody without the help of T-lymphocytes (T-independent). The responses to many other antigens are augmented by helper T-lymphocytes (T-dependent). PedvaxHIB is a PRP-conjugate vaccine in which the PRP is covalently bound to the OMPC carrier[29] producing an antigen which is postulated to convert the T-independent antigen (PRP alone) into a T-dependent antigen resulting in both an enhanced antibody response and immunologic memory.

Clinical Evaluation of PedvaxHIB

PedvaxHIB, in a lyophilized formulation (lyophilized PedvaxHIB), was initially evaluated in 3,486 Native American (Navajo) infants, who completed the primary two-dose regimen in a randomized, double-blind, placebo-controlled study (The Protective Efficacy Study). At the time of the study, this population had a much higher incidence of Hib disease than the United States population as a whole and also had a lower antibody response to Haemophilus b Conjugate Vaccines, including PedvaxHIB.[14,15,16,30,33]

Each infant in this study received two doses of either placebo or lyophilized PedvaxHIB with the first dose administered at a mean of 8 weeks of age and the second administered approximately two months later; DTP and OPV were administered concomitantly. Antibody levels were measured in a subset of each group (TABLE 1).

TABLE 1
Antibody Responses in Navajo Infants

Vaccine	No. of Subjects	Time	% Subjects with >0.15 mcg/mL	% Subjects with >1.0 mcg/mL	Anti-PRP GMT (mcg/mL)
Lyophilized PedvaxHIB*	416**	Pre-Vaccination	44	10	0.16
	416	Post-Dose 1	88	52	0.95
	416	Post-Dose 2	91	60	1.43
Placebo*	461**	Pre-Vaccination	44	9	0.16
	461	Post-Dose 1	21	2	0.09
	461	Post-Dose 2	14	1	0.08
Lyophilized PedvaxHIB	27†	Prebooster	70	33	0.51
	27	Postbooster††	100	89	8.39

* Post-Vaccination values obtained approximately 1–3 months after each dose.
** The Protective Efficacy Study
† Immunogenicity Trial[34]
†† Booster given at 12 months of age; Post-Vaccination values obtained 1 month after administration of booster dose.

Most subjects were initially followed until 15 to 18 months of age. During this time, 22 cases of invasive Hib disease occurred in the placebo group (8 cases after the first dose and 14 cases after the second dose) and only 1 case in the vaccine group (none after the first dose and 1 after the second dose). Following the primary two-dose regimen, the protective efficacy of lyophilized PedvaxHIB was calculated to be 93% with a 95% confidence interval of 57%-98% (p=0.001, two-tailed). In the two months between the first and second doses, the difference in number of cases of disease between placebo and vaccine recipients (8 vs. 0 cases, respectively) was statistically significant (p=0.008, two-tailed); however, a primary two-dose regimen is required for infants 2-14 months of age.

At termination of the study, placebo recipients were offered vaccine. All original participants were then followed two years and nine months from termination of the study. During this extended follow-up, invasive Hib disease occurred in an additional seven of the original placebo recipients prior to receiving vaccine and in one of the original vaccine recipients (who had received only one dose of vaccine). No cases of invasive Hib disease were observed in placebo recipients after they received at least one dose of vaccine. Efficacy for this follow-up period, estimated from person-days at risk, was 96.6% (95 C.I., 72.2-99.9%) in children under 18 months of age and 100% (95 C.I., 23.5-100%) in children over 18 months of age.[33]

Since protective efficacy with lyophilized PedvaxHIB was demonstrated in such a high risk population, it would be expected to be predictive of efficacy in other populations.

The safety and immunogenicity of lyophilized PedvaxHIB were evaluated in infants and children in other clinical studies that were conducted in various locations throughout the United States. PedvaxHIB was highly immunogenic in all age groups studied.[31,32]

Lyophilized PedvaxHIB induced antibody levels greater than 1.0 mcg/mL in children who were poor responders to nonconjugated PRP vaccines. In a study involving such a subpopulation,[33,34] 34 children ranging in age from 27 to 61 months who developed invasive Hib disease despite previous vaccination with nonconjugated PRP vaccines were randomly assigned to 2 groups. One group (n=14) was vaccinated with lyophilized PedvaxHIB and the other group (n=20) with a nonconjugated PRP vaccine at a mean interval of approximately 12 months after recovery from disease. All 14 children vaccinated with lyophilized PedvaxHIB but only 6 of 20 children re-vaccinated with a nonconjugated PRP vaccine achieved an antibody level of >1.0 mcg/mL. The 14 children who had not responded to revaccination with the nonconjugated PRP vaccine were then vaccinated with a single dose of lyophilized PedvaxHIB; following this vaccination, all achieved antibody levels of >1.0 mcg/mL.

In addition, lyophilized PedvaxHIB has been studied in children at high risk of Hib disease because of genetically-related deficiencies [Blacks who were Km(1) allotype negative and Caucasians who were G2m(23) allotype negative] and are considered hyporesponsive to nonconjugated PRP vaccines on this basis.[35] The hyporesponsive children had anti-PRP responses comparable to those of allotype positive children of similar age range when vaccinated with lyophilized PedvaxHIB. All children achieved anti-PRP levels of >1.0 mcg/mL.

The safety and immunogenicity of Liquid PedvaxHIB were compared with those of lyophilized PedvaxHIB in a randomized clinical study involving 903 infants 2 to 6 months of age from the general U.S. population. DTP and OPV were administered concomitantly to most subjects. The antibody responses induced by each formulation of PedvaxHIB were similar. TABLE 2 shows antibody responses from this clinical study in subjects who received their first dose at 2 to 3 months of age.

TABLE 2
Antibody Responses to Liquid and Lyophilized PedvaxHIB in Infants From the General U.S. Population

Formulation	Age (Months)	Time	No. of Subjects	% Subjects with anti-PRP >0.15 mcg/mL	% Subjects with anti-PRP >1.0 mcg/mL	Anti-PRP GMT (mcg/mL)
Liquid PedvaxHIB (7.5 mcg PRP)		Pre-Vaccination	487	32	7	0.12
	2-3	Post-Dose 1*	480	94	64	1.55
		Post-Dose 2**	393	97	80	3.22
	12-15	Prebooster	284	80	30	0.49
		Postbooster**	284	99	95	10.23
	24†	Persistence	94	97	55	1.29
Lyophilized PedvaxHIB (15 mcg PRP)		Pre-Vaccination	171	37	6	0.13
	2-3	Post-Dose 1*	169	97	72	1.88
		Post-Dose 2**	133	99	81	2.69
	12-15	Prebooster	87	71	28	0.39
		Postbooster**	87	99	91	7.64
	24†	Persistence	37	97	54	1.10

* Approximately two months Post-Vaccination
** Approximately one month Post-Vaccination
† Approximately

A booster dose of PedvaxHIB is required in infants who complete the primary two-dose regimen before 12 months of age. This booster dose will help maintain antibody levels during the first two years of life when children are at highest risk for invasive Hib disease. (See TABLE 2 and DOSAGE AND ADMINISTRATION.)

In four United States studies, antibody responses to lyophilized PedvaxHIB were evaluated in several subpopulations of infants initially vaccinated between 2 to 3 months of age. (See TABLE 3.)

TABLE 3
Antibody Responses*
After Two Doses of Lyophilized PedvaxHIB Among Infants Initially Vaccinated at 2–3 Months of Age By Racial/Ethnic Group

LYOPHILIZED				
Racial/Ethnic Groups	No. of Subjects	% Subjects With Anti-PRP >0.15 mcg/mL	% Subjects With Anti-PRP >1.0 mcg/mL	Anti-PRP GMT (mcg/mL)
Native American†	54	96	70	2.47
Caucasian	201	99	82	3.52
Hispanic	76	99	88	3.54
Black	23	100	96	5.40

* One month after the second dose
† Apache and Navajo

In two United States studies, antibody responses to Liquid PedvaxHIB were evaluated in several subpopulations of infants initially vaccinated between 2 to 3 months of age. (See TABLE 4.)

TABLE 4
Antibody Responses*
After Two Doses of Liquid PedvaxHIB Among Infants Initially Vaccinated at 2–3 Months of Age By Racial/Ethnic Group

LIQUID				
Racial/Ethnic Groups	No. of Subjects	% Subjects With Anti-PRP >0.15 mcg/mL	% Subjects With Anti-PRP >1.0 mcg/mL	Anti-PRP GMT (mcg/mL)
Native American**	90	97	78	2.76
Caucasian	143	94	72	2.16
Hispanic	184	98	85	4.34
Black	18	100	94	7.58

* One month after the second dose
** Apache and Navajo

Antibodies to the OMPC of *N. meningitidis* have been demonstrated in vaccinee sera, but the clinical relevance of these antibodies has not been established.[33]

Interchangeability of Licensed Haemophilus b Conjugate Vaccines and PedvaxHIB

Published studies have examined the interchangeability of other licensed Haemophilus b Conjugate Vaccines and PedvaxHIB.[42,43,44,45,52] According to the American Academy of Pediatrics, excellent immune responses have been achieved when different vaccines have been interchanged in the primary series. If PedvaxHIB is given in a series with one of the other products licensed for infants, the recommended number of doses to complete the series is determined by the other product and not by PedvaxHIB. PedvaxHIB may be interchanged with other licensed Haemophilus b Conjugate Vaccines for the booster dose.[52]

Use with Other Vaccines

Results from clinical studies indicate that Liquid PedvaxHIB can be administered concomitantly with DTP, OPV, eIPV (enhanced inactivated poliovirus vaccine), VARIVAX® [Varicella Virus Vaccine Live (Oka/Merck)], M-M-R® II (Measles, Mumps, and Rubella Virus Vaccine Live) or RECOMBIVAX HB® [Hepatitis B Vaccine (Recombinant)].[33] No impairment of immune response to individual tested vaccine antigens was demonstrated.

The type, frequency and severity of adverse experiences observed in these studies with PedvaxHIB were similar to those seen when the other vaccines were given alone.

In addition, a PRP-OMPC-containing product, COMVAX® [Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine], was given concomitantly with a booster dose of DTaP [diphtheria, tetanus, acellular pertussis] at approximately 15 months of age, using separate sites and syringes for injectable vaccines. No impairment of immune response to these individually tested vaccine antigens was demonstrated. COMVAX has also been administered concomitantly with the primary series of DTaP to a limited number of infants. PRP antibody responses are satisfactory for COMVAX, but immune responses are currently unavailable for DTaP (see Manufacturer's Product Circular for COMVAX). No serious vaccine-related adverse events were reported.[33]

INDICATIONS AND USAGE

Liquid PedvaxHIB is indicated for routine vaccination against invasive disease caused by *Haemophilus influenzae* type b in infants and children 2 to 71 months of age.

Liquid PedvaxHIB will not protect against disease caused by *Haemophilus influenzae* other than type b or against other microorganisms that cause invasive disease such as meningitis or sepsis. As with any vaccine, vaccination with Liquid PedvaxHIB may not result in a protective antibody response in all individuals given the vaccine.

BECAUSE OF THE POTENTIAL FOR IMMUNE TOLERANCE, Liquid PedvaxHIB IS NOT RECOMMENDED FOR USE IN INFANTS YOUNGER THAN 6 WEEKS OF AGE. (See PRECAUTIONS.)

Revaccination

Infants completing the primary two-dose regimen before 12 months of age should receive a booster dose (see DOSAGE AND ADMINISTRATION).

CONTRAINDICATIONS

Hypersensitivity to any component of the vaccine or the diluent.

Persons who develop symptoms suggestive of hypersensitivity after an injection should not receive further injections of the vaccine.

PRECAUTIONS

General

As for any vaccine, adequate treatment provisions, including epinephrine, should be available for immediate use should an anaphylactoid reaction occur.

Use caution when vaccinating latex-sensitive individuals since the vial stopper contains dry natural latex rubber that may cause allergic reactions.

Special care should be taken to ensure that the injection does not enter a blood vessel.

It is important to use a separate sterile syringe and needle for each patient to prevent transmission of hepatitis B or other infectious agents from one person to another.

As with other vaccines, Liquid PedvaxHIB may not induce protective antibody levels immediately following vaccination.

As reported with Haemophilus b Polysaccharide Vaccine[36] and another Haemophilus b Conjugate Vaccine[37], cases of Hib disease may occur in the week after vaccination, prior to the onset of the protective effects of the vaccines.

There is insufficient evidence that Liquid PedvaxHIB given immediately after exposure to natural *Haemophilus influenzae* type b will prevent illness.

The decision to administer or delay vaccination because of current or recent febrile illness depends on the severity of symptoms and on the etiology of the disease. The Advisory Committee on Immunization Practices (ACIP) has recommended that vaccination should be delayed during the course of an acute febrile illness. All vaccines can be administered to persons with minor illnesses such as diarrhea, mild upper-respiratory infection with or without low-grade fever, or other low-grade febrile illness. Persons with moderate or severe febrile illness should be vaccinated as soon as they have recovered from the acute phase of the illness.[46]

If PedvaxHIB is used in persons with malignancies or those receiving immunosuppressive therapy or who are otherwise immunocompromised, the expected immune response may not be obtained.

Instructions to Healthcare Provider

The healthcare provider should determine the current health status and previous vaccination history of the vaccinee.

The healthcare provider should question the patient, parent, or guardian about reactions to a previous dose of PedvaxHIB or other Haemophilus b Conjugate Vaccines.

Information for Patients

The healthcare provider should provide the vaccine information required to be given with each vaccination to the patient, parent, or guardian.

The healthcare provider should inform the patient, parent, or guardian of the benefits and risks associated with vaccination. For risks associated with vaccination, see ADVERSE REACTIONS.

Patients, parents, and guardians should be instructed to report any serious adverse reactions to their healthcare provider who in turn should report such events to the U. S. Department of Health and Human Services through the Vaccine Adverse Event Reporting System (VAERS), 1-800-822-7967.[47]

Laboratory Test Interactions

Sensitive tests (e.g., Latex Agglutination Kits) may detect PRP derived from the vaccine in urine of some vaccinees for at least 30 days following vaccination with lyophilized PedvaxHIB;[38] in clinical studies with lyophilized PedvaxHIB, such children demonstrated normal immune response to the vaccine.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Liquid PedvaxHIB has not been evaluated for carcinogenic or mutagenic potential, or potential to impair fertility.

Pregnancy

Animal reproduction studies have not been conducted with PedvaxHIB. Liquid PedvaxHIB is not recommended for use in individuals 6 years of age and older.

Pediatric Use

Safety and effectiveness in infants below the age of 2 months and in children 6 years of age and older have not been established. In addition, Liquid PedvaxHIB should not be used in infants younger than 6 weeks of age because this will lead to a reduced anti-PRP response and may lead to immune tolerance (impaired ability to respond to subsequent exposure to the PRP antigen).[49-51] Liquid PedvaxHIB is not recommended for use in individuals 6 years of age and older because they are generally not at risk of Hib disease.

Geriatric Use

This vaccine is NOT recommended for use in adult populations.

ADVERSE REACTIONS

Liquid PedvaxHIB

In a multicenter clinical study (n=903) comparing the effects of Liquid PedvaxHIB with those of lyophilized PedvaxHIB, 1,699 doses of Liquid PedvaxHIB were administered to 678 healthy infants 2 to 6 months of age from the general U.S. population. DTP and OPV were administered concomitantly to most subjects. Both formulations of PedvaxHIB were generally well tolerated and no serious vaccine-related adverse reactions were reported.

During a three-day period following primary vaccination with Liquid PedvaxHIB in these infants, the most frequently reported (>1%) adverse reactions, without regard to causality, excluding those shown in TABLE 5, in decreasing order of frequency, were: irritability, sleepiness, injection site pain/soreness, injection site erythema (≤2.5 cm diameter, see also TABLE 5), injection site swelling/induration (≤2.5 cm diameter, see also TABLE 5), unusual high-pitched crying, prolonged crying (>4 hr), diarrhea, vomiting, crying, pain, otitis media, rash, and upper respiratory infection.

Selected objective observations reported by parents over a 48-hour period in these infants following primary vaccination with Liquid PedvaxHIB are summarized in TABLE 5.

TABLE 5
Fever or Local Reactions in Subjects First Vaccinated at 2 to 6 Months of Age with Liquid PedvaxHIB*

Reaction	Post-Dose 1 (hr) No. of Subjects Evaluated	6	24	48	Post-Dose 2 (hr) No. of Subjects Evaluated	6	24	48
		Percentage				Percentage		
Fever** >38.3°C (≥101°F) Rectal	222	18.1	4.4	0.5	206	14.1	9.4	2.8
Erythema >2.5 cm diameter	674	2.2	1.0	0.5	562	1.6	1.1	0.4
Swelling >2.5 cm diameter	674	2.5	1.9	0.9	562	0.9	0.9	1.3

* DTP and OPV were administered concomitantly to most subjects.
** Fever was also measured by another method or reported as normal for an additional 345 infants after dose 1 and for an additional 249 infants after dose 2; however, these data are not included in this table.

Adverse reactions during a three-day period following administration of the booster dose were generally similar in type and frequency to those seen following primary vaccination.

Lyophilized PedvaxHIB

In The Protective Efficacy Study (see CLINICAL PHARMACOLOGY), 4,459 healthy Navajo infants 6 to 12 weeks of age received lyophilized PedvaxHIB or placebo. Most of these infants received DTP/OPV concomitantly. No differences were seen in the type and frequency of serious health problems expected in this Navajo population or in serious adverse experiences reported among those who received lyophilized PedvaxHIB and those who received placebo, and none was reported to be related to lyophilized PedvaxHIB. Only one serious reaction (tracheitis) was reported as possibly related to lyophilized PedvaxHIB and only one (diarrhea) as possibly related to placebo. Seizures occurred infrequently in both groups (9 occurred in vaccine recipients, 8 of whom also received DTP; 8 occurred in placebo recipients, 7 of whom also received DTP) and were not reported to be related to lyophilized PedvaxHIB.

In early clinical studies involving the administration of 8,086 doses of lyophilized PedvaxHIB alone to 5,027 healthy infants and children 2 months to 71 months of age, lyophilized PedvaxHIB was generally well tolerated. No serious adverse reactions were reported. In a subset of these infants, urticaria was reported in two children, and thrombocytopenia was seen in one child. A cause and effect relationship between these side effects and the vaccination has not been established.

Potential Adverse Reactions

The use of Haemophilus b Polysaccharide Vaccines and another Haemophilus b Conjugate Vaccine has been associated with the following additional adverse effects: early onset Hib disease and Guillain-Barré syndrome. A cause and effect relationship between these side effects and the vaccination was not established.[36,37,39,40,41,49]

Post-Marketing Adverse Reactions

The following additional adverse reactions have been reported with the use of the lyophilized and liquid formulations of PedvaxHIB:

Hemic and Lymphatic System

Lymphadenopathy

Hypersensitivity

Rarely, angioedema

Nervous System

Febrile seizures

Skin

Sterile injection site abscess

DOSAGE AND ADMINISTRATION

Liquid PedvaxHIB

FOR INTRAMUSCULAR ADMINISTRATION

DO NOT INJECT INTRAVENOUSLY

If there is an interruption or delay between doses in the primary series, there is no need to repeat the series, but dosing should be continued at the next clinic visit. (See CONTRAINDICATIONS and PRECAUTIONS.)

2 to 14 Months of Age

Infants 2 to 14 months of age should receive a 0.5 mL dose of vaccine ideally beginning at 2 months of age followed by a 0.5 mL dose 2 months later (or as soon as possible thereafter). When the primary two-dose regimen is completed before 12 months of age, a booster dose is required (see below and TABLE 6). Infants born prematurely, regardless of birth weight, should be vaccinated at the same chronological age and according to the same schedule and precautions as full-term infants and children.[46]

15 Months of Age and Older

Children 15 months of age and older previously unvaccinated against Hib disease should receive a single 0.5 mL dose of vaccine.

Booster Dose

In infants completing the primary two-dose regimen before 12 months of age, a booster dose (0.5 mL) should be administered at 12 to 15 months of age, but not earlier than 2 months after the second dose.

Vaccination regimens for Liquid PedvaxHIB by age group are outlined in TABLE 6.

TABLE 6
Vaccination Regimens for Liquid PedvaxHIB
By Age Groups

Age (Months) at First Dose	Primary	Age (Months) at Booster Dose
2–10	2 doses, 2 mo. apart	12–15
11–14	2 doses, 2 mo. apart	—
15–71	1 dose	—

Interchangeability

PedvaxHIB may be interchanged with other licensed Haemophilus b Conjugate Vaccines for the primary and booster doses.[52] (See CLINICAL PHARMACOLOGY.)

Use with Other Vaccines

Results from clinical studies indicate that Liquid PedvaxHIB can be administered concomitantly with DTP, OPV, eIPV (enhanced inactivated poliovirus vaccine), VARIVAX [Varicella Virus Vaccine Live (Oka/Merck)], M-M-R II (Measles, Mumps, and Rubella Virus Vaccine Live) or RECOMBIVAX HB [Hepatitis B Vaccine (Recombinant)]. No impairment of immune response to these individually tested vaccine antigens was demonstrated.

The type, frequency and severity of adverse experiences observed in these studies with PedvaxHIB were similar to those seen with the other vaccines when given alone. (See CLINICAL PHARMACOLOGY.)

In addition, a PRP-OMPC-containing product, COMVAX [Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine], was given concomitantly with a booster dose of DTaP [diphtheria, tetanus, acellular pertussis] at approximately 15 months of age, using separate sites and syringes for injectable vaccines. No impairment of immune response to these individually tested vaccine antigens was demonstrated. COMVAX has also been administered concomitantly with the primary series of DTaP to a limited number of infants. PRP antibody responses are satisfactory for COMVAX, but immune responses are currently unavailable for DTaP (see Manufacturer's Product Circular for COMVAX). No serious vaccine-related adverse events were reported.[33]

Parenteral drug products should be inspected visually for extraneous particulate matter and discoloration prior to administration whenever solution and container permit.

Liquid PedvaxHIB is a slightly opaque white suspension. (See DESCRIPTION.)

The vaccine should be used as supplied; no reconstitution is necessary.

Shake well before withdrawal and use. Thorough agitation is necessary to maintain suspension of the vaccine.

Inject 0.5 mL intramuscularly, preferably into the anterolateral thigh or the outer aspect of the upper arm. The buttocks should not be used for active vaccination of infants and children, because of the potential risk of injury to the sciatic nerve. Discard vial after use.

HOW SUPPLIED

Liquid PedvaxHIB is supplied as follows:

No. 4897 — A box of 10 single-dose vials of liquid vaccine, **NDC** 0006-4897-00.

Storage

Store vaccine at 2-8°C (36-46°F).

DO NOT FREEZE.

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Manuf. and Dist. by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

For patent information: www.merck.com/product/patent/home.html

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Hepatitis A

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use HAVRIX safely and effectively. See full prescribing information for HAVRIX.

HAVRIX (Hepatitis A Vaccine) injectable suspension, for intramuscular use
Initial U.S. Approval: 1995

---------- INDICATIONS AND USAGE----------
HAVRIX is a vaccine indicated for active immunization against disease caused by hepatitis A virus (HAV). HAVRIX is approved for use in persons 12 months of age and older. Primary immunization should be administered at least 2 weeks prior to expected exposure to HAV. (1)

----------DOSAGE AND ADMINISTRATION----------
- HAVRIX is administered by intramuscular injection. (2.2)
- Children and adolescents: A single 0.5-mL dose and a 0.5-mL booster dose administered between 6 to 12 months later. (2.3)
- Adults: A single 1-mL dose and a 1-mL booster dose administered between 6 to 12 months later. (2.3)

---------- DOSAGE FORMS AND STRENGTHS----------
- Suspension for injection available in the following presentations:
- 0.5-mL single-dose prefilled syringes. (3)
- 1-mL single-dose prefilled syringes. (3)

---------- CONTRAINDICATIONS ----------
Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis A-containing vaccine, or to any component of HAVRIX, including neomycin. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including HAVRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)

---------- ADVERSE REACTIONS ----------
- In studies of adults and children 2 years of age and older, the most common solicited adverse reactions were injection-site soreness (56% of adults and 21% of children) and headache (14% of adults and less than 9% of children). (6.1)
- In studies of children 11 to 25 months of age, the most frequently reported solicited local reactions were pain (32%) and redness (29%). Common solicited general adverse reactions were irritability (42%), drowsiness (28%), and loss of appetite (28%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- DRUG INTERACTIONS----------
Do not mix HAVRIX with any other vaccine or product in the same syringe . (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 9/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

HAVRIX is indicated for active immunization against disease caused by hepatitis A virus (HAV). HAVRIX is approved for use in persons 12 months of age and older. Primary immunization should be administered at least 2 weeks prior to expected exposure to HAV.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

Shake well before use. With thorough agitation, HAVRIX is a homogeneous, turbid, white suspension. Do not administer if it appears otherwise. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered. Attach a sterile needle to the prefilled syringe and administer intramuscularly.

2.2 Administration

HAVRIX should be administered by intramuscular injection only. HAVRIX should not be administered in the gluteal region; such injections may result in suboptimal response.

Do not administer this product intravenously, intradermally, or subcutaneously.

2.3 Recommended Dose and Schedule

Children and Adolescents (aged 12 months through 18 years)

Primary immunization for children and adolescents consists of a single 0.5-mL dose and a 0.5-mL booster dose administered anytime between 6 and 12 months later. The preferred sites for intramuscular injections are the anterolateral aspect of the thigh in young children or the deltoid muscle of the upper arm in older children.

Adults (aged 19 years and older)

Primary immunization for adults consists of a single 1-mL dose and a 1-mL booster dose administered anytime between 6 and 12 months later. In adults, the injection should be given in the deltoid region.

3 DOSAGE FORMS AND STRENGTHS

Suspension for injection available in the following presentations:

- 0.5-mL single-dose prefilled TIP-LOK syringes.
- 1-mL single-dose prefilled TIP-LOK syringes. *[See How Supplied/Storage and Handling (16).]*

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis A-containing vaccine, or to any component of HAVRIX, including neomycin, is a contraindication to administration of HAVRIX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including HAVRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Preventing and Managing Allergic Vaccine Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine *[see Contraindications (4)]*.

5.4 Altered Immunocompetence

Immunocompromised persons may have a diminished immune response to HAVRIX, including individuals receiving immunosuppressant therapy.

5.5 Limitations of Vaccine Effectiveness

Hepatitis A virus has a relatively long incubation period (15 to 50 days). HAVRIX may not prevent hepatitis A infection in individuals who have an unrecognized hepatitis A infection at the time of vaccination. Additionally, vaccination with HAVRIX may not protect all individuals.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of HAVRIX has been evaluated in 61 clinical trials involving approximately 37,000 individuals receiving doses of 360 EL.U. (n = 21,928 in 3- or 4-dose schedule), 720 EL.U. (n = 12,274 in 2- or 3-dose schedule), or 1440 EL.U. (n = 2,782 in 2- or 3-dose schedule).

Of solicited adverse reactions in clinical trials of adults, who received HAVRIX 1440 EL.U., and children (aged 2 years and older), who received either HAVRIX 360 EL.U. or 720 EL.U., the most frequently reported was injection-site soreness (56% of adults and 21% of children); less than 0.5% of soreness was reported as severe. Headache was reported by 14% of adults and less than 9% of children. Other solicited and unsolicited reactions occurring during clinical trials are listed below.

Incidence 1% to 10% of Injections

Metabolism and Nutrition Disorders: Anorexia.

Gastrointestinal Disorders: Nausea.

General Disorders and Administration Site Conditions: Fatigue; fever >99.5°F (37.5°C); induration, redness, and swelling of the injection site; malaise.

Incidence <1% of Injections

Infections and Infestations: Pharyngitis, upper respiratory tract infections.

Blood and Lymphatic System Disorders: Lymphadenopathy.

Psychiatric Disorders: Insomnia.

Nervous System Disorders: Dysgeusia, hypertonia.

Eye Disorders: Photophobia.

Ear and Labyrinth Disorders: Vertigo.

Gastrointestinal Disorders: Abdominal pain, diarrhea, vomiting.

Skin and Subcutaneous Tissue Disorders: Pruritus, rash, urticaria.

Musculoskeletal and Connective Tissue Disorders: Arthralgia, myalgia.

General Disorders and Administration Site Conditions: Injection site hematoma.

Investigations: Creatine phosphokinase increased.

Coadministration Studies of HAVRIX in Children Aged 11 to 25 Months

In 4 studies, 3,152 children aged 11 to 25 months received at least 1 dose of HAVRIX 720 EL.U. administered alone or concomitantly with other routine childhood vaccinations *[see Clinical Studies (14.2, 14.5)]*. The studies included HAV 210 (N = 1,084), HAV 232 (N = 394), HAV 220 (N = 433), and HAV 231 (N = 1,241).

In the largest of these studies (HAV 231) conducted in the U.S., 1,241 children aged 15 months were randomized to receive: Group 1) HAVRIX alone; Group 2) HAVRIX concomitantly with measles, mumps, and rubella (MMR) vaccine (manufactured by Merck and Co.) and varicella vaccine (manufactured by Merck and Co.); or Group 3) MMR and varicella vaccines. Subjects in Group 3 who received MMR and varicella vaccines received the first dose of HAVRIX 42 days later. A second dose of HAVRIX was administered to all subjects 6 to 9 months after the first dose of HAVRIX. Solicited local adverse reactions and general events were recorded by parents/guardians on diary cards for 4 days (Days 0 to 3) after vaccination. Unsolicited adverse events were recorded on the diary card for 31 days after vaccination. Telephone follow-up was conducted 6 months after the last vaccination to inquire about serious adverse events, new onset chronic illnesses, and medically significant events. A total of 1,035 children completed the 6-

month follow-up. Among subjects in all groups combined, 53% were male; 69% of subjects were White, 16% were Hispanic, 9% were Black, and 6% were other racial/ethnic groups.

Percentages of subjects with solicited local adverse reactions and general adverse reactions following HAVRIX administered alone (Group 1) or concomitantly with MMR and varicella vaccines (Group 2) are presented in Table 1. The solicited adverse reactions from the 3 additional coadministration studies conducted with HAVRIX were comparable to those from Study HAV 231.

Table 1. Solicited Local Adverse Reactions and General Adverse Reactions Occurring within 4 Days of Vaccination[a] in Children Aged 15 to 24 Months with HAVRIX Administered Alone or Concomitantly with MMR and Varicella Vaccines (TVC)

	Group 1 HAVRIX Dose 1 %	Group 2 HAVRIX+ MMR+V[b] Dose 1 %	Group 1 HAVRIX Dose 2 %	Group 2 HAVRIX Dose 2 %
Local (at injection site for HAVRIX)				
n	**298**	**411**	**272**	**373**
Pain, any	24	24	24	30
Redness, any	20	20	23	24
Swelling, any	9	10	10	10
General				
n	**300**	**417**	**271**	**375**
Irritability, any	33	44	31	27
Irritability, Grade 3	0	2	2	0
Drowsiness, any	22	35	21	21
Drowsiness, Grade 3	1	2	1	0
Loss of appetite, any	18	26	20	21
Loss of appetite, Grade 3	1	1	0	0
Fever ≥100.6°F (38.1°C)	3	5	3	3
Fever ≥101.5°F (38.6°C)	2	3	2	2
Fever ≥102.4°F (39.1°C)	1	1	0	1

Total vaccinated cohort (TVC) = all subjects who received at least 1 dose of vaccine.
n = Number of subjects who received at least 1 dose of vaccine and for whom diary card information was available.

Grade 3: Drowsiness defined as prevented normal daily activities; irritability/fussiness defined as crying that could not be comforted/prevented normal daily activities; loss of appetite defined as no eating at all.

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] MMR = Measles, mumps, and rubella vaccine; V = Varicella vaccine.

Serious Adverse Events in Children Aged 11 to 25 Months: Among these 4 studies, 0.9% (29/3,152) of subjects reported a serious adverse event within the 31-day period following vaccination with HAVRIX. Among subjects administered HAVRIX alone 1.0% (13/1,332) reported a serious adverse event. Among subjects who received HAVRIX concomitantly with other childhood vaccines, 0.9% (8/909) reported a serious adverse event. In these 4 studies, there were 4 reports of seizure within 31 days post-vaccination: these occurred 2, 9, and 27 days following the first dose of HAVRIX administered alone and 12 days following the second dose of HAVRIX. In 1 subject who received INFANRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) and Hib conjugate vaccine followed by HAVRIX 6 weeks later, bronchial hyperreactivity and respiratory distress were reported on the day of administration of HAVRIX alone.

6.2 Postmarketing Experience

The following adverse reactions have been identified during post-approval use of HAVRIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Infections and Infestations

Rhinitis.

Blood and Lymphatic System Disorders

Thrombocytopenia.

Immune System Disorders

Anaphylactic reaction, anaphylactoid reaction, serum sickness–like syndrome.

Nervous System Disorders

Convulsion, dizziness, encephalopathy, Guillain-Barré syndrome, hypoesthesia, multiple sclerosis, myelitis, neuropathy, paresthesia, somnolence, syncope.

Vascular Disorders

Vasculitis.

Respiratory, Thoracic, and Mediastinal Disorders

Dyspnea.

Hepatobiliary Disorders

Hepatitis, jaundice.

Skin and Subcutaneous Tissue Disorders

Angioedema, erythema multiforme, hyperhidrosis.

Congenital, Familial, and Genetic Disorders

Congenital anomaly.

Musculoskeletal and Connective Tissue Disorders

Musculoskeletal stiffness.

General Disorders and Administration Site Conditions

Chills, influenza-like symptoms, injection site reaction, local swelling.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Vaccines and Immune Globulin

In clinical studies HAVRIX was administered concomitantly with the following vaccines *[see Adverse Reactions (6.1), Clinical Studies (14.5)]*.

- INFANRIX (DTaP);
- Hib conjugate vaccine;
- pneumococcal 7-valent conjugate vaccine;
- MMR vaccine;
- varicella vaccine.

HAVRIX may be administered concomitantly with immune globulin.

When concomitant administration of other vaccines or immune globulin is required, they should be given with different syringes and at different injection sites. Do not mix HAVRIX with any other vaccine or product in the same syringe.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater-than-physiologic doses), may reduce the immune response to HAVRIX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no adequate and well-controlled studies of HAVRIX in pregnant women in the U.S. Available data do not suggest an increased risk of major birth defects and miscarriage in women who received HAVRIX during pregnancy *(see Data)*.

There are no animal studies with HAVRIX to inform use during pregnancy.

Data

Human Data: In pre- and post-licensure clinical studies of HAVRIX, 175 pregnant women (177 outcomes, including two sets of twins) were inadvertently administered HAVRIX following their last menstrual period. After excluding ectopic pregnancies (n = 2), molar pregnancies (n = 1), elective terminations (n = 22, including one of a fetus with a birth defect), those that were lost to follow-up (n = 9), and those with an unknown exposure timing (n = 5), there were 138 known pregnancy outcomes with exposure during the first or second trimester. Of these, miscarriage was reported in 11% of pregnancies exposed prior to 20 weeks gestation (15/136) and major birth defects were reported in 3.3% (4/123) of live births. The rates of miscarriage and major birth defects were consistent with estimated background rates.

8.2 Lactation

Risk Summary

There is no information regarding the presence of HAVRIX in human milk, the effects on the breastfed child, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for HAVRIX and any potential adverse effects on the breastfed child from HAVRIX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

The safety and effectiveness of HAVRIX, doses of 360 EL.U. or 720 EL.U., have been evaluated in more than 22,000 subjects aged 1 to 18 years.

The safety and effectiveness of HAVRIX have not been established in subjects younger than 12 months.

8.5 Geriatric Use

Clinical studies of HAVRIX did not include sufficient numbers of subjects aged 65 years and older to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in overall safety between these subjects and younger adult subjects.

8.6 Hepatic Impairment

Subjects with chronic liver disease had a lower antibody response to HAVRIX than healthy subjects *[see Clinical Studies (14.3)]*.

11 DESCRIPTION

HAVRIX (Hepatitis A Vaccine) is a sterile suspension of inactivated virus for intramuscular administration. The virus (strain HM175) is propagated in MRC-5 human diploid cells. After removal of the cell culture medium, the cells are lysed to form a suspension. This suspension is purified through ultrafiltration and gel permeation chromatography procedures. Treatment of this lysate with formalin ensures viral inactivation. Viral antigen activity is referenced to a standard using an enzyme linked immunosorbent assay (ELISA), and is therefore expressed in terms of ELISA Units (EL.U.).

Each 1-mL adult dose of vaccine contains 1440 EL.U. of viral antigen, adsorbed on 0.5 mg of aluminum as aluminum hydroxide.

Each 0.5-mL pediatric dose of vaccine contains 720 EL.U. of viral antigen, adsorbed onto 0.25 mg of aluminum as aluminum hydroxide.

HAVRIX contains the following excipients: Amino acid supplement (0.3% w/v) in a phosphate-buffered saline solution and polysorbate 20 (0.05 mg/mL). From the manufacturing process, HAVRIX also contains residual MRC-5 cellular proteins (not more than 5 mcg/mL), formalin (not more than 0.1 mg/mL), and neomycin sulfate (not more than 40 ng/mL), an aminoglycoside antibiotic included in the cell growth media.

HAVRIX is formulated without preservatives.

HAVRIX is available in prefilled syringes. The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The hepatitis A virus belongs to the picornavirus family. It is 1 of several hepatitis viruses that cause systemic disease with pathology in the liver.

The incubation period for hepatitis A averages 28 days (range: 15 to 50 days).[1] The course of hepatitis A infection is extremely variable, ranging from asymptomatic infection to icteric hepatitis and death.

The presence of antibodies to HAV confers protection against hepatitis A infection. However, the lowest titer needed to confer protection has not been determined.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

HAVRIX has not been evaluated for its carcinogenic potential, mutagenic potential, or potential for impairment of fertility.

14 CLINICAL STUDIES

14.1 Pediatric Effectiveness Studies

Protective efficacy with HAVRIX has been demonstrated in a double-blind, randomized controlled study in school children (aged 1 to 16 years) in Thailand who were at high risk of HAV infection. A total of 40,119 children were randomized to be vaccinated with either HAVRIX 360 EL.U. or ENGERIX-B [Hepatitis B Vaccine (Recombinant)] 10 mcg at 0, 1, and 12 months. Of these, 19,037 children received 2 doses of HAVRIX (0 and 1 months) and 19,120 children received 2 doses of control vaccine, ENGERIX-B (0 and 1 months). A total of 38,157 children entered surveillance at Day 138 and were observed for an additional 8 months. Using the protocol-defined endpoint (≥2 days absence from school, ALT level >45 U/mL, and a positive result in the HAVAB-M test), 32 cases of clinical hepatitis A occurred in the control group. In the group receiving HAVRIX, 2 cases were identified. These 2 cases were mild in terms of both biochemical and clinical indices of hepatitis A disease. Thus the calculated efficacy rate for prevention of clinical hepatitis A was 94% (95% Confidence Interval [CI]: 74, 98).

In outbreak investigations occurring in the trial, 26 clinical cases of hepatitis A (of a total of 34 occurring in the trial) occurred. No cases occurred in vaccinees who received HAVRIX.

Using additional virological and serological analyses post hoc, the efficacy of HAVRIX was confirmed. Up to 3 additional cases of mild clinical illness may have occurred in vaccinees. Using available testing, these illnesses could neither be proven nor disproven to have been caused by HAV. By including these as cases, the calculated efficacy rate for prevention of clinical hepatitis A would be 84% (95% CI: 60, 94).

14.2 Immunogenicity in Children and Adolescents

Immune Response to HAVRIX 720 EL.U./0.5 mL at Age 11 to 25 Months (Study HAV 210)

In this prospective, open-label, multicenter study, 1,084 children were administered study vaccine in 1 of 5 groups:

(1) Children aged 11 to 13 months who received HAVRIX on a 0- and 6-month schedule;

(2) Children aged 15 to 18 months who received HAVRIX on a 0- and 6-month schedule;

(3) Children aged 15 to 18 months who received HAVRIX coadministered with INFANRIX and Haemophilus b (Hib) conjugate vaccine (no longer U.S.-licensed) at Month 0 and HAVRIX at Month 6;

(4) Children aged 15 to 18 months who received INFANRIX coadministered with Hib conjugate vaccine at Month 0 and HAVRIX at Months 1 and 7;

(5) Children aged 23 to 25 months who received HAVRIX on a 0- and 6-month schedule.

Among subjects in all groups, 52% were male; 61% of subjects were White, 9% were Black, 3% were Asian, and 27% were other racial/ethnic groups. The anti-hepatitis A antibody vaccine responses and geometric mean antibody titers (GMTs), calculated on responders for Groups 1, 2, and 5 are presented in Table 2. Vaccine response rates were similar among the 3 age-groups that received HAVRIX. One month after the second dose of HAVRIX, the GMT in each of the younger age-groups (aged 11 to 13 months and 15 to 18 months) was shown to be similar to that achieved in the 23- to 25-month age-group.

Table 2. Anti-Hepatitis A Immune Response following 2 Doses of HAVRIX 720 EL.U./0.5 mL Administered 6 Months Apart in Children Given the First Dose of HAVRIX at Age 11 to 13 Months, 15 to 18 Months, or 23 to 25 Months

Age Group	n	Vaccine Response		GMT (mIU/mL)
		%	95% CI	
11-13 months (Group 1)	218	99	97, 100	1,461[a]
15-18 months (Group 2)	200	100	98, 100	1,635[a]
23-25 months (Group 5)	211	100	98, 100	1,911

Vaccine response = Seroconversion (anti-HAV ≥15 mIU/mL [lower limit of antibody measurement by assay]) in children initially seronegative or at least the maintenance of the pre-vaccination anti-HAV concentration in initially seropositive children.
CI = Confidence Interval; GMT = Geometric mean antibody titer.

[a] Calculated on vaccine responders 1 month post-dose 2. GMTs in children aged 11 to 13 months and 15 to 18 months were non-inferior (similar) to the GMT in children aged 23 to 25 months (i.e., the lower limit of the 2-sided 95% CI on the GMT ratio for Group 1/Group 5 and for Group 2/Group 5 were both ≥0.5).

In 3 additional clinical studies (HAV 232, HAV 220, and HAV 231), children received either 2 doses of HAVRIX alone or the first dose of HAVRIX concomitantly administered with other routinely recommended U.S.-licensed vaccines followed by a second dose of HAVRIX. After the second dose of HAVRIX, there was no evidence for interference with the anti-HAV response in the children who received concomitantly administered vaccines compared with those who received HAVRIX alone. *[See Adverse Reactions (6.1), Clinical Studies (14.5).]*

Immune Response to HAVRIX 360 EL.U. among Individuals Aged 2 to 18 Years

In 6 clinical studies, 762 subjects aged 2 to 18 years received 2 doses of HAVRIX (360 EL.U.) given 1 month apart (GMT ranged from 197 to 660 mIU/mL). Ninety-nine percent of subjects seroconverted following 2 doses. When a third dose of HAVRIX 360 EL.U. was administered 6 months following the initial dose, all subjects were seropositive (anti-HAV ≥20 mIU/mL) 1 month following the third dose, with GMTs rising to a range of 3,388 to 4,643 mIU/mL. In 1 study in which children were followed for an additional 6 months, all subjects remained seropositive.

Immune Response to HAVRIX 720 EL.U./0.5 mL among Individuals Aged 2 to 19 Years

In 4 clinical studies, 314 children and adolescents ranging from age 2 to 19 years were immunized with 2 doses of HAVRIX 720 EL.U./0.5 mL given 6 months apart. One month after the first dose, seroconversion (anti-HAV ≥20 mIU/mL [lower limit of antibody measurement by assay]) ranged from 96.8% to 100%, with GMTs of 194 mIU/mL to 305 mIU/mL. In studies in which sera were obtained 2 weeks following the initial dose, seroconversion ranged from 91.6% to 96.1%. One month following the booster dose at Month 6, all subjects were seropositive, with GMTs ranging from 2,495 mIU/mL to 3,644 mIU/mL.

In an additional study in which the booster dose was delayed until 1 year following the initial dose, 95.2% of the subjects were seropositive just prior to administration of the booster dose. One month later, all subjects were seropositive, with a GMT of 2,657 mIU/mL.

14.3 Immunogenicity in Adults

More than 400 healthy adults aged 18 to 50 years in 3 clinical studies were given a single 1440 EL.U. dose of HAVRIX. All subjects were seronegative for hepatitis A antibodies at baseline. Specific humoral antibodies against HAV were elicited in more than 96% of subjects when measured 1 month after vaccination. By Day 15, 80% to 98% of vaccinees had already seroconverted (anti-HAV ≥20 mIU/mL [lower limit of antibody measurement by assay]). GMTs of seroconverters ranged from 264 to 339 mIU/mL at Day 15 and increased to a range of 335 to 637 mIU/mL by 1 month following vaccination.

The GMTs obtained following a single dose of HAVRIX are at least several times higher than that expected following receipt of immune globulin.

In a clinical study using 2.5 to 5 times the standard dose of immune globulin (standard dose = 0.02 to 0.06 mL/kg), the GMT in recipients was 146 mIU/mL at 5 days post-administration, 77 mIU/mL at Month 1, and 63 mIU/mL at Month 2.

In 2 clinical trials in which a booster dose of 1440 EL.U. was given 6 months following the initial dose, 100% of vaccinees (n = 269) were seropositive 1 month after the booster dose, with GMTs ranging from 3,318 mIU/mL to 5,925 mIU/mL. The titers obtained from this additional dose approximate those observed several years after natural infection.

In a subset of vaccinees (n = 89), a single dose of HAVRIX 1440 EL.U. elicited specific anti-HAV neutralizing antibodies in more than 94% of vaccinees when measured 1 month after vaccination. These neutralizing antibodies persisted until Month 6. One hundred percent of vaccinees had neutralizing antibodies when measured 1 month after a booster dose given at Month 6.

Immunogenicity of HAVRIX was studied in subjects with chronic liver disease of various etiologies. One hundred eighty-nine healthy adults and 220 adults with either chronic hepatitis B (n = 46), chronic hepatitis C (n = 104), or moderate chronic liver disease of other etiology (n = 70) were vaccinated with HAVRIX 1440 EL.U. on a 0- and 6-month schedule. The last group consisted of alcoholic cirrhosis (n = 17), autoimmune hepatitis (n = 10), chronic hepatitis/cryptogenic cirrhosis (n = 9), hemochromatosis (n = 2), primary biliary cirrhosis (n = 15), primary sclerosing cholangitis (n = 4), and unspecified (n = 13). At each time point, GMTs were lower for subjects with chronic liver disease than for healthy subjects. At Month 7, the GMTs ranged from 478 mIU/mL (chronic hepatitis C) to 1,245 mIU/mL (healthy). One month after the first dose, seroconversion rates in adults with chronic liver disease were lower than in healthy adults. However, 1 month after the booster dose at Month 6, seroconversion rates were similar in all groups; rates ranged from 94.7% to 98.1%. The relevance of these data to the duration of protection afforded by HAVRIX is unknown.

In subjects with chronic liver disease, local injection site reactions with HAVRIX were similar among all 4 groups, and no serious adverse reactions attributed to the vaccine were reported in subjects with chronic liver disease.

14.4 Duration of Immunity

The duration of immunity following a complete schedule of immunization with HAVRIX has not been established.

14.5 Immune Response to Concomitantly Administered Vaccines

In 3 clinical studies HAVRIX was administered concomitantly with other routinely recommended U.S.-licensed vaccines: Study HAV 232: Diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (INFANRIX, DTaP) and Haemophilus b (Hib) conjugate vaccine (tetanus toxoid conjugate) (manufactured by Sanofi Pasteur SA); Study HAV 220: Pneumococcal 7-valent conjugate vaccine (PCV-7) (manufactured by Pfizer), and Study HAV 231: MMR and varicella vaccines. *[See Adverse Reactions (6.1).]*

Concomitant Administration with DTaP and Hib Conjugate Vaccine (Study HAV 232)

In this U.S. multicenter study, 468 subjects, children aged 15 months were randomized to receive: Group 1) HAVRIX coadministered with INFANRIX and Hib conjugate vaccine (n = 127); Group 2) INFANRIX and Hib conjugate vaccine alone followed by a first dose of HAVRIX 1 month later (n = 132); or Group 3) HAVRIX alone (n = 135). All subjects received a second dose of HAVRIX alone 6 to 9 months following the first dose. Among subjects in all

groups combined, 53% were male; 64% of subjects were White, 12% were Black, 6% were Hispanic, and 18% were other racial/ethnic groups.

There was no evidence for reduced antibody response to diphtheria and tetanus toxoids (percentage of subjects with antibody levels ≥0.1 mIU/mL to each antigen), pertussis antigens (percentage of subjects with seroresponse, antibody concentrations ≥5 EL.U./mL in seronegative subjects or post-vaccination antibody concentration ≥2 times the pre-vaccination antibody concentration in seropositive subjects, and GMTs), or Hib (percentage of subjects with antibody levels ≥1 mcg/mL to polyribosyl-ribitol phosphate, PRP) when HAVRIX was administered concomitantly with INFANRIX and Hib conjugate vaccine (Group 1) relative to INFANRIX and Hib conjugate vaccine administered together (Group 2).

Concomitant Administration with Pneumococcal 7-Valent Conjugate Vaccine (Study HAV 220)

In this U.S. multicenter study, 433 children aged 15 months were randomized to receive: Group 1) HAVRIX coadministered with PCV-7 vaccine (n = 137); Group 2) HAVRIX administered alone (n = 147); or Group 3) PCV-7 vaccine administered alone (n = 149) followed by a first dose of HAVRIX 1 month later. All subjects received a second dose of HAVRIX 6 to 9 months after the first dose. Among subjects in all groups combined, 53% were female; 61% of subjects were White, 16% were Hispanic, 15% were Black, and 8% were other racial/ethnic groups.

There was no evidence for reduced antibody response to PCV-7 (GMC to each serotype) when HAVRIX was administered concomitantly with PCV-7 vaccine (Group 1) relative to PCV-7 administered alone (Group 3).

Concomitant Administration with MMR and Varicella Vaccines (Study HAV 231)

In a U.S. multicenter study, there was no evidence for interference in the immune response to MMR and varicella vaccines (the percentage of subjects with pre-specified seroconversion/seroresponse levels) administered to subjects aged 15 months concomitantly with HAVRIX relative to the response when MMR and varicella vaccines are administered without HAVRIX. *[See Adverse Reactions (6.1).]*

15 REFERENCES

1. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR.* 2006;55(RR-7):1-23.

16 HOW SUPPLIED/STORAGE AND HANDLING

HAVRIX is available in single-dose prefilled disposable TIP-LOK syringes (packaged without needles) (Preservative-Free Formulation):

720 EL.U./0.5 mL

NDC 58160-825-43 Prefilled Syringe in Package of 10: NDC 58160-825-52

1440 EL.U./mL

NDC 58160-826-43 Prefilled Syringe in Package of 10: NDC 58160-826-52

Store refrigerated between 2° and 8°C (36° and 46°F). Do not freeze. Discard if the vaccine has been frozen. Do not dilute to administer.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipients and parents or guardians of the potential benefits and risks of immunization with HAVRIX.
- Emphasize, when educating vaccine recipients and parents or guardians regarding potential side effects, that HAVRIX contains non-infectious killed viruses and cannot cause hepatitis A infection.
- Instruct vaccine recipients and parents or guardians to report any adverse events to their healthcare provider.
- Give vaccine recipients and parents or guardians the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by **GlaxoSmithKline Biologicals**
Rixensart, Belgium, U.S. License No. 1617
Distributed by **GlaxoSmithKline**
Durham, NC 27701

HVX:47PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use TWINRIX safely and effectively. See full prescribing information for TWINRIX.

TWINRIX [Hepatitis A & Hepatitis B (Recombinant) Vaccine] injectable suspension, for intramuscular use
Initial U.S. Approval: 2001

---------- INDICATIONS AND USAGE----------
TWINRIX is a vaccine indicated for active immunization against disease caused by hepatitis A virus and infection by all known subtypes of hepatitis B virus. TWINRIX is approved for use in persons 18 years of age or older. (1)

----------DOSAGE AND ADMINISTRATION----------
- TWINRIX is administered by intramuscular injection. (2.2)
- Standard Dosing: A series of 3 doses (1 mL each) given on a 0-, 1-, and 6-month schedule. (2.3)
- Accelerated Dosing: A series of 4 doses (1-mL each) given on Days 0, 7, and 21 to 30 followed by a booster dose at Month 12. (2.3)

---------- DOSAGE FORMS AND STRENGTHS----------
Suspension for injection available in 1-mL prefilled syringes. (3, 11, 16)

---------- CONTRAINDICATIONS ----------
Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis A-containing or hepatitis B-containing vaccine, or to any component of TWINRIX, including yeast and neomycin. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including TWINRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)

---------- ADVERSE REACTIONS ----------
Following any dose of TWINRIX, the most common (≥10%) solicited injection site reactions were injection site soreness (35% to 41%) and redness (8% to 11%); the most common solicited systemic adverse reactions were headache (13% to 22%) and fatigue (11% to 14%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- DRUG INTERACTIONS----------
Do not mix TWINRIX with any other vaccine or product in the same syringe. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 12/2018

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

TWINRIX is indicated for active immunization against disease caused by hepatitis A virus and infection by all known subtypes of hepatitis B virus. TWINRIX is approved for use in persons 18 years of age or older.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

The vaccine should be re-suspended before use. When re-suspended, the vaccine will have a uniform hazy white appearance.

Upon storage, a fine white deposit with a clear colorless layer above may be present. Re-suspend the vaccine following the steps below.

1. Hold the syringe upright in a closed hand.
2. Shake the syringe by tipping it upside down and back upright again.
3. Repeat this action vigorously for at least 15 seconds.
4. Inspect the vaccine again:
    - If the vaccine appears as a uniform hazy white suspension, it is ready to use – the appearance should not be clear.
    - If the vaccine still does not appear as a uniform hazy white suspension, tip upside down and back upright again for at least another 15 seconds then inspect again.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Attach a sterile needle to the prefilled syringe and administer intramuscularly.

2.2 Administration

TWINRIX should be administered by intramuscular injection only as a 1-mL dose. Administer in the deltoid region. Do not administer in the gluteal region; such injections may result in a suboptimal response.

Do not administer this product intravenously, intradermally, or subcutaneously.

2.3 Recommended Dose and Schedule

Standard dosing schedule consists of 3 doses (1-mL each), given intramuscularly at 0, 1, and 6 months. Alternatively, an accelerated schedule of 4 doses (1-mL each), given intramuscularly on Days 0, 7, and 21 to 30 followed by a booster dose at Month 12 may be used.

3 DOSAGE FORMS AND STRENGTHS

Suspension for injection available in 1-mL prefilled TIP-LOK syringes *[see Description (11), How Supplied/Storage and Handling (16)]*.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis A-containing or hepatitis B-containing vaccine, or to any component of TWINRIX, including yeast and neomycin, is a contraindication to administration of TWINRIX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including TWINRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Preventing and Managing Allergic Vaccine Reactions

Prior to immunization, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions to allow an assessment of benefits and risks. Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine. *[See Contraindications (4).]*

5.4 Moderate or Severe Acute Illness

To avoid diagnostic confusion between manifestations of an acute illness and possible vaccine adverse effects, vaccination with TWINRIX should be postponed in persons with moderate or severe acute febrile illness unless they are at immediate risk of hepatitis A or hepatitis B infection.

5.5 Altered Immunocompetence

Immunocompromised persons, including individuals receiving immunosuppressive therapy, may have a diminished immune response to TWINRIX.

5.6 Multiple Sclerosis

Results from 2 clinical studies indicate that there is no association between hepatitis B vaccination and the development of multiple sclerosis,[1] and that vaccination with hepatitis B vaccine does not appear to increase the short-term risk of relapse in multiple sclerosis.[2]

5.7 Limitations of Vaccine Effectiveness

Hepatitis A and hepatitis B have relatively long incubation periods. The vaccine may not prevent hepatitis A or hepatitis B infection in individuals who have an unrecognized hepatitis A or hepatitis B infection at the time of vaccination. Additionally, vaccination with TWINRIX may not protect all individuals.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

Following any dose of TWINRIX, the most common (≥10%) solicited injection site reactions were injection site soreness (35% to 41%) and redness (8% to 11%); the most common solicited systemic adverse reactions were headache (13% to 22%) and fatigue (11% to 14%).

The safety of TWINRIX has been evaluated in clinical trials involving the administration of approximately 7,500 doses to more than 2,500 individuals.

In a U.S. study, 773 subjects (aged 18 to 70 years) were randomized 1:1 to receive TWINRIX (0-, 1-, and 6-month schedule) or concurrent administration of ENGERIX-B (0-, 1-, and 6-month schedule) and HAVRIX (0- and 6-month schedule). Solicited local adverse reactions and systemic adverse events were recorded by parents/guardians on diary cards for 4 days (Days 0 to 3) after vaccination. Unsolicited adverse events were recorded for 31 days after vaccination. Solicited reactions reported following the administration of TWINRIX or ENGERIX-B and HAVRIX are presented in Table 1.

Table 1. Rates of Local Adverse Reactions and Systemic Adverse Reactions within 4 Days of Vaccination[a] with TWINRIX[b] or ENGERIX-B and HAVRIX[c]

	TWINRIX			ENGERIX-B			HAVRIX	
	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2
	(n = 385)	(n = 382)	(n = 374)	(n = 382)	(n = 376)	(n = 369)	(n = 382)	(n = 369)
Local	%	%	%	%	%	%	%	%
Soreness	37	35	41	41	25	30	53	47
Redness	8	9	11	6	7	9	7	9
Swelling	4	4	6	3	5	5	5	5

	TWINRIX			ENGERIX-B and HAVRIX		
	Dose 1	Dose 2	Dose 3	Dose 1[d]	Dose 2[e]	Dose 3[d]
	(n = 385)	(n = 382)	(n = 374)	(n = 382)	(n = 376)	(n = 369)
Systemic	%	%	%	%	%	%
Headache	22	15	13	19	12	14
Fatigue	14	13	11	14	9	10
Diarrhea	5	4	6	5	3	3
Nausea	4	3	2	7	3	5
Fever	4	3	2	4	2	4
Vomiting	1	1	0	1	1	1

[a] Within 4 days of vaccination defined as day of vaccination and the next 3 days.

[b] 389 subjects received at least 1 dose of TWINRIX.
[c] 384 subjects received at least 1 dose each of ENGERIX-B and HAVRIX.
[d] Doses 1 and 3 included ENGERIX-B and HAVRIX in the control group receiving separate vaccinations.
[e] Dose 2 included only ENGERIX-B in the control group receiving separate vaccinations.

Most solicited local adverse reactions and systemic adverse reactions seen with TWINRIX were considered by the subjects as mild and self-limiting and did not last more than 48 hours.

In a clinical trial in which TWINRIX was given on a 0-, 7-, and 21- to 30-day schedule followed by a booster dose at 12 months, solicited local adverse reactions or systemic adverse reactions were comparable to those seen in other clinical trials of TWINRIX given on a 0-, 1-, and 6-month schedule.

Among 2,299 subjects in 14 clinical trials, the following adverse reactions were reported to occur within 30 days following vaccination:

Incidence 1% to 10% of Injections, Seen in Clinical Trials with TWINRIX

Infections and Infestations: Upper respiratory tract infections.

General Disorders and Administration Site Conditions: Injection site induration.

Incidence <1% of Injections, Seen in Clinical Trials with TWINRIX

Infections and Infestations: Respiratory tract illnesses.

Metabolism and Nutrition Disorders: Anorexia.

Psychiatric Disorders: Agitation, insomnia.

Nervous System Disorders: Dizziness, migraine, paresthesia, somnolence, syncope.

Ear and Labyrinth Disorders: Vertigo.

Vascular Disorders: Flushing.

Gastrointestinal Disorders: Abdominal pain, vomiting.

Skin and Subcutaneous Tissue Disorders: Erythema, petechiae, rash, sweating, urticaria.

Musculoskeletal and Connective Tissue Disorders: Arthralgia, back pain, myalgia.

General Disorders and Administration Site Conditions: Injection site ecchymosis, injection site pruritus, influenza-like symptoms, irritability, weakness.

Incidence <1% of Injections, Seen in Clinical Trials with HAVRIX and/or ENGERIX-B

Blood and Lymphatic System Disorders: Lymphadenopathy.[a+b]

Nervous System Disorders: Dysgeusia,[a] hypertonia,[a] tingling.[b]

Eye Disorders: Photophobia.[a]

Vascular Disorders: Hypotension.[b]

Gastrointestinal Disorders: Constipation.[b]

Investigations: Creatine phosphokinase increased.[a]

[a+b] Following either HAVRIX or ENGERIX-B.

[a] Following HAVRIX.

[b] Following ENGERIX-B.

Adverse reactions within 30 days of vaccination in the U.S. clinical trial of TWINRIX given on a 0-, 7-, and 21- to 30-day schedule followed by a booster dose at 12 months were comparable to those reported in other clinical trials.

6.2 Postmarketing Experience

The following adverse reactions have been identified during post-approval use of TWINRIX, HAVRIX, or ENGERIX-B. Because these reactions are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Postmarketing Experience with TWINRIX

Infections and Infestations: Herpes zoster, meningitis.

Blood and Lymphatic System Disorders: Thrombocytopenia, thrombocytopenic purpura.

Immune System Disorders: Allergic reaction, anaphylactoid reaction, anaphylaxis, serum sickness–like syndrome days to weeks after vaccination (including arthralgia/arthritis, usually transient; fever; urticaria; erythema multiforme; ecchymoses; and erythema nodosum).

Nervous System Disorders: Bell's palsy, convulsions, encephalitis, encephalopathy, Guillain-Barré syndrome, hypoesthesia, myelitis, multiple sclerosis, neuritis, neuropathy, optic neuritis, paralysis, paresis, transverse myelitis.

Eye Disorders: Conjunctivitis, visual disturbances.

Ear and Labyrinth Disorders: Earache, tinnitus.

Cardiac Disorders: Palpitations, tachycardia.

Vascular Disorders: Vasculitis.

Respiratory, Thoracic, and Mediastinal Disorders: Bronchospasm, including asthma-like symptoms; dyspnea.

Gastrointestinal Disorders: Dyspepsia.

Hepatobiliary Disorders: Hepatitis, jaundice.

Skin and Subcutaneous Tissue Disorders: Alopecia, angioedema, eczema, erythema multiforme, erythema nodosum, hyperhidrosis, lichen planus.

Musculoskeletal and Connective Tissue Disorders: Arthritis, muscular weakness.

General Disorders and Administration Site Conditions: Chills; immediate injection site pain, stinging, and burning sensation; injection site reaction; malaise.

Investigations: Abnormal liver function tests.

Postmarketing Experience with HAVRIX and/or ENGERIX-B

The following list includes adverse reactions for HAVRIX and/or ENGERIX-B not already reported above for TWINRIX.

Eye Disorders: Keratitis.[a]

Skin and Subcutaneous Tissue Disorders: Stevens-Johnson syndrome.[a]

Congenital, Familial, and Genetic Disorders: Congenital abnormality.[b]

[a] Following ENGERIX-B.

[b] Following HAVRIX.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Vaccines and Immune Globulin

Do not mix TWINRIX with any other vaccine or product in the same syringe.

When concomitant administration of immunoglobulin is required, it should be given with a different syringe and at a different injection site.

There are no data to assess the concomitant use of TWINRIX with other vaccines.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater-than-physiologic doses), may reduce the immune response to TWINRIX.

7.3 Interference with Laboratory Tests

Hepatitis B surface antigen (HBsAg) derived from hepatitis B vaccines has been transiently detected in blood samples following vaccination. Serum HBsAg detection may not have diagnostic value within 28 days after receipt of a hepatitis B vaccine, including TWINRIX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no adequate and well-controlled studies of TWINRIX in pregnant women in the U.S. Available data do not suggest an increased risk of major birth defects and miscarriage in women who received TWINRIX within 28 days prior to conception or during pregnancy *(see Data)*.

A developmental toxicity study was performed in female rats administered TWINRIX prior to mating and during gestation (0.2 mL at each occasion). This study revealed no adverse effects on fetal or pre-weaning development *(see Data)*.

Data

Human Data: A pregnancy exposure registry was maintained from 2001 to 2015. The registry prospectively enrolled 245 women who received a dose of TWINRIX during pregnancy or within 28 days prior to conception. After excluding induced abortions (n = 6, including one of a fetus with congenital anomalies), those lost to follow-up (n = 142), those with exposure in the third trimester (n = 1), and those with an unknown exposure timing (n = 9), there were 87 pregnancies with known outcomes with exposure within 28 days prior to conception, or in the first or second trimesters. Miscarriage was reported for 9.6% of pregnancies with exposure to TWINRIX prior to 20 weeks gestation (8/83). Major birth defects were reported for 3.8% of live born infants whose mothers were exposed within 28 days prior to conception or during the first or second trimester (3/80). The rates of miscarriage and major birth defects were consistent with estimated background rates.

In pre- and post-licensure clinical studies of TWINRIX, 45 pregnant women were inadvertently administered TWINRIX following their last menstrual period. Among such pregnancies, after excluding elective terminations (n = 1) and those lost to follow-up (n = 1), there were 43 pregnancies with known outcomes all with exposure in the first trimester. Miscarriage was reported in 16% of pregnancies (7/43) and major birth defects were reported in 2.6% of live births (1/38). The rates of miscarriage and major birth defects were consistent with estimated background rates.

Animal Data: In a developmental toxicity study, female rats were administered TWINRIX by intramuscular injection on Day 30 prior to mating and on gestation Days 6, 8, 11, and 15. The total dose was 0.2 mL (divided) at each occasion (a single human dose is 1 mL). No adverse effects on pre-weaning development up to post-natal Day 25 were observed. There were no fetal malformations or variations.

8.2 Lactation

Risk Summary

There is no information regarding the presence of TWINRIX in human milk, the effects on the breastfed child, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TWINRIX and any potential adverse effects on the breastfed child from TWINRIX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness in pediatric patients younger than 18 years have not been established.

8.5 Geriatric Use

Clinical studies of TWINRIX did not include sufficient numbers of subjects aged 65 years and older to determine whether they respond differently from younger subjects *[see Clinical Studies (14.1, 14.3)]*.

11 DESCRIPTION

TWINRIX [Hepatitis A & Hepatitis B (Recombinant) Vaccine] is a bivalent vaccine containing the antigenic components used in producing HAVRIX (Hepatitis A Vaccine) and ENGERIX-B [Hepatitis B Vaccine (Recombinant)]. TWINRIX is a sterile suspension for intramuscular administration that contains inactivated hepatitis A virus (strain HM175) and noninfectious HBsAg. The hepatitis A virus is propagated in MRC-5 human diploid cells and inactivated with formalin. The purified HBsAg is obtained by culturing genetically engineered *Saccharomyces cerevisiae* yeast cells, which carry the surface antigen gene of the hepatitis B virus. Bulk preparations of each antigen are adsorbed separately onto aluminum salts and then pooled during formulation.

A 1-mL dose of vaccine contains 720 ELISA Units of inactivated hepatitis A virus and 20 mcg of recombinant HBsAg protein. One dose of vaccine also contains 0.45 mg of aluminum in the form of aluminum phosphate and aluminum hydroxide as adjuvants, amino acids, sodium chloride, phosphate buffer, polysorbate 20, and Water for Injection. From the manufacturing process, each 1-mL dose of TWINRIX also contains residual formalin (not more than 0.1 mg), MRC-5 cellular proteins (not more than 2.5 mcg), neomycin sulfate (an aminoglycoside antibiotic included in the cell growth media; not more than 20 ng), and yeast protein (no more than 5%).

TWINRIX is available in prefilled syringes. The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex.

TWINRIX is formulated without preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Hepatitis A

The course of infection with hepatitis A virus (HAV) is extremely variable, ranging from asymptomatic infection to fulminant hepatitis.[3]

The presence of antibodies to HAV (anti-HAV) confers protection against hepatitis A disease. However, the lowest titer needed to confer protection has not been determined. Natural infection provides lifelong immunity even when antibodies to hepatitis A are undetectable. Seroconversion is defined as antibody titers equal to or greater than the assay cut-off (cut-off values vary depending on the assay used) in those previously seronegative.

Hepatitis B

Infection with hepatitis B virus (HBV) can have serious consequences including acute massive hepatic necrosis and chronic active hepatitis. Chronically infected persons are at increased risk for cirrhosis and hepatocellular carcinoma.

Antibody concentrations ≥10 mIU/mL against HBsAg are recognized as conferring protection against hepatitis B virus infection.[4]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

TWINRIX has not been evaluated for its carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Vaccination of female rats with TWINRIX had no effect on fertility. *[See Use in Specific Populations (8.1).]*

14 CLINICAL STUDIES

14.1 Immunogenicity: Standard 0-, 1-, and 6-Month Dosing Schedule

In 11 clinical trials, sera from 1,551 healthy adults aged 17 to 70 years, including 555 male subjects and 996 female subjects, were analyzed following administration of 3 doses of TWINRIX on a 0-, 1-, and 6-month schedule. Seroconversion (defined as equal to or greater than assay cut-off depending on assay used) for antibodies against HAV was elicited in 99.9% of vaccinees, and protective antibodies (defined as ≥10 mIU/mL) against HBV surface antigen were detected in 98.5% of vaccinees, 1 month after completion of the 3-dose series (Table 2).

Table 2. Seroconversion and Seroprotection Rates in Worldwide Clinical Trials

Dose of TWINRIX	n	% Seroconversion for Hepatitis A[a]	% Seroprotection for Hepatitis B[b]
1	1,587	93.8	30.8
2	1,571	98.8	78.2
3	1,551	99.9	98.5

[a] Anti-HAV titer ≥assay cut-off: 20 mIU/mL (HAVAB Test) or 33 mIU/mL (ENZYMUN-TEST).

[b] Anti-HBsAg titer ≥10 mIU/mL (AUSAB Test).

One of the 11 trials was a comparative trial conducted in a U.S. population given either TWINRIX (on a 0-, 1-, and 6-month schedule) or HAVRIX (0- and 6-month schedule) and ENGERIX-B (0-, 1-, and 6-month schedule). The monovalent vaccines were given concurrently in opposite arms. Of the 773 adults (aged 18 to 70 years) enrolled in this trial, an immunogenicity analysis was performed in 533 subjects who completed the study according to protocol. Of these, 264 subjects received TWINRIX and 269 subjects received HAVRIX and ENGERIX-B. Seroconversion rates against HAV and seroprotection rates against HBV are presented in Table 3; geometric mean titers (GMTs) are presented in Table 4. The absolute difference in anti-HAV seropositivity rates between groups was 0.36% (90% CI: -1.8, 3.1). Non-inferiority in terms of anti-HAV response was demonstrated (lower limit of the 90% CI was higher than the pre-specified non-inferiority criterion of -4.3%). The absolute difference in anti-HBsAg seroprotection rates between groups was 2.8% (90% CI: -1.3, 7.7). Non-inferiority in terms of anti-HBV response was demonstrated (lower limit of the 90% CI was higher than the pre-specified non-inferiority criterion of -9.4%).

Table 3. Seroconversion and Seroprotection Rates in a U.S. Clinical Trial

Vaccine	n	Timepoint	% Seroconversion for Hepatitis A[a] (95% CI)	% Seroprotection for Hepatitis B[b] (95% CI)
TWINRIX	264	Month 1	91.6	17.9
		Month 2	97.7	61.2
		Month 7	99.6 (97.9, 100.0)	95.1 (91.7, 97.4)
HAVRIX and ENGERIX-B	269	Month 1	98.1	7.5
		Month 2	98.9	50.4
		Month 7	99.3 (97.3, 99.9)	92.2 (88.3, 95.1)

CI = Confidence Interval.

[a] Anti-HAV titer ≥assay cut-off: 33 mIU/mL (ENZYMUN-TEST).

[b] Anti-HBsAg titer ≥10 mIU/mL (AUSAB Test).

Table 4. Geometric Mean Titers in a U.S. Clinical Trial

Vaccine	n	Timepoint	GMT to Hepatitis A (95% CI)	GMT to Hepatitis B (95% CI)
TWINRIX	263	Month 1	335	8
	259	Month 2	636	23
	264	Month 7	4756 (4152, 5448)	2099 (1663, 2649)
HAVRIX and ENGERIX-B	268	Month 1	444	6
	269	Month 2	257	18
	269	Month 7	2948 (2638, 3294)	1871 (1428, 2450)

GMT = Geometric mean titer; CI = Confidence Interval.

Since the immune responses to hepatitis A and hepatitis B induced by TWINRIX were non-inferior to the monovalent vaccines, efficacy is expected to be similar to the efficacy for each of the monovalent vaccines.

The antibody titers achieved 1 month after the final dose of TWINRIX were higher than titers achieved 1 month after the final dose of HAVRIX in this clinical trial. This may have been due to a difference in the recommended dosage regimens for these 2 vaccines, whereby vaccinees receiving TWINRIX received 3 doses of 720 EL.U. of hepatitis A antigen at 0, 1, and 6 months, whereas vaccinees receiving HAVRIX received 2 doses of 1440 EL.U. of the same antigen (at 0 and 6 months). However, these differences in peak titer have not been shown to be clinically significant.

14.2 Immunogenicity: Accelerated Dosing Schedule (Day 0, 7, and 21 to 30, Month 12)

In 496 healthy adults, the safety and immunogenicity of TWINRIX given on a 0-, 7-, and 21- to 30-day schedule followed by a booster dose at 12 months (n = 250), was compared with separate vaccinations with monovalent hepatitis A vaccine (HAVRIX at 0 and 12 months) and hepatitis B vaccine (ENGERIX-B at 0, 1, 2, and 12 months) as a control group (n = 246).

Following a booster dose at Month 12, seroprotection rates for hepatitis B and seroconversion rates for hepatitis A at Month 13 following TWINRIX were non-inferior to the control group. The absolute difference in anti-HBs seroprotection rates between groups (HAVRIX + ENGERIX-B minus TWINRIX) was -2.99 (95% CI: -7.80, 1.49). Non-inferiority was demonstrated as the upper limit of the 95% CI was lower than the pre-defined limit of 7%. The absolute difference in anti-HAV seroprotection rates between groups (HAVRIX + ENGERIX-B minus TWINRIX) was 0 (95% CI: -1.91, 1.94). Non-inferiority was demonstrated as the upper limit of the 95% CI was lower than the pre-defined limit of 7%. The immune responses are presented in Table 5.

Table 5. Seroconversion and Seroprotection Rates up to 1 Month after the Last Dose of Vaccines (According-to-Protocol Cohort)

	Timepoint	TWINRIX[a] (n = 194-204)	HAVRIX and ENGERIX-B[b] (n = 197-207)
% Seroconversion for Hepatitis A[c] (95% CI)	Day 37	98.5 (95.8, 99.7)	98.6 (95.8, 99.7)
	Day 90	100 (98.2, 100)	95.6 (91.9, 98.0)
	Month 12	96.9 (93.4, 98.9)	86.9 (81.4, 91.2)
	Month 13	100 (98.1, 100)	100 (98.1, 100)
% Seroprotection for Hepatitis B[d] (95% CI)	Day 37	63.2 (56.2, 69.9)	43.5 (36.6, 50.5)
	Day 90	83.2 (77.3, 88.1)	76.7 (70.3, 82.3)
	Month 12	82.1 (75.9, 87.2)	77.8 (71.3, 83.4)
	Month 13	96.4 (92.7, 98.5)	93.4 (89.0, 96.4)

CI = Confidence Interval.

[a] TWINRIX given on a 0-, 7-, and 21- to 30-day schedule followed by a booster at Month 12.

[b] HAVRIX 1440 EL.U./1 mL given on a 0- and 12-month schedule and ENGERIX-B 20 mcg/1 mL given on a 0-, 1-, 2-, and 12-month schedule.

[c] Anti-HAV titer ≥assay cut-off: 15 mIU/mL (anti-HAV Behring Test).

[d] Anti-HBsAg titer ≥10 mIU/mL (AUSAB Test).

14.3 Immunogenicity in Adults Older than 40 Years

The effect of age on immune response to TWINRIX was studied in 2 trials. The first trial evaluated subjects aged 41 to 63 years (N = 72; mean age = 50). All subjects were seropositive for anti-HAV antibodies following the third dose of TWINRIX. For the hepatitis B response, 94% of subjects were seroprotected after the third dose of TWINRIX.

The second trial included subjects aged 19 years and older with a comparison between those older than 40 years (n = 183, aged 41 to 70 years; mean age: 48) and those aged 40 years or younger (n = 191; aged 19 to 40 years; mean age: 33). More than 99% of subjects in both age groups achieved a seropositive response for anti-HAV antibodies, and GMTs were comparable between the age groups. In the older subjects who received TWINRIX, 92.9% (95% CI: 88.2, 96.2) achieved seroprotection against hepatitis B compared with 96.9% (95% CI: 93.3, 98.8) of the younger subjects. The GMT was 1,890 mIU/mL in the older subjects compared with 2,285 mIU/mL in the younger subjects.

14.4 Duration of Immunity

Two clinical trials involving a total of 129 subjects demonstrated that antibodies to both HAV and HBV surface antigen persisted for at least 4 years after the first vaccine dose in a 3-dose series of TWINRIX, given on a 0-, 1-, and 6-month schedule. For comparison, after the recommended immunization regimens for HAVRIX and ENGERIX-B, respectively, similar

studies involving a total of 114 subjects have shown that seropositivity to HAV and HBV also persists for at least 4 years.

15 REFERENCES

1. Ascherio A, Zhang SM, Hernán MA, et al. Hepatitis B vaccination and the risk of multiple sclerosis. *N Engl J Med.* 2001;344(5):327-332.
2. Confavreux C, Suissa S, Saddier P, et al. Vaccination and the risk of relapse in multiple sclerosis. *N Engl J Med.* 2001;344(5):319-326.
3. Lemon SM. Type A viral hepatitis: new developments in an old disease. *N Engl J Med.* 1985;313(17):1059-1067.
4. Frisch-Niggemeyer W, Ambrosch F, Hofmann H. The assessment of immunity against hepatitis B after vaccination. *J Bio Stand.* 1986;14(3):255-258.

16 HOW SUPPLIED/STORAGE AND HANDLING

TWINRIX is available in 1-mL single-dose prefilled disposable TIP-LOK syringes (packaged without needles) (Preservative-Free Formulation):

NDC 58160-815-43 Syringe in Package of 10: NDC 58160-815-52

Store refrigerated between 2° and 8°C (36° and 46°F). Do not freeze; discard if product has been frozen.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipients of the potential benefits and risks of immunization with TWINRIX.
- Emphasize, when educating vaccine recipients regarding potential side effects, that components of TWINRIX cannot cause hepatitis A or hepatitis B infection.
- Instruct vaccine recipients to report any adverse events to their healthcare provider.
- Give vaccine recipients the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

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Rixensart, Belgium, U.S. License No. 1617
Distributed by **GlaxoSmithKline**
Research Triangle Park, NC 27709

TWR:27PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use VAQTA safely and effectively. See full prescribing information for VAQTA.

VAQTA® (Hepatitis A Vaccine, Inactivated)
Suspension for Intramuscular Injection
Initial U.S. Approval: 1996

----------INDICATIONS AND USAGE----------
VAQTA is a vaccine indicated for the prevention of disease caused by hepatitis A virus (HAV) in persons 12 months of age and older. The primary dose should be given at least 2 weeks prior to expected exposure to HAV. (1.1)

----------DOSAGE AND ADMINISTRATION----------
- For intramuscular administration only. (2)
- Children/Adolescents: vaccination consists of a 0.5-mL primary dose administered intramuscularly, and a 0.5-mL booster dose administered intramuscularly 6 to 18 months later. (2.1)
- Adults: vaccination consists of a 1-mL primary dose administered intramuscularly, and a 1-mL booster dose administered intramuscularly 6 to 18 months later. (2.1)

----------DOSAGE FORMS AND STRENGTHS----------
Suspension supplied in four presentations:
- 0.5-mL pediatric dose in single-dose vials and prefilled syringes. (3, 11, 16)
- 1-mL adult dose in single-dose vials and prefilled syringes. (3, 11, 16)

----------CONTRAINDICATIONS----------
Do not administer VAQTA to individuals with a history of immediate and/or severe allergic or hypersensitivity reactions (*e.g.*, anaphylaxis) after a previous dose of any hepatitis A vaccine or with an anaphylactic reaction to neomycin. (4, 11)

---------- WARNINGS AND PRECAUTIONS ----------
- The vial stopper and the syringe plunger stopper and tip cap contain dry natural latex rubber that may cause allergic reactions in latex-sensitive individuals. (5.2)

---------- ADVERSE REACTIONS ----------
The most common local adverse reactions and systemic adverse events (≥15%) reported in different clinical trials across different age groups when VAQTA was administered alone or concomitantly were:
- Children — 12 through 23 months of age: injection-site pain/tenderness (37.0%), injection-site erythema (21.2%), fever (16.4% when administered alone, and 27.0% when administered concomitantly) (6.1)
- Children/Adolescents — 2 through 18 years of age: injection-site pain (18.7%) (6.1)
- Adults — 19 years of age and older: injection-site pain, tenderness, or soreness (67.0%), injection-site warmth (18.2%) and headache (16.1%) (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------DRUG INTERACTIONS----------
- Do not mix VAQTA with any other vaccine in the same syringe or vial. (7.1)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 10/2020

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Indications and Use

VAQTA® [Hepatitis A Vaccine, Inactivated] is indicated for the prevention of disease caused by hepatitis A virus (HAV) in persons 12 months of age and older. The primary dose should be given at least 2 weeks prior to expected exposure to HAV.

2 DOSAGE AND ADMINISTRATION

FOR INTRAMUSCULAR ADMINISTRATION ONLY.

2.1 Dosage and Schedule

Children/Adolescents (12 months through 18 years of age): The vaccination schedule consists of a primary 0.5-mL dose administered intramuscularly, and a 0.5-mL booster dose administered intramuscularly 6 to 18 months later.

Adults (*19 years of age and older*): The vaccination schedule consists of a primary 1-mL dose administered intramuscularly, and a 1-mL booster dose administered intramuscularly 6 to 18 months later.

Booster Immunization Following Another Manufacturer's Hepatitis A Vaccine: A booster dose of VAQTA may be given at 6 to 12 months following a primary dose of HAVRIX *[see Clinical Studies (14.6)].*

2.2 Preparation and Administration

Shake the single-dose vial or single-dose prefilled syringe well to obtain a slightly opaque, white suspension before withdrawal and use. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.
Discard if the suspension does not appear homogenous or if extraneous particulate matter remains or discoloration is observed.

For single-dose vials, withdraw and administer entire dose of VAQTA intramuscularly using a sterile needle and syringe. Discard vial after use.

For single-dose prefilled syringes, securely attach a needle by twisting in a clockwise direction and administer dose of VAQTA intramuscularly. Discard syringe after use.

For adults, adolescents, and children older than 2 years of age, the deltoid muscle is the preferred site for intramuscular injection. For children 12 through 23 months of age, the anterolateral area of the thigh is the preferred site for intramuscular injection.

3 DOSAGE FORMS AND STRENGTHS

Suspension for injection available in four presentations:

- 0.5-mL pediatric dose in single-dose vials and prefilled syringes
- 1-mL adult dose in single-dose vials and prefilled syringes

[See Description (11) for listing of vaccine components and How Supplied/Storage and Handling (16).]

4 CONTRAINDICATIONS

Do not administer VAQTA to individuals with a history of immediate and/or severe allergic or hypersensitivity reactions (*e.g.*, anaphylaxis) after a previous dose of any hepatitis A vaccine, or to individuals who have had an anaphylactic reaction to any component of VAQTA, including neomycin *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Prevention and Management of Allergic Vaccine Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine *[see Contraindications (4)]*.

5.2 Hypersensitivity to Latex

The vial stopper and the syringe plunger stopper and tip cap contain dry natural latex rubber that may cause allergic reactions in latex-sensitive individuals *[see How Supplied/Storage and Handling (16)]*.

5.3 Altered Immunocompetence

Immunocompromised persons, including individuals receiving immunosuppressive therapy, may have a diminished immune response to VAQTA and may not be protected against HAV infection after vaccination *[see Use in Specific Populations (8.6)]*.

5.4 Limitations of Vaccine Effectiveness

Hepatitis A virus has a relatively long incubation period (approximately 20 to 50 days). VAQTA may not prevent hepatitis A infection in individuals who have an unrecognized hepatitis A infection at the time of vaccination. Vaccination with VAQTA may not result in a protective response in all susceptible vaccinees.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of VAQTA has been evaluated in over 10,000 subjects 1 year to 85 years of age. Subjects were given one or two doses of the vaccine. The second (booster dose) was given 6 months or more after the first dose.

The most common local adverse reactions and systemic adverse events (≥15%) reported in different clinical trials across different age groups when VAQTA was administered alone or concomitantly were:

- Children — 12 through 23 months of age: injection-site pain/tenderness (37.0%), injection-site erythema (21.2%), fever (16.4% when administered alone, and 27.0% when administered concomitantly).
- Children/Adolescents — 2 through 18 years of age: injection-site pain (18.7%)
- Adults — 19 years of age and older: injection-site pain, tenderness, or soreness (67.0%), injection-site warmth (18.2%) and headache (16.1%)

Allergic Reactions

Local and/or systemic allergic reactions that occurred in <1% of over 10,000 children/adolescents or adults in clinical trials regardless of causality included: injection-site pruritus and/or rash; bronchial constriction; asthma; wheezing; edema/swelling; rash; generalized erythema; urticaria; pruritus; eye irritation/itching; dermatitis *[see Contraindications (4) and Warnings and Precautions (5.1)]*.

Children — 12 through 23 Months of Age

Across five clinical trials, 4374 children 12 to 23 months of age received one or two 25U doses of VAQTA, including 3885 children who received 2 doses of VAQTA and 1250 children who received VAQTA concomitantly with one or more other vaccines, including Measles, Mumps, and Rubella Virus Vaccine, Live (M-M-R II®), Varicella Vaccine, Live (VARIVAX®), Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine, Adsorbed (Tripedia or INFANRIX), Measles, Mumps, Rubella, and Varicella Vaccine, Live (ProQuad®), Pneumococcal 7-valent Conjugate Vaccine (Diphtheria $CRM_{197,}$ Prevnar), or Haemophilus B Conjugate Vaccine (Meningococcal Protein Conjugate, PedvaxHIB®). Overall, the race distribution of study subjects was as follows: 64.7% Caucasian; 15.7% Hispanic-American; 12.3% Black; 4.8% other; 1.4% Asian; and 1.1% Native American. The distribution of subjects by gender was 51.8% male and 48.2% female.

In an open-label clinical trial, 653 children 12 to 23 months of age were randomized to receive a first dose of VAQTA with ProQuad and Prevnar concomitantly (N=330) or a first dose of ProQuad and pneumococcal 7-valent conjugate vaccine concomitantly, followed by a first dose of VAQTA 6 weeks later (N=323). Approximately 6 months later, subjects received either the second doses of ProQuad and VAQTA concomitantly or the second doses of ProQuad and VAQTA separately. The race distribution of the study subjects was as follows: 60.3% Caucasian; 21.6% African-American; 9.5% Hispanic-American; 7.2% other; 1.1% Asian; and 0.3% Native American. The distribution of subjects by gender was 50.7% male and 49.3% female.

Table 1 presents rates of solicited local reactions at the VAQTA injection site and rates of elevated temperatures (≥100.4°F and ≥102.2°F) that occurred within 5 days following each dose of VAQTA and elevated temperatures >98.6°F for a total of 14 days after vaccination; occurrences of these events were recorded daily on diary cards. Table 2 presents rates of unsolicited systemic adverse events that occurred within 14 days at ≥5% in any group following each dose of VAQTA.

Table 1: Incidences of Solicited Local Adverse Reactions at the VAQTA Injection Site and Elevated Temperatures Following Each Dose of VAQTA in Healthy Children 12-23 Months of Age Receiving VAQTA Alone or Concomitantly With ProQuad and PREVNAR*

	Dose 1		Dose 2	
Adverse reaction: Days 1-5 unless noted	**VAQTA alone**	**VAQTA + ProQuad + Prevnar concomitantly**	**VAQTA alone**	**VAQTA + ProQuad concomitantly**
Injection site adverse reactions	N=274	N=311	N=251	N=263
Injection site erythema	11.7%	9.6%	12.7%	9.5%
Injection site pain/tenderness	15.3%	20.9%	20.3%	17.5%
Injection site swelling	9.5%	6.8%	7.6%	6.1%
Temperature > 98.6°F or feverish (Days 1-14)	12.4%	35.7%	10.8%	10.3%
	N=243	N=285	N=221	N=237
Temperature ≥ 100.4°F	10.3%	16.8%	10%	4.2%
Temperature ≥ 102.2 °F	2.1%	3.5%	2.3%	2.5%

*Pneumococcal 7-valent Conjugate Vaccine
N=number of subjects for whom data are available.

Table 2: Incidences of Unsolicited Systemic Adverse Events ≥5% in Any Group Following Each Dose of VAQTA in Healthy Children 12-23 Months of Age Receiving VAQTA Alone or Concomitantly With ProQuad and PREVNAR*

	Dose 1		Dose 2	
Adverse Event: Days 1-14	**VAQTA alone**	**VAQTA + ProQuad + PREVNAR concomitantly**	**VAQTA alone**	**VAQTA + ProQuad concomitantly**
	N=274	N=311	N=251	N=263
General Disorders and Administration Site Conditions				
Irritability	3.6%	6.1%	2.8%	2.7%
Infections and Infestations				
Upper respiratory tract infection	3.3%	6.1%	4.8%	5.7%
Skin and Subcutaneous Tissue Disorders				
Dermatitis diaper	1.1%	6.1%	2.4%	3.4%

*Pneumococcal 7-valent Conjugate Vaccine

In Stage I of an open, multicenter, randomized study, children 15 months of age were randomized to receive the first dose of VAQTA alone (N=151) or concomitantly with PedvaxHIB and INFANRIX (N=155); another group of children 15 months of age were randomized to receive the first dose of VAQTA alone (N=152) or concomitantly with PedvaxHIB (N=159). All groups received the second dose of VAQTA alone at least 6 months following the first dose. The race distribution of Stage I study subjects was: 63.9% Caucasian; 17.5% Hispanic-American; 14.7% Black; 2.6% other; and 1.3% Asian. The distribution of subjects by gender was 54.0% male and 46.0% female. In Stage II of this study, an additional 654 children 12-17 months of age received the first dose of VAQTA alone followed by the second dose of VAQTA 6 months later. The race distribution of Stage II of the study subjects was: 66.1% Caucasian; 10.6% Hispanic-American; 16.8% Black; 4.7% other; and 1.5% Asian. The distribution of subjects by gender was 51.2% male and 48.8% female.

Table 3 presents rates of solicited local reactions at the VAQTA injection-site and rates of elevated temperatures (≥100.4°F and ≥102.2°F) that occurred within 5 days following each dose of VAQTA and elevated temperatures >98.6°F for a total of 14 days following each dose of VAQTA. Occurrences of these events were recorded daily on diary cards. Table 4 presents rates of unsolicited systemic adverse events that occurred within 14 days at ≥5% following each dose of VAQTA.

Table 3: Incidences of Solicited Local Adverse Reactions at the VAQTA Injection Site and Elevated Temperatures Following Each Dose of VAQTA in Healthy Children 12-23 Months of Age Receiving VAQTA Alone or Concomitantly with PedvaxHIB With or Without INFANRIX (Stage I) and those Receiving VAQTA Alone at Both Doses (Stage II)

	Stage I			Stage II	
	Dose 1		Dose 2	Dose 1	Dose 2
Adverse Reaction: Days 1-5 unless noted	**VAQTA alone**	**VAQTA + PedvaxHIB and Infanrix or VAQTA + PedvaxHIB concomitantly**	**VAQTA alone**	**VAQTA alone**	**VAQTA alone**
Injection site adverse reactions	N=256	N=302	N=503	N=647	N=599
Injection site erythema	18.0%	19.9%	21.5%	11.7%	16.2%
Injection site pain/tenderness	21.9%	36.4%	27.4%	20.1%	22.9%
Injection site swelling	10.2%	14.2%	10.1%	7.1%	7.0%
Temperature > 98.6°F or feverish (Days 1-14)	10.2%	17.2%	10.7%	10.0%	8.2%
	N=234	N=290	N=473	N=631	N=591
Temperature ≥ 100.4°F	9.0%	16.9%	9.1%	9.4%	8.6%
Temperature ≥ 102.2 °F	3.8%	3.1%	3.2%	2.9%	2.4%

N= number of subjects for whom data is available

Table 4: Incidences of Unsolicited Systemic Adverse Events ≥5% in Any Group Following Each Dose of VAQTA in Healthy Children 12-23 Months of Age Receiving VAQTA Alone or Concomitantly with PedvaxHIB With or Without INFANRIX (Stage I) and Those Receiving VAQTA Alone at Both Doses (Stage II)

	Stage I			Stage II	
	Dose 1		Dose 2	Dose 1	Dose 2
Adverse Event: Days 1-14	**VAQTA alone**	**VAQTA + PedvaxHIB and Infanrix or VAQTA + PedvaxHIB concomitantly**	**VAQTA alone**	**VAQTA alone**	**VAQTA alone**
	N=256	N=302	N=503	N=647	N=599
Gastrointestinal Disorders					
Diarrhea	3.9%	8.3%	3.8%	4.6%	3.8%
Teething	3.1%	2.3%	1.4%	5.7%	4.3%
General Disorders and Administration Site Conditions					
Irritability	6.3%	9.6%	4.0%	8.8%	6.5%
Infections and Infestations					
Upper respiratory tract infection	2.3%	3.3%	3.0%	4.9%	5.2%
Respiratory, Thoracic and Mediastinal Disorders					
Rhinorrhea	2.0%	4.0%	3.8%	6.2%	3.8%

Data presented in Tables 1 through 4 on solicited local reactions, and solicited and unsolicited systemic adverse events with incidence ≥5% following each dose of VAQTA are representative of other clinical trials of VAQTA in children 12 through 23 months of age. Across the five studies conducted in children 12-23 months of age, ≥39.9% of subjects experienced local adverse reactions and ≥55.7% of subjects experienced systemic adverse events. The majority of local and systemic adverse events were mild to moderate in intensity.

The following additional unsolicited local adverse reactions and systemic adverse events were observed at a common frequency of ≥1% to <10% in any individual clinical study. This listing includes only the

adverse reactions not reported elsewhere in the label. These local adverse reactions and systemic adverse events occurred among recipients of VAQTA alone or VAQTA given concomitantly within 14 days following any dose of VAQTA across four clinical studies.

Eye disorders: Conjunctivitis

Gastrointestinal disorders: Constipation; vomiting

General disorders and administration site conditions: Injection-site bruising; injection-site ecchymosis

Infections and infestations: Otitis media; nasopharyngitis; rhinitis; viral infection; croup; pharyngitis streptococcal; laryngotracheobronchitis; viral exanthema; gastroenteritis viral; roseola

Metabolism and nutrition disorders: Anorexia

Psychiatric disorders: Insomnia; crying

Respiratory, thoracic and mediastinal disorders: Cough; nasal congestion; respiratory congestion

Skin and subcutaneous tissue disorders: Rash vesicular; measles-like/rubella-like rash; varicella-like rash; rash morbilliform

Serious Adverse Events (Children 12 through 23 Months of Age): Across the five studies conducted in subjects 12-23 months of age, 0.7% (32/4374) of subjects reported a serious adverse event following any dose of VAQTA, and 0.1% (5/4374) of subjects reported a serious adverse event judged to be vaccine related by the study investigator. The serious adverse events were collected over the period defined in each protocol (14, 28, or 42 days). Vaccine-related serious adverse events which occurred following any dose of VAQTA with or without concomitant vaccines included febrile seizure (0.05%), dehydration (0.02%), gastroenteritis (0.02%), and cellulitis (0.02%).

Children/Adolescents — 2 Years through 18 Years of Age

In 11 clinical trials, 2615 healthy children 2 years through 18 years of age received at least one dose of VAQTA. These studies included administration of VAQTA in varying doses and regimens (1377 children received one or more 25U doses). The race distribution of the study subjects who received at least one dose of VAQTA in these studies was as follows: 84.7% Caucasian; 10.6% American Indian; 2.3% African-American; 1.5% Hispanic-American; 0.6% other; 0.2% Oriental. The distribution of subjects by gender was 51.2% male and 48.8% female.

In a double-blind, placebo-controlled efficacy trial (i.e. The Monroe Efficacy Study), 1037 healthy children and adolescents 2 through 16 years of age were randomized to receive a primary dose of 25U of VAQTA and a booster dose of VAQTA 6, 12, or 18 months later, or placebo (alum diluent). All study subjects were Caucasian: 51.5% were male and 48.5% were female. Subjects were followed days 1 to 5 postvaccination for fever and local adverse reactions and days 1 to 14 for systemic adverse events. The most common adverse events/reactions were injection-site reactions, reported by 6.4% of subjects. Table 5 summarizes local adverse reactions and systemic adverse events reported in ≥1% of subjects. There were no significant differences in the rates of any adverse events or adverse reactions between vaccine and placebo recipients after Dose 1.

Table 5: Local Adverse Reactions and Systemic Adverse Events (≥1%) in Healthy Children and Adolescents from the Monroe Efficacy Study

Adverse Event	VAQTA (N=519)		Placebo (Alum Diluent)*,†,‡ (N=518) Rate (Percent)
	Dose 1* Rate (Percent)	Booster Rate (Percent)	
Injection Site§	n=515	n=475	n=510
Pain	6.4%	3.4%	6.3%
Tenderness	4.9%	1.7%	6.1%
Erythema	1.9%	0.8%	1.8%
Swelling	1.7%	1.5%	1.6%
Warmth	1.7%	0.6%	1.6%
Systemic¶	n=519	n=475	n=518
Abdominal pain	1.2%	1.1%	1.0%
Pharyngitis	1.2%	0%	0.8%
Headache	0.4%	0.8%	1.0%

N=Number of subjects enrolled/randomized.
Percent=percentage of subjects for whom data are available with adverse event
n=number of subjects for whom adverse events available

* No statistically significant differences between the two groups.
† Second injection of placebo not administered because code for the trial was broken.
‡ Placebo (Alum diluent) = amorphous aluminum hydroxyphosphate sulfate.
§ Adverse Reactions at the injection site (VAQTA) Days 1-5 after vaccination with VAQTA
¶ Systemic adverse events reported Days 1-15 after vaccination, regardless of causality.

Adults — 19 Years of Age and Older

In an open-label clinical trial, 240 healthy adults 18 to 54 years of age were randomized to receive either VAQTA (50U/1-mL) with Typhim Vi (Typhoid Vi polysaccharide vaccine) and YF-Vax (yellow fever vaccine) concomitantly (N=80), typhoid Vi polysaccharide and yellow fever vaccines concomitantly (N=80), or VAQTA alone (N=80). Approximately 6 months later, subjects who received VAQTA were administered a second dose of VAQTA. The race distribution of the study subjects who received VAQTA with or without typhoid Vi polysaccharide and yellow fever vaccine was as follows: 78.3% Caucasian; 14.2% Oriental; 3.3% other; 2.1% African-American; 1.7% Indian; 0.4% Hispanic-American. The distribution of subjects by gender was 40.8% male and 59.2% female. Subjects were monitored for local adverse reactions and fever for 5 days and systemic adverse events for 14 days after each vaccination. In the 14 days after the first dose of VAQTA, the proportion of subjects with adverse events was similar between recipients of VAQTA given concomitantly with typhoid Vi polysaccharide and yellow fever vaccines compared to recipients of typhoid Vi polysaccharide and yellow fever vaccines without VAQTA. Table 6 summarizes solicited local adverse reactions and Table 7 summarizes unsolicited systemic adverse events reported in ≥5% in adults who received one or two doses of VAQTA alone and for subjects who received VAQTA concomitantly with typhoid Vi polysaccharide and yellow fever vaccines. There were no solicited systemic complaints reported at a rate ≥5%. Fever ≥101°F occurred in 1.3% of subjects in each group.

Table 6: Incidences of Solicited Local Adverse Reactions in Healthy Adults ≥19 Years of Age Occurring at ≥5% After Any Dose

Adverse Event	VAQTA administered alone (N=80)	VAQTA + ViCPS* and Yellow Fever vaccines administered concomitantly† (N=80)
	Rate (Percent)	
Injection-site‡		
Pain/tenderness/soreness	78.8%	70.3%
Warmth	23.7%	23.7%
Swelling	16.2%	8.8%
Erythema	17.5%	6.3%

N=Number of subjects enrolled/randomized.

Percent=percentage of subjects with adverse event.
*ViCPS=Typhoid Vi polysaccharide vaccine.
†VAQTA administered concomitantly with typhoid Vi polysaccharide (ViCPS) and yellow fever vaccines.
‡ Adverse Reactions at the injection site (VAQTA) Days 1-5 after vaccination

Table 7: Incidences of Unsolicited Systemic Adverse Events in Adults ≥19 Years of Age Occurring at ≥5% After Any Dose

Body System **Adverse Event**	**VAQTA administered alone (N=80)**	**VAQTA + ViCPS* and Yellow Fever vaccines administered concomitantly† (N=80)**
	Rate (Percent)	
General disorders and administration site reactions‡		
Asthenia/fatigue	7.5%	11.3%
Chills	1.3%	7.5%
Gastrointestinal disorders‡		
Nausea	7.5%	12.5%
Musculoskeletal and connective tissue disorders‡		
Myalgia	5.0%	10.0%
Arm pain	0.0%	6.3%
Nervous system disorders‡		
Headache	23.8%	26.3%
Infections and infestations‡		
Upper respiratory infection	7.5%	3.8%
Pharyngitis	2.5%	6.3%

N=Number of subjects enrolled/randomized with data available.
Percent=percentage of subjects with adverse event for whom data are available.
*ViCPS=Typhoid Vi polysaccharide vaccine.
†VAQTA administered concomitantly with typhoid Vi polysaccharide (ViCPS) and yellow fever vaccines.
‡Systemic Adverse Events reported Days 1-15 after vaccination, regardless of causality.

In four clinical trials involving 1645 healthy adults 19 years of age and older who received one or more 50U doses of hepatitis A vaccine, subjects were followed for fever and local adverse reactions 1 to 5 days postvaccination and for systemic adverse events 1 to 14 days postvaccination. One single-blind study evaluated doses of VAQTA with varying amounts of viral antigen and/or alum content in healthy adults ≥170 pounds and ≥30 years of age (N=210 adults administered 50U/1-mL dose). One open-label study evaluated VAQTA given with immune globulin (IG) or alone (N=164 adults who received VAQTA alone). A third study was single-blind and evaluated 3 different lots of VAQTA (N=1112). The fourth study that was also single-blind evaluated doses of VAQTA with varying amounts of viral antigen in healthy adults ≥170 pounds and ≥30 years of age (N=159 adults administered the 50U/1-mL dose). Overall, the race distribution of the study subjects who received at least one dose of VAQTA was as follows: 94.2% Caucasian; 2.2% Black; 1.5% Hispanic; 1.5% Oriental; 0.4% other; 0.2% American Indian. 47.6% of subjects were male and 52.4% were female. The most common adverse event/reaction was injection-site pain/soreness/tenderness reported by 67.0% of subjects. Of all reported injection-site reactions 99.8% were mild (*i.e.,* easily tolerated with no medical intervention) or moderate (*i.e.,* minimally interfered with usual activity possibly requiring little medical intervention). Listed below in Table 8 are the local adverse reactions and systemic adverse events reported by ≥5% of subjects, in decreasing order of frequency within each body system.

Table 8: Incidences of Local Adverse Reactions and Systemic Adverse Events ≥5% in Adults 19 Years of Age and Older

Body System **Adverse Events**	**VAQTA (Any Dose) (N=1645)** **Rate (n/total n)**
Nervous system disorders*	n=1641
Headache	16.1%
General disorders and administration site reactions†	n=1640
Injection-site	67.0%

pain/tenderness/soreness	
Injection-site warmth	18.2%
Injection-site swelling	14.7%
Injection-site erythema	13.7%

N=Number of subjects enrolled/randomized.
n=Number of subjects in each category with data available.
Percent=percentage of subjects for whom data are available with adverse event.
*Systemic Adverse Events reported Days 1 to 14 after vaccination, regardless of causality.
†Adverse Reactions at the injection site (VAQTA) and measured fever Days 1 to 5 after vaccination.

The following additional unsolicited systemic adverse events were observed among recipients of VAQTA that occurred within 14 days at a common frequency of ≥1% to <10% following any dose not reported elsewhere in the label. These adverse reactions have been reported across 4 clinical studies.

Musculoskeletal and connective tissue disorders: Back pain; stiffness

Reproductive system and breast disorders: Menstruation disorders

6.2 Post-Marketing Experience

The following additional adverse events have been reported with use of the marketed vaccine. Because these reactions are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to a vaccine exposure.

Blood and lymphatic disorders: Thrombocytopenia.
Nervous system disorders: Guillain-Barré syndrome; cerebellar ataxia; encephalitis.

Post-Marketing Observational Safety Study

In a post-marketing, 60-day safety surveillance study, conducted at a large health maintenance organization in the United States, a total of 42,110 individuals ≥2 years of age received 1 or 2 doses of VAQTA (13,735 children/adolescents and 28,375 adult subjects). Safety was passively monitored by electronic search of the automated medical records database for emergency room and outpatient visits, hospitalizations, and deaths. Medical charts were reviewed when an event was considered to be possibly vaccine-related by the investigator. None of the serious adverse events identified were assessed as being related to vaccine by the investigator. Diarrhea/gastroenteritis, resulting in outpatient visits, was determined by the investigator to be the only vaccine-related nonserious adverse reaction in the study. There was no vaccine-related adverse reaction identified that had not been reported in earlier clinical trials with VAQTA.

7 DRUG INTERACTIONS

7.1 Use with Other Vaccines

Do not mix VAQTA with any other vaccine in the same syringe or vial. Use separate injection sites and syringes for each vaccine. Please refer to package inserts of coadministered vaccines.
In clinical trials in children, VAQTA was concomitantly administered with one or more of the following US licensed vaccines: Measles, Mumps, and Rubella Virus Vaccine, Live; Varicella Vaccine, Live; Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine, Adsorbed; Measles, Mumps, Rubella, and Varicella Vaccine, Live; Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197}); and Haemophilus B Conjugate Vaccine (Meningococcal Protein Conjugate). Safety and immunogenicity were similar for concomitantly administered vaccines compared to separately administered vaccines.

In clinical trials in adults, VAQTA was concomitantly administered with typhoid Vi polysaccharide and yellow fever vaccines *[see Adverse Reactions (6.1) and Clinical Studies (14.2, 14.7)]*. Safety and immunogenicity were similar for concomitantly administered vaccines compared to separately administered vaccines.

7.2 Use with Immune Globulin
VAQTA may be administered concomitantly with Immune Globulin, human, using separate sites and syringes. The recommended vaccination regimen for VAQTA should be followed. Consult the manufacturer's product circular for the appropriate dosage of Immune Globulin. A booster dose of VAQTA should be administered at the appropriate time as outlined in the recommended regimen for VAQTA *[see Clinical Studies (14.5)].*

7.3 Immunosuppressive Therapy
If VAQTA is administered to a person receiving immunosuppressive therapy, an adequate immunologic response may not be obtained.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy
Risk Summary
All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no adequate and well-controlled studies designed to evaluate VAQTA in pregnant women. Available post-approval data do not suggest an increased risk of miscarriage or major birth defects in women who received VAQTA during pregnancy.

Developmental toxicity studies have not been conducted with VAQTA in animals.

Data
Human Data
Post-approval adverse reactions are reported voluntarily from a population of uncertain size. It is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

In prospectively reported spontaneous post-approval reports from 1995 to 2018, 36 women with a known pregnancy outcome were exposed to VAQTA during pregnancy following the last menstrual period. After excluding induced abortions (n=4) and those with exposure in the third trimester (n=2), there were 30 pregnancies with known outcomes with exposures in the first or second trimester. Miscarriage was reported for 3 of 30 (10%) pregnancies. Major birth defects were reported for 1 of 27 (3.7%) live born infants. The rates of miscarriage and major birth defects were consistent with estimated background rates.

8.2 Lactation
Risk Summary
It is not known whether VAQTA is excreted in human milk. Data are not available to assess the effects of VAQTA on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother’s clinical need for VAQTA and any potential adverse effects on the breastfed child from VAQTA or from the underlying maternal condition. For preventive vaccines the underlying condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use
The safety of VAQTA has been evaluated in 4374 children 12 through 23 months of age, and 2615 children/adolescents 2 through 18 years of age who received at least one 25U dose of VAQTA *[see Adverse Reactions (6) and Dosage and Administration (2)]*.
Safety and effectiveness in infants below 12 months of age have not been established.

8.5 Geriatric Use

In the post-marketing observational safety study which included 42,110 persons who received VAQTA *[see Adverse Reactions (6.2)]*, 4769 persons were 65 years of age or older and 1073 persons were 75 years of age or older. There were no adverse events judged by the investigator to be vaccine-related in the geriatric study population. In other clinical studies, 68 subjects 65 years of age or older were vaccinated with VAQTA, 10 of whom were 75 years of age or older. No overall differences in safety and immunogenicity were observed between these subjects and younger subjects; however, greater sensitivity of some older individuals cannot be ruled out. Other reported clinical experience has not identified differences in responses between the elderly and younger subjects.

8.6 Immunocompromised Individuals

Immunocompromised persons may have a diminished immune response to VAQTA and may not be protected against HAV infection.

11 DESCRIPTION

VAQTA is an inactivated whole virus vaccine derived from hepatitis A virus grown in cell culture in human MRC-5 diploid fibroblasts. It contains inactivated virus of a strain which was originally derived by further serial passage of a proven attenuated strain. The virus is grown, harvested, purified by a combination of physical and high performance liquid chromatographic techniques developed at the Merck Research Laboratories, formalin inactivated, and then adsorbed onto amorphous aluminum hydroxyphosphate sulfate.

VAQTA is a sterile suspension for intramuscular injection. One milliliter of the vaccine contains approximately 50U of hepatitis A virus antigen, which is purified and formulated without a preservative. Within the limits of current assay variability, the 50U dose of VAQTA contains less than 0.1 mcg of non-viral protein, less than 4 x 10^{-6} mcg of DNA, less than 10^{-4} mcg of bovine albumin, and less than 0.8 mcg of formaldehyde. Other process chemical residuals are less than 10 parts per billion (ppb), including neomycin.

Each 0.5-mL pediatric dose contains 25U of hepatitis A virus antigen and adsorbed onto approximately 0.225 mg of aluminum provided as amorphous aluminum hydroxyphosphate sulfate, and 35 mcg of sodium borate as a pH stabilizer, in 0.9% sodium chloride.

Each 1-mL adult dose contains 50U of hepatitis A virus antigen and adsorbed onto approximately 0.45 mg of aluminum provided as amorphous aluminum hydroxyphosphate sulfate, and 70 mcg of sodium borate as a pH stabilizer, in 0.9% sodium chloride.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

VAQTA has been shown to elicit antibodies to hepatitis A as measured by ELISA.

Protection from hepatitis A disease has been shown to be related to the presence of antibody. However, the lowest titer needed to confer protection has not been determined.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

VAQTA has not been evaluated for its carcinogenic or mutagenic potential, or its potential to impair fertility. *[See Use in Specific Populations (8).]*

14 CLINICAL STUDIES

14.1 Efficacy of VAQTA: The Monroe Clinical Study

The immunogenicity and protective efficacy of VAQTA were evaluated in a randomized, double-blind, placebo-controlled study involving 1037 susceptible healthy children and adolescents 2 through 16 years of age in a U.S. community with recurrent outbreaks of hepatitis A (The Monroe Efficacy Study). All of these children were Caucasian, and there were 51.5% male and 48.5% female. Each child received an intramuscular dose of VAQTA (25U) (N=519) or placebo (alum diluent) (N=518). Among those individuals who were initially seronegative (measured by a modification of the HAVAB radioimmunoassay [RIA]),

seroconversion was achieved in >99% of vaccine recipients within 4 weeks after vaccination. The onset of seroconversion following a single dose of VAQTA was shown to parallel the onset of protection against clinical hepatitis A disease.
Because of the long incubation period of the disease (approximately 20 to 50 days, or longer in children), clinical efficacy was based on confirmed cases[1] of hepatitis A occurring ≥50 days after vaccination in order to exclude any children incubating the infection before vaccination. In subjects who were initially seronegative, the protective efficacy of a single dose of VAQTA was observed to be 100% with 21 cases of clinically confirmed hepatitis A occurring in the placebo group and none in the vaccine group ($p<0.001$). The number of clinically confirmed cases of hepatitis A ≥30 days after vaccination were also compared. In this analysis, 28 cases of clinically confirmed hepatitis A occurred in the placebo group while none occurred in the vaccine group ≥30 days after vaccination. In addition, it was observed in this trial that no cases of clinically confirmed hepatitis A occurred in the vaccine group after day 16.[2] Following demonstration of protection with a single dose and termination of the study, a booster dose was administered to a subset of vaccinees 6, 12, or 18 months after the primary dose.
No cases of clinically confirmed hepatitis A disease ≥50 days after vaccination have occurred in those vaccinees from The Monroe Efficacy Study monitored for up to 9 years.

14.2 Other Clinical Studies
The efficacy of VAQTA in other age groups was based upon immunogenicity measured 4 to 6 weeks following vaccination. VAQTA was found to be immunogenic in all age groups.

Children — 12 through 23 Months of Age
In a clinical trial, children 12 through 23 months of age were randomized to receive the first dose of VAQTA with or without M-M-R II and VARIVAX (N=617) and the second dose of VAQTA with or without Tripedia and optionally either oral poliovirus vaccine (no longer licensed in the US) or IPOL (N=555). The race distribution of study subjects who received at least one dose of VAQTA was as follows: 56.7% Caucasian; 17.5% Hispanic-American; 14.3% African-American; 7.0% Native American; 3.4% other; 0.8% Oriental; 0.2% Asian; and 0.2% Indian. The distribution of subjects by gender was 53.6% male and 46.4% female. In the analysis population, there were 471 initially seronegative children 12 through 23 months of age, who received the first dose of VAQTA with (N=237) or without (N=234) M-M-R II and VARIVAX of whom 96% (95% CI: 93.7%, 97.5%) seroconverted (defined as having an anti-HAV titer ≥10 mIU/mL) post dose 1 with an anti-HAV geometric mean titer (GMT) of 48 mIU/mL (95% CI: 44.7, 51.6). There were 343 children in the analysis population who received the second dose of VAQTA with (N=168) or without (N=175) Tripedia and optional oral poliovirus vaccine or IPOL of whom 100% (95% CI: 99.3%, 100%) seroconverted post dose 2 with an anti-HAV GMT of 6920 mIU/mL (95% CI: 6136, 7801). Of children who received only VAQTA at both visits, 100% (n=97) seroconverted after the second dose of VAQTA.
In a clinical trial involving 653 healthy children 12 to 15 months of age, 330 were randomized to receive VAQTA, ProQuad, and pneumococcal 7-valent conjugate vaccine concomitantly, and 323 were randomized to receive ProQuad and pneumococcal 7-valent conjugate vaccine concomitantly followed by VAQTA 6 weeks later. The race distribution of the study subjects was as follows: 60.3% Caucasian; 21.6% African-American; 9.5% Hispanic-American; 7.2% other; 1.1% Asian/Pacific; and 0.3% Native American. The distribution of subjects by gender was 50.7% male and 49.3% female. In the analysis population, the seropositivity rate for hepatitis A antibody (defined as the percent of subjects with an anti-HAV titer ≥10 mIU/mL) was 100% (n=182; 95% CI: 98.0%, 100%) post dose 2 with an anti-HAV GMT of 4977 mIU/mL (95% CI: 4068, 6089) when VAQTA was given with ProQuad and pneumococcal 7-valent conjugate vaccine and 99.4% (n=159, 95% CI: 96.5%, 100%) post dose 2 with an anti-HAV GMT of 6123 mIU/mL (95% CI: 4826, 7770) when VAQTA alone was given. These seropositivity rates were similar

[1]The clinical case definition included all of the following occurring at the same time: 1) one or more typical clinical signs or symptoms of hepatitis A (*e.g.*, jaundice, malaise, fever ≥38.3°C); 2) elevation of hepatitis A IgM antibody (HAVAB-M); 3) elevation of alanine transferase (ALT) ≥2 times the upper limit of normal.

[2]One vaccinee did not meet the pre-defined criteria for clinically confirmed hepatitis A but did have positive hepatitis A IgM and borderline liver enzyme (ALT) elevations on days 34, 50, and 58 after vaccination with mild clinical symptoms observed on days 49 and 50.

whether VAQTA was administered with or without ProQuad and pneumococcal 7-valent conjugate vaccine.
In an open, multicenter, randomized study involving 617 children 15 months of age, 306 were randomized to receive VAQTA with or without PedvaxHIB and INFANRIX, and 311 were randomized to receive VAQTA with or without PedvaxHIB. The race distribution of the study subjects was as follows: 63.9% Caucasian; 17.5% Hispanic-American; 14.7% Black; 2.6% other; and 1.3% Asian. The distribution of subjects by gender was 54.0% male and 46.0% female. The seropositivity rate for hepatitis A antibody (defined as the percent of subjects with an anti-HAV titer ≥ 10 mIU/mL) 4 weeks post dose 2 was 100% (n=208, 95% CI: 98.2%, 100.0%) in those who received VAQTA concomitantly with PedvaxHIB and INFANRIX or concomitantly with PedvaxHIB. In those subjects who received VAQTA alone, the seropositivity rate for hepatitis A antibody was 100% (n=183, 95% CI: 98.0%, 100.0%), regardless of baseline hepatitis A serostatus. Overall, the anti-HAV GMT in the concomitant groups was 3616.5 mIU/mL (95% CI: 3084.5, 4240.2). The anti-HAV GMT in the nonconcomitant groups was 4712.6 mIU/mL (95% CI: 3996.8, 5556.8). Comparable responses were observed in both the initially seronegative and seropositive subjects.
In three combined clinical studies 1022 initially seronegative subjects received 2 doses of VAQTA alone or concomitantly with other vaccines. Of the seronegative subjects, 99.9% achieved an anti-HAV titer ≥10 mIU/mL (95% CI: 99.5%, 100%) and an anti-HAV GMT of 5392.1 mIU/mL (95% CI: 4996.5, 5819.0) 4 weeks following dose 2 of VAQTA.

Children/Adolescents — 2 Years through 18 Years of Age
Immunogenicity data were combined from eleven randomized clinical studies in children and adolescents 2 through 18 years of age who received VAQTA (25U/0.5 mL). These included administration of VAQTA in varying doses and regimens (N=404 received 25U/0.5 mL), the Monroe Efficacy Study (N=973), and comparison studies for process and formulation changes (N=1238). The race distribution of the study subjects who received at least one dose of VAQTA in these studies was as follows: 84.8% Caucasian; 10.6% American Indian; 2.3% African-American; 1.5% Hispanic-American; 0.6% other; 0.2% Oriental. The distribution of subjects by gender was 51.2% male and 48.8% female. The proportions of subjects who seroconverted 4 weeks after the first and second doses administered 6 months apart were 97% (n=1230; 95% CI: 96%, 98%) and 100% (n=1057; 95% CI: 99.5%, 100%) of subjects with anti-HAV GMTs of 43 mIU/mL (95% CI: 40, 45) and 10,077 mIU/mL (95% CI: 9394, 10,810), respectively.

Adults — 19 Years of Age and Older
Immunogenicity data were combined from five randomized clinical studies in adults 19 years of age and older who received VAQTA (50U/1-mL). One single-blind study evaluated doses of VAQTA with varying amounts of viral antigen and/or alum content in healthy adults ≥170 pounds and ≥30 years of age (N=208 adults administered 50U/1-mL dose). One open-label study evaluated VAQTA given with immune globulin or alone (N=164 adults who received VAQTA alone). A third study was single-blind and evaluated 3 different lots of VAQTA (N=1112). The fourth study was single-blind and evaluated doses of VAQTA with varying amounts of viral antigen in healthy adults ≥170 pounds and ≥30 years of age (N=159 adults administered the 50U/1-mL dose). The fifth study was an open-label study to evaluate various regimens for time of administration of the booster dose of VAQTA (6, 12, and 18 months post dose 1, N=354). The race distribution of the study subjects who received at least one dose of VAQTA in these studies was as follows: 93.2% Caucasian; 2.5% African-American; 2.1% Hispanic-American; 1.4% Oriental; 0.5% other; 0.3% American Indian. The distribution of subjects by gender was 44.8% male and 55.2% female. The proportion of subjects who seroconverted 4 weeks after the first and second doses administered 6 months apart was 95% (n=1411; 95% CI: 94%, 96%) and 99.9% (n=1244; 95% CI: 99.4%, 100%) with GMTs of 37 mIU/mL (95% CI: 35, 38) and 6013 mIU/mL (95% CI: 5592, 6467), respectively. Furthermore, at 2 weeks postvaccination, 69.2% (n=744; 95% CI: 65.7%, 72.5%) of adults seroconverted with an anti-HAV GMT of 16 mIU/mL after a single dose of VAQTA.

14.3 Timing of Booster Dose Administration
Children/Adolescents — 2 through 18 Years of Age
In the Monroe Efficacy Study, children were administered a second dose of VAQTA (25U/0.5 mL) 6, 12, or 18 months following the initial dose. For subjects who received both doses of VAQTA, the GMTs and

proportions of subjects who seroconverted 4 weeks after the booster dose administered 6, 12, and 18 months after the first dose are presented in Table 9.

Table 9: Children/Adolescents from the Monroe Efficacy Study
Seroconversion Rates (%) and Geometric Mean Titers (GMT) for Cohorts of Initially Seronegative Vaccinees at the Time of the Booster(25U) and 4 Weeks Later

Months Following Initial 25U Dose	Cohort* (n=960) 0 and 6 Months	Cohort* (n=35) 0 and 12 Months	Cohort* (n=39) 0 and 18 Months
	Seroconversion Rate GMT (mIU/mL) (95% CI)		
6	97% 107 (98, 117)	—	—
7	100% 10433 (9681, 11243)	—	—
12	—	91% 48 (33, 71)	—
13	—	100% 12308 (9337, 16226)	—
18	—	—	90% 50 (28, 89)
19	—	—	100% 9591 (7613, 12082)

* Blood samples were taken at prebooster and postbooster time points.

Adults — 19 years of age and older
Among the 5 randomized clinical studies in adults 19 years of age and older described in Section 14.2, there were additional data in which a booster dose of VAQTA (50U/1-mL) was administered 12 or 18 months after the first dose. For subjects in these studies who received both doses of VAQTA, the proportions who seroconverted 4 weeks after the booster dose administered 6, 12, and 18 months after the first dose were 100% of 1201 subjects, 98% of 91 subjects, and 100% of 84 subjects, respectively. GMTs in mIU/mL one month after the subjects received the booster dose at 6, 12, or 18 months after the primary dose were 5987 mIU/mL (95% CI: 5561, 6445), 4896 mIU/mL (95% CI: 3589, 6679), and 6043 mIU/mL (95% CI: 4687, 7793), respectively.

14.4 Duration of Immune Response

In follow-up of subjects in The Monroe Efficacy Study, in children (≥2 years of age) and adolescents who received two doses (25U) of VAQTA, detectable levels of anti-HAV antibodies (≥10 mIU/mL) were present in 100% of subjects for at least 10 years postvaccination. In subjects who received VAQTA at 0 and 6 months, the GMT was 819 mIU/mL (n=175) at 2.5 to 3.5 years and 505 mIU/mL (n=174) at 5 to 6 years, and 574 mIU/mL (n=114) at 10 years postvaccination. In subjects who received VAQTA at 0 and 12 months, the GMT was 2224 mIU/mL (n=49) at 2.5 to 3.5 years, 1191 mIU/mL (n=47) at 5 to 6 years, and 1005 mIU/mL (n=36) at 10 years postvaccination. In subjects who received VAQTA at 0 and 18 months, the GMT was 2501 mIU/mL (n=53) at 2.5 to 3.5 years, 1614 mIU/mL (n=56) at 5 to 6 years, and 1507 mIU/mL (n=41) at 10 years postvaccination.
In adults that were administered VAQTA at 0 and 6 months, the hepatitis A antibody response to date has been shown to persist at least 6 years. Detectable levels of anti-HAV antibodies (≥10 mIU/mL) were present in 100% (378/378) of subjects with a GMT of 1734 mIU/mL at 1 year, 99.2% (252/254) of subjects with a GMT of 687 mIU/mL at 2 to 3 years, 99.1% (219/221) of subjects with a GMT of 605 mIU/mL at 4 years, and 99.4% (170/171) of subjects with a GMT of 684 mIU/mL at 6 years postvaccination.
The total duration of the protective effect of VAQTA in healthy vaccinees is unknown at present.

14.5 Concomitant Administration of VAQTA and Immune Globulin

The concurrent use of VAQTA (50U) and immune globulin (IG, 0.06 mL/kg) was evaluated in an open-label, randomized clinical study involving 294 healthy adults 18 to 39 years of age. Adults were randomized to receive 2 doses of VAQTA 24 weeks apart (N=129), the first dose of VAQTA concomitant with a dose of IG followed by the second dose of VAQTA alone 24 weeks later (N=135), or IG alone

(N=30). The race distribution of the study subjects who received at least one dose of VAQTA or IG in this study was as follows: 92.3% Caucasian; 4.0% Hispanic-American; 3.0% African-American; 0.3% Native American; 0.3% Asian/Pacific. The distribution of subjects by gender was 28.7% male and 71.3% female. Table 10 provides seroconversion rates and GMTs at 4 and 24 weeks after the first dose in each treatment group and at one month after a booster dose of VAQTA (administered at 24 weeks) *[see Drug Interactions (7.2)].*

Table 10: Seroconversion Rates (%) and Geometric Mean Titers (GMT) After Vaccination with VAQTA Plus IG, VAQTA Alone, and IG Alone

	VAQTA plus IG	VAQTA	IG
Weeks	**Seroconversion Rate GMT (mIU/mL) (95% CI)**		
4	100% 42 (39, 45) (n=129)	96% 38 (33, 42) (n=135)	87% 19 (15, 23) (n=30)
24	92% 83 (65, 105) (n=125)	97%* 137* (112, 169) (n=132)	0% Undetectable† (n=28)
28	100% 4872 (3716, 6388) (n=114)	100% 6498 (5111, 8261) (n=128)	N/A

*The seroconversion rate and the GMT in the group receiving VAQTA alone were significantly higher than in the group receiving VAQTA plus IG (p=0.05, p<0.001, respectively).
†Undetectable is defined as <10mIU/mL.
N/A = Not Applicable.

14.6 Interchangeability of the Booster Dose

A randomized, double-blind clinical study in 537 healthy adults, 18 to 83 years of age, evaluated the immune response to a booster dose of VAQTA and HAVRIX given at 6 or 12 months following an initial dose of HAVRIX. Subjects were randomized to receive VAQTA (50U) as a booster dose 6 months (N=232) or 12 months (N=124) following an initial dose of HAVRIX or HAVRIX (1440 EL. U) as a booster dose 6 months (N=118) or 12 months (N=63) following an initial dose of HAVRIX. The race distribution of the study subjects who received the booster dose of VAQTA or HAVRIX in this study was as follows: 87.2% Caucasian; 8.0% African-American; 1.9% Hispanic-American; 1.3% Oriental; 0.9% Asian; 0.4% Indian; 0.4% other. The distribution of subjects by gender was 44.9% male and 55.1% female. When VAQTA was given as a booster dose following HAVRIX, the vaccine produced an adequate immune response (see Table 11) *[see Dosage and Administration (2.1)].*

Table 11: Seropositivity Rate, Booster Response Rate* and Geometric Mean Titer 4 Weeks Following a Booster Dose of VAQTA or HAVRIX Administered 6 to 12 Months After First Dose of HAVRIX†

First Dose	Booster Dose	Seropositivity Rate	Booster Response Rate*	Geometric Mean Titer
HAVRIX 1440 EL.U.	VAQTA 50 U	99.7% (n=313)	86.1% (n=310)	3272 (n=313)
HAVRIX 1440 EL.U.	HAVRIX 1440 EL.U.	99.3% (n=151)	80.1% (n=151)	2423 (n=151)

*Booster Response Rate is defined as greater than or equal to a tenfold rise from prebooster to postbooster titer and postbooster titer ≥100 mIU/mL.
†Study conducted in adults 18 years of age and older.

14.7 Immune Response to Concomitantly Administered Vaccines

Clinical Studies of VAQTA with M-M-R II, VARIVAX, and Tripedia

In the clinical trial in which children 12 months of age received the first dose of VAQTA concomitantly with M-M-R II and VARIVAX described in Section 14.2, rates of seroprotection to hepatitis A were similar between the two groups who received VAQTA with or without M-M-R II and VARIVAX. Measles, mumps, and rubella immune responses were tested in 241 subjects, 263 subjects, and 270 subjects, respectively. Seropositivity rates were 98.8% [95% CI: 96.4%, 99.7%] for measles, 99.6% [95% CI: 97.9%, 100%] for mumps, and 100% [95% CI: 98.6%, 100%] for rubella, which were similar to observed historical rates (seropositivity rates 99% for all three antigens, with lower bound of the 95% CI >89%) following

vaccination with a first dose of M-M-R II in this age group. Data from this study were insufficient to adequately assess the immune response to VARIVAX administered concomitantly with VAQTA. In this same study, the second dose of VAQTA at 18 months of age was given with or without Tripedia (DTaP). Seropositivity rates for diphtheria and tetanus were similar to those in historical controls. However, data from this study were insufficient to assess the pertussis response of DTaP when administered with VAQTA. Rates of seroprotection to hepatitis A were similar between the two groups who received VAQTA with or without M-M-R II and VARIVAX, and between the two groups who received VAQTA with or without DTaP.

Clinical Studies of VAQTA with ProQuad and Prevnar
In the clinical trial of concomitant use of VAQTA with ProQuad and pneumococcal 7-valent conjugate vaccine in children 12 to 15 months of age described in Section 14.2, the antibody GMTs for *S. pneumoniae* types 4, 6B, 9V, 14, 18C, 19F, and 23F 6 weeks after vaccination with pneumococcal 7-valent conjugate vaccine administered concomitantly with ProQuad and VAQTA were non-inferior as compared to GMTs observed in the group given pneumococcal 7-valent conjugate vaccine with ProQuad alone (the lower bounds of the 95% CI around the fold-difference for the 7 serotypes excluded 0.5). For the varicella component of ProQuad, in subjects with baseline antibody titers <1.25 gpELISA units/mL, the proportion with a titer ≥5 gpELISA units/mL 6 weeks after their first dose of ProQuad was non-inferior (defined as -10 percentage point change) when ProQuad was administered with VAQTA and pneumococcal 7-valent conjugate vaccine as compared to the proportion with a titer ≥5 gpELISA units/mL when ProQuad was administered with pneumococcal 7-valent conjugate vaccine alone (difference in seroprotection rate -5.1% [95% CI: -9.3, -1.4%]). Hepatitis A responses were similar when compared between the two groups who received VAQTA with or without ProQuad and pneumococcal 7-valent conjugate vaccine. Seroconversion rates and antibody titers for varicella and *S. pneumoniae* types 4, 6B, 9V, 14, 18C, 19F, and 23F were similar between groups at 6 weeks postvaccination.

Clinical Studies of VAQTA with INFANRIX and PedvaxHIB
In the clinical trial of concomitant administration of VAQTA with INFANRIX and PedvaxHIB in children 15 months of age, described in Section 14.2, when the first dose of VAQTA was administered concomitantly with either INFANRIX and PedvaxHIB or PedvaxHIB, there was no interference in immune response to hepatitis A as measured by seropositivity rates after dose 2 of VAQTA compared to administration of both doses of VAQTA alone. When dose 1 of VAQTA was administered concomitantly with either PedvaxHIB and INFANRIX or PedvaxHIB, there was no interference in immune response to *Haemophilus influenzae b* (as measured by the proportion of subjects who attained an anti-polyribosylribitol phosphate antibody titer >1.0 mcg/mL at 4 weeks after vaccination), compared to subjects receiving either PedvaxHIB and INFANRIX or PedvaxHIB. When VAQTA was administered concomitantly with INFANRIX and PedvaxHIB, there was no interference in immune responses at 4 weeks after vaccination to the pertussis antigens (PT, FHA, or pertactin, as measured by GMTs) and no interference in immune responses to diphtheria toxoid or tetanus toxoid (as measured by the proportion of subjects achieving an antibody titer >0.1 IU/mL) compared to administration of INFANRIX and PedvaxHIB.

Clinical Studies of VAQTA with Typhoid Vi Polysaccharide Vaccine and Yellow Fever Vaccine, Live Attenuated
In the clinical trial of concomitant use of VAQTA with typhoid Vi polysaccharide and yellow fever vaccines in adults 18-54 years of age described in Section 6.1, the antibody response rates for typhoid Vi polysaccharide and yellow fever were adequate when typhoid Vi polysaccharide and yellow fever vaccines were administered concomitantly with (N=80) and nonconcomitantly without VAQTA (N=80). The seropositivity rate for hepatitis A when VAQTA, typhoid Vi polysaccharide, and yellow fever vaccines were administered concomitantly was generally similar to when VAQTA was given alone *[see Drug Interactions (7.1)].*

Data are insufficient to assess the immune response to VAQTA and poliovirus vaccine when administered concomitantly.

16 HOW SUPPLIED/STORAGE AND HANDLING

VAQTA is available in single-dose vials and prefilled Luer-Lok® syringes.

Pediatric/Adolescent Formulations

25U/0.5 mL in single-dose vials and prefilled Luer-Lok® syringes.
NDC 0006-4831-41 – box of ten 0.5-mL single dose vials.
NDC 0006-4095-02 – carton of ten 0.5-mL prefilled single-dose Luer-Lok® syringes with tip caps.

Adult Formulations

50U/1-mL in single-dose vials and prefilled Luer-Lok® syringes.
NDC 0006-4841-00 – 1-mL single dose vial.
NDC 0006-4841-41 – box of ten 1-mL single dose vials.
NDC 0006-4096-02 – carton of ten 1-mL prefilled single-dose Luer-Lok® syringes with tip caps.

Store vaccine at 2-8°C (36-46°F).
DO NOT FREEZE since freezing destroys potency.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Information for Vaccine Recipients and Parents or Guardians

- Inform the patient, parent or guardian of the potential benefits and risks of the vaccine.
- Question the vaccine recipient, parent, or guardian about the occurrence of any symptoms and/or signs of an adverse reaction after a previous dose of hepatitis A vaccine.
- Inform the patient, parent, or guardian about the potential for adverse events that have been temporally associated with administration of VAQTA.
- Tell the patient, parent, or guardian accompanying the recipient, to report adverse events to the physician or clinic where the vaccine was administered.
- Prior to vaccination, give the patient, parent, or guardian the Vaccine Information Statements which are required by the National Childhood Vaccine Injury Act of 1986. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).
- Tell the patient, parent, or guardian that the United States Department of Health and Human Services has established a Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine, including but not limited to the reporting of events required by the National Childhood Vaccine Injury Act of 1986. The VAERS toll-free number is 1-800-822-7967. Reporting forms may also be obtained at the VAERS website at (www.//vaers.hhs.gov/).

Manuf. and Dist. by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

For patent information: www.merck.com/product/patent/home.html

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Hepatitis B

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ENGERIX-B safely and effectively. See full prescribing information for ENGERIX-B.

ENGERIX-B [Hepatitis B Vaccine (Recombinant)] injectable suspension, for intramuscular use
Initial U.S. Approval: 1989

---------- INDICATIONS AND USAGE----------
ENGERIX-B is a vaccine indicated for immunization against infection caused by all known subtypes of hepatitis B virus. (1)

----------DOSAGE AND ADMINISTRATION----------
For intramuscular administration. (2, 2.2)
- Persons from birth through 19 years of age: A series of 3 doses (0.5 mL each) on a 0-, 1-, 6-month schedule. (2.3)
- Persons 20 years of age and older: A series of 3 doses (1 mL each) on a 0-, 1-, 6-month schedule. (2.3)
- Adults on hemodialysis: A series of 4 doses (2 mL each) as a single 2-mL dose or as two 1-mL doses on a 0-, 1-, 2-, 6-month schedule. (2.3)

---------- DOSAGE FORMS AND STRENGTHS----------
ENGERIX-B is a sterile suspension available in the following presentations:
- 0.5-mL (10 mcg) prefilled syringes (3)
- 1-mL (20 mcg) single-dose vials and prefilled syringes (3)

---------- CONTRAINDICATIONS ----------
Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis B-containing vaccine, or to any component of ENGERIX-B, including yeast. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including ENGERIX-B. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)
- Temporarily defer vaccination of infants with a birth weight less than 2,000 g born to hepatitis B surface antigen (HBsAg)-negative mothers. (5.3)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including ENGERIX-B, to infants born prematurely should be based on consideration of the infant's medical status, and the potential benefits and possible risks of vaccination. (5.4)

---------- ADVERSE REACTIONS ----------
The most common solicited adverse reactions were injection-site soreness (22%) and fatigue (14%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

---------- DRUG INTERACTIONS----------
Do not mix ENGERIX-B with any other vaccine or product in the same syringe or vial. (7.1)

---------- USE IN SPECIFIC POPULATIONS ----------
Antibody responses are lower in persons older than 60 years than in younger adults. (8.5)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 06/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ENGERIX-B is indicated for immunization against infection caused by all known subtypes of hepatitis B virus.

2 DOSAGE AND ADMINISTRATION

For intramuscular administration. See Section 2.2 for subcutaneous administration in persons at risk of hemorrhage.

2.1 Preparation for Administration

Shake well before use. With thorough agitation, ENGERIX-B is a homogeneous, turbid white suspension. Do not administer if it appears otherwise. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

For the prefilled syringes, attach a sterile needle and administer intramuscularly.

For the vials, use a sterile needle and sterile syringe to withdraw the vaccine dose and administer intramuscularly. Changing needles between drawing vaccine from a vial and injecting it into a recipient is not necessary unless the needle has been damaged or contaminated. Use a separate sterile needle and syringe for each individual.

2.2 Administration

ENGERIX-B should be administered by intramuscular injection. The preferred administration site is the anterolateral aspect of the thigh for infants younger than 1 year and the deltoid muscle in older children (whose deltoid is large enough for an intramuscular injection) and adults. ENGERIX-B should not be administered in the gluteal region; such injections may result in suboptimal response.

ENGERIX-B may be administered subcutaneously to persons at risk of hemorrhage (e.g., hemophiliacs). However, hepatitis B vaccines administered subcutaneously are known to result in a lower antibody response. Additionally, when other aluminum-adsorbed vaccines have been administered subcutaneously, an increased incidence of local reactions including subcutaneous nodules has been observed. Therefore, subcutaneous administration should be used only in persons who are at risk of hemorrhage with intramuscular injections.

Do not administer this product intravenously or intradermally.

2.3 Recommended Dose and Schedule

Persons from Birth through 19 Years

Primary immunization for infants (born of hepatitis B surface antigen [HBsAg]-negative or HBsAg-positive mothers), children (birth through 10 years), and adolescents (aged 11 through 19 years) consists of a series of 3 doses (0.5 mL each) given on a 0-, 1-, and 6-month schedule.

Persons Aged 20 Years and Older

Primary immunization for persons aged 20 years and older consists of a series of 3 doses (1 mL each) given on a 0-, 1-, and 6-month schedule.

Adults on Hemodialysis

Primary immunization consists of a series of 4 doses (2-mL each) given as a single 2-mL dose or two 1-mL doses on a 0-, 1-, 2-, and 6-month schedule. In hemodialysis patients, antibody response is lower than in healthy persons and protection may persist only as long as antibody levels remain above 10 mIU/mL. Therefore, the need for booster doses should be assessed by annual antibody testing. A 2-mL booster dose (as a single 2-mL dose or two 1-mL doses) should be given when antibody levels decline below 10 mIU/mL.[1] *[See Clinical Studies (14.2).]*

Table 1. Recommended Dosage and Administration Schedules

Group	Dose[a]	Schedules
Infants born of:		
HBsAg-negative mothers	0.5 mL	0, 1, 6 months
HBsAg-positive mothers[b]	0.5 mL	0, 1, 6 months
Children:		
Birth through 10 years	0.5 mL	0, 1, 6 months
Adolescents:		
Aged 11 through 19 years	0.5 mL	0, 1, 6 months
Adults:		
Aged 20 years and older	1 mL	0, 1, 6 months
Adults on hemodialysis	2 mL[c]	0, 1, 2, 6 months

HBsAg = Hepatitis B surface antigen.

[a] 0.5 mL (10 mcg); 1 mL (20 mcg).

[b] Infants born to HBsAg-positive mothers should receive vaccine and hepatitis B immune globulin (HBIG) within 12 hours after birth *[see Dosage and Administration (2.6)]*.

[c] Given as a single 2-mL dose or as two 1-mL doses.

2.4 Alternate Dosing Schedules

There are alternate dosing and administration schedules which may be used for specific populations (e.g., neonates born of hepatitis B–infected mothers, persons who have or might have been recently exposed to the virus, and travelers to high-risk areas) (Table 2). For some of these alternate schedules, an additional dose at 12 months is recommended for prolonged maintenance of protective titers.

Table 2. Alternate Dosage and Administration Schedules

Group	Dose[a]	Schedules
Infants born of:		
HBsAg-positive mothers[b]	0.5 mL	0, 1, 2, 12 months
Children:		
Birth through 10 years	0.5 mL	0, 1, 2, 12 months
Aged 5 through 10 years	0.5 mL	0, 12, 24 months[c]
Adolescents:		
Aged 11 through 16 years	0.5 mL	0, 12, 24 months[c]
Aged 11 through 19 years	1 mL	0, 1, 6 months
Aged 11 through 19 years	1 mL	0, 1, 2, 12 months
Adults:		
Aged 20 years and older	1 mL	0, 1, 2, 12 months

HBsAg = Hepatitis B surface antigen.

[a] 0.5 mL (10 mcg); 1 mL (20 mcg).

[b] Infants born to HBsAg-positive mothers should receive vaccine and hepatitis B immune globulin (HBIG) within 12 hours after birth *[see Dosage and Administration (2.6)]*.

[c] For children and adolescents for whom an extended administration schedule is acceptable based on risk of exposure.

2.5 Booster Vaccinations

Whenever administration of a booster dose is appropriate, the dose of ENGERIX-B is 0.5 mL for children aged 10 years and younger and 1 mL for persons aged 11 years and older. Studies have demonstrated a substantial increase in antibody titers after booster vaccination with ENGERIX-B. See Section 2.3 for information on booster vaccination for adults on hemodialysis.

2.6 Known or Presumed Exposure to Hepatitis B Virus

Persons with known or presumed exposure to the hepatitis B virus (e.g., neonates born of infected mothers, persons who experienced percutaneous or permucosal exposure to the virus) should be given hepatitis B immune globulin (HBIG) in addition to ENGERIX-B in accordance with Advisory Committee on Immunization Practices recommendations and with the package insert for HBIG. ENGERIX-B can be given on either dosing schedule (0, 1, and 6 months or 0, 1, 2, and 12 months).

3 DOSAGE FORMS AND STRENGTHS

ENGERIX-B is a sterile suspension available in the following presentations:

- 0.5-mL (10 mcg) prefilled TIP-LOK syringes
- 1-mL (20 mcg) single-dose vials and prefilled TIP-LOK syringes

[See Description (11), How Supplied/Storage and Handling (16).]

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis B-containing vaccine, or to any component of ENGERIX-B, including yeast, is a contraindication to administration of ENGERIX-B *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including ENGERIX-B. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Infants Weighing Less than 2,000 g at Birth

Hepatitis B vaccine should be deferred for infants with a birth weight <2,000 g if the mother is documented to be HBsAg negative at the time of the infant's birth. Vaccination can commence at chronological age 1 month or hospital discharge. Infants born weighing <2,000 g to HBsAg-positive mothers should receive vaccine and HBIG within 12 hours after birth. Infants born weighing <2,000 g to mothers of unknown HBsAg status should receive vaccine and HBIG within 12 hours after birth if the mother's HBsAg status cannot be determined within the first 12 hours of life. The birth dose in infants born weighing <2,000 g should not be counted as the first dose in the vaccine series and it should be followed with a full 3-dose standard regimen (total of 4 doses).[2] *[See Dosage and Administration (2).]*

5.4 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including ENGERIX-B, to infants born prematurely should be based on consideration of the infant's medical status, and the potential benefits and possible risks of vaccination. For ENGERIX-B, this assessment should include consideration of the mother's hepatitis B antigen status and the high probability of maternal transmission of hepatitis B virus to infants born of mothers who are HBsAg positive if vaccination is delayed.

5.5 Preventing and Managing Allergic Vaccine Reactions

Prior to immunization, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions to allow an assessment of benefits and risks. Epinephrine and other appropriate agents used for the control of

immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur. *[See Contraindications (4).]*

5.6 Moderate or Severe Acute Illness

To avoid diagnostic confusion between manifestations of an acute illness and possible vaccine adverse effects, vaccination with ENGERIX-B should be postponed in persons with moderate or severe acute febrile illness unless they are at immediate risk of hepatitis B infection (e.g., infants born of HBsAg-positive mothers).

5.7 Altered Immunocompetence

Immunocompromised persons may have a diminished immune response to ENGERIX-B, including individuals receiving immunosuppressant therapy.

5.8 Multiple Sclerosis

Results from 2 clinical studies indicate that there is no association between hepatitis B vaccination and the development of multiple sclerosis,[3] and that vaccination with hepatitis B vaccine does not appear to increase the short-term risk of relapse in multiple sclerosis.[4]

5.9 Limitations of Vaccine Effectiveness

Hepatitis B has a long incubation period. ENGERIX-B may not prevent hepatitis B infection in individuals who had an unrecognized hepatitis B infection at the time of vaccine administration. Additionally, it may not prevent infection in individuals who do not achieve protective antibody titers.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The most common solicited adverse reactions were injection site soreness (22%) and fatigue (14%).

In 36 clinical studies, a total of 13,495 doses of ENGERIX-B were administered to 5,071 healthy adults and children who were initially seronegative for hepatitis B markers, and healthy neonates. All subjects were monitored for 4 days post-administration. Frequency of adverse reactions tended to decrease with successive doses of ENGERIX-B.

Using a symptom checklist, the most frequently reported adverse reactions were injection site soreness (22%) and fatigue (14%). Other reactions are listed below. Parent or guardian completed forms for children and neonates. Neonatal checklist did not include headache, fatigue, or dizziness.

Incidence 1% to 10% of Injections

Nervous System Disorders: Dizziness, headache.

General Disorders and Administration Site Conditions: Fever (>37.5°C), injection site erythema, injection site induration, injection site swelling.

Incidence <1% of Injections

Infections and Infestations: Upper respiratory tract illnesses.

Blood and Lymphatic System Disorders: Lymphadenopathy.

Metabolism and Nutrition Disorders: Anorexia.

Psychiatric Disorders: Agitation, insomnia.

Nervous System Disorders: Somnolence, tingling.

Vascular Disorders: Flushing, hypotension.

Gastrointestinal Disorders: Abdominal pain/cramps, constipation, diarrhea, nausea, vomiting.

Skin and Subcutaneous Tissue Disorders: Erythema, petechiae, pruritus, rash, sweating, urticaria.

Musculoskeletal and Connective Tissue Disorders: Arthralgia, back pain, myalgia, pain/stiffness in arm, shoulder, or neck.

General Disorders and Administration Site Conditions: Chills, influenza-like symptoms, injection site ecchymosis, injection site pain, injection site pruritus, irritability, malaise, weakness.

In a clinical trial, 416 adults with type 2 diabetes and 258 control subjects without type 2 diabetes who were seronegative for hepatitis B markers received at least 1 dose of ENGERIX-B. Subjects were monitored for solicited adverse reactions for 4 days following each vaccination. The most frequently reported solicited adverse reactions in the entire study population were injection site pain (reported in 39% of diabetic subjects and 45% of control subjects) and fatigue (reported in 29% of diabetic subjects and 27% of control subjects). Serious adverse events were monitored through 30 days following the last vaccination. Serious adverse events (SAEs) occurred in 3.8% of diabetic subjects and 1.6% of controls. No SAEs were deemed related to ENGERIX-B.

6.2 Postmarketing Experience

The following adverse reactions have been identified during post-approval use of ENGERIX-B. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Infections and Infestations

Herpes zoster, meningitis.

Blood and Lymphatic System Disorders

Thrombocytopenia.

Immune System Disorders

Allergic reaction, anaphylactoid reaction, anaphylaxis. An apparent hypersensitivity syndrome (serum sickness-like) of delayed onset has been reported days to weeks after vaccination, including: arthralgia/arthritis (usually transient), fever, and dermatologic reactions such as urticaria, erythema multiforme, ecchymoses, and erythema nodosum.

Nervous System Disorders

Encephalitis; encephalopathy; migraine; multiple sclerosis; neuritis; neuropathy including hypoesthesia, paresthesia, Guillain-Barré syndrome and Bell's palsy; optic neuritis; paralysis; paresis; seizures; syncope; transverse myelitis.

Eye Disorders

Conjunctivitis, keratitis, visual disturbances.

Ear and Labyrinth Disorders

Earache, tinnitus, vertigo.

Cardiac Disorders

Palpitations, tachycardia.

Vascular Disorders

Vasculitis.

Respiratory, Thoracic, and Mediastinal Disorders

Apnea, bronchospasm including asthma-like symptoms.

Gastrointestinal Disorders

Dyspepsia.

Skin and Subcutaneous Tissue Disorders

Alopecia, angioedema, eczema, erythema multiforme including Stevens-Johnson syndrome, erythema nodosum, lichen planus, purpura.

Musculoskeletal and Connective Tissue Disorders

Arthritis, muscular weakness.

General Disorders and Administration Site Conditions

Injection site reaction.

Investigations

Abnormal liver function tests.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Vaccines and Immune Globulin

ENGERIX-B may be administered concomitantly with immune globulin.

When concomitant administration of other vaccines or immune globulin is required, they should be given with different syringes and at different injection sites. Do not mix ENGERIX-B with any other vaccine or product in the same syringe or vial.

7.2 Interference with Laboratory Tests

HBsAg derived from hepatitis B vaccines has been transiently detected in blood samples following vaccination. Serum HBsAg detection may not have diagnostic value within 28 days after receipt of a hepatitis B vaccine, including ENGERIX-B.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no adequate and well-controlled studies of ENGERIX-B in pregnant women in the U.S. Available data do not suggest an increased risk of major birth defects and miscarriage in women who received ENGERIX-B during pregnancy *(see Data)*.

There are no animal studies with ENGERIX-B to inform use during pregnancy. A developmental toxicity study was performed in female rats administered a vaccine with the same hepatitis B surface antigen component and quantity as ENGERIX-B prior to mating and during gestation (0.2 mL at each occasion). This study revealed no adverse effects on fetal or pre-weaning development (*see Data*).

Data

Human Data: In an evaluation of pre- and post-licensure clinical trials of ENGERIX-B, 58 pregnant women were inadvertently administered ENGERIX-B following their last menstrual period. After excluding elective terminations (n = 6), those with an unknown outcome (n = 3), those with exposure in the third trimester (n = 1), and those with an unknown exposure timing

(n = 22), there were 26 pregnancies with known outcomes with exposure in the first or second trimester. Miscarriage was reported in 11.5% of pregnancies with exposure prior to 20 weeks of gestation (3/26) and major birth defects were reported in 0% (0/23) of live births born to women with exposure during the first or second trimester. The rates of miscarriage and major birth defects were consistent with estimated background rates.

No pregnancy registry for ENGERIX-B was conducted. TWINRIX [Hepatitis A & Hepatitis B (Recombinant) Vaccine] is a bivalent vaccine containing the same hepatitis B surface antigen component and quantity as used in ENGERIX-B. Therefore, clinical data accrued with TWINRIX are relevant to ENGERIX-B. A pregnancy exposure registry was maintained for TWINRIX from 2001 to 2015. The registry prospectively enrolled 245 women who received a dose of TWINRIX during pregnancy or within 28 days prior to conception. After excluding induced abortions (n = 6, including one of a fetus with congenital anomalies), those lost to follow-up (n = 142), those with exposure in the third trimester (n = 1), and those with an unknown exposure timing (n = 9), there were 87 pregnancies with known outcomes with exposure within 28 days prior to conception, or in the first or second trimesters. Miscarriage was reported for 9.6% of pregnancies with exposure to TWINRIX prior to 20 weeks gestation (8/83). Major birth defects were reported for 3.8% of live born infants whose mothers were exposed within 28 days prior to conception or during the first or second trimester (3/80). The rates of miscarriage and major birth defects were consistent with estimated background rates.

Animal Data: In a developmental toxicity study, female rats were administered TWINRIX, which contains the same hepatitis B surface antigen component and quantity as ENGERIX-B, by intramuscular injection on Day 30 prior to mating and on gestation Days 6, 8, 11, and 15. The total dose was 0.2 mL (divided) at each occasion (a single human dose is 1 mL). No adverse effects on pre-weaning development up to post-natal Day 25 were observed. There were no fetal malformations or variations.

8.2 Lactation

Risk Summary

There is no information regarding the presence of ENGERIX-B in human milk, the effects on the breastfed child, or the effects on milk production. The developmental and health benefits of breastfeeding should be considered along with the mother’s clinical need for ENGERIX-B and any potential adverse effects on the breastfed child from ENGERIX-B or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of ENGERIX-B have been established in all pediatric age-groups. Maternally transferred antibodies do not interfere with the active immune response to the vaccine. *[See Adverse Reactions (6), Clinical Studies (14.1, 14.3, 14.4).]*

The timing of the first dose in infants weighing less than 2,000 g at birth depends on the HBsAg status of the mother. *[See Warnings and Precautions (5.3).]*

8.5 Geriatric Use

Clinical studies of ENGERIX-B used for licensure did not include sufficient numbers of subjects aged 65 years and older to determine whether they respond differently from younger subjects. However, in later studies it has been shown that a diminished antibody response and seroprotective levels can be expected in persons older than 60 years.[5] *[See Clinical Studies (14.2).]*

11 DESCRIPTION

ENGERIX-B [Hepatitis B Vaccine (Recombinant)] is a sterile suspension of noninfectious HBsAg for intramuscular administration. It contains purified surface antigen of the virus obtained by culturing genetically engineered *Saccharomyces cerevisiae* cells, which carry the surface antigen gene of the hepatitis B virus. The HBsAg expressed in the cells is purified by several physicochemical steps and formulated as a suspension of the antigen adsorbed on aluminum hydroxide. The procedures used to manufacture ENGERIX-B result in a product that contains no more than 5% yeast protein.

Each 0.5-mL pediatric/adolescent dose contains 10 mcg of HBsAg adsorbed on 0.25 mg aluminum as aluminum hydroxide.

Each 1-mL adult dose contains 20 mcg of HBsAg adsorbed on 0.5 mg aluminum as aluminum hydroxide.

ENGERIX-B contains the following excipients: Sodium chloride (8 mg/mL) and phosphate buffers (disodium phosphate dihydrate, 0.9 mg/mL; sodium dihydrogen phosphate dihydrate, 0.7 mg/mL).

ENGERIX-B is available in vials (adult dose only) and prefilled syringes. The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex. The vial stoppers are not made with natural rubber latex.

ENGERIX-B is formulated without preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Infection with hepatitis B virus can have serious consequences including acute massive hepatic necrosis and chronic active hepatitis. Chronically infected persons are at increased risk for cirrhosis and hepatocellular carcinoma.

Antibody concentrations ≥10 mIU/mL against HBsAg are recognized as conferring protection against hepatitis B virus infection.[1] Seroconversion is defined as antibody titers ≥1 mIU/mL.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

ENGERIX-B has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Vaccination of female rats with TWINRIX, which contains the same HBsAg component and quantity as ENGERIX-B, had no effect on fertility. *[See Use in Specific Populations (8.1).]*

14 CLINICAL STUDIES

14.1 Efficacy in Neonates

Protective efficacy with ENGERIX-B has been demonstrated in a clinical trial in neonates at high risk of hepatitis B infection.[6,7] Fifty-eight neonates born of mothers who were both HBsAg-positive and hepatitis B "e" antigen (HBeAg)-positive were given ENGERIX-B (10 mcg/0.5 mL) at 0, 1, and 2 months, without concomitant hepatitis B immune globulin (HBIG). Two infants became chronic carriers in the 12-month follow-up period after initial inoculation. Assuming an expected carrier rate of 70%, the protective efficacy rate against the chronic carrier state during the first 12 months of life was 95%.

14.2 Efficacy and Immunogenicity in Specific Populations

Homosexual Men

ENGERIX-B (20 mcg/1 mL) given at 0, 1, and 6 months was evaluated in homosexual men aged 16 to 59 years. Four of 244 subjects became infected with hepatitis B during the period prior to completion of the 3-dose immunization schedule. No additional subjects became infected during the 18-month follow-up period after completion of the immunization course.

Adults with Chronic Hepatitis C

In a clinical trial of 67 adults aged 25 to 67 years with chronic hepatitis C, ENGERIX-B (20 mcg/1 mL) was given at 0, 1, and 6 months. Of the subjects assessed at Month 7 (n = 31), 100% responded with seroprotective titers. The geometric mean antibody titer (GMT) was 1,260 mIU/mL (95% Confidence Interval [CI]: 709, 2,237).

Adults on Hemodialysis

Hemodialysis patients given hepatitis B vaccines respond with lower titers, which remain at protective levels for shorter durations than in normal subjects. In a clinical trial of 56 adults who had been on hemodialysis for a mean period of 56 months, ENGERIX-B (40 mcg/2 mL given as two 1-mL doses) was given at 0, 1, 2, and 6 months. Two months after the fourth dose, 67% (29/43) of patients had seroprotective antibody levels (≥10 mIU/mL) and the GMT among seroconverters was 93 mIU/mL.

Adults with Type 2 Diabetes Mellitus

In a descriptive study, 674 adult subjects with type 2 diabetes (diagnosed within the preceding 5 years) or without type 2 diabetes were enrolled and stratified by age and body mass index (BMI). The per-protocol immunogenicity cohort included 378 diabetic subjects and 189 matched control subjects who received ENGERIX-B (20 mcg/1 mL) at 0, 1, and 6 months. Among these subjects, the mean age was 54 years (range: 20 to 82 years); mean BMI was 32 kg/m^2 (range: 17 to 64 kg/m^2); 51% were male; 88% were white, 3% were American Indian or Alaskan Native, 3% were black, 2% were Asian, 4% were other racial groups; 2% were Hispanic or Latino.

The overall seroprotection rates (1 month after the third dose) were 75% (95% CI: 71, 80) in patients with diabetes and 82% (95% CI: 76, 87) in control subjects. The seroprotection rates in those with diabetes aged 20 to 39 years, 40 to 49 years, 50 to 59 years, and at least 60 years were 89%, 81%, 83%, and 58%, respectively. The seroprotection rates in those without diabetes in these same age-groups were 100%, 86%, 82%, and 70%, respectively. Subjects with diabetes and a BMI of at least 30 kg/m^2 had a seroprotection rate of 72% compared with 80% in diabetic subjects with lower BMIs. In control subjects, seroprotection rates were 82% in those with a BMI of at least 30 kg/m^2 and 83% in those with lower BMIs.

14.3 Immunogenicity in Neonates

In clinical studies, neonates were given ENGERIX-B (10 mcg/0.5 mL) at age 0, 1, and 6 months or at age 0, 1, and 2 months. The immune response to vaccination was evaluated in sera obtained 1 month after the third dose of ENGERIX-B.

Among infants administered ENGERIX-B at age 0, 1, and 6 months, 100% of evaluable subjects (n = 52) seroconverted by Month 7. The GMT was 713 mIU/mL. Of these, 97% had seroprotective levels (≥10 mIU/mL).

Among infants enrolled (n = 381) to receive ENGERIX-B at age 0, 1, and 2 months, 96% had seroprotective levels (≥10 mIU/mL) by Month 4. The GMT among seroconverters (n = 311) (antibody titer ≥1 mIU/mL) was 210 mIU/mL. A subset of these children received a fourth dose of ENGERIX-B at age 12 months. One month following this dose, seroconverters (n = 126) had a GMT of 2,941 mIU/mL.

14.4 Immunogenicity in Children and Adults

Persons Aged 6 Months through 10 Years

In clinical trials, children (N = 242) aged 6 months through 10 years were given ENGERIX-B (10 mcg/0.5 mL) at 0, 1, and 6 months. One to 2 months after the third dose, the seroprotection rate was 98% and the GMT of seroconverters was 4,023 mIU/mL.

Persons Aged 5 through 16 Years

In a separate clinical trial including both children and adolescents aged 5 through 16 years, ENGERIX-B (10 mcg/0.5 mL) was administered at 0, 1, and 6 months (n = 181) or 0, 12, and

24 months (n = 161). Immediately before the third dose of vaccine, seroprotection was achieved in 92.3% of subjects vaccinated on the 0-, 1-, and 6-month schedule and 88.8% of subjects on the 0-, 12-, and 24-month schedule (GMT: 118 mIU/mL versus 162 mIU/mL, respectively, $P = 0.18$). One month following the third dose, seroprotection was achieved in 99.5% of children vaccinated on the 0-, 1-, and 6-month schedule compared with 98.1% of those on the 0-, 12-, and 24-month schedule. GMTs were higher ($P = 0.02$) for children receiving vaccine on the 0-, 1-, and 6-month schedule compared with those on the 0-, 12-, and 24-month schedule (5,687 mIU/mL versus 3,159 mIU/mL, respectively).

Persons Aged 11 through 19 Years

In clinical trials with healthy adolescent subjects aged 11 through 19 years, ENGERIX-B (10 mcg/0.5 mL) given at 0, 1, and 6 months produced a seroprotection rate of 97% at Month 8 (n = 119) with a GMT of 1,989 mIU/mL (n = 118, 95% CI: 1,318, 3,020). Immunization with ENGERIX-B (20 mcg/1 mL) at 0, 1, and 6 months produced a seroprotection rate of 99% at Month 8 (n = 122) with a GMT of 7,672 mIU/mL (n = 122, 95% CI: 5,248, 10,965).

Persons Aged 16 through 65 Years

Clinical trials in healthy adult and adolescent subjects (aged 16 through 65 years) have shown that following a course of 3 doses of ENGERIX-B (20 mcg/1 mL) given at 0, 1, and 6 months, the seroprotection (antibody titers ≥10 mIU/mL) rate for all individuals was 79% at Month 6 (5 months after second dose) and 96% at Month 7 (1 month after third dose); the GMT for seroconverters was 2,204 mIU/mL at Month 7 (n = 110).

An alternate 3-dose schedule (20 mcg/1 mL given at 0, 1, and 2 months) designed for certain populations (e.g., individuals who have or might have been recently exposed to the virus and travelers to high-risk areas) was also evaluated. At Month 3 (1 month after third dose), 99% of all individuals were seroprotected and remained protected through Month 12. On the alternate schedule, a fourth dose of ENGERIX-B (20 mcg/1 mL) at 12 months produced a GMT of 9,163 mIU/mL at Month 13 (1 month after fourth dose) (n = 373).

Persons Aged 40 Years and Older

Among subjects aged 40 years and older given ENGERIX-B (20 mcg/1 mL) at 0, 1, and 6 months, the seroprotection rate 1 month after the third dose was 88% and the GMT for seroconverters was 610 mIU/mL (n = 50). In adults aged older than 40 years, ENGERIX-B produced anti-HBsAg antibody titers that were lower than those in younger adults.

14.5 Interchangeability with Other Hepatitis B Vaccines

A controlled study (N = 48) demonstrated that completion of a course of immunization with 1 dose of ENGERIX-B (20 mcg/1 mL) at Month 6 following 2 doses of RECOMBIVAX HB [Hepatitis B Vaccine (Recombinant)] (10 mcg) at Months 0 and 1 produced a similar GMT (4,077 mIU/mL) to immunization with 3 doses of RECOMBIVAX HB (10 mcg) at Months 0, 1,

and 6 (GMT: 2,654 mIU/mL). Thus, ENGERIX-B can be used to complete a vaccination course initiated with RECOMBIVAX HB.[8]

15 REFERENCES

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16 HOW SUPPLIED/STORAGE AND HANDLING

ENGERIX-B (preservative-free formulation) is available in prefilled disposable TIP-LOK syringes (packaged without needles) (pediatric/adolescent and adult doses) and single-dose vials (adult dose only):

10 mcg/0.5 mL Pediatric/Adolescent Dose

NDC 58160-820-43 Syringe in Package of 10: NDC 58160-820-52

20 mcg/mL Adult Dose

NDC 58160-821-01 Vial in Package of 10: NDC 58160-821-11

NDC 58160-821-43 Syringe in Package of 10: NDC 58160-821-52

Store refrigerated between 2° and 8°C (36° and 46°F). Do not freeze; discard if product has been frozen. Do not dilute to administer.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipients and parents or guardians of the potential benefits and risks of immunization with ENGERIX-B.
- Emphasize, when educating vaccine recipients and parents or guardians regarding potential side effects, that ENGERIX-B contains non-infectious purified HBsAg and cannot cause hepatitis B infection.
- Instruct vaccine recipients and parents or guardians to report any adverse events to their healthcare provider.
- Give vaccine recipients and parents or guardians the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

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Research Triangle Park, NC 27709

ENG:60PI

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use HEPLISAV-B® safely and effectively. See full prescribing information for HEPLISAV-B.

HEPLISAV-B [Hepatitis B Vaccine (Recombinant), Adjuvanted] Solution for Intramuscular Injection

Initial US Approval: 2017

----------INDICATIONS AND USAGE ----------

HEPLISAV-B is indicated for prevention of infection caused by all known subtypes of hepatitis B virus. HEPLISAV-B is approved for use in adults 18 years of age and older. (1)

---------- DOSAGE AND ADMINISTRATION ----------

For intramuscular administration

Administer two doses (0.5 mL each) of HEPLISAV-B intramuscularly one month apart. (2.1, 2.2)

----------DOSAGE FORMS AND STRENGTHS ----------

HEPLISAV-B is a solution for injection supplied as a single-dose prefilled syringe. A single dose of HEPLISAV-B is 0.5 mL. (3)

----------CONTRAINDICATIONS----------

Severe allergic reaction, such as anaphylaxis, after a previous dose of any hepatitis B vaccine or to any component of HEPLISAV-B, including yeast. (4)

----------ADVERSE REACTIONS ----------

The most common local reaction was injection site pain (23% - 39%). The most common systemic reactions were fatigue (11% - 17%) and headache (8% - 17%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Dynavax at 1-844-889-8753 or VAERS at 1-800-822-7967 and www.vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS ----------

A pregnancy registry is available for HEPLISAV-B. Women who receive HEPLISAV-B during pregnancy are encouraged to contact 1-844-443-7734. (8.1)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 05/2020

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

HEPLISAV-B is indicated for prevention of infection caused by all known subtypes of hepatitis B virus. HEPLISAV-B is approved for use in adults 18 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular administration.

2.1 Dose and Regimen

Administer two doses (0.5 mL each) of HEPLISAV-B one month apart.

2.2 Administration

HEPLISAV-B is a clear to slightly opalescent, colorless to slightly yellow solution.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Administer HEPLISAV-B by intramuscular injection in the deltoid region using a sterile needle and syringe.

3 DOSAGE FORMS AND STRENGTHS

HEPLISAV-B is a sterile solution for injection available in 0.5 mL single-dose prefilled syringes. [see *How Supplied/Storage and Handling* (16.1)].

4 CONTRAINDICATIONS

Do not administer HEPLISAV-B to individuals with a history of severe allergic reaction (e.g. anaphylaxis) after a previous dose of any hepatitis B vaccine or to any component of HEPLISAV-B, including yeast [see *Description* (11)].

5 WARNINGS AND PRECAUTIONS

5.1 Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of HEPLISAV-B.

5.2 Immunocompromised Individuals

Immunocompromised persons, including individuals receiving immunosuppressant therapy, may have a diminished immune response to HEPLISAV-B.

5.3 Limitations of Vaccine Effectiveness

Hepatitis B has a long incubation period. HEPLISAV-B may not prevent hepatitis B infection in individuals who have an unrecognized hepatitis B infection at the time of vaccine administration.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

A total of 9597 individuals 18 through 70 years of age received at least 1 dose of HEPLISAV-B in 5 clinical trials conducted in the United States, Canada, and Germany. Data from 3 of these trials are provided below.

Study 1 in Subjects 18 through 55 Years of Age

Study 1 was a randomized, observer-blind, active-controlled, multicenter study in Canada and Germany in which 1810 subjects received at least 1 dose of HEPLISAV-B and 605 subjects received at least 1 dose of Engerix-B® [Hepatitis B Vaccine (Recombinant)]. Enrolled subjects had no history of hepatitis B vaccination or infection. HEPLISAV-B was given as a 2-dose regimen at 0 and 1 month followed by saline placebo at 6 months. Engerix-B was given at 0, 1, and 6 months. In the total study population, the mean age was 40 years; 46% of the subjects were men; 93% were white, 2% black, 3% Asian and 3% Hispanic; 26% were obese, 10% had hypertension, 8% had dyslipidemia, and 2% had diabetes mellitus. These demographic and baseline characteristics were similar in both vaccine groups.

Solicited Local and Systemic Adverse Reactions

Subjects were monitored for local and systemic adverse reactions using diary cards for a 7-day period starting on the day of vaccination. The percentages of subjects who reported local and systemic reactions are shown in Table 1.

Table 1
Study 1: Percent of Subjects Who Reported Local or Systemic Reactions Within 7 Days of Vaccination

	HEPLISAV-B %		Engerix-B %		
	Post-Dose*		Post-Dose*		
Reaction	**1**	**2**	**1**	**2**	**3**
Local	N=1810	N=1798	N=605	N=603	N=598
Injection Site Pain	38.5	34.8	33.6	24.7	20.2
Injection Site Redness†	4.1	2.9	0.5	1.0	0.7
Injection Site Swelling†	2.3	1.5	0.7	0.5	0.5
Systemic					
Fatigue	17.4	13.8	16.7	11.9	10.0
Headache	16.9	12.8	19.2	12.3	9.5
Malaise	9.2	7.6	8.9	6.5	6.4
	N=1784	N=1764	N=596	N=590	N=561
Fever‡	1.1	1.5	1.8	1.7	1.8

Note: only subjects having data are included. Clinical trial number: NCT00435812
*HEPLISAV-B was given as a 2-dose regimen at 0 and 1 month followed by saline placebo at 6 months. Engerix-B was given at 0, 1, and 6 months
† Redness and swelling ≥ 2.5 cm.
‡ Oral temperature ≥ 100.4°F (38.0°C).

Unsolicited Adverse Events:

Unsolicited adverse events within 28 days following any injection, including placebo, were reported by 42.0% of HEPLISAV-B recipients and 41.3% of Engerix-B recipients.

Serious Adverse Events (SAEs)

Subjects were monitored for serious adverse events for 7 months after the first dose of vaccine. The percentage of subjects reporting serious adverse events was 1.5% in the HEPLISAV-B group and 2.1% in the Engerix-B group. No acute myocardial infarctions were reported. No deaths were reported.

Potentially Immune-mediated Adverse Events
Potentially immune-mediated adverse events that occurred within 7 months of the first dose of vaccine were reported in 0.2% (n = 4) of HEPLISAV-B recipients and 0.7% (n = 4) of Engerix-B recipients. The following events were reported in the HEPLISAV-B group in one subject each: granulomatosis with polyangiitis, lichen planus, Guillain-Barré syndrome, and Grave's disease. The following events were reported in the Engerix-B group in one subject each: Bell's palsy, Raynaud's phenomenon, and Grave's disease. One additional Engerix-B recipient with a history of mixed connective tissue disease had p-ANCA-positive vasculitis.

Study 2 in Subjects 40 through 70 Years of Age

Study 2 was a randomized, observer-blind, active-controlled, multicenter study in Canada and the United States in which 1968 subjects received at least 1 dose of HEPLISAV-B and 481 subjects received at least 1 dose of Engerix-B. HEPLISAV-B was given as a 2-dose regimen at 0 and 1 month followed by saline placebo at 6 months. Enrolled subjects had no history of hepatitis B vaccination or infection. Engerix-B was given at 0, 1, and 6 months. In the total population, the mean age was 54 years; 48% of subjects were men; 82% were white, 15% black, 1% Asian and 6% Hispanic; 44% were obese, 30% had hypertension, 30% had dyslipidemia, and 8% had diabetes mellitus. These demographic and baseline characteristics were similar in both vaccine groups.

Solicited Local and Systemic Adverse Reactions
Subjects were monitored for local and systemic adverse reactions using diary cards for a 7-day period starting on the day of vaccination. The percentages of subjects who experienced local and systemic reactions are shown in Table 2.

Table 2
Study 2: Percent of Subjects Who Reported Local or Systemic Reactions Within 7 Days of Vaccination

	HEPLISAV-B %		Engerix-B %		
	Post-Dose*		Post-Dose*		
Reaction	**1**	**2**	**1**	**2**	**3**
Local	N=1952	N=1905	N=477	N=464	N=448
Injection Site Pain	23.7	22.8	18.4	15.9	13.8
Injection Site Redness†	0.9	0.7	0.6	0.2	0.2
Injection Site Swelling†	0.9	0.6	0.6	0.6	0.2
Systemic					
Fatigue	12.6	10.8	12.8	12.1	9.4
Headache	11.8	8.1	11.9	9.5	8.5
Malaise	7.7	7.0	8.6	7.1	5.1
Myalgia	8.5	6.4	9.6	8.0	4.5
	N=1923	N=1887	N=472	N=459	N=438
Fever‡	0.6	0.6	0.6	0.9	0.7

Note: only subjects having data are included. Clinical Trial Number: NCT01005407
*HEPLISAV-B was given as a 2-dose regimen at 0 and 1 month followed by saline placebo at 6 months. Engerix-B was given at 0, 1, and 6 months
† Redness and swelling ≥2.5 cm
‡ Oral temperature ≥ 100.4°F (38.0°C).

Unsolicited Adverse Events:
Unsolicited adverse events within 28 days following any injection, including placebo, were reported by 35.4% of HEPLISAV-B recipients and 36.2% of Engerix-B recipients.

Serious Adverse Events
Subjects were monitored for serious adverse events for 12 months after the first dose of vaccine. The percentage of subjects reporting serious adverse events was 3.9% in the HEPLISAV-B group and 4.8% in the Engerix-B group. Acute myocardial infarction occurred in 0.1% (n=2) of HEPLISAV-B recipients and 0.2% (n=1) of Engerix-B recipients.

Autoimmune Adverse Events
Subjects were monitored for the occurrence of new-onset potentially immune-mediated adverse events

for 12 months after the first dose of vaccine. Events were adjudicated as to whether they were autoimmune by an external group of experts blinded to treatment assignment. As determined by the adjudicators, new-onset autoimmune adverse events were reported in 0.2% (n=3) of HEPLISAV-B recipients: two subjects with hypothyroidism and one subject with vitiligo. None of these events was considered related to vaccination by the expert group. No new-onset autoimmune adverse events were reported in the Engerix-B group. Although not referred to the external group of experts, one HEPLISAV-B recipient was determined to have Tolosa-Hunt syndrome which is presumed to have an immune-mediated etiology. This event was not considered related to vaccination.

Deaths
One subject (0.05%) died of a pulmonary embolism in the HEPLISAV-B group and 1 subject (0.2%) died of heart failure in the Engerix-B group. Neither death was considered related to vaccination.

Study 3 in Subjects 18 through 70 Years of Age

Study 3 was a randomized, observer-blind, active-controlled, multicenter study in the United States in which 5587 subjects received at least 1 dose of HEPLISAV-B and 2781 subjects received at least 1 dose of Engerix-B. Enrolled subjects had no history of hepatitis B vaccination or infection. HEPLISAV-B was given as a 2-dose regimen at 0 and 1 month followed by saline placebo at 6 months. Engerix-B was given at 0, 1, and 6 months. In the total study population, the mean age was 50 years; 51% were men; 71% were white, 26% black, 1% Asian, and 9% Hispanic; 48% were obese, 36% had hypertension, 32% had dyslipidemia, and 14% had type 2 diabetes mellitus. These demographic and baseline characteristics were similar in both vaccine groups.

Unsolicited Medically-Attended Adverse Events
Subjects were monitored for unsolicited medically-attended adverse events, those for which a subject sought medical care, for 13 months after the first dose of vaccine. Overall, medically-attended adverse events were reported in 46.0% of HEPLISAV-B recipients and 46.2% of Engerix-B recipients. Herpes zoster was reported in 0.7% of HEPLISAV-B recipients and 0.3% of Engerix-B recipients. Unsolicited medically-attended adverse events within 28 days following any injection, including placebo, were reported by 20.1% of both HEPLISAV-B and Engerix-B recipients.

Serious Adverse Events
Subjects were monitored for serious adverse events for 13 months after the first dose of vaccine. The percentage of subjects who reported serious adverse events was 6.2% in the HEPLISAV-B group and 5.3% in the Engerix-B group. Acute myocardial infarction (AMI) was reported in 0.25% (n=14) of HEPLISAV-B recipients and 0.04% (n=1) of Engerix-B recipients. An analysis of serious adverse events likely representing myocardial infarction (MI) was conducted using the standard Medical Dictionary for Regulatory Activities (MedDRA) query (SMQ) for MI. This analysis identified a total of 19 HEPLISAV-B subjects (0.3%) and 3 Engerix-B subjects (0.1%) with events included in the SMQ for MI (these events include the 15 reports of AMI). Additional evidence, including information on temporal relationship and baseline risk factors, does not support a causal relationship between HEPLISAV-B administration and AMI. Among the 19 events identified as MI in HEPLISAV-B recipients, three occurred within 14 days, nine occurred within 53-180 days, and seven occurred more than 180 days following any dose of HEPLISAV-B. Among the three events identified as MI in Engerix-B recipients, one each occurred 13, 115, and 203 days following any dose. All 19 HEPLISAV-B recipients and 3 Engerix-B recipients reported one or more baseline risk factors for cardiovascular disease.

Autoimmune Adverse Events
Subjects were monitored for the occurrence of new-onset potentially immune-mediated adverse events for 13 months after the first dose of vaccine. Events were adjudicated as to whether they were autoimmune by an external group of experts who were blinded to treatment assignment. As determined by the adjudicators, new-onset autoimmune adverse events were reported in 0.1% (n=4) of HEPLISAV-B recipients [one each of: alopecia areata, polymyalgia rheumatica, ulcerative colitis, and autoimmune thyroiditis (with concurrent diagnosis of papillary thyroid carcinoma)]. None of these events was considered to be related to vaccination by the external experts. No new-onset autoimmune adverse events were reported in the Engerix-B group.

Deaths
During the study death was reported in 25 subjects (0.4%) in the HEPLISAV-B group and 7 subjects (0.3%) in the Engerix-B group. No death was considered related to vaccination.

7 DRUG INTERACTIONS

7.1 Use with Immune Globulin

There are no data to assess the concomitant use of HEPLISAV-B with immune globulin. When concomitant administration of HEPLISAV-B and immune globulin is required, they should be given with different syringes at different injection sites.

7.2 Interference with Laboratory Tests

Hepatitis B surface antigen (HBsAg) derived from hepatitis B vaccines has been transiently detected in blood samples following vaccination. Serum HBsAg detection may not have diagnostic value within 28 days after receipt of HEPLISAV-B.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry
There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to HEPLISAV-B during pregnancy. Women who receive HEPLISAV-B during pregnancy are encouraged to contact 1-844-443-7734.

Risk Summary
All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In clinically recognized pregnancies in the US general population, the estimated background risk of major birth defects is 2% to 4% and of miscarriage is 15% to 20%.

There are no clinical studies of HEPLISAV-B in pregnant women. Available human data on HEPLISAV-B administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

In a developmental toxicity study, 0.3 mL of a vaccine formulation containing 2.5 mcg HBsAg and 3000 mcg cytosine phosphoguanine (CpG) 1018 adjuvant was administered to female rats prior to mating and during gestation. These animal studies revealed no evidence of harm to the fetus due to this vaccine formulation *[see Data]*.

Data
Animal data
Developmental toxicity studies were conducted in female rats. Animals were administered 0.3 mL of a vaccine formulation containing 2.5 mcg HBsAg and 3000 mcg CpG 1018 adjuvant twice prior to mating, and on gestation days 6 and 18 (a single human dose of HEPLISAV-B contains 20 mcg HBsAg and 3000 mcg CpG 1018 adjuvant). No adverse effects on pre-natal and post-natal development up to the time of weaning were observed. There were no vaccine-related fetal malformations or variations observed.

8.2 Lactation

Risk Summary
It is not known whether HEPLISAV-B is excreted in human milk. Data are not available to assess the effects of HEPLISAV-B on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for HEPLISAV-B and any potential adverse effects on the breastfed child from HEPLISAV-B or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of HEPLISAV-B have not been established in individuals less than 18 years of age.

8.5 Geriatric Use

Clinical trials included 909 adults 65 through 70 years of age who received HEPLISAV-B.

Among subjects who received HEPLISAV-B, a seroprotective level of antibody to HBsAg was achieved in 90% of those 65 through 70 years of age compared to 96% of those aged 18 through 64 years of age.

Safety and effectiveness of HEPLISAV-B in adults older than 70 years of age were extrapolated from findings in subjects younger than 70 years of age.

8.6 Adults on Hemodialysis

Safety and effectiveness of HEPLISAV-B have not been established in adults on hemodialysis.

11 DESCRIPTION

HEPLISAV-B [Hepatitis B Vaccine (Recombinant), Adjuvanted] is a sterile solution for intramuscular injection.

The HBsAg is expressed in a recombinant strain of *Hansenula polymorpha* yeast. The fermentation process involves growth of the recombinant *H. polymorpha* on chemically-defined fermentation media containing vitamins and mineral salts.

The HBsAg is expressed intra-cellularly in the yeast cells. It is released from the yeast cells by cell disruption and purified by a series of physicochemical steps. Each dose may contain residual amounts of yeast protein (≤5.0% of total protein), yeast DNA (<20 picogram), and deoxycholate (<0.9 ppm) from the HBsAg manufacturing process.

HEPLISAV-B is prepared by combining the purified HBsAg together with the CpG 1018 adjuvant, a 22-mer phosphorothioate linked oligodeoxynucleotide in a phosphate buffered saline (sodium chloride, 9.0 mg/mL; sodium phosphate, dibasic dodecahydrate, 1.75 mg/mL; sodium phosphate, monobasic dihydrate, 0.48 mg/mL; and polysorbate 80, 0.1 mg/mL).

Each 0.5-mL dose is formulated to contain 20 mcg of HBsAg and 3000 mcg of CpG 1018 adjuvant.

HEPLISAV-B is available in prefilled syringes. The tip caps and stoppers of the prefilled syringes are not made with natural rubber latex.

HEPLISAV-B is formulated without preservatives. [see *How Supplied/Storage and Handling (16)*].

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Infection with hepatitis B virus can have serious consequences including acute massive hepatic necrosis and chronic active hepatitis. Chronically infected persons are at increased risk for cirrhosis and hepatocellular carcinoma.

Antibody concentrations ≥10 mIU/mL against HBsAg are recognized as conferring protection against hepatitis B virus infection.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

HEPLISAV-B has not been evaluated for carcinogenicity, mutagenic potential or male infertility in animals. Vaccination of female rats with a vaccine formulation containing 2.5 mcg HBsAg and 3000 mcg CpG 1018 adjuvant had no effect on fertility [see *Use in Specific Populations (8)*].

14 CLINICAL STUDIES

14.1 Evaluation of Seroprotection

The immunogenicity of HEPLISAV-B was evaluated in comparison with a licensed hepatitis B vaccine (Engerix-B) in 3 randomized, active controlled, observer-blinded, multi-center Phase 3 clinical trials of

adults. HEPLISAV-B was given as a 2-dose regimen at 0 and 1 months followed by saline placebo at 6 months. Engerix-B was given at 0, 1, and 6 months.

The trials compared the seroprotection rates (% with antibody concentration ≥ 10 mIU/mL) induced by HEPLISAV-B and Engerix-B. Noninferiority was met if the lower bound of the 95% confidence interval of the difference in seroprotection rates (HEPLISAV-B minus Engerix-B) was greater than -10%.

Study 1: Seroprotection in Adults 18 through 55 Years of Age

In Study 1, the immunogenicity population comprised 1511 participants who received HEPLISAV-B and 521 who received Engerix-B. The mean age was 40 years for both groups. The primary analysis compared the seroprotection rate at Week 12 for HEPLISAV-B with that at Week 28 for Engerix-B. Non-inferiority of the seroprotection rate induced by HEPLISAV-B compared to Engerix-B was demonstrated (Table 3).

Table 3
Study 1: Seroprotection Rate of HEPLISAV-B and Engerix-B (ages 18 through 55 years)

Timepoint	HEPLISAV-B N = 1511	Engerix-B N = 521	Difference in SPRs (HEPLISAV-B minus Engerix-B)
	SPR (95% CI)	SPR (95% CI)	Difference (95% CI)
Week 12 (HEPLISAV-B) Week 28 (Engerix-B)	95% (93.9, 96.1)	81.3% (77.8, 84.6)	13.7% (10.4, 17.5)*

CI = confidence interval; N = number of subjects in the analysis population in the group; SPR = seroprotection rate (% with anti-HBs ≥ 10 mIU/mL).
* Noninferiority was met because the lower bound of the 95% confidence interval of the difference in SPRs was greater than -10%.
Clinical trial number: NCT00435812

Study 2: Seroprotection in Adults 40 through 70 Years of Age

In Study 2, the immunogenicity population comprised 1121 subjects who received HEPLISAV-B and 353 subjects who received Engerix-B. The mean age was 54 years for both groups. The primary analysis compared the seroprotection rate at Week 12 for HEPLISAV-B with that at Week 32 for Engerix-B. Non-inferiority of the seroprotection rate induced by HEPLISAV-B compared to Engerix-B was demonstrated (Table 4).

Table 4
Study 2: Seroprotection Rate of HEPLISAV-B and Engerix-B (ages 40 through 70 years)

Timepoint	HEPLISAV-B N = 1121	Engerix-B N = 353	Difference in SPRs (HEPLISAV-B minus Engerix-B)
	SPR (95% CI)	SPR (95% CI)	Difference (95% CI)
Week 12 (HEPLISAV-B) Week 32 (Engerix-B)	90.1% (88.2, 91.8)	70.5% (65.5, 75.2)	19.6% (14.7, 24.8)*

CI = confidence interval; N = number of subjects in the analysis population in the group; SPR = seroprotection rate (% with anti-HBs ≥ 10 mIU/mL).
* Noninferiority was met because the lower bound of the 95% confidence interval of the difference in SPRs was greater than -10%. The SPR following HEPLISAV-B was statistically significantly higher than following Engerix-B (lower bound of the 95% confidence interval of the difference in SPRs was greater than 0%).
Clinical trial number: NCT01005407

Study 3: Seroprotection in Adults 18 through 70 Years of Age Including those with Type 2 Diabetes Mellitus

In Study 3, the immunogenicity population comprised 4537 subjects who received HEPLISAV-B and 2289 subjects who received Engerix-B. The mean age was 51 years and 14% of subjects had type 2 diabetes mellitus (defined as having a clinical diagnosis of type 2 diabetes and taking at least an oral or non-insulin injectable hypoglycemic agent and/or insulin).

The primary analysis compared the seroprotection rate at Week 28 for HEPLISAV-B (n= 640) with that at Week 28 for Engerix-B (n= 321) in subjects with type 2 diabetes mellitus. Non-inferiority of the seroprotection rate induced by HEPLISAV-B compared to Engerix-B was demonstrated (Table 5).

Timepoint	Table 5 Study 3: Seroprotection Rate of HEPLISAV-B and Engerix-B (subjects with type 2 diabetes mellitus ages 18 through 70 years)		
	HEPLISAV-B N = 640	Engerix-B N = 321	Difference in SPRs (HEPLISAV-B minus Engerix-B)
	SPR (95% CI)	SPR (95% CI)	Difference (95% CI)
Week 28	90.0% (87.4, 92.2)	65.1% (59.6, 70.3)	24.9% (19.3, 30.7)*

CI = confidence interval; N = number of subjects in the analysis population in the group; SPR = seroprotection rate (% with anti-HBs ≥ 10 mIU/mL).
* Noninferiority was met because the lower bound of the 95% confidence interval of the difference in SPRs was greater than -10%. The SPR following HEPLISAV-B was statistically significantly higher than following Engerix-B (lower bound of the 95% confidence interval of the difference in SPRs was greater than 0%).
Clinical trial number: NCT02117934

A secondary analysis compared the seroprotection rate at Week 24 for HEPLISAV-B with that at Week 28 for Engerix-B in the total study population. Non-inferiority of the seroprotection rate induced by HEPLISAV-B compared to Engerix-B was demonstrated (Table 6).

Timepoint	Table 6 Study 3: Seroprotection Rate of HEPLISAV-B and Engerix-B (total study population ages 18 through 70 years)		
	HEPLISAV-B N = 4376	Engerix-B N = 2289	Difference in SPRs (HEPLISAV-B minus Engerix-B)
	SPR (95% CI)	SPR (95% CI)	Difference (95% CI)
Week 24 (HEPLISAV-B) Week 28 (Engerix-B)	95.4% (94.8, 96.0)	81.3% (79.6, 82.8)	14.2% (12.5, 15.9)*

CI = confidence interval; N = number of subjects in the analysis population in the group; SPR = seroprotection rate (% with anti-HBs ≥ 10 mIU/mL).
Clinical trial number: NCT02117934
*Noninferiority was met because the lower bound of the 95% confidence interval of the difference in SPRs was greater than -10%. The SPR following HEPLISAV-B was statistically significantly higher than following Engerix-B (lower bound of the 95% confidence interval of the difference in SPRs was greater than 0%.).

Another secondary analysis compared the seroprotection rate at Week 24 for HEPLISAV-B with that at Week 28 for Engerix-B, by age group. For each age stratum non-inferiority of the seroprotection rate induced by HEPLISAV-B compared to Engerix-B was demonstrated (Table 7).

Age (years)	Table 7 Study 3: Seroprotection Rates of HEPLISAV-B and Engerix-B[a] (ages 18 - 70 years)				
	HEPLISAV-B[a]		Engerix-B[a]		Difference in SPRs (HEPLISAV-B minus Engerix-B)
	N	SPR (95% CI)	N	SPR (95% CI)	Difference (95% CI)
18-29	174	100.0% (97.9, 100.0)	99	93.9% (87.3, 97.7)	6.1% (2.8, 12.6)*
30-39	632	98.9% (97.7, 99.6)	326	92.0% (88.5, 94.7)	6.9% (4.2, 10.4)*
40-49	974	97.2% (96.0, 98.2)	518	84.2% (80.7, 87.2)	13.1% (9.9, 16.6)*
50-59	1439	95.2% (94.0, 96.3)	758	79.7% (76.6, 82.5)	15.5% (12.6, 18.7)*
60-70	1157	91.6% (89.9, 93.1)	588	72.6% (68.8, 76.2)	19.0% (15.2, 23.0)*

CI = confidence interval; N = number of subjects in the analysis population in the group; SPR = seroprotection rate (% with anti-HBs ≥ 10 mIU/mL).
[a] Week 24 for HEPLISAV-B and Week 28 for Engerix-B
Clinical trial number: NCT02117934
*Noninferiority was met because the lower bound of the 95% confidence interval of the difference in SPRs was greater than -10%. The SPR following HEPLISAV-B was statistically significantly higher than following Engerix-B (lower bound of the 95% confidence interval of the difference in SPRs was greater than 0%).

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

- Prefilled syringe, 1 dose (0.5 mL) - (NDC number: 43528-003-01)
- Package of 5 single dose prefilled syringes - (NDC number: 43528-003-05)

The tip caps and stoppers of the prefilled syringes are not made with natural rubber latex.

16.2 Storage Conditions

Store in a refrigerator at 2°C to 8°C (36°F to 46°F).
Do not freeze; discard if the vaccine has been frozen.
Do not use the vaccine after the expiration date shown on the prefilled syringe label.

17. PATIENT COUNSELING INFORMATION

- Inform vaccine recipient of the potential benefits and risks associated with vaccination, as well as the importance of completing the immunization series.
- Emphasize that HEPLISAV-B contains non-infectious purified HBsAg and cannot cause hepatitis B infection.
- Advise vaccine recipient to report any adverse events to their healthcare provider or to the Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967 and www.vaers.hhs.gov.
- Provide the Vaccine Information Statements, which are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

DYNAVAX

Manufactured by:
Dynavax Technologies Corporation
Emeryville, CA 94608 USA

US-19-02-00030

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PREHEVBRIO safely and effectively. See full prescribing information for PREHEVBRIO.

PREHEVBRIO [Hepatitis B Vaccine (Recombinant)]
Injectable suspension, for intramuscular use
Initial U.S. Approval: 2021

INDICATIONS AND USAGE

PREHEVBRIO is indicated for prevention of infection caused by all known subtypes of hepatitis B virus. PREHEVBRIO is approved for use in adults 18 years of age and older. (1)

DOSAGE AND ADMINISTRATION

For intramuscular injection.
Administer a series of three doses (1.0 mL each) of PREHEVBRIO on a 0-, 1- and 6-month schedule. (2.1, 2.2)

DOSAGE FORMS AND STRENGTHS

PREHEVBRIO is an injectable suspension, for intramuscular use supplied as a single-dose vial. A single dose of PREHEVBRIO is 1.0 mL (3)

CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis B vaccine or to any component of PREHEVBRIO. (4)

ADVERSE REACTIONS

Individuals 18 through 44 years of age: The most common local reactions following each dose of PREHEVBRIO were injection site pain (52.0 – 58.3%) and tenderness (52.6 – 59.6%). The most common systemic reactions following each dose of PREHEVBRIO were headache (17.2 – 25.8%), fatigue (20.1- 28.3%) and myalgia (22.2 – 29.9%).

Individuals 45 through 64 years of age: The most common local reactions following each dose of PREHEVBRIO were injection site pain (42.2 – 48.8%) and tenderness (43.2 – 50.5%). The most common systemic reactions following each dose of PREHEVBRIO were headache (13.8 – 21.3%), fatigue (14.3 – 19.7%) and myalgia (16.7 – 24.1%).

Individuals ≥ 65 years of age: The most common local reactions following each dose of PREHEVBRIO were injection site pain (26.7 – 34.8%) and tenderness (30.2 – 32.8%). The most common systemic reactions following each dose of PREHEVBRIO were headache (7.3 – 12.2%), fatigue (11.5 – 14.5%) and myalgia (11.5 - 16.6%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact VBI Vaccines at 1-888-421-8808 (toll-free) or VAERS at 1-800-822-7967 or www.vaers.hhs.gov .

See 17 for PATIENT COUNSELING INFORMATION

Revised: 11/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

***Sections or subsections omitted from the full prescribing information are not listed.**

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

PREHEVBRIO is indicated for prevention of infection caused by all known subtypes of hepatitis B virus.

PREHEVBRIO is approved for use in adults 18 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection.

2.1 Dosage and Schedule

Administer a series of three doses (1.0 mL each) of PREHEVBRIO on a 0-, 1- and 6-month schedule.

2.2 Administration

Shake the vial of PREHEVBRIO well to obtain a slightly opaque, white suspension.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Administer PREHEVBRIO by intramuscular injection.

3 DOSAGE FORMS AND STRENGTHS

PREHEVBRIO is an injectable suspension, for intramuscular use supplied as a single-dose vial. A single dose of PREHEVBRIO is 1.0 mL [see *How Supplied/Storage and Handling* (16.1)].

4 CONTRAINDICATIONS

Do not administer PREHEVBRIO to individuals with a history of severe allergic reaction (e.g., anaphylaxis) after a previous dose of any hepatitis B vaccine or to any component of PREHEVBRIO [see *Description* (11)].

5 WARNINGS AND PRECAUTIONS

5.1 Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of PREHEVBRIO.

5.2 Immunocompromised Individuals

Immunocompromised persons, including individuals receiving immunosuppressant therapy, may have a diminished immune response to PREHEVBRIO.

5.3 Limitations of Vaccine Effectiveness

Hepatitis B has a long incubation period. PREHEVBRIO may not prevent hepatitis B infection in individuals who have an unrecognized hepatitis B infection at the time of vaccine administration.

6 ADVERSE REACTIONS

Individuals 18 through 44 years of age: The most common local reactions following each dose of PREHEVBRIO were injection site pain (52.0 – 58.3%) and tenderness (52.6 – 59.6%). The most common systemic reactions following each dose of PREHEVBRIO were headache (17.2 – 25.8%), fatigue (20.1- 28.3%) and myalgia (22.2 – 29.9%).

Individuals 45 through 64 years of age: The most common local reactions following each dose of PREHEVBRIO were injection site pain (42.2 – 48.8%) and tenderness (43.2 – 50.5%). The most common systemic reactions following each dose of PREHEVBRIO were headache (13.8 – 21.3%), fatigue (14.3 – 19.7%) and myalgia (16.7 – 24.1%).

Individuals ≥ 65 years of age: The most common local reactions following each dose of PREHEVBRIO were injection site pain (26.7 – 34.8%) and tenderness (30.2 – 32.8%). The most common systemic reactions following each dose of PREHEVBRIO were headache (7.3 – 12.2%), fatigue (11.5 – 14.5%) and myalgia (11.5 - 16.6%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of PREHEVBRIO was evaluated in 2 active-controlled clinical studies (Studies 1 and 2) involving 4,443 subjects who received at least 1 dose of PREHEVBRIO (n = 2,920) or Engerix-B [Hepatitis B Vaccine (Recombinant)] (n = 1,523) administered according to a 0-, 1- and 6-months schedule.

Study 1 in adults ≥18 years of age

Study 1 was a randomized, double-blind, active-controlled, multicenter study that enrolled subjects in the United States (US), Canada, Belgium and Finland in which 796 subjects received at least 1 dose of PREHEVBRIO and 811 subjects received at least 1 dose of Engerix-B. In the total study population at baseline the mean age was 57 years, 81% were age ≥45 years; 62% were women; 90% were White, 8% Black, 1% Asian, and 10% Hispanic/Latino; 37% were obese (body mass index [BMI] >30 kg/m^2), 14% were current smokers and 8% had Type 2 diabetes mellitus. Demographic and baseline characteristics were similar in both vaccine groups.

Solicited Local and Systemic Adverse Reactions

Subjects were monitored for local and systemic adverse reactions using diary cards for a 7-day period starting on the day of vaccination. The percentages of subjects who reported local and systemic reactions in Study 1 are shown by age subgroup in Table 1 to Table 3.

Table 1: **Study 1: Percent of Subjects Who Reported Local or Systemic Reactions Within 7 Days of Vaccination (18 through 44 years of age)**

	PREHEVBRIO Dose 1 (N=145) %	PREHEVBRIO Dose 2 (N=141) %	PREHEVBRIO Dose 3 (N= 134) %	Engerix-B Dose 1 (N=154) %	Engerix-B Dose 2 (N=152) %	Engerix-B Dose 3 (N=148) %
Local Reaction						
Pain	58.6	50.4	46.3	33.8	28.9	31.8
Pain, Grade 3 or greater [a]	0	0	0	0	0	0
Tenderness	53.8	50.4	42.5	32.5	32.2	36.5
Tenderness, Grade 3 or greater [b]	0.7	0	0.7	0.6	0.7	0.7
Itching	2.1	3.5	6.0	7.1	3.9	7.4
Itching, Grade 3 or greater [c]	0	0	0	0	0.7	1.4
Redness (≥ 2.5 cm)	0.7	1.4	1.5	0.6	1.3	0
Redness, Grade 3 or greater [d]	0	0	0	0	0	0
Swelling (≥ 2.5 cm)	2.8	1.4	0.7	0	1.3	2.0
Swelling, Grade 3 or greater [e]	0	0	0	0	0.7	1.4
Systemic Reaction						
Headache	33.8	24.1	20.9	29.9	19.1	13.5
Headache, Grade 3 or greater [a]	1.4	0.7	0	1.3	0.7	0
Fatigue	29.7	22.0	22.4	31.8	20.4	20.3
Fatigue, Grade 3 or greater [c]	1.4	0.7	0	0.6	2.0	1.4
Myalgia	27.6	24.1	21.6	20.8	11.8	10.1
Myalgia, Grade 3 or greater [c]	0.7	0	0	0	1.3	0
Diarrhea	9.7	5.7	4.5	9.7	5.9	7.4
Diarrhea, Grade 3 or greater [f]	0.7	0	0	0	0.7	0
Nausea/Vomiting	8.3	4.3	4.5	7.8	6.6	6.1
Nausea/Vomiting, Grade 3 or greater [f]	0	0.7	0	0	0.7	0
Fever (≥100.4°F)	0.7	0.7	0	1.3	0	0.7
Fever, Grade 3 or greater (≥102.1°F)	0.7	0	0	0	0	0

[a] Grade 3 or greater pain and headache: defined as use of narcotic pain reliever or prevents daily activity; or ER visit or hospitalization

[b] Grade 3 or greater tenderness: defined as significant discomfort at rest; or ER visit or hospitalization

[c] Grade 3 or greater itching, fatigue and myalgia: defined as prevents daily activity; or ER visit or hospitalization

[d] Grade 3 or greater redness: defined as > 10 cm or skin necrosis or exfoliative dermatitis

[e] Grade 3 or greater swelling: defined as > 10 cm or prevents daily activity; or skin necrosis.

[f] Grade 3 or greater diarrhea and nausea/vomiting: defined as prevents daily activity or requires outpatient IV hydration; or ER visit or hospitalization.

Table 2: Study 1: Percent of Subjects Who Reported Local or Systemic Reactions Within 7 Days of Vaccination (45 through 64 years of age)

	PREHEVBRIO Dose 1 (N=355) %	PREHEVBRIO Dose 2 (N=350) %	PREHEVBRIO Dose 3 (N=343) %	Engerix-B Dose 1 (N=361) %	Engerix-B Dose 2 (N=357) %	Engerix-B Dose 3 (N=349) %
Local Reaction						
Pain	46.8	44.9	39.4	22.2	15.4	17.2
Pain, Grade 3 or greater [a]	0	0	0.3	0	0	0
Tenderness	48.7	42.6	40.5	23.8	16.5	17.5
Tenderness, Grade 3 or greater [b]	0.8	0.6	0.3	0	0	0.3
Itching	4.5	3.1	3.8	3.9	2.0	3.4
Itching, Grade 3 or greater [c]	0	0.3	0	0	0	0
Redness (≥ 2.5 cm)	1.7	0.6	0.3	1.1	0.3	1.1
Redness, Grade 3 or greater [d]	0	0	0	0.8	0.3	0.6
Swelling (≥ 2.5 cm)	1.4	0.3	0.9	0	0.6	0.3
Swelling, Grade 3 or greater [e]	0	0	0.3	0	0	0

	PREHEVBRIO Dose 1 (N=355) %	PREHEVBRIO Dose 2 (N=350) %	PREHEVBRIO Dose 3 (N=343) %	Engerix-B Dose 1 (N=361) %	Engerix-B Dose 2 (N=357) %	Engerix-B Dose 3 (N=349) %
Systemic Reaction						
Headache	21.4	13.7	15.7	20.5	11.2	14.0
Headache, Grade 3 or greater [a]	0	0	0.3	0.3	0.3	0.3
Fatigue	16.6	16.9	12.5	22.2	11.5	12.3
Fatigue, Grade 3 or greater [c]	0.6	0	0.3	0.6	0.3	0.6
Myalgia	21.4	20.0	15.5	16.1	8.4	9.5
Myalgia, Grade 3 or greater [c]	0.6	0	0	0	0	0
Diarrhea	4.8	4.0	3.2	6.4	3.6	3.7
Diarrhea, Grade 3 or greater [f]	0	0	0	0	0	0
Nausea/Vomiting	4.2	2.9	2.3	6.4	3.6	2.6
Nausea/Vomiting, Grade 3 or greater [f]	0	0	0	0	0	0
Fever (≥100.4°F)	0.6	0	0	0.3	0.3	0.6
Fever, Grade 3 or greater (≥102.1°F)	0	0	0	0	0	0.3

[a] Grade 3 or greater pain and headache: defined as use of narcotic pain reliever or prevents daily activity; or ER visit or hospitalization
[b] Grade 3 or greater tenderness: defined as significant discomfort at rest; or ER visit or hospitalization
[c] Grade 3 or greater itching, fatigue and myalgia: defined as prevents daily activity; or ER visit or hospitalization
[d] Grade 3 or greater redness: defined as > 10 cm or skin necrosis or exfoliative dermatitis
[e] Grade 3 or greater swelling: defined as > 10 cm or prevents daily activity; or skin necrosis.
[f] Grade 3 or greater diarrhea and nausea/vomiting: defined as prevents daily activity or requires outpatient IV hydration; or ER visit or hospitalization.

Table 3: Study 1: Percent of Subjects Who Reported Local or Systemic Reactions Within 7 Days of Vaccination (Age ≥ 65 years of age)

	PREHEVBRIO Dose 1 (N=296) %	PREHEVBRIO Dose 2 (N=288) %	PREHEVBRIO Dose 3 (N=281) %	Engerix-B Dose 1 (N=296) %	Engerix-B Dose 2 (N=292) %	Engerix-B Dose 3 (N= 288) %
Local Reaction						
Pain	34.8	28.8	26.7	16.2	12.0	11.1
Pain, Grade 3 or greater [a]	0	0	0	0.3	0	0
Tenderness	32.8	30.2	31.0	14.2	12.0	10.1
Tenderness, Grade 3 or greater [b]	0	0	0	0	0	0
Itching	6.1	3.8	5.0	4.1	1.4	2.4
Itching, Grade 3 or greater [c]	0	0	0	0	0	0
Redness (≥ 2.5 cm)	1.0	0.3	1.4	0.7	0.3	0
Redness, Grade 3 or greater [d]	0.3	0	0.4	0	0.3	0
Swelling (≥ 2.5 cm)	1.0	0.7	1.1	1.4	0.3	0.3
Swelling, Grade 3 or greater [e]	0.3	0	0	0	0.3	0
Systemic Reaction						
Headache	12.2	7.3	7.8	12.8	5.8	6.9
Headache, Grade 3 or greater [a]	0	0	0	0	0	0
Fatigue	14.5	11.5	12.5	17.9	9.9	10.1
Fatigue, Grade 3 or greater [c]	0	0	0	0.7	0	0.3
Myalgia	16.6	11.5	13.2	12.8	8.2	6.9
Myalgia, Grade 3 or greater [c]	0	0	0	0	0.3	0
Diarrhea	6.4	4.2	1.1	6.4	2.4	3.5
Diarrhea, Grade 3 or greater [f]	0.3	0	0	0.3	0	0
Nausea/Vomiting	3.7	0.7	1.1	1.7	1.7	0.7
Nausea/Vomiting, Grade 3 or greater [f]	0	0	0	0.3	0	0.3
Fever (≥100.4°F)	0	0	0.7	0	0	0.7
Fever, Grade 3 or greater (≥102.1°F)	0	0	0	0	0	0

[a] Grade 3 or greater pain and headache: defined as use of narcotic pain reliever or prevents daily activity; or ER visit or hospitalization
[b] Grade 3 or greater tenderness: defined as significant discomfort at rest; or ER visit or hospitalization
[c] Grade 3 or greater itching, fatigue and myalgia: defined as prevents daily activity; or ER visit or hospitalization
[d] Grade 3 or greater redness: defined as > 10 cm or skin necrosis or exfoliative dermatitis
[e] Grade 3 or greater swelling: defined as > 10 cm or prevents daily activity; or skin necrosis.
[f] Grade 3 or greater diarrhea and nausea/vomiting: defined as prevents daily activity or requires outpatient IV hydration; or ER visit or hospitalization.

The median duration of local and systemic solicited adverse reactions was 1-2 days in both treatment groups. Among all subjects who received PREHEVBRIO, the frequencies of the most commonly reported solicited reactions extending beyond the 7-day assessment period were as follows: fatigue (4.1%), injection site pain (2.0%), headache (1.9%) and myalgia (1.9%).

Study 2 in adults 18 through 45 years of age

Study 2 was a randomized, double-blind, active-controlled, multicenter study that enrolled subjects in the US, Canada, Belgium, Finland, Germany and the United Kingdom in which 2,124 subjects received at least 1 dose of PREHEVBRIO and 712 subjects received at least 1 dose of Engerix-B. In the total study population at baseline, the mean age was 34 years; 58% were women; 92% were White, 6% Black, 2% Asian, and 10% Hispanic/Latino; 18% were obese (BMI >30 kg/m^2) and 19% were current smokers. Demographic and baseline characteristics were similar in both vaccine groups.

Solicited Local and Systemic Adverse Reactions

Subjects were monitored for local and systemic adverse reactions using diary cards for a 7-day period starting on the day of vaccination. The percentages of subjects who reported local and systemic reactions in Study 2 are shown in Table 4.

Table 4: Study 2: Percent of Subjects Who Reported Local or Systemic Reactions Within 7 Days of Vaccination (18 through 45 years of age)

	PREHEVBRIO Dose 1 (N=2122) [a] %	PREHEVBRIO Dose 2 (N=2071) %	PREHEVBRIO Dose 3 (N=1967) %	Engerix-B Dose 1 (N=712) %	Engerix-B Dose 2 (N=701) %	Engerix-B Dose 3 (N=671) %
Local Reaction						
Pain	58.2	52.2	52.5	35.1	29.2	32.5
Pain, Grade 3 or greater [b]	0.3	0.3	0.4	0.1	0	0.3
Tenderness	59.9	52.9	55.5	37.6	30.4	33.8
Tenderness, Grade 3 or greater [c]	0.8	0.9	0.8	0.6	0.1	0.1
Itching	5.7	5.7	6.7	6.6	5.3	5.4
Itching, Grade 3 or greater [d]	0	0	0.1	0.3	0.1	0
Redness (≥ 2.5 cm)	1.1	1.1	1.3	0.6	0.4	1.0
Redness, Grade 3 or greater [e]	0.2	0	0.2	0.1	0.1	0.1
Swelling (≥ 2.5 cm)	1.2	0.9	1.1	0.6	0	0.4
Swelling, Grade 3 or greater [f]	0.1	0	0.1	0	0	0
Systemic Reaction						
Headache	25.1	16.7	17.4	24.2	15.0	18.3
Headache, Grade 3 or greater [b]	0.3	0.2	0.5	0.4	0.4	0.6
Fatigue	28.4	19.8	20.2	27.1	17.8	22.1
Fatigue, Grade 3 or greater [d]	0.5	0.8	0.6	0.4	0.6	0.6
Myalgia	30.3	21.9	23.6	17.7	13.0	18.5
Myalgia, Grade 3 or greater [d]	0.3	0.6	0.5	0.4	0.1	0.4
Diarrhea	7.4	5.0	4.4	9.6	4.9	5.4
Diarrhea, Grade 3 or greater [g]	0.2	0.1	0.1	0	0	0
Nausea/Vomiting	6.7	3.7	4.7	7.0	3.6	3.9
Nausea/Vomiting, Grade 3 or greater [g]	0	0	0.2	0	0.1	0
Fever (≥100.4°F)	0.3	0.3	0.6	0.4	0.3	0.9
Fever, Grade 3 or greater (≥102.1°F)	0	0.1	0.1	0.1	0	0

[a] Two subjects without solicited adverse event data following dose 1 of PREHEVBRIO were excluded from this analysis.
[b] Grade 3 or greater pain and headache: defined as use of narcotic pain reliever or prevents daily activity; or ER visit or hospitalization
[c] Grade 3 or greater tenderness: defined as significant discomfort at rest; or ER visit or hospitalization
[d] Grade 3 or greater itching, fatigue and myalgia: defined as prevents daily activity; or ER visit or hospitalization
[e] Grade 3 or greater redness: defined as > 10 cm or skin necrosis or exfoliative dermatitis
[f] Grade 3 or greater swelling: defined as > 10 cm or prevents daily activity; or skin necrosis
[g] Grade 3 or greater diarrhea and nausea/vomiting: defined as prevents daily activity or requires outpatient IV hydration; or ER visit or hospitalization.

The median duration of local and systemic solicited adverse reactions was 1-2 days in both treatment groups. Among all subjects who received PREHEVBRIO, the frequencies of the most commonly reported solicited reactions extending beyond the 7-day assessment period were as follows: fatigue (3.5%), injection site pain (2.0%), headache (1.9%) and myalgia (1.8%).

Unsolicited Adverse Events (AEs)

In both studies, unsolicited adverse events, including serious and non-serious events, that occurred within 28 days following each vaccination were recorded on a diary card by all subjects.

In both studies combined, unsolicited AEs that occurred within 28 days of any vaccination were reported by 48.3% and 48.4% of subjects who received PREHEVBRIO or Engerix-B, respectively. Unsolicited AEs in subjects who received PREHEVBRIO for which available information suggests a causal relationship to vaccination include injection site bruising (1.4%), dizziness/vertigo (1.1%), general pruritus/itchiness (0.2%), arthralgia (0.2%), urticaria/hives (0.2%) and lymphadenopathy/lymph node pain (0.1%).

Serious Adverse Events (SAEs)

In both studies, SAEs were collected from first vaccination through 6 months following the last vaccination. In both studies combined, SAEs were reported by 0.9% and 0.6% within 28 days of vaccination with PREHEVBRIO or Engerix-B, respectively. SAEs were reported by 2.5% of subjects in the PREHEVBRIO group and 1.6% in the Engerix-B group from the first vaccination through 6 months following the third vaccination. There were no notable patterns or numerical imbalances between vaccination groups for specific categories of serious adverse events that would suggest a causal relationship to PREHEVBRIO.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Immune Globulin

There are no data to assess the concomitant use of PREHEVBRIO with immune globulin. When concomitant administration of PREHEVBRIO and immune globulin is required, they should be given with different syringes at different injection sites.

7.2 Interference with Laboratory Tests

Hepatitis B surface antigen (HBsAg) derived from hepatitis B vaccines has been transiently detected in blood samples following vaccination. Serum HBsAg detection may not have diagnostic value within 28 days after receipt of PREHEVBRIO.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to PREHEVBRIO during pregnancy. Women who receive PREHEVBRIO during pregnancy are encouraged to contact 1-888-421-8808 (toll-free).

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In clinically recognized pregnancies in the US general population, the estimated background risk of major birth defects is 2% to 4% and of miscarriage is 15% to 20%.

There are no adequate and well-controlled studies of PREHEVBRIO in pregnant women. Available human data on PREHEVBRIO administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study has been performed in female rats administered the equivalent of a single human dose of PREHEVBRIO on four occasions; twice prior to mating, twice during gestation. The study revealed no evidence of harm to the fetus due to the vaccine [see Animal Data below].

Data

Animal Data

A developmental toxicity study has been performed in female rats using a dose equivalent to the adult human dose. In the study, female rats received 0.5 mL (2 x 0.25 mL injections) of a vaccine formulation containing 10 mcg HBsAg (S, pre-S1, pre-S2) adsorbed on to aluminum hydroxide by intramuscular injection 30 days and 15 days prior to mating and on gestation days 4 and 15. No adverse effects of pre-weaning development were observed. There was no evidence of fetal malformations or variations.

8.2 Lactation

Risk Summary

It is not known whether PREHEVBRIO is excreted in human milk. Data are not available to assess the effects of PREHEVBRIO on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for PREHEVBRIO and any potential adverse effects on the breastfed child from PREHEVBRIO or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of PREHEVBRIO have not been established in individuals less than 18 years of age.

8.5 Geriatric Use

Study 1 included 296 adults 65 through 86 years of age who received PREHEVBRIO. Among subjects who received PREHEVBRIO, a seroprotective level of antibody to HBsAg was achieved in 83.6% of those ≥ 65 years of age compared to 94.8% in adults 45 through 64 years of age and 99.2% in adults 18 through 44 years of age [see *Evaluation of Immunogenicity* (14.1)].

Frequencies of local and systemic solicited adverse reactions were generally lower in elderly subjects ≥65 years of age than in younger subjects [see *Adverse Reactions* (6)].

8.6 Adults on Hemodialysis

Safety and effectiveness of PREHEVBRIO have not been established in adults on hemodialysis.

11 DESCRIPTION

PREHEVBRIO [Hepatitis B Vaccine (Recombinant)] is a sterile suspension for intramuscular injection.

PREHEVBRIO contains the small (S), middle (pre-S2) and large (pre-S1) hepatitis B surface antigens, co-purified from genetically modified CHO (Chinese Hamster Ovary) cells cultured in growth medium containing vitamins, amino acids, minerals, and fetal bovine serum.

The hepatitis B surface antigens are co-purified from the supernatant of CHO cells by a series of physicochemical steps as virus-like particles containing CHO cell membrane lipids.

Each 1.0 mL dose is formulated to contain 10 mcg hepatitis B surface antigens (S, pre-S1 and pre-S2) adsorbed on aluminum hydroxide [$Al(OH)_3$] as an adjuvant (aluminum content of 0.5 mg/mL).

Each 1.0 mL dose of PREHEVBRIO also contains sodium chloride (NaCl) (8.45 mg/dose), potassium chloride (KCl) (0.02 mg/dose), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$) (0.38 mg/dose), potassium dihydrogen phosphate anhydrous (KH_2PO_4) (0.02 mg/dose) and water for injections (WFI). Each dose may contain residual amounts of CHO cell proteins (up to 2.5 ng/dose), CHO cell DNA (up to 10 pg/dose), Bovine Serum Albumin (up to 2.5 ng/dose) and Formaldehyde (up to 500 ng/dose) from the manufacturing process.

PREHEVBRIO does not contain a preservative.

The vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

PREHEVBRIO induces antibodies to HBsAg. Antibody concentrations ≥10 mIU/mL against HBsAg are recognized as conferring protection against hepatitis B virus infection.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

PREHEVBRIO has not been evaluated for carcinogenic, mutagenic potential or male infertility in animals. In a developmental toxicity study in rats with a vaccine formulation containing 10 mcg HBsAg (S, pre-S1, pre-S2) adsorbed on to aluminum hydroxide there were no effects on female fertility [see *Animal Data* (8.1)].

14 CLINICAL STUDIES

14.1 Evaluation of Immunogenicity

The immunogenicity of PREHEVBRIO was evaluated in comparison with a US-licensed hepatitis B vaccine (Engerix-B) in 2 randomized, active controlled, double-blind, multi-center Phase 3 clinical trials in adults. PREHEVBRIO and Engerix-B were administered according to a 0-, 1- and 6-month schedule. For subject baseline characteristics, see section 6.1.

The trials compared the seroprotection rates (SPR), defined as the proportion of participants with anti-HBs titers ≥ 10 mIU/mL, induced by PREHEVBRIO and Engerix-B. Non-inferiority was met if the lower bound of the 95% confidence interval (CI) of the difference in SPR (PREHEVBRIO minus Engerix-B) was greater than -5%.

Study 1 in adults ≥18 years of age

The immunogenicity population included 718 subjects who received PREHEVBRIO and 723 subjects who received Engerix-B. The mean age was 57 years in both groups. The primary analysis compared the SPR, 4 weeks after receiving the third dose of PREHEVBRIO or Engerix-B in subjects ≥ 18 years of age. The SPR induced by PREHEVBRIO compared to Engerix-B was non-inferior in subjects ≥ 18 years of age (Table 5).

Table 5: Study 1: Seroprotection Rate (SPR) 4 Weeks After Receiving the Third Dose of PREHEVBRIO or Engerix-B

Study Population	PREHEVBRIO N	PREHEVBRIO SPR (95% CI)	Engerix-B N	Engerix-B SPR (95% CI)	Difference in SPR; PREHEVBRIO – Engerix-B (95% CI)
All Adults (Age 18+) [a]	718	91.4 (89.1, 93.3)	723	76.5 (73.2, 79.5)	14.9 (11.2, 18.6) [c]
Age 45+ [b]	625	89.4 (86.8, 91.7)	627	73.1 (69.4, 76.5)	16.4 (12.2, 20.7) [d]
Age 18-44	125	99.2 (95.6, 100.0)	135	91.1 (85.0. 95.3)	- [e]
Age 45-64	325	94.8 (91.8, 96.6)	322	80.1 (75.3, 84.3)	- [e]
Age 65 +	268	83.6 (78.6, 87.8)	266	64.7 (58.6, 70.4)	- [e]

Abbreviations: N=number of subjects in the analysis set; SPR= Seroprotection Rate (percent of subjects with anti-HBs titers ≥10 mIU/mL)
[a] Per-protocol set (PPS). PPS included all subjects in the full analysis set who received all 3 vaccinations, had an evaluable serum immunogenicity sample at baseline and at the time point of interest, were seronegative at baseline, and had no major protocol violations leading to exclusion.
[b] Full analysis set (FAS). FAS included all subjects who received at least 1 vaccination and provided at least 1 evaluable serum immunogenicity sample both at baseline and after baseline. Subjects were seronegative at baseline.
[c] Non-inferiority was met because the lower bound of the 95% CI of the difference in SPR (PREHEVBRIO - Engerix-B) was > -5%.
[d] The SPR following PREHEVBRIO was statistically significantly higher than following Engerix-B (lower bound of the 95% CI of the difference in SPR was > 0%).
[e] Exploratory analysis

Study 2 in adults 18 through 45 years of age

The immunogenicity population included 1,753 subjects who received PREHEVBRIO and 592 subjects who received Engerix-B. The mean age was 34 years in the PREHEVBRIO group and 33 years in the Engerix-B group. The study compared the SPR, 4 weeks after receiving the third dose of PREHEVBRIO or Engerix-B in all subjects. The SPR induced by PREHEVBRIO compared to Engerix-B was non-inferior (Table 6).

Table 6: Study 2: Seroprotection Rate (SPR) 4 Weeks After Receiving the Third Dose of PREHEVBRIO or Engerix-B

Study Population	PREHEVBRIO N	PREHEVBRIO SPR (95% CI)	Engerix-B N	Engerix-B SPR (95% CI)	Difference in SPR; PREHEVBRIO –Engerix-B (95% CI)
Age 18-45	1753	99.3 (98.7. 99.6)	592	94.8 (92.7, 96.4)	4.5 (2.9, 6.6) *

SPR= Seroprotection Rate (percent of subjects with anti-HBs titers ≥10 mIU/mL)
*Non-inferiority was met because the lower bound of the 95% CI of the difference in SPR (PREHEVBRIO - Engerix-B) was > -5%.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Single dose vial, 1.0 mL (NDC number 75052-001-01)

Supplied as a package of 10 single dose vials (NDC number: 75052-001-10)

The vial stoppers are not made with natural rubber latex.

16.2 Storage Conditions

Store in a refrigerator at 2°C to 8°C (36°F to 46°F). Protect from light.

Do not freeze; discard if the vaccine has been frozen.

Do not use the vaccine after the expiration date shown on the vial label.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipient of the potential benefits and risks associated with vaccination with PREHEVBRIO, as well as the importance of completing the immunization series.
- Emphasize that PREHEVBRIO contains non-infectious purified HBsAg and cannot cause hepatitis B infection.
- Advise vaccine recipient to report any adverse events to their healthcare provider or to the Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967 and www.vaers.hhs.gov.
- Provide the Vaccine Information Statements, which are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use RECOMBIVAX HB safely and effectively. See full prescribing information for RECOMBIVAX HB.

RECOMBIVAX HB® Hepatitis B Vaccine (Recombinant)
Suspension for intramuscular injection
Initial U.S. Approval: 1986

----------INDICATIONS AND USAGE----------
RECOMBIVAX HB is a vaccine indicated for prevention of infection caused by all known subtypes of hepatitis B virus. RECOMBIVAX HB is approved for use in individuals of all ages. RECOMBIVAX HB Dialysis Formulation is approved for use in predialysis and dialysis patients 18 years of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------
RECOMBIVAX HB
- Persons from birth through 19 years of age: A series of 3 doses (0.5 mL each) given on a 0-, 1-, and 6-month schedule. (2.1)
- Adolescents 11 through 15 years of age: A series of either 3 doses (0.5 mL each) given on a 0-, 1-, and 6-month schedule or a series of 2 doses (1.0 mL) on a 0- and 4- to 6-month schedule). (2.1)
- Persons 20 years of age and older: A series of 3 doses (1.0 mL each) given on a 0-, 1-, and 6-month schedule. (2.1)

RECOMBIVAX HB Dialysis Formulation
- Adults on predialysis or dialysis: A series of 3 doses (1.0 mL each) given on a 0-, 1-, and 6-month schedule. (2.1)

----------DOSAGE FORMS AND STRENGTHS----------
RECOMBIVAX HB is a sterile suspension available in the following presentations:
- 0.5 mL (5 mcg) Pediatric/Adolescent Formulation single-dose vials and prefilled syringes (3, 11, 16.1)
- 1 mL (10 mcg) Adult Formulation single-dose vials and prefilled syringes (3, 11, 16.1)

RECOMBIVAX HB Dialysis Formulation is a sterile suspension available in the following presentation:
- 1 mL (40 mcg) single-dose vials (3, 11, 16.1)

----------CONTRAINDICATIONS----------
Severe allergic or hypersensitivity reactions (e.g., anaphylaxis) after a previous dose of any hepatitis B-containing vaccine, or to any component of RECOMBIVAX HB, including yeast. (4, 11)

---------- WARNINGS AND PRECAUTIONS ----------
The vial stopper, the syringe plunger stopper, and tip cap contain dry natural latex rubber which may cause allergic reactions in latex-sensitive individuals. (5.1)

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including RECOMBIVAX HB, to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.2)

---------- ADVERSE REACTIONS ----------
In healthy infants and children (up to 10 years of age), the most frequently reported systemic adverse reactions (>1% injections), in decreasing order of frequency, were irritability, fever, diarrhea, fatigue/weakness, diminished appetite, and rhinitis. (6.1)
In healthy adults, injection site reactions and systemic adverse reactions were reported following 17% and 15% of the injections, respectively. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------DRUG INTERACTIONS----------
Do not mix RECOMBIVAX HB with any other vaccine in the same syringe or vial. (7.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 02/2020

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

RECOMBIVAX HB® [Hepatitis B Vaccine, Recombinant] is indicated for prevention of infection caused by all known subtypes of hepatitis B virus. RECOMBIVAX HB is approved for use in individuals of all ages. RECOMBIVAX HB Dialysis Formulation is approved for use in adult predialysis and dialysis patients 18 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular administration. See Section 2.2 for subcutaneous administration in persons with hemophilia.

RECOMBIVAX HB should be administered as soon as possible after being removed from refrigeration *[see How Supplied/Storage and Handling (16)]*.

2.1 Dosage and Schedule

RECOMBIVAX HB:

Persons from birth through 19 years of age: A series of 3 doses (0.5 mL each) given on a 0-, 1-, and 6-month schedule.

Adolescents 11 through 15 years of age: A series of 3 doses (0.5 mL each) given on a 0-, 1-, and 6-month schedule or a series of 2 doses (1.0 mL each) on a 0- and 4- to 6-month schedule.

Persons 20 years of age and older: A series of 3 doses (1.0 mL each) given on a 0-, 1-, and 6-month schedule.

RECOMBIVAX HB Dialysis Formulation:

Adults on predialysis and dialysis: A series of 3 doses (1.0 mL each) given on a 0-, 1-, and 6-month schedule.

Table 1 summarizes the dose and formulation of RECOMBIVAX HB for specific populations, regardless of the risk of infection with hepatitis B virus.

Table 1: RECOMBIVAX HB Recommended Dose and Administration Schedules

Group	Dose/Regimen
Infants*, Children and Adolescents 0-19 years of age (Pediatric/Adolescent Formulation)	5 mcg (0.5 mL) 3 doses at 0, 1, and 6 months
Adolescents† 11 through 15 years of age (Adult Formulation)	10 mcg‡ (1.0 mL) 2 doses at 0 and 4-6 months
Adults ≥20 years of age (Adult Formulation)	10 mcg‡ (1.0 mL) 3 doses at 0, 1, and 6 months
Predialysis and Dialysis Patients§ (Dialysis Formulation)	40 mcg (1.0 mL) 3 doses at 0, 1, and 6 months

* For specific recommendations for infants see ACIP recommendations.{1}

† Adolescents (11 through 15 years of age) may receive either regimen: 3 x 5 mcg (Pediatric Formulation) or 2 x 10 mcg (Adult Formulation).

‡ If the suggested dose (10 mcg) is not available, the appropriate dosage can be achieved with two 5 mcg doses. However, the Dialysis Formulation may be used only for adult predialysis/dialysis patients.

§ See also recommendations for revaccination of predialysis and dialysis patients in *[Dosage and Administration (2.4)]*.

2.2 Preparation and Administration

Shake the single-dose vial or single-dose prefilled syringe well to obtain a slightly opaque, white suspension before withdrawal and use. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Discard if the suspension does not appear homogeneous or if extraneous particulate matter remains or if discoloration is observed.

For single-dose vials, withdraw and administer entire dose of RECOMBIVAX HB intramuscularly using a sterile needle and syringe. Discard vial after use.

For single-dose prefilled syringes, securely attach a needle by twisting in a clockwise direction and administer dose of RECOMBIVAX HB intramuscularly. Discard syringe after use.

The deltoid muscle is the preferred site for intramuscular injection for adults, adolescents and children 1 year of age and older whose deltoid is large enough for intramuscular injection. The anterolateral aspect of the thigh is the preferred site for intramuscular injection for infants younger than 1

year of age. RECOMBIVAX HB should not be administered in the gluteal region, as injections given in the buttocks have resulted in lower seroconversion rates than expected.{2}

RECOMBIVAX HB may be administered subcutaneously to persons at risk for hemorrhage following intramuscular injections (e.g., hemophiliacs). However, hepatitis B vaccines are known to result in lower antibody response when administered subcutaneously.{3} Additionally, when other aluminum-adsorbed vaccines have been administered subcutaneously, an increased incidence of local reactions including subcutaneous nodules has been observed. Therefore, consider subcutaneous administration only in persons who are at risk of hemorrhage following intramuscular injections.

Do not administer intravenously or intradermally.

2.3 Known or Presumed Exposure to Hepatitis B Virus

Known or Presumed Exposure to HBsAg

Refer to recommendations of the Advisory Committee on Immunization Practices (ACIP) and to the package insert for hepatitis B immune globulin (HBIG) for management of persons with known or presumed exposure to the hepatitis B virus (e.g., neonates born of infected mothers or persons who experienced percutaneous or permucosal exposure to the virus). When recommended, administer RECOMBIVAX HB and HBIG intramuscularly at separate sites (e.g., opposite anterolateral thighs for exposed neonates) as soon as possible after exposure. Administer additional doses of RECOMBIVAX HB (to complete a vaccination series) in accordance with ACIP recommendations.

2.4 Booster Vaccinations

The duration of the protective effect of RECOMBIVAX HB in healthy vaccinees is unknown at present and the need for booster doses is not yet defined. The ACIP provides recommendations for use of a booster dose or revaccination series in previously vaccinated individuals with known or presumed exposure to Hepatitis B Virus.

Consider a booster dose or revaccination with RECOMBIVAX HB Dialysis Formulation (blue color code) in predialysis/dialysis patients if the anti-HBs level is less than 10 mIU/mL at 1 to 2 months after the third dose. Assess the need for a booster dose annually by antibody testing, and give a booster dose when the anti-HBs level declines to less than 10 mIU/mL.{3}

3 DOSAGE FORMS AND STRENGTHS

RECOMBIVAX HB is a sterile suspension available in the following presentations:

- 0.5 mL (5 mcg) Pediatric/Adolescent Formulation single-dose vials and prefilled syringes
- 1 mL (10 mcg) Adult Formulation single-dose vials and prefilled syringes

RECOMBIVAX HB DIALYSIS FORMULATION is a sterile suspension available in the following presentation:

- 1 mL (40 mcg) single-dose vial *[see Description (11) and How Supplied/Storage and Handling (16)]*

4 CONTRAINDICATIONS

Do not administer RECOMBIVAX HB to individuals with a history of severe allergic or hypersensitivity reactions (*e.g.*, anaphylaxis) after a previous dose of any hepatitis B-containing vaccine or to any component of RECOMBIVAX HB, including yeast *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity to Latex

The vial stopper and the syringe plunger stopper and tip cap contain dry natural latex rubber, which may cause allergic reactions in latex-sensitive individuals.

5.2 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including RECOMBIVAX HB, to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. For RECOMBIVAX HB, this assessment should include consideration of the mother's hepatitis B antigen status and the high probability of maternal

transmission of hepatitis B virus to infants born to mothers who are HBsAg positive if vaccination is delayed.

5.3 Infants Weighing Less Than 2000 g

Hepatitis B vaccination should be delayed until 1 month of age or hospital discharge in infants weighing <2000 g if the mother is documented to be HBsAg negative at the time of the infant's birth. Infants weighing <2000 g born to HBsAg positive or HBsAg unknown mothers should receive vaccine and hepatitis B immune globulin (HBIG) in accordance with ACIP recommendations if HBsAg status cannot be determined{3} *[see Dosage and Administration (2)].*

5.4 Prevention and Management of Allergic Vaccine Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration *[see Contraindications (4)].*

5.5 Limitations of Vaccine Effectiveness

Hepatitis B virus has a long incubation period. RECOMBIVAX HB may not prevent hepatitis B infection in individuals who have an unrecognized hepatitis B infection at the time of vaccination. Additionally, vaccination with RECOMBIVAX HB may not protect all individuals.

6 ADVERSE REACTIONS

In healthy infants and children (up to 10 years of age), the most frequently reported systemic adverse reactions (>1% injections), in decreasing order of frequency, were irritability, fever, diarrhea, fatigue/weakness, diminished appetite, and rhinitis. In healthy adults, injection site reactions and systemic adverse reactions were reported following 17% and 15% of the injections, respectively.

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

In three clinical studies, 434 doses of RECOMBIVAX HB, 5 mcg, were administered to 147 healthy infants and children (up to 10 years of age) who were monitored for 5 days after each dose. Injection site reactions and systemic adverse reactions were reported following 0.2% and 10.4% of the injections, respectively. The most frequently reported systemic adverse reactions (>1% injections), in decreasing order of frequency, were irritability, fever (≥101°F oral equivalent), diarrhea, fatigue/weakness, diminished appetite, and rhinitis.

In a study that compared the three-dose regimen (5 mcg) with the two-dose regimen (10 mcg) of RECOMBIVAX HB in adolescents, the overall frequency of adverse reactions was generally similar.

In a group of studies, 3258 doses of RECOMBIVAX HB, 10 mcg, were administered to 1252 healthy adults who were monitored for 5 days after each dose. Injection site reactions and systemic adverse reactions were reported following 17% and 15% of the injections, respectively. The following adverse reactions were reported:

Incidence Equal To or Greater Than 1% of Injections

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS

Injection site reactions consisting principally of soreness, and including pain, tenderness, pruritus, erythema, ecchymosis, swelling, warmth, nodule formation.

The most frequent systemic complaints include fatigue/weakness; headache; fever (≥100°F); malaise.

GASTROINTESTINAL DISORDERS

Nausea; diarrhea

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS

Pharyngitis; upper respiratory infection

Incidence Less Than 1% of Injections

GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS

Sweating; achiness; sensation of warmth; lightheadedness; chills; flushing

GASTROINTESTINAL DISORDERS

Vomiting; abdominal pains/cramps; dyspepsia; diminished appetite

RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS

Rhinitis; influenza; cough

NERVOUS SYSTEM DISORDERS

Vertigo/dizziness; paresthesia

SKIN AND SUBCUTANEOUS TISSUE DISORDERS
Pruritus; rash (non-specified); angioedema; urticaria
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS
Arthralgia including monoarticular; myalgia; back pain; neck pain; shoulder pain; neck stiffness
BLOOD AND LYMPHATIC DISORDERS
Lymphadenopathy
PSYCHIATRIC DISORDERS
Insomnia/disturbed sleep
EAR AND LABYRINTH DISORDERS
Earache
RENAL AND URINARY DISORDERS
Dysuria
CARDIAC DISORDERS
Hypotension

6.2 Post-Marketing Experience

The following additional adverse reactions have been reported with use of the marketed vaccine. Because these reactions are reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to a vaccine exposure.

Immune System Disorders
Hypersensitivity reactions including anaphylactic/anaphylactoid reactions, bronchospasm, and urticaria have been reported within the first few hours after vaccination. An apparent hypersensitivity syndrome (serum-sickness-like) of delayed onset has been reported days to weeks after vaccination, including: arthralgia/arthritis (usually transient), fever, and dermatologic reactions such as urticaria, erythema multiforme, ecchymoses and erythema nodosum *[see Warnings and Precautions (5.1)]*. Autoimmune diseases including systemic lupus erythematosus (SLE), lupus-like syndrome, vasculitis, and polyarteritis nodosa have also been reported.
Gastrointestinal Disorders
Elevation of liver enzymes; constipation
Nervous System Disorders
Guillain-Barré syndrome; multiple sclerosis; exacerbation of multiple sclerosis; myelitis including transverse myelitis; seizure; febrile seizure; peripheral neuropathy including Bell's Palsy; radiculopathy; herpes zoster; migraine; muscle weakness; hypesthesia; encephalitis
Skin and Subcutaneous Disorders
Stevens-Johnson syndrome; alopecia; petechiae; eczema
Musculoskeletal and Connective Tissue Disorders
Arthritis
Pain in extremity
Blood and Lymphatic System Disorders
Increased erythrocyte sedimentation rate; thrombocytopenia
Psychiatric Disorders
Irritability; agitation; somnolence
Eye Disorders
Optic neuritis; tinnitus; conjunctivitis; visual disturbances; uveitis
Cardiac Disorders
Syncope; tachycardia

The following adverse reaction has been reported with another Hepatitis B Vaccine (Recombinant) but not with RECOMBIVAX HB: keratitis.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

Do not mix RECOMBIVAX HB with any other vaccine in the same syringe or vial. Use separate injection sites and syringes for each vaccine.

In clinical trials in children, RECOMBIVAX HB was concomitantly administered with one or more of the following US licensed vaccines: Diphtheria, Tetanus and whole cell Pertussis; oral Poliomyelitis vaccine;

Measles, Mumps, and Rubella Virus Vaccine, Live; Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)] or a booster dose of Diphtheria, Tetanus, acellular Pertussis. Safety and immunogenicity were similar for concomitantly administered vaccines compared to separately administered vaccines.

In another clinical trial, a related HBsAg-containing product, Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) combination product (no longer licensed), was given concomitantly with eIPV (enhanced inactivated Poliovirus vaccine) or VARIVAX® [Varicella Virus Vaccine Live (Oka/Merck)], using separate sites and syringes for injectable vaccines. No serious vaccine-related adverse events were reported, and no impairment of immune response to these individually tested vaccine antigens was demonstrated.

The Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) combination product (no longer licensed) has also been administered concomitantly with the primary series of DTaP to a limited number of infants. No serious vaccine-related adverse events were reported.

7.2 Concomitant Administration with Immune Globulin

RECOMBIVAX HB may be administered concomitantly with HBIG. The first dose of RECOMBIVAX HB may be given at the same time as HBIG, but the injections should be administered at different sites.

7.3 Interference with Laboratory Tests

Hepatitis B surface antigen (HBsAg) derived from hepatitis B vaccines has been transiently detected in blood samples following vaccination. Serum HBsAg detection may not have diagnostic value within 28 days after receipt of a hepatitis B vaccine, including RECOMBIVAX HB.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4%, and 15% to 20%, respectively.

There are no adequate and well-controlled studies designed to evaluate RECOMBIVAX HB in pregnant women. Available post-approval data do not suggest an increased risk of miscarriage or major birth defects in women who received RECOMBIVAX HB during pregnancy.

Developmental toxicity studies have not been conducted with the vaccine in animals.

Data

Human Data

In post-licensure clinical studies of RECOMBIVAX HB, 26 pregnant women were inadvertently administered RECOMBIVAX HB following their last menstrual period. Among these pregnancies, after excluding elective terminations (n=3), there were 23 pregnancies with known outcomes all with exposure in the first trimester. Miscarriage was reported in 4 of 23 (17%) pregnancies and major birth defects were reported in 0 of 19 (0%) live births. The rates of miscarriage and major birth defects were consistent with estimated background rates.

Post-approval adverse reactions are reported voluntarily from a population of uncertain size. It is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

In prospectively reported spontaneous post-approval reports from 1986 to 2018, 105 women with known pregnancy outcomes were exposed to RECOMBIVAX HB during pregnancy following the last menstrual period. After excluding induced abortions (n=5), those with exposure in the third trimester (n=4), and those with an unknown exposure timing (n=6), there were 90 pregnancies with known outcomes with exposures in the first or second trimester. Miscarriage was reported for 7 of 90 (7.8%) pregnancies. Major birth defects were reported for 2 of 83 (2.4%) live born infants. The rates of miscarriage and major birth defects were consistent with estimated background rates.

8.2 Lactation

Risk Summary

It is not known whether RECOMBIVAX HB is excreted in human milk. Data are not available to assess the effects of RECOMBIVAX HB on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for RECOMBIVAX HB and any potential adverse effects on the breastfed child from

RECOMBIVAX HB or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to the disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of RECOMBIVAX HB have been established in all pediatric age groups. Maternally transferred antibodies do not interfere with the active immune response to the vaccine. *[See Adverse Reactions (6.1) and Clinical Studies (14.1 and 14.2).]* The safety and effectiveness of RECOMBIVAX HB Dialysis Formulation in children have not been established.

8.5 Geriatric Use

Clinical studies of RECOMBIVAX HB used for licensure did not include sufficient numbers of subjects 65 years of age and older to determine whether they respond differently from younger subjects. However, in later studies it has been shown that a diminished antibody response can be expected in persons older than 60 years of age.

11 DESCRIPTION

RECOMBIVAX HB Hepatitis B Vaccine (Recombinant) is a sterile suspension of non-infectious subunit viral vaccine derived from HBsAg produced in yeast cells. A portion of the hepatitis B virus gene, coding for HBsAg, is cloned into yeast, and the vaccine for hepatitis B is produced from cultures of this recombinant yeast strain according to methods developed in the Merck Research Laboratories.

The antigen is harvested and purified from fermentation cultures of a recombinant strain of the yeast *Saccharomyces cerevisiae* containing the gene for the *adw* subtype of HBsAg. The fermentation process involves growth of *Saccharomyces cerevisiae* on a complex fermentation medium which consists of an extract of yeast, soy peptone, dextrose, amino acids and mineral salts. The HBsAg protein is released from the yeast cells by cell disruption and purified by a series of physical and chemical methods. The purified protein is treated in phosphate buffer with formaldehyde and then coprecipitated with alum (potassium aluminum sulfate) to form bulk vaccine adjuvanted with amorphous aluminum hydroxyphosphate sulfate. Each dose contains less than 1% yeast protein. The vaccine produced by the Merck method has been shown to be comparable to the plasma-derived vaccine in terms of animal potency (mouse, monkey, and chimpanzee) and protective efficacy (chimpanzee and human).

The vaccine against hepatitis B, prepared from recombinant yeast cultures, is free of association with human blood or blood products.

RECOMBIVAX HB Hepatitis B Vaccine (Recombinant) is supplied in three formulations. *[See How Supplied/Storage and Handling (16).]*

Pediatric/Adolescent Formulation (Without Preservative), 10 mcg/mL: each 0.5 mL dose contains 5 mcg of hepatitis B surface antigen.

Adult Formulation (Without Preservative), 10 mcg/mL: each 1 mL dose contains 10 mcg of hepatitis B surface antigen.

Dialysis Formulation (Without Preservative), 40 mcg/mL: each 1 mL dose contains 40 mcg of hepatitis B surface antigen.

All formulations contain approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate, previously referred to as aluminum hydroxide) per mL of vaccine. In each formulation, hepatitis B surface antigen is adsorbed onto approximately 0.5 mg of aluminum (provided as amorphous aluminum hydroxyphosphate sulfate) per mL of vaccine. The vaccine contains <15 mcg/mL residual formaldehyde. The vaccine is of the *adw* subtype.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

RECOMBIVAX HB has been shown to elicit antibodies to hepatitis B virus as measured by ELISA.

Antibody concentrations ≥10mIU/mL against HBsAg are recognized as conferring protection against hepatitis B infection.{2}

Infection with hepatitis B virus can have serious consequences including acute massive hepatic necrosis and chronic active hepatitis. Chronically infected persons are at increased risk for cirrhosis and hepatocellular carcinoma.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

RECOMBIVAX HB has not been evaluated for its carcinogenic or mutagenic potential, or its potential to impair fertility *[see Use in Specific Populations (8)]*.

14 CLINICAL STUDIES

14.1 Efficacy in Neonates with Peripartum Exposure to Hepatitis B

The protective efficacy of three 5 mcg doses of RECOMBIVAX HB has been demonstrated in neonates born of mothers positive for both HBsAg and HBeAg (a core-associated antigenic complex which correlates with high infectivity). In a clinical study of infants who received one dose of HBIG at birth followed by the recommended three-dose regimen of RECOMBIVAX HB, chronic infection had not occurred in 96% of 130 infants after nine months of follow-up.{4} The estimated efficacy in prevention of chronic hepatitis B infection was 95% as compared to the infection rate in untreated historical controls.{5} Significantly fewer neonates became chronically infected when given one dose of HBIG at birth followed by the recommended three-dose regimen of RECOMBIVAX HB when compared to historical controls who received only a single dose of HBIG.{6} As demonstrated in the above study, HBIG, when administered simultaneously with RECOMBIVAX HB at separate body sites, did not interfere with the induction of protective antibodies against hepatitis B virus elicited by the vaccine.{6}

14.2 Immunogenicity of a Three-Dose Regimen in Healthy Infants, Children, and Adolescents

Three 5 mcg doses of RECOMBIVAX HB induced a protective level of antibody in 100% of 92 infants, 99% of 129 children, and in 99% of 112 adolescents *[see Dosage and Administration (2.3)].*

14.3 Immunogenicity of a Two-Dose Regimen in Healthy Adolescents 11 through 15 Years of Age

For adolescents (11 through 15 years of age), the immunogenicity of a two-dose regimen (10 mcg at 0 and 4-6 months) was compared with that of the standard three-dose regimen (5 mcg at 0, 1, and 6 months) in an open, randomized, multicenter study. The proportion of adolescents receiving the two-dose regimen who developed a protective level of antibody one month after the last dose (99% of 255 subjects) appears similar to that among adolescents who received the three-dose regimen (98% of 121 subjects). After adolescents (11 through 15 years of age) received the first 10-mcg dose of the two-dose regimen, the proportion who developed a protective level of antibody was approximately 72%.

14.4 Immunogenicity in Healthy Adults

Clinical studies have shown that RECOMBIVAX HB when injected into the deltoid muscle induced protective levels of antibody in 96% of 1213 healthy adults who received the recommended three-dose regimen. Antibody responses varied with age; a protective level of antibody was induced in 98% of 787 young adults 20-29 years of age, 94% of 249 adults 30-39 years of age and in 89% of 177 adults ≥40 years of age.

14.5 Efficacy and Immunogenicity in Specific Populations

Chronic Hepatitis C Infection

In one published study, the seroprotection rates in individuals with chronic hepatitis C virus (HCV) infection given the standard regimen of RECOMBIVAX HB was approximately 70%.{7} In a second published study of intravenous drug users given an accelerated schedule of RECOMBIVAX HB, infection with HCV did not affect the response to RECOMBIVAX HB.{8}

Predialysis and Dialysis Adult Patients

Predialysis and dialysis adult patients respond less well to hepatitis B vaccines than do healthy individuals; however, vaccination of adult patients early in the course of their renal disease produces higher seroconversion rates than vaccination after dialysis has been initiated.{9} In addition, the responses to these vaccines may be lower if the vaccine is administered as a buttock injection. When 40 mcg of Hepatitis B Vaccine (Recombinant), was administered in the deltoid muscle, 89% of 28 participants developed anti-HBs with 86% achieving levels ≥10 mIU/mL. However, when the same dosage of this vaccine was administered inappropriately either in the buttock or a combination of buttock and deltoid, 62% of 47 participants developed anti-HBs with 55% achieving levels of ≥10 mIU/mL.

15 REFERENCES

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Persons with Discrete Identifiable Exposures to Hepatitis B Virus (HBV) and http://www.cdc.gov/hepatitis/hbv/pdfs/correctedtable4.pdf

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9. Recommendations of the Advisory Committee on Immunization Practices (ACIP): Hepatitis B Virus Infection: A Comprehensive Strategy to Eliminate Transmission in the United States, 1996 update, MMWR (draft January 13, 1996).

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

RECOMBIVAX HB (pediatric and adult) FORMULATION is available in single-dose vials and prefilled Luer-Lok® syringes.

RECOMBIVAX HB DIALYSIS FORMULATION is available in single-dose vials.

Pediatric/Adolescent Formulation (PRESERVATIVE FREE)

0.5 mL (5 mcg) in single-dose vials and prefilled Luer-Lok® syringes

NDC 0006-4981-00 – box of ten 0.5-mL single-dose vials

Color coded with a yellow cap and stripe on the vial labels and cartons and an orange banner on the vial labels and cartons

NDC 0006-4093-02 – carton of 10 prefilled single-dose Luer-Lok® syringes with tip caps

Color coded with a yellow plunger rod

Adult Formulation *(PRESERVATIVE FREE)*

1 mL (10mcg) in single-dose vials and prefilled Luer-Lok® syringes

NDC 0006-4995-00 – 1-mL single dose vial

Color coded with a green cap and stripe

NDC 0006-4995-41 – box of ten 1-mL single-dose vials

Color coded with a green cap and stripe

NDC 0006-4094-02 – carton of 10 pre-filled single-dose syringes with tip caps

Color coded with a green plunger rod

RECOMBIVAX HB DIALYSIS FORMULATION

1 mL (40mcg) in single-dose vials

NDC 0006-4992-00 – 1-mL single-dose vial

Color coded with a blue cap and stripe

16.2 Storage and Handling

- Protect from light.
- Store vials and syringes at 2-8°C (36-46°F).
- *Do not freeze since freezing destroys potency*.
- RECOMBIVAX HB is stable at temperatures from 0° to 25° C (32° to 77°F) for 72 hours. These data are not recommendations for shipping or storage but may guide decisions for use in case of temporary temperature excursions.

17 PATIENT COUNSELING INFORMATION

Information for Vaccine Recipients and Parents/Guardians

- Inform the patient, parent or guardian of the potential benefits and risks associated with vaccination, as well as the importance of completing the immunization series.
- Question the vaccine recipient, parent or guardian about the occurrence of any symptoms and/or signs of adverse reaction after a previous dose of hepatitis B vaccine.
- Tell the patient, parent or guardian to report adverse events to the physician or clinic where the vaccine was administered.
- Prior to vaccination, give the patient, parent or guardian the Vaccine Information Statements which are required by the National Vaccine Injury Act of 1986. The materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).
- Tell the patient, parent or guardian that the United States Department of Health and Human Services has established a Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine, including but not limited to the reporting of events by the National Childhood Vaccine Injury Act of 1986. The VAERS toll-free number is 1-800-822-7967. Reporting forms may also be obtained at the VAERS website at (www.vaers.hhs.gov).

Manuf. and Dist. by: Merck Sharp & Dohme Corp., a subsidiary of **MERCK & CO., INC.,** Whitehouse Station, NJ 08889, USA

For patent information: www.merck.com/product/patent/home.html

uspi-v232-i-2002r442

Human Papillomavirus (HPV)

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use GARDASIL 9 safely and effectively. See full prescribing information for GARDASIL 9.

GARDASIL®9
(Human Papillomavirus 9-valent Vaccine, Recombinant)
Suspension for intramuscular injection
Initial U.S. Approval: 2014

----------INDICATIONS AND USAGE----------

GARDASIL 9 is a vaccine indicated in girls and women 9 through 45 years of age for the prevention of the following diseases:

- Cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers caused by Human Papillomavirus (HPV) types 16, 18, 31, 33, 45, 52, and 58. (1.1)
- Genital warts (condyloma acuminata) caused by HPV types 6 and 11. (1.1)

And the following precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58:

- Cervical intraepithelial neoplasia (CIN) grade 2/3 and cervical adenocarcinoma *in situ* (AIS). (1.1)
- Cervical intraepithelial neoplasia (CIN) grade 1. (1.1)
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3. (1.1)
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3. (1.1)
- Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3. (1.1)

GARDASIL 9 is indicated in boys and men 9 through 45 years of age for the prevention of the following diseases:

- Anal, oropharyngeal and other head and neck cancers caused by HPV types 16, 18, 31, 33, 45, 52, and 58. (1.2)
- Genital warts (condyloma acuminata) caused by HPV types 6 and 11. (1.2)

And the following precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58:

- Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3. (1.2)

The oropharyngeal and head and neck cancer indication is approved under accelerated approval based on effectiveness in preventing HPV-related anogenital disease. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial (1).

Limitations of Use and Effectiveness:

- Vaccination with GARDASIL 9 does not eliminate the necessity for vaccine recipients to undergo screening for cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers as recommended by a health care provider. (1.3, 17)
- GARDASIL 9 has not been demonstrated to provide protection against disease caused by:
  - HPV types not covered by the vaccine
  - HPV types to which a person has previously been exposed through sexual activity. (1.3)
- Not all vulvar, vaginal, anal, oropharyngeal and other head and neck cancers are caused by HPV, and GARDASIL 9 protects only against those vulvar, vaginal, anal, oropharyngeal and other head and neck cancers caused by HPV 16, 18, 31, 33, 45, 52, and 58. (1.3)
- GARDASIL 9 is not a treatment for external genital lesions; cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers; CIN; VIN; VaIN; or AIN. (1.3)
- Vaccination with GARDASIL 9 may not result in protection in all vaccine recipients. (1.3)

---------- DOSAGE AND ADMINISTRATION ----------

For intramuscular administration only. (2)
Each dose of GARDASIL 9 is 0.5-mL
Administer GARDASIL 9 as follows: (2.1)

Age	Regimen	Schedule
9 through 14 years	2-dose	0, 6 to 12 months*
	3-dose	0, 2, 6 months
15 through 45 years	3-dose	0, 2, 6 months

*If the second dose is administered earlier than 5 months after the first dose, administer a third dose at least 4 months after the second dose. (14.2 and 14.6)

---------- DOSAGE FORMS AND STRENGTHS ----------

- 0.5-mL suspension for injection as a single-dose vial and prefilled syringe. (3, 11)

----------CONTRAINDICATIONS----------

Hypersensitivity, including severe allergic reactions to yeast (a vaccine component), or after a previous dose of GARDASIL 9 or GARDASIL®. (4, 11)

---------- WARNINGS AND PRECAUTIONS ----------

Because vaccinees may develop syncope, sometimes resulting in falling with injury, observation for 15 minutes after administration is recommended. Syncope, sometimes associated with tonic-clonic movements and other seizure-like activity, has been reported following HPV vaccination. When syncope is associated with tonic-clonic movements, the activity is usually transient and typically responds to restoring cerebral perfusion by maintaining a supine or Trendelenburg position. (5.1)

---------- ADVERSE REACTIONS ----------

The most common (≥10%) local and systemic adverse reactions reported:

- In girls and women 16 through 26 years of age: injection-site pain (89.9%), injection-site swelling (40.0%), injection-site erythema (34.0%) and headache (14.6%). (6.1)
- In girls 9 through 15 years of age: injection-site pain (89.3%), injection-site swelling (47.8%), injection-site erythema (34.1%) and headache (11.4%). (6.1)
- In women 27 through 45 years of age: injection-site pain (82.8%), injection-site swelling (23.3%), injection-site erythema (16.9%), and headache (13.6%) (6.1)
- In boys and men 16 through 26 years of age: injection-site pain (63.4%), injection-site swelling (20.2%) and injection-site erythema (20.7%). (6.1)
- In boys 9 through 15 years of age: injection-site pain (71.5%), injection-site swelling (26.9%), and injection-site erythema (24.9%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 08/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Girls and Women

GARDASIL®9 is a vaccine indicated in girls and women 9 through 45 years of age for the prevention of the following diseases:

- Cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers caused by Human Papillomavirus (HPV) types 16, 18, 31, 33, 45, 52, and 58
- Genital warts (condyloma acuminata) caused by HPV types 6 and 11

And the following precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58:

- Cervical intraepithelial neoplasia (CIN) grade 2/3 and cervical adenocarcinoma *in situ* (AIS)
- Cervical intraepithelial neoplasia (CIN) grade 1
- Vulvar intraepithelial neoplasia (VIN) grade 2 and grade 3
- Vaginal intraepithelial neoplasia (VaIN) grade 2 and grade 3
- Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3

1.2 Boys and Men

GARDASIL 9 is indicated in boys and men 9 through 45 years of age for the prevention of the following diseases:

- Anal, oropharyngeal and other head and neck cancers caused by HPV types 16, 18, 31, 33, 45, 52, and 58
- Genital warts (condyloma acuminata) caused by HPV types 6 and 11

And the following precancerous or dysplastic lesions caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58:

- Anal intraepithelial neoplasia (AIN) grades 1, 2, and 3

The oropharyngeal and head and neck cancer indication is approved under accelerated approval based on effectiveness in preventing HPV-related anogenital disease *[see Clinical Studies (14.4)].* Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

1.3 Limitations of Use and Effectiveness

- Vaccination with GARDASIL 9 does not eliminate the necessity for vaccine recipients to undergo screening for cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers as recommended by a health care provider.
- GARDASIL 9 has not been demonstrated to provide protection against disease caused by:
  - HPV types not covered by the vaccine *[see Description (11)],*
  - HPV types to which a person has previously been exposed through sexual activity.
- Not all vulvar, vaginal, anal, oropharyngeal and other head and neck cancers are caused by HPV, and GARDASIL 9 protects only against those vulvar, vaginal, anal, oropharyngeal and other head and neck cancers caused by HPV 16, 18, 31, 33, 45, 52, and 58.
- GARDASIL 9 is not a treatment for external genital lesions; cervical, vulvar, vaginal, anal, oropharyngeal and other head and neck cancers; CIN; VIN; VaIN; or AIN.
- Vaccination with GARDASIL 9 may not result in protection in all vaccine recipients.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only

2.1 Dosage

Each dose of GARDASIL 9 is 0.5-mL.
Administer GARDASIL 9 as follows:

Age	Regimen	Schedule
9 through 14 years	2-dose	0, 6 to 12 months*
	3-dose	0, 2, 6 months
15 through 45 years	3-dose	0, 2, 6 months

*If the second dose is administered earlier than 5 months after the first dose, administer a third dose at least 4 months after the second dose. *[See Clinical Studies (14.2 and 14.6).]*

2.2 Method of Administration

- Do not dilute or mix GARDASIL 9 with other vaccines.
- Shake well immediately before use to maintain suspension of the vaccine.
- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use the product if particulates are present or if it appears discolored. After thorough agitation, GARDASIL 9 is a white cloudy liquid.
- Administer intramuscularly in the deltoid or anterolateral area of the thigh.
- Observe patients for 15 minutes after administration *[see Warnings and Precautions (5)].*

Single-Dose Vial Use

Withdraw the 0.5-mL dose of vaccine from the single-dose vial using a sterile needle and syringe and use promptly. Discard vial after use.

Prefilled Syringe Use

This package does not contain a needle. Shake well before use. Attach a needle by twisting in a clockwise direction until the needle fits securely on the syringe. Administer the entire dose as per standard protocol. Discard syringe after use.

2.3 Administration of GARDASIL 9 in Individuals Who Have Been Previously Vaccinated with GARDASIL®

Safety and immunogenicity were assessed in individuals who completed a three-dose vaccination series with GARDASIL 9 and had previously completed a three-dose vaccination series with GARDASIL *[see Adverse Reactions (6.1) and Clinical Studies (14.5)].* Studies using a mixed regimen of HPV vaccines to assess interchangeability were not performed for GARDASIL 9.

3 DOSAGE FORMS AND STRENGTHS

GARDASIL 9 is a suspension for intramuscular administration available in 0.5-mL single-dose vials and prefilled syringes. *[*See *Description (11)]* for the complete listing of ingredients.

4 CONTRAINDICATIONS

Hypersensitivity, including severe allergic reactions to yeast (a vaccine component), or after a previous dose of GARDASIL 9 or GARDASIL *[see Description (11)].*

5 WARNINGS AND PRECAUTIONS

5.1 Syncope

Because vaccinees may develop syncope, sometimes resulting in falling with injury, observation for 15 minutes after administration is recommended. Syncope, sometimes associated with tonic-clonic movements and other seizure-like activity, has been reported following HPV vaccination. When syncope is associated with tonic-clonic movements, the activity is usually transient and typically responds to restoring cerebral perfusion by maintaining a supine or Trendelenburg position.

5.2 Managing Allergic Reactions

Appropriate medical treatment and supervision must be readily available in case of anaphylactic reactions following the administration of GARDASIL 9.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of GARDASIL 9 was evaluated in seven clinical studies that included 15,703 individuals who received at least one dose of GARDASIL 9 and had safety follow-up. Study 1 and Study 3 also included 7,378 individuals who received at least one dose of GARDASIL as a control and had safety follow-up. The vaccines were administered on the day of enrollment and the subsequent doses administered approximately two and six months thereafter. Safety was evaluated using vaccination report card (VRC)-aided surveillance for 14 days after each injection of GARDASIL 9 or GARDASIL.

The individuals who were monitored using VRC-aided surveillance included 9,097 girls and women 16 through 26 years of age, 1,394 boys and men 16 through 26 years of age, and 5,212 girls and boys 9 through 15 years of age (3,436 girls and 1,776 boys) at enrollment who received GARDASIL 9; and 7,078 girls and women 16 through 26 years of age and 300 girls 9 through 15 years of age at enrollment who received GARDASIL. The race distribution of the integrated safety population for GARDASIL 9 was similar between girls and women 16 through 26 years of age (56.8% White; 25.2% Other Races or Multiracial; 14.1% Asian; 3.9% Black), girls and boys 9 through 15 years of age (62.0% White; 19.2% Other Races or Multiracial; 13.5% Asian; 5.4% Black), and boys and men 16 through 26 years of age (62.1% White; 22.6% Other Races or Multiracial; 9.8% Asian; 5.5% Black). The safety of GARDASIL 9 was compared directly to the safety of GARDASIL in two studies (Study 1 and Study 3) for which the overall race distribution of the GARDASIL cohorts (57.0% White; 26.3% Other Races or Multiracial; 13.6% Asian; 3.2% Black) was similar to that of the GARDASIL 9 cohorts.

Safety of GARDASIL 9 in women 27 through 45 years of age was evaluated in a clinical trial comparing 640 women 27 through 45 years of age and 570 girls and women 16 through 26 years of age. The race distribution was similar between women 27 through 45 years of age (97.7% White, 1.6% Asian, 0.3% Other or Multiracial, 0.5% Black) and girls and women 16 through 26 years of age (94.6% White, 3.0% Asian, 1.6% Other or Multiracial, 0.9% Black).

Safety of GARDASIL 9 in men 27 through 45 years of age is inferred from the safety data of GARDASIL 9 in boys and men 9 through 26 years of age and girls and women 9 through 45 years of age and GARDASIL in individuals 9 through 45 years of age.

Injection-Site and Systemic Adverse Reactions

Injection-site reactions (pain, swelling, and erythema) and oral temperature were solicited using VRC-aided surveillance for five days after each injection of GARDASIL 9 during the clinical studies. The rates and severity of these solicited adverse reactions that occurred within five days following each dose of GARDASIL 9 compared with GARDASIL in Study 1 (girls and women 16 through 26 years of age) and Study 3 (girls 9 through 15 years of age) are presented in Table 1. Among subjects who received GARDASIL 9, the rates of injection-site pain were approximately equal across the three reporting time periods. Rates of injection-site swelling and injection-site erythema increased following each successive dose of GARDASIL 9. Recipients of GARDASIL 9 had numerically higher rates of injection-site reactions compared with recipients of GARDASIL.

Table 1: Rates (%) and Severity of Solicited Injection-Site and Systemic Adverse Reactions Occurring within Five Days of Each Vaccination with GARDASIL 9 Compared with GARDASIL (Studies 1 and 3)

	GARDASIL 9				GARDASIL			
	Post-dose 1	Post-dose 2	Post-dose 3	Post any dose	Post-dose 1	Post-dose 2	Post-dose 3	Post any dose
Girls and Women 16 through 26 Years of Age								
Injection-Site Adverse Reactions	**N=7069**	**N=6997**	**N=6909**	**N=7071**	**N=7076**	**N=6992**	**N=6909**	**N=7078**
Pain, Any	70.7	73.5	71.6	89.9	58.2	62.2	62.6	83.5
Pain, Severe	0.7	1.7	2.6	4.3	0.4	1.0	1.7	2.6
Swelling, Any	12.5	23.3	28.3	40.0	9.3	14.6	18.7	28.8
Swelling, Severe	0.6	1.5	2.5	3.8	0.3	0.5	1.0	1.5
Erythema, Any	10.6	18.0	22.6	34.0	8.1	12.9	15.6	25.6
Erythema, Severe	0.2	0.5	1.1	1.6	0.2	0.2	0.4	0.8
Systemic Adverse Reactions	**n=6995**	**n=6913**	**n=6743**	**n=7022**	**n=7003**	**n=6914**	**n=6725**	**n=7024**
Temperature ≥100°F	1.7	2.6	2.7	6.0	1.7	2.4	2.5	5.9
Temperature ≥102°F	0.3	0.3	0.4	1.0	0.2	0.3	0.3	0.8
Girls 9 through 15 Years of Age								
Injection-Site Adverse Reactions	**N=300**	**N=297**	**N=296**	**N=299**	**N=299**	**N=299**	**N=294**	**N=300**
Pain, Any	71.7	71.0	74.3	89.3	66.2	66.2	69.4	88.3
Pain, Severe	0.7	2.0	3.0	5.7	0.7	1.3	1.7	3.3
Swelling, Any	14.0	23.9	36.1	47.8	10.4	17.7	25.2	36.0
Swelling, Severe	0.3	2.4	3.7	6.0	0.7	2.7	4.1	6.3
Erythema, Any	7.0	15.5	21.3	34.1	9.7	14.4	18.4	29.3
Erythema, Severe	0	0.3	1.4	1.7	0	0.3	1.7	2.0
Systemic Adverse Reactions	**n=300**	**n=294**	**n=295**	**n=299**	**n=299**	**n=297**	**n=291**	**n=300**
Temperature ≥100°F	2.3	1.7	3.0	6.7	1.7	1.7	0	3.3
Temperature ≥102°F	0	0.3	1.0	1.3	0.3	0.3	0	0.7

The data for girls and women 16 through 26 years of age are from Study 1 (NCT00543543), and the data for girls 9 through 15 years of age are from Study 3 (NCT01304498).
N=number of subjects vaccinated with safety follow-up
n=number of subjects with temperature data
Pain, Any=mild, moderate, severe or unknown intensity
Pain, Severe=incapacitating with inability to work or do usual activity
Swelling, Any=any size or size unknown
Swelling, Severe=maximum size greater than 2 inches
Erythema, Any=any size or size unknown
Erythema, Severe=maximum size greater than 2 inches

Unsolicited injection-site and systemic adverse reactions (assessed as vaccine-related by the investigator) observed among recipients of either GARDASIL 9 or GARDASIL in Studies 1 and 3 at a frequency of at least 1% are shown in Table 2. Few individuals discontinued study participation due to adverse experiences after receiving either vaccine (GARDASIL 9 = 0.1% vs. GARDASIL <0.1%).

Table 2: Rates (%) of Unsolicited Injection-Site and Systemic Adverse Reactions Occurring among ≥1.0% of Individuals after Any Vaccination with GARDASIL 9 Compared with GARDASIL (Studies 1 and 3)

	Girls and Women 16 through 26 Years of Age		Girls 9 through 15 Years of Age	
	GARDASIL 9 N=7071	GARDASIL N=7078	GARDASIL 9 N=299	GARDASIL N=300
Injection-Site Adverse Reactions (1 to 5 Days Post-Vaccination, Any Dose)				
Pruritus	5.5	4.0	4.0	2.7
Bruising	1.9	1.9	0	0
Hematoma	0.9	0.6	3.7	4.7
Mass	1.3	0.6	0	0
Hemorrhage	1.0	0.7	1.0	2.0
Induration	0.8	0.2	2.0	1.0
Warmth	0.8	0.5	0.7	1.7
Reaction	0.6	0.6	0.3	1.0
Systemic Adverse Reactions (1 to 15 Days Post-Vaccination, Any Dose)				
Headache	14.6	13.7	11.4	11.3
Pyrexia	5.0	4.3	5.0	2.7
Nausea	4.4	3.7	3.0	3.7
Dizziness	3.0	2.8	0.7	0.7
Fatigue	2.3	2.1	0	2.7
Diarrhea	1.2	1.0	0.3	0
Oropharyngeal pain	1.0	0.6	2.7	0.7
Myalgia	1.0	0.7	0.7	0.7
Abdominal pain, upper	0.7	0.8	1.7	1.3
Upper respiratory tract infection	0.1	0.1	0.3	1.0

The data for girls and women 16 through 26 years of age are from Study 1 (NCT00543543), and the data for girls 9 through 15 years of age are from Study 3 (NCT01304498).
N=number of subjects vaccinated with safety follow-up

In an uncontrolled clinical trial with 639 boys and 1,878 girls 9 through 15 years of age (Study 2), the rates and severity of solicited adverse reactions following each dose of GARDASIL 9 were similar between boys and girls. Rates of solicited and unsolicited injection-site and systemic adverse reactions in boys 9 through 15 years of age were similar to those among girls 9 through 15 years of age. Solicited and unsolicited adverse reactions reported by boys in this study are shown in Table 3.

In another uncontrolled clinical trial with 1,394 boys and men and 1,075 girls and women 16 through 26 years of age (Study 7), the rates of solicited and unsolicited adverse reactions following each dose of GARDASIL 9 among girls and women 16 through 26 years of age were similar to those reported in Study 1. Rates of solicited and unsolicited adverse reactions reported by boys and men 16 through 26 years of age in this study are shown in Table 3.

In an uncontrolled clinical trial with 640 women 27 through 45 years of age and 570 girls and women 16 through 26 years of age (Study 9), the rates of solicited and unsolicited adverse reactions following each dose of GARDASIL 9 among girls and women 16 through 26 years of age were similar to those reported in Study 1. Rates of solicited and unsolicited adverse reactions reported by women 27 through 45 years of age in this study are shown in Table 3.

Table 3: Rates (%) of Solicited and Unsolicited* Injection-Site and Systemic Adverse Reactions among Boys 9 through 15 Years of Age, among Boys and Men 16 through 26 Years of Age and Women 27 through 45 Years of Age Who Received GARDASIL 9 (Studies 2, 7, and 9)

	GARDASIL 9
Boys and Men 16 through 26 Years of Age	**N=1394**
Solicited Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)	
Injection-Site Pain, Any	63.4
Injection-Site Pain, Severe	0.6
Injection-Site Erythema, Any	20.7
Injection-Site Erythema, Severe	0.4
Injection-Site Swelling, Any	20.2
Injection-Site Swelling, Severe	1.1
Oral Temperature ≥100.0°F†	4.4
Oral Temperature ≥102°F	0.6
Unsolicited Injection-Site Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)	
Injection-Site Hypersensitivity	1.0
Injection-Site Pruritus	1.0
Unsolicited Systemic Adverse Reactions (1-15 Days Post-Vaccination, Any Dose)	
Headache	7.3
Pyrexia	2.4
Fatigue	1.4
Dizziness	1.1
Nausea	1.0
Boys 9 through 15 Years of Age	**N=639**
Solicited Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)	
Injection-Site Pain, Any	71.5
Injection-Site Pain, Severe	0.5
Injection-Site Erythema, Any	24.9
Injection-Site Erythema, Severe	1.9
Injection-Site Swelling, Any	26.9
Injection-Site Swelling, Severe	5.2
Oral Temperature ≥100.0°F†	10.4
Oral Temperature ≥102°F	1.4
Unsolicited Injection-Site Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)	
Injection-Site Hematoma	1.3
Injection-Site Induration	1.1
Unsolicited Systemic Adverse Reactions (1-15 Days Post-Vaccination, Any Dose)	
Headache	9.4
Pyrexia	8.9
Nausea	1.3
Women 27 through 45 Years of Age	**N=640**
Solicited Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)	
Injection-Site Pain, Any	82.8
Injection-Site Pain, Severe	1.9
Injection-Site Erythema, Any	16.9
Injection-Site Erythema, Severe	0.5
Injection-Site Swelling, Any	23.3
Injection-Site Swelling, Severe	1.9
Oral Temperature ≥100.0°F†	2.5
Oral Temperature ≥102°F	0.3
Unsolicited Injection-Site Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)	
Injection-Site Pruritus	1.6
Injection-Site Hematoma	1.3
Unsolicited Systemic Adverse Reactions (1-15 Days Post-Vaccination, Any Dose)	
Headache	13.6
Fatigue	3.4
Pyrexia	1.7
Nausea	1.7
Oropharyngeal pain	1.1

The data for GARDASIL 9 boys 9 through 15 years of age are from Study 2 (NCT00943722). The data for boys and men 16 through 26 years of age for GARDASIL 9 are from Study 7 (NCT01651949). The data for women 27 through 45 years of age are from Study 9 (NCT03158220).

*Unsolicited adverse reactions reported by ≥1% of individuals
N=number of subjects vaccinated with safety follow-up
†For oral temperature: number of subjects with temperature data for boys 9 through 15 years of age N=637; for boys and men 16 through 26 years of age N=1,386; for women 27 through 45 years of age N=640
Pain, Any=mild, moderate, severe or unknown intensity
Pain, Severe=incapacitating with inability to work or do usual activity
Swelling, Any=any size or size unknown
Swelling, Severe=maximum size greater than 2 inches
Erythema, Any=any size or size unknown
Erythema, Severe=maximum size greater than 2 inches

Serious Adverse Events in Clinical Studies

Serious adverse events were collected throughout the entire study period (range one month to 48 months post-last dose) for the seven clinical studies for GARDASIL 9. Out of the 15,705 individuals who were administered GARDASIL 9 and had safety follow-up, 354 reported a serious adverse event; representing 2.3% of the population. As a comparison, of the 7,378 individuals who were administered GARDASIL and had safety follow-up, 185 reported a serious adverse event; representing 2.5% of the population. Four GARDASIL 9 recipients each reported at least one serious adverse event that was determined to be vaccine-related. The vaccine-related serious adverse reactions were pyrexia, allergy to vaccine, asthmatic crisis, and headache.

Deaths in the Entire Study Population

Across the clinical studies, ten deaths occurred (five each in the GARDASIL 9 and GARDASIL groups); none were assessed as vaccine-related. Causes of death in the GARDASIL 9 group included one automobile accident, one suicide, one case of acute lymphocytic leukemia, one case of hypovolemic septic shock, and one unexplained sudden death 678 days following the last dose of GARDASIL 9. Causes of death in the GARDASIL control group included one automobile accident, one airplane crash, one cerebral hemorrhage, one gunshot wound, and one stomach adenocarcinoma.

Systemic Autoimmune Disorders

In all of the clinical trials with GARDASIL 9 subjects were evaluated for new medical conditions potentially indicative of a systemic autoimmune disorder. In total, 2.2% (351/15,703) of GARDASIL 9 recipients and 3.3% (240/7,378) of GARDASIL recipients reported new medical conditions potentially indicative of systemic autoimmune disorders, which were similar to rates reported following GARDASIL, AAHS control, or saline placebo in historical clinical trials.

Clinical Trials Experience for GARDASIL 9 in Individuals Who Have Been Previously Vaccinated with GARDASIL

A clinical study (Study 4) evaluated the safety of GARDASIL 9 in 12- through 26-year-old girls and women who had previously been vaccinated with three doses of GARDASIL. The time interval between the last injection of GARDASIL and the first injection of GARDASIL 9 ranged from approximately 12 to 36 months. Individuals were administered GARDASIL 9 or saline placebo and safety was evaluated using VRC-aided surveillance for 14 days after each injection of GARDASIL 9 or saline placebo in these individuals. The individuals who were monitored included 608 individuals who received GARDASIL 9 and 305 individuals who received saline placebo. Few (0.5%) individuals who received GARDASIL 9 discontinued due to adverse reactions. The vaccine-related adverse experiences that were observed among recipients of GARDASIL 9 at a frequency of at least 1.0% and also at a greater frequency than that observed among saline placebo recipients are shown in Table 4. Overall the safety profile was similar between individuals vaccinated with GARDASIL 9 who were previously vaccinated with GARDASIL and those who were naïve to HPV vaccination with the exception of numerically higher rates of injection-site swelling and erythema among individuals who were previously vaccinated with GARDASIL (Tables 1 and 4).

Table 4: Rates (%) of Solicited and Unsolicited* Injection-Site and Systemic Adverse Reactions among Individuals Previously Vaccinated with GARDASIL Who Received GARDASIL 9 or Saline Placebo (Girls and Women 12 through 26 Years of Age) (Study 4)

	GARDASIL 9 N=608	Saline Placebo N=305
Solicited Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)		
Injection-Site Pain	90.3	38.0
Injection-Site Erythema	42.3	8.5
Injection-Site Swelling	49.0	5.9
Oral Temperature ≥100.0°F†	6.5	3.0
Unsolicited Injection-Site Adverse Reactions (1-5 Days Post-Vaccination, Any Dose)		
Injection-Site Pruritus	7.7	1.3
Injection-Site Hematoma	4.8	2.3
Injection-Site Reaction	1.3	0.3
Injection-Site Mass	1.2	0.7
Unsolicited Systemic Adverse Reactions (1-15 Days Post-Vaccination, Any Dose)		
Headache	19.6	18.0
Pyrexia	5.1	1.6
Nausea	3.9	2.0
Dizziness	3.0	1.6
Abdominal pain, upper	1.5	0.7
Influenza	1.2	1.0

The data for GARDASIL 9 and saline placebo are from Study 4 (NCT01047345).
*Unsolicited adverse reactions reported by ≥1% of individuals
N=number of subjects vaccinated with safety follow-up
†For oral temperature: number of subjects with temperature data GARDASIL 9 N=604; Saline Placebo N=304

Safety in Concomitant Use with Menactra and Adacel

In Study 5, the safety of GARDASIL 9 when administered concomitantly with Menactra [Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine] and Adacel [Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap)] was evaluated in a randomized study of 1,241 boys (n = 620) and girls (n = 621) with a mean age of 12.2 years *[see Clinical Studies (14.7)]*.

Of the 1,237 boys and girls vaccinated, 1,220 had safety follow-up for injection-site adverse reactions. The rates of injection-site adverse reactions were similar between the concomitant group and non-concomitant group (vaccination with GARDASIL 9 separated from vaccination with Menactra and Adacel by 1 month) with the exception of an increased rate of swelling reported at the injection site for GARDASIL 9 in the concomitant group (14.4%) compared to the non-concomitant group (9.4%). The majority of injection-site swelling adverse reactions were reported as being mild to moderate in intensity.

6.2 Postmarketing Experience

The postmarketing adverse experiences were reported voluntarily from a population of uncertain size, therefore, it is not possible to reliably estimate their frequency or to establish a causal relationship to vaccine exposure.

The safety profile of GARDASIL 9 and GARDASIL are similar. The postmarketing safety experience with GARDASIL is relevant to GARDASIL 9 since the vaccines are manufactured similarly and contain the same L1 HPV proteins of four of the same HPV types.

GARDASIL 9

In addition to the adverse reactions reported in the clinical studies, the following adverse experiences have been spontaneously reported during post-approval use of GARDASIL 9:

Gastrointestinal disorders: Vomiting
Skin and subcutaneous tissue disorders: Urticaria

GARDASIL

Additionally, the following postmarketing adverse experiences have been spontaneously reported for GARDASIL:

Blood and lymphatic system disorders: Autoimmune hemolytic anemia, idiopathic thrombocytopenic purpura, lymphadenopathy.
Respiratory, thoracic and mediastinal disorders: Pulmonary embolus.
Gastrointestinal disorders: Pancreatitis.
General disorders and administration site conditions: Asthenia, chills, death, malaise.
Immune system disorders: Autoimmune diseases, hypersensitivity reactions including anaphylactic/anaphylactoid reactions, bronchospasm.
Musculoskeletal and connective tissue disorders: Arthralgia, myalgia.
Nervous system disorders: Acute disseminated encephalomyelitis, Guillain-Barré syndrome, motor neuron disease, paralysis, seizures, transverse myelitis.
Infections and infestations: Cellulitis.
Vascular disorders: Deep venous thrombosis.

7 DRUG INTERACTIONS

7.1 Use with Systemic Immunosuppressive Medications

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune responses to vaccines *[see Use in Specific Populations (8.6)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry to monitor pregnancy outcomes in women exposed to GARDASIL 9 during pregnancy. To enroll in or obtain information about the registry, call Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-800-986-8999.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no adequate and well-controlled studies of GARDASIL 9 in pregnant women. Available human data do not demonstrate vaccine-associated increase in risk of major birth defects and miscarriages when GARDASIL 9 is administered during pregnancy.

In one developmental toxicity study, 0.5 mL of a vaccine formulation containing between 1 and 1.5 – fold of each of the 9 HPV antigen types was administered to female rats prior to mating and during gestation. In a second study, animals were administered a single human dose (0.5 mL) of GARDASIL 9 prior to mating, during gestation and during lactation. These animal studies revealed no evidence of harm to the fetus due to GARDASIL 9 *[see Data]*.

Data

Human Data

In pre-licensure clinical studies of GARDASIL 9, women underwent pregnancy testing immediately prior to administration of each dose of GARDASIL 9 or control vaccine (GARDASIL). (Data from GARDASIL are relevant to GARDASIL 9 because both vaccines are manufactured using the same process and have overlapping compositions.) Subjects who were determined to be pregnant were instructed to defer vaccination until the end of their pregnancy. Despite this pregnancy screening regimen, some subjects were vaccinated very early in pregnancy before human chorionic gonadotropin (HCG) was detectable. An analysis was conducted to evaluate pregnancy outcomes for pregnancies with onset within 30 days before or after vaccination with GARDASIL 9 or GARDASIL. Among such pregnancies, there were 62 and 55 with known outcomes (excluding ectopic pregnancies and elective terminations) for GARDASIL 9 and GARDASIL, respectively, including 44 and 48 live births, respectively. The rates of pregnancies that resulted in a miscarriage were 27.4% (17/62) and 12.7% (7/55) in subjects

who received GARDASIL 9 or GARDASIL, respectively. The rates of live births with major birth defects were 0% (0/44) and 2.1% (1/48) in subjects who received GARDASIL 9 or GARDASIL, respectively.

A five-year pregnancy registry enrolled 2,942 women who were inadvertently exposed to GARDASIL within one month prior to the last menstrual period (LMP) or at any time during pregnancy, 2,566 of whom were prospectively followed. After excluding elective terminations (n=107), ectopic pregnancies (n=5) and those lost to follow-up (n=814), there were 1,640 pregnancies with known outcomes. Rates of miscarriage and major birth defects were 6.8% of pregnancies (111/1,640) and 2.4% of live born infants (37/1,527), respectively. These rates of assessed outcomes in the prospective population were consistent with estimated background rates.

In two postmarketing studies of GARDASIL (one conducted in the U.S., and the other in Nordic countries), pregnancy outcomes among subjects who received GARDASIL during pregnancy were evaluated retrospectively. Among the 1,740 pregnancies included in the U.S. study database, outcomes were available to assess the rates of major birth defects and miscarriage. Among the 499 pregnancies included in the Nordic study database, outcomes were available to assess the rates of major birth defects. In both studies, rates of assessed outcomes did not suggest an increased risk with the administration of GARDASIL during pregnancy.

Animal Data

Developmental toxicity studies were conducted in female rats. In one study, animals were administered 0.5 mL of a vaccine formulation containing between 1 and 1.5 –fold of each of the 9 HPV antigen types 5 and 2 weeks prior to mating, and on gestation day 6. In a second study, animals were administered a single human dose (0.5 mL) of GARDASIL 9, 5 and 2 weeks prior to mating, on gestation day 6, and on lactation day 7. No adverse effects on pre- and post-weaning development were observed. There were no vaccine-related fetal malformations or variations.

8.2 Lactation

Risk Summary

Available data are not sufficient to assess the effects of GARDASIL 9 on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for GARDASIL 9 and any potential adverse effects on the breastfed child from GARDASIL 9 or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness have not been established in pediatric patients below 9 years of age.

8.5 Geriatric Use

The safety and effectiveness of GARDASIL 9 have not been evaluated in a geriatric population, defined as individuals aged 65 years and over.

8.6 Immunocompromised Individuals

The immunologic response to GARDASIL 9 may be diminished in immunocompromised individuals *[see Drug Interactions (7.1)]*.

11 DESCRIPTION

GARDASIL 9, Human Papillomavirus 9-valent Vaccine, Recombinant, is a non-infectious recombinant 9-valent vaccine prepared from the purified virus-like particles (VLPs) of the major capsid (L1) protein of HPV Types 6, 11, 16, 18, 31, 33, 45, 52, and 58. The L1 proteins are produced by separate fermentations using recombinant *Saccharomyces cerevisiae* and self-assembled into VLPs. The fermentation process involves growth of *S. cerevisiae* on chemically-defined fermentation media which include vitamins, amino acids, mineral salts, and carbohydrates. The VLPs are released from the yeast cells by cell disruption and purified by a series of chemical and physical methods. The purified VLPs are adsorbed on preformed aluminum-containing adjuvant (Amorphous Aluminum Hydroxyphosphate Sulfate or AAHS). The 9-valent HPV VLP vaccine is a sterile liquid suspension that is prepared by combining the adsorbed VLPs of each HPV type and additional amounts of the aluminum-containing adjuvant and the final purification buffer.

GARDASIL 9 is a sterile suspension for intramuscular administration. Each 0.5-mL dose contains approximately 30 mcg of HPV Type 6 L1 protein, 40 mcg of HPV Type 11 L1 protein, 60 mcg of HPV Type 16 L1 protein, 40 mcg of HPV Type 18 L1 protein, 20 mcg of HPV Type 31 L1 protein, 20 mcg of HPV Type 33 L1 protein, 20 mcg of HPV Type 45 L1 protein, 20 mcg of HPV Type 52 L1 protein, and 20 mcg of HPV Type 58 L1 protein.

Each 0.5-mL dose of the vaccine also contains approximately 500 mcg of aluminum (provided as AAHS), 9.56 mg of sodium chloride, 0.78 mg of L-histidine, 50 mcg of polysorbate 80, 35 mcg of sodium borate, <7 mcg yeast protein, and water for injection. The product does not contain a preservative or antibiotics.

After thorough agitation, GARDASIL 9 is a white, cloudy liquid.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

HPV only infects human beings. Animal studies with analogous animal papillomaviruses suggest that the efficacy of L1 VLP vaccines may involve the development of humoral immune responses. Efficacy of GARDASIL 9 against anogenital diseases related to the vaccine HPV types in human beings is thought to be mediated by humoral immune responses induced by the vaccine, although the exact mechanism of protection is unknown.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

GARDASIL 9 has not been evaluated for the potential to cause carcinogenicity, genotoxicity or impairment of male fertility. GARDASIL 9 administered to female rats had no effects on fertility *[see Pregnancy (8.1)]*.

14 CLINICAL STUDIES

In these studies, seropositive is defined as anti-HPV titer greater than or equal to the pre-specified serostatus cutoff for a given HPV type. Seronegative is defined as anti-HPV titer less than the pre-specified serostatus cutoff for a given HPV type. The serostatus cutoff is the antibody titer level above the assay's lower limit of quantification that reliably distinguishes sera samples classified by clinical likelihood of HPV infection and positive or negative status by previous versions of competitive Luminex Immunoassay (cLIA). The lower limits of quantification and serostatus cutoffs for each of the 9 vaccine HPV types are shown in Table 5 below. PCR positive is defined as DNA detected for a given HPV type. PCR negative is defined as DNA not detected for a given HPV type. The lower limit of detection for the multiplexed HPV PCR assays ranged from 5 to 34 copies per test across the 9 vaccine HPV types.

Table 5: Competitive Luminex Immunoassay (cLIA) Limits of Quantification and Serostatus Cutoffs for GARDASIL 9 HPV Types

HPV Type	cLIA Lower Limit of Quantification (mMU*/mL)	cLIA Serostatus Cutoff (mMU*/mL)
HPV 6	16	30
HPV 11	6	16
HPV 16	12	20
HPV 18	8	24
HPV 31	4	10
HPV 33	4	8
HPV 45	3	8
HPV 52	3	8
HPV 58	4	8

*mMU=milli-Merck Units

14.1 Efficacy and Effectiveness Data for GARDASIL

Efficacy and effectiveness of GARDASIL are relevant to GARDASIL 9 since the vaccines are manufactured similarly and contain four of the same HPV L1 VLPs.

Individuals 16 through 26 Years of Age

Efficacy of GARDASIL was assessed in five AAHS-controlled, double-blind, randomized clinical trials evaluating 24,596 individuals 16 through 26 years of age (20,541 girls and women and 4,055 boys and men). The results of these trials are shown in Table 6 below.

Table 6: Analysis of Efficacy of GARDASIL in the PPE* Population for Vaccine HPV Types

Disease Endpoints	GARDASIL		AAHS Control		% Efficacy (95% CI)
	N	Number of cases	N	Number of cases	
16- through 26-Year-Old Girls and Women†					
HPV 16- or 18-related CIN 2/3 or AIS	8493	2	8464	112	98.2 (93.5, 99.8)
HPV 16- or 18-related VIN 2/3	7772	0	7744	10	100.0 (55.5, 100.0)
HPV 16- or 18-related VaIN 2/3	7772	0	7744	9	100.0 (49.5, 100.0)
HPV 6-, 11-, 16-, or 18-related CIN (CIN 1, CIN 2/3) or AIS	7864	9	7865	225	96.0 (92.3, 98.2)
HPV 6-, 11-, 16-, or 18-related Genital Warts	7900	2	7902	193	99.0 (96.2, 99.9)
HPV 6- and 11-related Genital Warts	6932	2	6856	189	99.0 (96.2, 99.9)
16- through 26-Year-Old Boys and Men					
External Genital Lesions HPV 6-, 11-, 16-, or 18-related					
External Genital Lesions	1394	3	1404	32	90.6 (70.1, 98.2)
Condyloma	1394	3	1404	28	89.3 (65.3, 97.9)
PIN 1/2/3	1394	0	1404	4	100.0 (-52.1, 100.0)
HPV 6-, 11-, 16-, or 18-related Endpoint					
AIN 1/2/3	194	5	208	24	77.5 (39.6, 93.3)
AIN 2/3	194	3	208	13	74.9 (8.8, 95.4)
AIN 1	194	4	208	16	73.0 (16.3, 93.4)
Condyloma Acuminatum	194	0	208	6	100.0 (8.2, 100.0)
Non-acuminate	194	4	208	11	60.4 (-33.5, 90.8)

*The PPE population consisted of individuals who received all three vaccinations within one year of enrollment, did not have major deviations from the study protocol, were naïve (PCR negative and seronegative) to the relevant HPV type(s) (Types 6, 11, 16, and 18) prior to dose 1 and who remained PCR negative to the relevant HPV type(s) through one month post-dose 3 (Month 7).
†Analyses of the combined trials were prospectively planned and included the use of similar study entry criteria.
N=Number of individuals with at least one follow-up visit after Month 7
CI=Confidence Interval
Note 1: Point estimates and confidence intervals are adjusted for person-time of follow-up.
Note 2: Table 6 does not include cases due to HPV types not covered by the vaccine.
AAHS = Amorphous Aluminum Hydroxyphosphate Sulfate, CIN = Cervical Intraepithelial Neoplasia, VIN = Vulvar Intraepithelial Neoplasia, VaIN=Vaginal Intraepithelial Neoplasia, PIN=Penile Intraepithelial Neoplasia, AIN=Anal Intraepithelial Neoplasia, AIS=Adenocarcinoma *In Situ*

In an extension study in females 16 through 26 years of age at enrollment, prophylactic efficacy of GARDASIL through Month 60 against overall cervical and genital disease related to HPV 6, 11, 16, and 18 was 100% (95% CI: 12.3%, 100%) compared to AAHS control.

An extension study in girls and women 16 through 23 years of age used national health care registries in Denmark, Iceland, Norway, and Sweden to monitor endpoint cases of HPV 6-, 11-, 16-, or 18-related CIN (any grade), AIS, cervical cancer, vulvar cancer, or vaginal cancer among 2,650 girls and women 16 through 23 years of age at enrollment who were randomized to vaccination with GARDASIL. An interim analysis of the per-protocol effectiveness population included 1,902 subjects who completed the GARDASIL vaccination series within one year, were naïve to the relevant HPV type through 1 month post-dose 3, had no protocol violations, and had follow-up data available. The median follow-up from the first dose of vaccine was 6.7 years with a range of 2.8 to 8.4 years. At the time of interim analysis, no cases of HPV 6-, 11-, 16-, or 18-related CIN (any grade), AIS, cervical cancer, vulvar cancer, or vaginal cancer were observed over a total of 5,765 person-years at risk.

Girls and Boys 9 through 15 Years of Age

An extension study of 614 girls and 565 boys 9 through 15 years of age at enrollment who were randomized to vaccination with GARDASIL actively followed subjects for endpoint cases of HPV 6-, 11-, 16-, or 18-related persistent infection, CIN (any grade), AIS, VIN, VaIN, cervical cancer, vulvar cancer,

vaginal cancer, and external genital lesions from the initiation of sexual activity or age 16 onwards. An interim analysis of the per-protocol effectiveness population included 246 girls and 168 boys who completed the GARDASIL vaccination series within one year, were seronegative to the relevant HPV type at initiation of the vaccination series, and had not initiated sexual activity prior to receiving the third dose of GARDASIL. The median follow-up from the first dose of vaccine was 7.2 years with a range of 0.5 to 8.5 years. At the time of interim analysis, no cases of persistent infection of at least 12 months' duration and no cases of HPV 6-, 11-, 16-, or 18-related CIN (any grade), AIS, VIN, VaIN, cervical cancer, vulvar cancer, vaginal cancer, or external genital lesions were observed over a total 1,105 person-years at risk. There were 4 cases of HPV 6-, 11-, 16-, or 18-related persistent infection of at least 6 months' duration, including 3 cases related to HPV 16 and 1 case related to HPV 6, none of which persisted to 12 months' duration.

Individuals 27 through 45 Years of Age

A clinical trial evaluated efficacy of GARDASIL in 3,253 women 27 through 45 years of age, based on a combined endpoint of HPV 6-, 11-, 16- or 18-related persistent infection, genital warts, vulvar and vaginal dysplastic lesions of any grade, CIN of any grade, AIS, and cervical cancer. These women were randomized 1:1 to receive either GARDASIL or AAHS control. The clinical trial was conducted in two phases: a base study and a long-term study extension. The per-protocol efficacy (PPE) population received all three vaccinations within one year of enrollment, did not have major deviations from the study protocol, were naïve (PCR negative and seronegative) to the relevant HPV type(s) (Types 6, 11, 16 and 18) prior to dose 1 and remained PCR negative to the relevant HPV type(s) through one month post-dose 3 (Month 7).

In the base study (median duration of follow-up of 3.5 years post-dose 3), the efficacy of GARDASIL against the combined incidence of HPV 6-, 11-, 16-, and 18-related persistent infection, genital warts, VIN, VaIN, vulvar cancer, vaginal cancer, cervical dysplasia (any grade CIN), AIS and cervical cancer in the PPE population was 87.7% (95% CI: 75.4%, 94.6%). The efficacy estimate for the combined endpoint was driven primarily by prevention of persistent infection. The efficacy of GARDASIL against the combined incidence of HPV 6-, 11-, 16-, and 18-related genital warts or cervical dysplasia was 95.0% (95% CI: 68.7%, 99.9%) in the PPE population. While no statistically significant efficacy was demonstrated for GARDASIL in the base study for prevention of cervical intraepithelial neoplasia grades 2 and 3 (CIN 2/3), adenocarcinoma *in situ* (AIS) or cervical cancer related to HPV types 16 and 18, there was 1 case of CIN 2/3 observed in the GARDASIL group and 5 cases in the placebo group. The CIN 2 case in the GARDASIL group tested positive by PCR for HPV 16 and HPV 51.

In the long-term extension of this study, subjects from Colombia (n=600) randomized to the GARDASIL group in the base study were monitored for HPV 6-, 11-, 16-, and 18-related genital warts or cervical dysplasia. The median follow-up post-dose 3 was 8.9 years with a range of 0.1 to 10.1 years over a total of 3,518 person-years. During the long-term extension phase, no cases of HPV 6-, 11-, 16-, or 18-related CIN (any grade) or genital warts were observed in the PPE population.

Effectiveness of GARDASIL in men 27 through 45 years of age is inferred from efficacy data in women 27 through 45 years of age as described above and supported by immunogenicity data from a clinical trial in which 150 men, 27 through 45 years of age, received a 3-dose regimen of GARDASIL (0, 2, 6 months). A cross-study analysis of per-protocol immunogenicity populations compared Month 7 anti-HPV 6, 11, 16, and 18 GMTs of these 27- through 45-year-old men (Study A) to those of 16- through 26-year old boys and men (Study B) in whom efficacy of GARDASIL had been established (see Table 6). GMT ratios (Study A/Study B) for HPV 6, 11, 16, and 18 were 0.82 (95%CI: 0.65, 1.03), 0.79 (95%CI: 0.66, 0.93), 0.91 (95%CI: 0.72, 1.13), and 0.74 (95%CI: 0.59, 0.92), respectively.

14.2 Clinical Trials for GARDASIL 9

Efficacy and/or immunogenicity of the 3-dose regimen of GARDASIL 9 were assessed in seven clinical trials. Study 1 evaluated the efficacy of GARDASIL 9 to prevent HPV-related cervical, vulvar, and vaginal disease using GARDASIL as a comparator.

The analysis of efficacy for GARDASIL 9 was evaluated in the per-protocol efficacy (PPE) population of 16- through 26-year-old girls and women, who received all three vaccinations within one year of enrollment, did not have major deviations from the study protocol, and were naïve to the relevant HPV type(s) by serology and PCR of cervicovaginal specimens prior to dose one and who remained PCR

negative for the relevant HPV type(s) through one month post-dose 3 (Month 7). Overall, approximately 52% of subjects were negative to all vaccine HPV types by both PCR and serology at Day 1.

The primary analysis of efficacy against HPV Types 31, 33, 45, 52, and 58 is based on a combined endpoint of Cervical Intraepithelial Neoplasia (CIN) 2, CIN 3, Adenocarcinoma *in situ* (AIS), invasive cervical carcinoma, Vulvar Intraepithelial Neoplasia (VIN) 2/3, Vaginal Intraepithelial Neoplasia (VaIN) 2/3, vulvar cancer, or vaginal cancer. Other endpoints evaluated include cervical, vulvar and vaginal disease of any grade, persistent infection, cytological abnormalities and invasive procedures. For all endpoints, the efficacy against the HPV Types 31, 33, 45, 52 and 58 in GARDASIL 9 was evaluated compared with GARDASIL. Efficacy of GARDASIL 9 against anal lesions caused by HPV Types 31, 33, 45, 52, and 58 was not assessed due to low incidence. Effectiveness of GARDASIL 9 against anal lesions was inferred from the efficacy of GARDASIL against anal lesions caused by HPV types 6, 11, 16 and 18 in men and antibody responses elicited by GARDASIL 9 against the HPV types covered by the vaccine.

Effectiveness against disease caused by HPV Types 6, 11, 16, and 18 was assessed by comparison of geometric mean titers (GMTs) of type-specific antibodies following vaccination with GARDASIL 9 with those following vaccination with GARDASIL (Study 1 and Study 3). The effectiveness of GARDASIL 9 in girls and boys 9 through 15 years old and in boys and men 16 through 26 years old was inferred based on a comparison of type-specific antibody GMTs to those of 16 through 26-year-old girls and women following vaccination with GARDASIL 9. Immunogenicity analyses were performed in the per-protocol immunogenicity (PPI) population consisting of individuals who received all three vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met pre-defined day range for serum collection for assessment of antibody response and were naïve [PCR negative (in girls and women 16 through 26 years of age; Studies 1 and 2) and seronegative (Studies 1, 2, 3, 5, 7 and 8)] to the relevant HPV type(s) prior to dose 1 and among 16- through 26-year-old girls and women (Studies 1 and 2) remained PCR negative to the relevant HPV type(s) through Month 7. Pre-defined day ranges for vaccinations were relative to Day 1 (dose 1). For the 3-dose schedule, dose 2 was at 2 months (± 3 weeks) and dose 3 was at 6 months (± 4 weeks). For the 2-dose schedule, dose 2 was at 6 or 12 months (± 4 weeks). Pre-defined day range for serum collection for assessment of antibody response was 21 to 49 days after the last dose.

Study 1 evaluated immunogenicity of GARDASIL 9 and efficacy to prevent infection and disease caused by HPV types 6, 11, 16, 18, 31, 33, 45, 52, and 58 in 16- through 26-year-old girls and women. Study 2 evaluated immunogenicity of GARDASIL 9 in girls and boys 9 through 15 years of age and women 16 through 26 years of age. Study 3 evaluated immunogenicity of GARDASIL 9 compared with GARDASIL in girls 9 through 15 years of age. Study 4 evaluated administration of GARDASIL 9 to girls and women 12 through 26 years of age previously vaccinated with GARDASIL. Study 5 evaluated GARDASIL 9 concomitantly administered with Menactra and Adacel in girls and boys 11 through 15 years of age. Together, these five clinical trials evaluated 12,233 individuals who received GARDASIL 9 (8,048 girls and women 16 through 26 years of age at enrollment with a mean age of 21.8 years; 2,927 girls 9 through 15 years of age at enrollment with a mean age of 11.9 years; and 1,258 boys 9 through 15 years of age at enrollment with a mean age of 11.9 years. Study 7 evaluated immunogenicity of GARDASIL 9 in boys and men, including 1,106 self-identified as heterosexual men (HM) and 313 self-identified as men having sex with men (MSM), 16 through 26 years of age at enrollment (mean ages 20.8 years and 22.2 years, respectively) and 1,101 girls and women 16 through 26 years of age at enrollment (mean age 21.3 years). Study 9 evaluated immunogenicity of GARDASIL 9 in 640 women 27 through 45 years of age and 570 girls and women 16 through 26 years of age (mean ages 35.8 years and 21.6 years, respectively).

The race distribution of the 16- through 26-year-old girls and women in the clinical trials was as follows: 56.8% White; 25.2% Other; 14.1% Asian; and 3.9% Black. The race distribution of the 9- through 15-year-old girls in the clinical trials was as follows: 60.3% White; 19.3% Other; 13.5% Asian; and 7.0% Black. The race distribution of the 9- through 15-year-old boys in the clinical trials was as follows: 46.6% White; 34.3% Other; 13.3% Asian; and 5.9% Black. The race distribution of the 16- through 26-year-old boys and men in the clinical trials was as follows: 62.1% White; 22.6% Other; 9.8% Asian; and 5.5% Black.

In Study 9 the race distribution of 27- through 45-year-old women was as follows: 97.7% White, 1.6% Asian, 0.3% Other or Multiracial, and 0.5% Black. The race distribution of girls and women 16 through 26

years of age in this study was as follows: 94.6% White, 3.0% Asian, 1.6% Other or Multiracial, and 0.9% Black.

One clinical trial (Study 8) assessed the 2-dose regimen of GARDASIL 9. Study 8 evaluated the immunogenicity of 2 doses of GARDASIL 9 in girls and boys 9 through 14 years of age and 3 doses of GARDASIL 9 in girls 9 through 14 years of age and women 16 through 26 years of age; (N=1,518; 753 girls; 451 boys and 314 women). The mean age for the girls and boys 9 through 14 years of age was 11.5 years; the mean age for girls and women 16 through 26 years of age was 21.0 years. In Study 8, the race distribution was as follows: 61.1% White; 16.3% Asian; 13.3% Other; and 8.9% Black.

14.3 Efficacy – HPV Types 31, 33, 45, 52 and 58 in Girls and Women 16 through 26 Years of Age

Studies Supporting the Efficacy of GARDASIL 9 against HPV Types 31, 33, 45, 52, and 58

The efficacy of GARDASIL 9 in 16- through 26-year-old girls and women was assessed in an active comparator-controlled, double-blind, randomized clinical trial (Study 1) that included a total of 14,204 women (GARDASIL 9 = 7,099; GARDASIL = 7,105) who were enrolled and vaccinated without pre-screening for the presence of HPV infection. Subjects were followed up with a median duration of 40 months (range 0 to 64 months) after the last vaccination.

The primary efficacy evaluation was conducted in the PPE population based on a composite clinical endpoint of HPV 31-, 33-, 45-, 52-, and 58-related cervical cancer, vulvar cancer, vaginal cancer, CIN 2/3 or AIS, VIN 2/3, and VaIN 2/3. Efficacy was further evaluated with the clinical endpoints of HPV 31-, 33-, 45-, 52-, and 58-related CIN 1, vulvar and vaginal disease of any grade, and persistent infection. In addition, the study also evaluated the impact of GARDASIL 9 on the rates of HPV 31-, 33-, 45-, 52-, and 58-related abnormal Papanicolaou (Pap) tests, cervical and external genital biopsy, and definitive therapy [including loop electrosurgical excision procedure (LEEP) and conization]. Efficacy for all endpoints was measured starting after the Month 7 visit.

GARDASIL 9 prevented HPV 31-, 33-, 45-, 52-, and 58-related persistent infection and disease and also reduced the incidence of HPV 31-, 33-, 45-, 52-, and 58-related Pap test abnormalities, cervical and external genital biopsy, and definitive therapy (Table 7).

Table 7: Analysis of Efficacy of GARDASIL 9 against HPV Types 31, 33, 45, 52, and 58 in the PPE* Population of 16- through 26-Year-old Girls and Women (Study 1)

Disease Endpoint	GARDASIL 9 N†=7099		GARDASIL N†=7105		GARDASIL 9 Efficacy % (95% CI)
	n‡	Number of cases	n‡	Number of cases	
HPV 31-, 33-, 45-, 52-, 58-related CIN 2/3, AIS, Cervical Cancer, VIN 2/3, VaIN 2/3, Vulvar Cancer, and Vaginal Cancer	6016	1	6017	30	96.7 (80.9, 99.8)
HPV 31-, 33-, 45-, 52-, 58-related CIN 1	5948	1	5943	69	98.6 (92.4, 99.9)
HPV 31-, 33-, 45-, 52-, 58-related CIN 2/3 or AIS	5948	1	5943	27	96.3 (79.5, 99.8)
HPV 31-, 33-, 45-, 52-, 58-related Vulvar or Vaginal Disease	6009	1	6012	16	93.8 (61.5, 99.7)
HPV 31-, 33-, 45-, 52-, 58-related Persistent Infection ≥6 Months§	5939	26	5953	642	96.2 (94.4, 97.5)
HPV 31-, 33-, 45-, 52-, 58-related Persistent Infection ≥12 Months¶	5939	15	5953	375	96.1 (93.7, 97.9)
HPV 31-, 33-, 45-, 52-, 58-related ASC-US HR-HPV Positive or Worse Pap# Abnormality	5881	35	5882	462	92.6 (89.7, 94.8)
HPV 31-, 33-, 45-, 52-, 58-related Biopsy	6016	7	6017	222	96.9 (93.6, 98.6)
HPV 31-, 33-, 45-, 52-, 58-related Definitive Therapyᵇ	6012	4	6014	32	87.5 (65.7, 96.0)

*The PPE population consisted of individuals who received all three vaccinations within one year of enrollment, did not have major deviations from the study protocol, were naïve (PCR negative and seronegative) to the relevant HPV type(s) (Types 31, 33, 45, 52, and 58) prior to dose 1, and who remained PCR negative to the relevant HPV type(s) through one month post-dose 3 (Month 7); data from Study 1 (NCT00543543).
†N=Number of individuals randomized to the respective vaccination group who received at least one injection
‡n=Number of individuals contributing to the analysis
§Persistent infection detected in samples from two or more consecutive visits at least six months apart
¶Persistent infection detected in samples from two or more consecutive visits over 12 months or longer
#Papanicolaou test
ᵇIncluding loop electrosurgical excision procedure (LEEP) and conization
CI=Confidence Interval
CIN=Cervical Intraepithelial Neoplasia, VIN=Vulvar Intraepithelial Neoplasia, VaIN=Vaginal Intraepithelial Neoplasia, AIS=Adenocarcinoma *In Situ*, ASC-US=Atypical squamous cells of undetermined significance
HR=High Risk

14.4 Effectiveness in Prevention of HPV-Related Oropharyngeal and Other Head and Neck Cancers

The effectiveness of GARDASIL 9 against oropharyngeal and other head and neck cancers caused by HPV types 16, 18, 31, 33, 45, 52 and 58, is based on the effectiveness of GARDASIL and GARDASIL 9 to prevent anogenital disease caused by HPV types covered by the vaccine *[see Clinical Studies (14.1, 14.2, 14.3)]*.

14.5 Immunogenicity of a 3-Dose Regimen

The minimum anti-HPV titer that confers protective efficacy has not been determined.

Type-specific immunoassays (i.e., cLIA) with type-specific standards were used to assess immunogenicity to each vaccine HPV type. These assays measured antibodies against neutralizing epitopes for each HPV type. The scales for these assays are unique to each HPV type; thus, comparisons across types and to other assays are not appropriate. Immunogenicity was measured by (1) the percentage of individuals who were seropositive for antibodies against the relevant vaccine HPV type, and (2) the Geometric Mean Titer (GMT).

Studies Supporting the Effectiveness of GARDASIL 9 against HPV Types 6, 11, 16, and 18

Effectiveness of GARDASIL 9 against persistent infection and disease related to HPV Types 6, 11, 16, or 18 was inferred from non-inferiority comparisons in Study 1 (16- through 26-year-old girls and women) and Study 3 (9- through 15-year-old girls) of GMTs following vaccination with GARDASIL 9 with those following vaccination with GARDASIL. A low number of efficacy endpoint cases related to HPV types 6,

11, 16 and 18 in both vaccination groups precluded a meaningful assessment of efficacy using disease endpoints associated with these HPV types. The primary analyses were conducted in the per-protocol population, which included subjects who received all three vaccinations within one year of enrollment, did not have major deviations from the study protocol, and were HPV-naïve. HPV-naïve individuals were defined as seronegative to the relevant HPV type(s) prior to dose 1 and among female subjects 16 through 26 years of age in Study 1 PCR negative to the relevant HPV type(s) in cervicovaginal specimens prior to dose 1 through Month 7.

Anti-HPV 6, 11, 16 and 18 GMTs at Month 7 for GARDASIL 9 among girls 9 through 15 years of age and young women 16 through 26 years of age were non-inferior to those among the corresponding populations for GARDASIL (Table 8). At least 99.7% of individuals included in the analyses for each HPV type became seropositive by Month 7.

Table 8: Comparison of Immune Responses (Based on cLIA) Between GARDASIL 9 and GARDASIL for HPV Types 6, 11, 16, and 18 in the PPI* Population of 9- through 26-Year-Old Girls and Women (Studies 1 and 3)

Population	GARDASIL 9		GARDASIL		GARDASIL 9/ GARDASIL	
	N† (n‡)	GMT mMU§/mL	N† (n‡)	GMT mMU§/mL	GMT Ratio	(95% CI)¶
Anti-HPV 6						
9- through 15-year-old girls	300 (273)	1679.4	300 (261)	1565.9	1.07	(0.93, 1.23)
16- through 26-year-old girls and women	6792 (3993)	893.1	6795 (3975)	875.2	1.02	(0.99, 1.06)
Anti-HPV 11						
9- through 15-year-old girls	300 (273)	1315.6	300 (261)	1417.3	0.93	(0.80, 1.08)
16- through 26-year-old girls and women	6792 (3995)	666.3	6795 (3982)	830.0	0.80	(0.77, 0.83)
Anti-HPV 16						
9- through 15-year-old girls	300 (276)	6739.5	300 (270)	6887.4	0.97	(0.85, 1.11)
16- through 26-year-old girls and women	6792 (4032)	3131.1	6795 (4062)	3156.6	0.99	(0.96, 1.03)
Anti-HPV 18						
9- through 15-year-old girls	300 (276)	1956.6	300 (269)	1795.6	1.08	(0.91, 1.29)
16- through 26-year-old girls and women	6792 (4539)	804.6	6795 (4541)	678.7	1.19	(1.14, 1.23)

*The PPI population consisted of individuals who received all three vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met predefined criteria for the interval between the Month 6 and Month 7 visit, were naïve (PCR negative [among 16- through 26-year old girls and women] and seronegative) to the relevant HPV type(s) (types 6, 11, 16, and 18) prior to dose 1, and among 16- through 26-year-old girls and women remained PCR negative to the relevant HPV type(s) through one month post-dose 3 (Month 7). The data for 16- through 26-year-old girls and women are from Study 1 (NCT00543543), and the data for 9- through 15-year-old girls are from Study 3 (NCT01304498).

†N=Number of individuals randomized to the respective vaccination group who received at least one injection

‡n=Number of individuals contributing to the analysis

§mMU=milli-Merck Units

¶Demonstration of non-inferiority required that the lower bound of the 95% CI of the GMT ratio be greater than 0.67

CI=Confidence Interval

GMT=Geometric Mean Titer

cLIA=competitive Luminex Immunoassay

Study Supporting the Effectiveness of GARDASIL 9 against Vaccine HPV Types in 9- through 15-Year-Old Girls and Boys

Effectiveness of GARDASIL 9 against persistent infection and disease related to vaccine HPV types in 9- through 15-year-old girls and boys was inferred from non-inferiority comparison conducted in the PPI population in Study 2 of GMTs following vaccination with GARDASIL 9 among 9- through 15-year-old girls and boys with those among 16- through 26-year-old girls and women. Anti-HPV GMTs at Month 7 among 9- through 15-year-old girls and boys were non-inferior to anti-HPV GMTs among 16- through 26-year-old girls and women (Table 9).

Table 9: Comparison of Immune Responses (Based on cLIA) between the PPI* Populations of 16- through 26-Year-Old

Girls and Women, 9- through 15-Year-Old Girls, and 9- through 15-Year-Old Boys for All GARDASIL 9 Vaccine HPV Types (Study 2)

Population	N[†]	n[‡]	GMT mMU[§]/mL	GMT Ratio relative to 16- through 26-year-old girls and women (95% CI)[¶]
Anti-HPV 6				
9- through 15-year-old girls	630	503	1703.1	1.89 (1.68, 2.12)
9- through 15-year-old boys	641	537	2083.4	2.31 (2.06, 2.60)
16- through 26-year-old girls and women	463	328	900.8	1
Anti-HPV 11				
9- through 15-year-old girls	630	503	1291.5	1.83 (1.63, 2.05)
9- through 15-year-old boys	641	537	1486.3	2.10 (1.88, 2.36)
16- through 26-year-old girls and women	463	332	706.6	1
Anti-HPV 16				
9- through 15-year-old girls	630	513	6933.9	1.97 (1.75, 2.21)
9- through 15-year-old boys	641	546	8683.0	2.46 (2.20, 2.76)
16- through 26-year-old girls and women	463	329	3522.6	1
Anti-HPV 18				
9- through 15-year-old girls	630	516	2148.3	2.43 (2.12, 2.79)
9- through 15-year-old boys	641	544	2855.4	3.23 (2.83, 3.70)
16- through 26-year-old girls and women	463	345	882.7	1
Anti-HPV 31				
9- through 15-year-old girls	630	506	1894.7	2.51 (2.21, 2.86)
9- through 15-year-old boys	641	543	2255.3	2.99 (2.63, 3.40)
16- through 26-year-old girls and women	463	340	753.9	1
Anti-HPV 33				
9- through 15-year-old girls	630	518	985.8	2.11 (1.88, 2.37)
9- through 15-year-old boys	641	544	1207.4	2.59 (2.31, 2.90)
16- through 26-year-old girls and women	463	354	466.8	1
Anti-HPV 45				
9- through 15-year-old girls	630	518	707.7	2.60 (2.25, 3.00)
9- through 15-year-old boys	641	547	912.1	3.35 (2.90, 3.87)
16- through 26-year-old girls and women	463	368	272.2	1
Anti-HPV 52				
9- through 15-year-old girls	630	517	962.2	2.21 (1.96, 2.49)
9- through 15-year-old boys	641	545	1055.5	2.52 (2.22, 2.84)
16- through 26-year-old girls and women	463	337	419.6	1
Anti-HPV 58				
9- through 15-year-old girls	630	516	1288.0	2.18 (1.94, 2.46)
9- through 15-year-old boys	641	544	1593.3	2.70 (2.40, 3.03)
16- through 26-year-old girls and women	463	332	590.5	1

*The PPI population consisted of individuals who received all three vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met predefined criteria for the interval between the Month 6 and Month 7 visit, were naïve (PCR negative [among 16- through 26-year old girls and women] and seronegative) to the relevant HPV type(s) prior to dose 1 and among 16- through 26-year-old girls and women remained PCR negative to the relevant HPV types through one month post-dose 3 (Month 7). The data are from Study 2 (NCT00943722).

[†]N=Number of individuals randomized to the respective vaccination group who received at least one injection

[‡]n=Number of individuals contributing to the analysis

[§]mMU=milli-Merck Units

[¶]Demonstration of non-inferiority required that the lower bound of the 95% CI of the GMT ratio be greater than 0.67

cLIA=competitive Luminex Immunoassay

CI=Confidence Interval

GMT=Geometric Mean Titer

Study Supporting the Effectiveness of GARDASIL 9 against Vaccine HPV Types in 16- through 26-Year-Old Boys and Men

Effectiveness of GARDASIL 9 against persistent infection and disease related to vaccine HPV types in 16- through 26-year-old boys and men was inferred from non-inferiority comparison conducted in the PPI population in Study 7 of GMTs following vaccination with GARDASIL 9 among 16- through 26-year-old HM with those among 16- through 26-year-old girls and women. Anti-HPV GMTs at Month 7 among 16- through 26-year-old HM were non-inferior to anti-HPV GMTs among 16- through 26-year-old girls and women (Table 10). Study 7 also enrolled 313 16- through 26-year-old HIV-negative MSM. At Month 7, anti-HPV GMT ratios for MSM relative to HM ranged from 0.6 to 0.8, depending on HPV type. The GMT ratios for MSM relative to HM were generally similar to those previously observed in clinical trials with GARDASIL.

Table 10: Comparison of Immune Responses (Based on cLIA) between the PPI* Populations of 16- through 26-Year-Old Girls and Women and 16- through 26-Year-Old Boys and Men Self-Identified as Heterosexual (HM) for All GARDASIL 9 Vaccine HPV Types (Study 7)

Population	N†	n‡	GMT mMU§/mL	GMT Ratio relative to 16- through 26-year-old girls and women (95% CI)¶
Anti-HPV 6				
16- through 26-year-old HM	1103	847	782.0	1.11 (1.02, 1.21)
16- through 26-year-old girls and women	1099	708	703.9	1
Anti-HPV 11				
16- through 26-year-old HM	1103	851	616.7	1.09 (1.00, 1.19)
16- through 26-year-old girls and women	1099	712	564.9	1
Anti-HPV 16				
16- through 26-year-old HM	1103	899	3346.0	1.20 (1.10, 1.30)
16- through 26-year-old girls and women	1099	781	2788.3	1
Anti-HPV 18				
16- through 26-year-old HM	1103	906	808.2	1.19 (1.08, 1.31)
16- through 26-year-old girls and women	1099	831	679.8	1
Anti-HPV 31				
16- through 26-year-old HM	1103	908	708.5	1.24 (1.13, 1.37)
16- through 26-year-old girls and women	1099	826	570.1	1
Anti-HPV 33				
16- through 26-year-old HM	1103	901	384.8	1.19 (1.10, 1.30)
16- through 26-year-old girls and women	1099	853	322.0	1
Anti-HPV 45				
16- through 26-year-old HM	1103	909	235.6	1.27 (1.14, 1.41)
16- through 26-year-old girls and women	1099	871	185.7	1
Anti-HPV 52				
16- through 26-year-old HM	1103	907	386.8	1.15 (1.05, 1.26)
16- through 26-year-old girls and women	1099	849	335.2	1
Anti-HPV 58				
16- through 26-year-old HM	1103	897	509.8	1.25 (1.14, 1.36)
16- through 26-year-old girls and women	1099	839	409.3	1

*The PPI population consisted of individuals who received all three vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met predefined criteria for the interval between the Month 6 and Month 7 visit, and were seronegative to the relevant HPV type(s) (types 6, 11, 16, 18, 31, 33, 45, 52, and 58) prior to dose 1. The data are from Study 7 (NCT01651949).

†Number of individuals randomized to the respective vaccination group who received at least one injection

‡Number of individuals contributing to the analysis

§mMU=milli-Merck Units

¶Demonstration of non-inferiority required that the lower bound of the 95% CI of the GMT ratio be greater than 0.67

cLIA=competitive Luminex Immunoassay

CI=Confidence Interval

GMT=Geometric Mean Titer

Study Supporting the Effectiveness of GARDASIL 9 against Vaccine HPV Types in 27- through 45-Year-Old Women

Effectiveness of GARDASIL 9 against persistent infection and disease related to vaccine HPV types in 27- through 45-year-old women was supported by immunobridging comparisons conducted in the PPI population in Study 9. In Study 9, the GMT ratios of anti-HPV responses at Month 7 among 27- through

45-year-old women relative to anti-HPV responses among 16- through 26-year-old girls and women met the success criteria of having the lower bound of the 95% CI of the GMT ratios greater than 0.50 for HPV 16, 18, 31, 33, 45, 52, and 58 (Table 11).

Table 11: Comparison of Immune Responses (Based on cLIA) Between the PPI* Populations of 27- through 45 Year-Old Women and 16- through 26-Year-Old Girls and Women for GARDASIL 9 Vaccine HPV Types (Study 9)

Population	N[†]	n[‡]	GMT mMU[§]/mL	GMT Ratio relative to 16-through 26-year-old girls and women (95% CI)[¶]
Anti-HPV 6				
27- through 45-year-old women	640	448	638.4	N.D[#]
16- through 26-year-old girls and women	570	421	787.8	N.D[#]
Anti-HPV 11				
27- through 45-year-old women	640	448	453.5	N.D[#]
16- through 26-year-old girls and women	570	421	598.7	N.D[#]
Anti-HPV 16				
27- through 45-year-old women	640	448	2,147.5	0.70 (0.63, 0.77)
16- through 26-year-old girls and women	570	436	3,075.8	1
Anti-HPV 18				
27- through 45-year-old women	640	471	532.1	0.71 (0.64, 0.80)
16- through 26-year-old girls and women	570	421	744.5	1
Anti-HPV 31				
27- through 45-year-old women	640	488	395.7	0.66 (0.60, 0.74)
16- through 26-year-old girls and women	570	447	596.1	1
Anti-HPV 33				
27- through 45-year-old women	640	493	259.0	0.73 (0.67, 0.80)
16- through 26-year-old girls and women	570	457	354.5	1
Anti-HPV 45				
27- through 45-year-old women	640	515	145.6	0.68 (0.60, 0.76)
16- through 26-year-old girls and women	570	470	214.9	1
Anti-HPV 52				
27- through 45-year-old women	640	496	244.7	0.71 (0.64, 0.78)
16- through 26-year-old girls and women	570	456	346.5	1
Anti-HPV 58				
27- through 45-year-old women	640	478	296.4	0.69 (0.63, 0.76)
16- through 26-year-old girls and women	570	451	428.0	1

*The PPI population consisted of individuals who received all 3 vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met predefined criteria for the interval between the Month 6 and Month 7 visit, and were seronegative to the relevant HPV type(s) (types 16, 18, 31, 33, 45, 52, and 58) prior to dose 1. The data are from Study 9 (NCT03158220).

[†]Number of individuals randomized to the respective vaccination group who received at least 1 injection

[‡]Number of individuals contributing to the analysis

[§]mMU=milli-Merck Units

[¶]Immunobridging required that the lower bound of the 95% CI of the GMT ratio be greater than 0.50

[#]N.D=Not Determined. GMT ratios were not calculated because immunobridging comparison was not specified in the study protocol for HPV types 6 and 11.

cLIA=Competitive Luminex Immunoassay

CI=Confidence Interval

GMT=Geometric Mean Titers

Immune Response to GARDASIL 9 across All Clinical Trials

Across all clinical trials, at least 99.2% of individuals included in the analyses for each of the nine vaccine HPV types became seropositive by Month 7. Anti-HPV GMTs at Month 7 among 9- through 15-year-old girls and boys and 16- through 26-year-old boys and men were comparable to anti-HPV responses among 16- through 26-year-old girls and women in the combined database of immunogenicity studies for GARDASIL 9.

Persistence of Immune Response to GARDASIL 9

The duration of immunity following a 3-dose schedule of vaccination with GARDASIL 9 has not been established. The peak anti-HPV GMTs for each vaccine HPV type occurred at Month 7. Proportions of individuals who remained seropositive to each vaccine HPV type at Month 24 were similar to the corresponding seropositive proportions at Month 7.

Administration of GARDASIL 9 to Individuals Previously Vaccinated with GARDASIL

Study 4 evaluated the immunogenicity of 3 doses of GARDASIL 9 in 921 girls and women (12 through 26 years of age) who had previously been vaccinated with 3 doses of GARDASIL. Prior to enrollment in the study, over 99% of subjects had received three injections of GARDASIL within a one year period. The time interval between the last injection of GARDASIL and the first injection of GARDASIL 9 ranged from approximately 12 to 36 months.

Seropositivity to HPV Types 6, 11, 16, 18, 31, 33, 45, 52, and 58 in the per protocol population ranged from 98.3 to 100% by Month 7 in individuals who received GARDASIL 9. The anti-HPV 31, 33, 45, 52 and 58 GMTs for the population previously vaccinated with GARDASIL were 25-63% of the GMTs in the combined populations from Studies 1, 2, 3, and 5, who had not previously received GARDASIL, although the clinical relevance of these differences is unknown. Efficacy of GARDASIL 9 in preventing infection and disease related to HPV Types 31, 33, 45, 52, and 58 in individuals previously vaccinated with GARDASIL has not been assessed.

Concomitant Use of Hormonal Contraceptives

Among 7,269 female recipients of GARDASIL 9 (16 through 26 years of age), 60.2% used hormonal contraceptives during the vaccination period of clinical studies 1 and 2. Use of hormonal contraceptives did not appear to affect the type specific immune responses to GARDASIL 9.

14.6 Immune Responses to GARDASIL 9 Using a 2-Dose Regimen in Individuals 9 through 14 Years of Age

Effectiveness of GARDASIL 9 against persistent infection and disease related to vaccine HPV types in 9- through 14-year-old girls and boys who received a 2-dose regimen was inferred from non-inferiority comparison conducted in the PPI population in Study 8 of GMTs following vaccination with GARDASIL 9 among 9- through 14-year-old girls and boys who received a 2-dose regimen (at 0, 6 months or 0, 12 months) with those among 16- through 26-year-old girls and women who received a 3-dose regimen (at 0, 2, 6 months). Anti-HPV GMTs at one month after the last dose among 9- through 14-year-old girls and boys who received 2 doses of GARDASIL 9 were non-inferior to anti-HPV GMTs among 16- through 26-year-old girls and women who received 3 doses of GARDASIL 9 (Table 12).

One month following the last dose of the assigned regimen, between 97.9% and 100% of subjects across all groups became seropositive for antibodies against the 9 vaccine HPV types (Table 12).

In the same study, in girls and boys 9 through 14 years old, GMTs at one month after the last vaccine dose were numerically lower for some vaccine types after a 2-dose schedule than in girls 9 through 14 years old after a 3-dose schedule (HPV types 18, 31, 45, and 52 after 0, 6 months and HPV type 45 after 0, 12 months; Table 12). The clinical relevance of these findings is unknown.

Duration of immunity of a 2-dose schedule of GARDASIL 9 has not been established.

Table 12: Summary of Anti-HPV cLIA Geometric Mean Titers in the PPI* Population at One Month After the Last Vaccine Dose Among Subjects Who Received 2 Doses† or 3 Doses† of GARDASIL 9 (Study 8)

Population (Regimen)	N	n	GMT mMU‡/mL	GMT Ratio relative to 3-dose regimen in 16- through 26-year-old girls and women (95% CI)
Anti-HPV 6				
9- to 14-year-old girls (0, 6)†	301	258	1657.9	2.15 (1.83, 2.53)§
9- to 14-year-old boys (0, 6)†	301	263	1557.4	2.02 (1.73, 2.36)§
9- to 14-year-old girls and boys (0, 12)†	300	257	2678.8	3.47 (2.93, 4.11)§
9- to 14-year-old girls (0, 2, 6)†	300	254	1496.1	1.94 (1.65, 2.29)¶
16- to 26-year-old women (0, 2, 6)†	314	238	770.9	1
Anti-HPV 11				
9- to 14-year-old girls (0, 6)†	301	258	1388.9	2.39 (2.03, 2.82)§
9- to 14-year-old boys (0, 6)†	301	264	1423.9	2.45 (2.09, 2.88)§
9- to 14-year-old girls and boys (0, 12)†	300	257	2941.8	5.07 (4.32, 5.94)§
9- to 14-year-old girls (0, 2, 6)†	300	254	1306.3	2.25 (1.90, 2.66)¶
16- to 26-year-old women (0, 2, 6)†	314	238	580.5	1
Anti-HPV 16				
9- to 14-year-old girls (0, 6)†	301	272	8004.9	2.54 (2.14, 3.00)§
9- to 14-year-old boys (0, 6)†	301	273	8474.8	2.69 (2.29, 3.15)§
9- to 14-year-old girls and boys (0, 12)†	300	264	14329.3	4.54 (3.84, 5.37)§
9- to 14-year-old girls (0, 2, 6)†	300	269	6996.0	2.22 (1.89, 2.61)¶
16- to 26-year-old women (0, 2, 6)†	314	249	3154.0	1
Anti-HPV 18				
9- to 14-year-old girls (0, 6)†	301	272	1872.8	2.46 (2.05, 2.96)§
9- to 14-year-old boys (0, 6)†	301	272	1860.9	2.44 (2.04, 2.92)§
9- to 14-year-old girls and boys (0, 12)†	300	266	2810.4	3.69 (3.06, 4.45)§
9- to 14-year-old girls (0, 2, 6)†	300	270	2049.3	2.69 (2.24, 3.24)¶
16- to 26-year-old women (0, 2, 6)†	314	267	761.5	1
Anti-HPV 31				
9- to 14-year-old girls (0, 6)†	301	272	1436.3	2.51 (2.10, 3.00)§
9- to 14-year-old boys (0, 6)†	301	271	1498.2	2.62 (2.20, 3.12)§
9- to 14-year-old girls and boys (0, 12)†	300	268	2117.5	3.70 (3.08, 4.45)§
9- to 14-year-old girls (0, 2, 6)†	300	271	1748.3	3.06 (2.54, 3.67)¶
16- to 26-year-old women (0, 2, 6)†	314	264	572.1	1
Anti-HPV 33				
9- to 14-year-old girls (0, 6)†	301	273	1030.0	2.96 (2.50, 3.50)§
9- to 14-year-old boys (0, 6)†	301	271	1040.0	2.99 (2.55, 3.50)§
9- to 14-year-old girls and boys (0, 12)†	300	269	2197.5	6.31 (5.36, 7.43)§
9- to 14-year-old girls (0, 2, 6)†	300	275	796.4	2.29 (1.95, 2.68)¶
16- to 26-year-old women (0, 2, 6)†	314	279	348.1	1
Anti-HPV 45				
9- to 14-year-old girls (0, 6)†	301	274	357.6	1.67 (1.38, 2.03)§
9- to 14-year-old boys (0, 6)†	301	273	352.3	1.65 (1.37, 1.99)§
9- to 14-year-old girls and boys (0, 12)†	300	268	417.7	1.96 (1.61, 2.37)§
9- to 14-year-old girls (0, 2, 6)†	300	275	661.7	3.10 (2.54, 3.77)¶
16- to 26-year-old women (0, 2, 6)†	314	280	213.6	1
Anti-HPV 52				
9- to 14-year-old girls (0, 6)†	301	272	581.1	1.60 (1.36, 1.87)§
9- to 14-year-old boys (0, 6)†	301	273	640.4	1.76 (1.51, 2.05)§
9- to 14-year-old girls and boys (0, 12)†	300	268	1123.4	3.08 (2.64, 3.61)§
9- to 14-year-old girls (0, 2, 6)†	300	275	909.9	2.50 (2.12, 2.95)¶
16- to 26-year-old women (0, 2, 6)†	314	271	364.2	1
Anti-HPV 58				
9- to 14-year-old girls (0, 6)†	301	270	1251.2	2.55 (2.15, 3.01)§
9- to 14-year-old boys (0, 6)†	301	270	1325.7	2.70 (2.30, 3.16)§
9- to 14-year-old girls and boys (0, 12)†	300	265	2444.6	4.98 (4.23, 5.86)§
9- to 14-year-old girls (0, 2, 6)†	300	273	1229.3	2.50 (2.11, 2.97)¶

16- to 26-year-old women (0, 2, 6)†	314	261	491.1	1

*The PPI population consisted of individuals who received all assigned vaccinations within pre-defined day ranges, did not have major deviations from the study protocol, met predefined criteria for the interval between the last vaccination dose and blood collection for immunogenicity assessment, and were seronegative to the relevant HPV type(s) (types 6, 11, 16, 18, 31, 33, 45, 52, and 58) prior to dose 1.

†2-dose regimen (0, 6): vaccination at Day 1 and Month 6; 2-dose regimen (0, 12): vaccination at Day 1 and Month 12; 3-dose regimen (0, 2, 6): vaccination at Day 1, Month 2, and Month 6. The data are from Study 8 (NCT01984697).

‡mMU=milli-Merck Units

§Demonstration of non-inferiority required that the lower bound of the 95% CI of the GMT ratio be greater than 0.67

¶Exploratory analysis; criterion for non-inferiority was not pre-specified

N = Number of individuals randomized to the respective vaccination group who received at least 1 injection

n = Number of individuals contributing to the analysis

CI=Confidence Interval

cLIA=competitive Luminex Immunoassay

GMT=Geometric Mean Titer

14.7 Studies with Menactra and Adacel

In Study 5, the safety and immunogenicity of co-administration of GARDASIL 9 with Menactra [Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine] and Adacel [Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap)] (same visit, injections at separate sites) were evaluated in 1,237 boys and girls 11 through 15 years of age at enrollment.

One group received GARDASIL 9 in one limb and both Menactra and Adacel, as separate injections, in the opposite limb concomitantly on Day 1 (n = 619). The second group received the first dose of GARDASIL 9 on Day 1 in one limb then Menactra and Adacel, as separate injections, at Month 1 in the opposite limb (n = 618). Subjects in both vaccination groups received the second dose of GARDASIL 9 at Month 2 and the third dose at Month 6. Immunogenicity was assessed for all vaccines one month post vaccination (one dose for Menactra and Adacel and three doses for GARDASIL 9).

Assessments of post-vaccination immune responses included type-specific antibody GMTs for each of the vaccine HPV types at four weeks following the last dose of GARDASIL 9; GMTs for anti-filamentous hemagglutinin, anti-pertactin, and anti-fimbrial antibodies at four weeks following Adacel; percentage of subjects with anti-tetanus toxin and anti-diphtheria toxin antibody concentrations ≥0.1 IU/mL at four weeks following Adacel; and percentage of subjects with ≥4-fold rise from pre-vaccination baseline in antibody titers against *N. meningitidis* serogroups A, C, Y, and W-135 at four weeks following Menactra. Based on these measures, concomitant administration of GARDASIL 9 with Menactra and Adacel did not interfere with the antibody responses to any of the vaccines when compared with non-concomitant administration of GARDASIL 9 with Menactra and Adacel.

15 REFERENCES

1. Study 1 NCT00543543
2. Study 2 NCT00943722
3. Study 3 NCT01304498
4. Study 4 NCT01047345
5. Study 5 NCT00988884
6. Study 6 NCT01073293
7. Study 7 NCT01651949
8. Study 8 NCT01984697
9. Study A NCT01432574
10. Study B NCT00090285
11. Study 9 NCT03158220

16 HOW SUPPLIED/STORAGE AND HANDLING

GARDASIL 9 is supplied in vials and syringes.
Carton of ten 0.5-mL single-dose vials. NDC 0006-4119-03
Carton of ten 0.5-mL single-dose prefilled Luer Lock syringes with tip caps. NDC 0006-4121-02
Store refrigerated at 2 to 8°C (36 to 46°F). Do not freeze. Protect from light.

GARDASIL 9 should be administered as soon as possible after being removed from refrigeration. GARDASIL 9 can be administered provided total (cumulative multiple excursion) time out of refrigeration (at temperatures between 8°C and 25°C) does not exceed 72 hours. Cumulative multiple excursions between 0°C and 2°C are also permitted as long as the total time between 0°C and 2°C does not exceed 72 hours. These are not, however, recommendations for storage.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Inform the patient, parent, or guardian:

- Vaccination does not eliminate the necessity for women to continue to undergo recommended cervical cancer screening. Women who receive GARDASIL 9 should continue to undergo cervical cancer screening per standard of care.
- Recipients of GARDASIL 9 should not discontinue anal cancer screening if it has been recommended by a health care provider.
- GARDASIL 9 has not been demonstrated to provide protection against disease from vaccine and non-vaccine HPV types to which a person has previously been exposed through sexual activity.
- Since syncope has been reported following HPV vaccination sometimes resulting in falling with injury, observation for 15 minutes after administration is recommended.
- Vaccine information is required to be given with each vaccination to the patient, parent, or guardian.
- Provide information regarding benefits and risks associated with vaccination.
- Safety and effectiveness of GARDASIL 9 have not been established in pregnant women. A pregnancy registry is available. Women exposed to GARDASIL 9 around the time of conception or during pregnancy are encouraged to register by calling 1-800-986-8999. *[See Use in Specific Populations (8.1).]*
- It is important to complete the full vaccination series unless contraindicated.
- Report any adverse reactions to their health care provider.

Manuf. and Dist. by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

For patent information: www.merck.com/product/patent/home.html

uspi-v503-i-2108r013

Influenza

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use AFLURIA® QUADRIVALENT safely and effectively. See full prescribing information for AFLURIA QUADRIVALENT.

AFLURIA QUADRIVALENT, Influenza Vaccine
Suspension for Intramuscular Injection
2021-2022 Formula
Initial U.S. Approval (AFLURIA QUADRIVALENT): 2016

----------RECENT MAJOR CHANGES----------
Dosage and Administration (2) 08/2020

----------INDICATIONS AND USAGE----------
- AFLURIA QUADRIVALENT is an inactivated influenza vaccine indicated for active immunization against influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. (1)
- AFLURIA QUADRIVALENT is approved for use in persons 6 months of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------

For intramuscular injection only, by needle and syringe (6 months and older) or by PharmaJet®Stratis® Needle-Free Injection System (18 through 64 years). (2)

Age	Dose	Schedule
6 months through 35 months	One or two doses[a], 0.25 mL each	If 2 doses, administer at least 1 month apart
36 months through 8 years	One or two doses[a], 0.5 mL each	If 2 doses, administer at least 1 month apart
9 years and older	One dose, 0.5 mL	Not Applicable

[a]1 or 2 doses depends on vaccination history as per Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines. (2)

----------DOSAGE FORMS AND STRENGTHS----------
AFLURIA QUADRIVALENT is a suspension for injection supplied in three presentations:
- 0.25 mL pre-filled syringe (single dose) (3, 11)
- 0.5 mL pre-filled syringe (single dose) (3, 11)
- 5 mL multi-dose vial (0.25 mL or 0.5 mL) (3, 11)

----------CONTRAINDICATIONS----------
- Severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine including egg protein, or to a previous dose of any influenza vaccine. (4, 11)

----------WARNINGS AND PRECAUTIONS----------
- If Guillain-Barré Syndrome (GBS) has occurred within 6 weeks of previous influenza vaccination, the decision to give AFLURIA QUADRIVALENT should be based on careful consideration of the potential benefits and risks. (5.1)
- Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine. (5.2)

----------ADVERSE REACTIONS----------
AFLURIA QUADRIVALENT administered by needle and syringe:
- In adults 18 through 64 years, the most commonly reported injection-site adverse reaction was pain (≥ 40%). The most common systemic adverse events were myalgia and headache (≥ 20%). (6.1)
- In adults 65 years of age and older, the most commonly reported injection-site adverse reaction was pain (≥ 20%). The most common systemic adverse event was myalgia (≥ 10%). (6.1)
- In children 5 through 8 years, the most commonly reported injection-site adverse reactions were pain (≥ 50%), redness and swelling (≥ 10%). The most common systemic adverse event was headache (≥ 10%). (6.1)
- In children 9 through 17 years, the most commonly reported injection-site adverse reactions were pain (≥ 50%), redness and swelling (≥ 10%). The most common systemic adverse events were headache, myalgia, and malaise and fatigue (≥ 10%). (6.1)
- In children 6 months through 35 months of age, the most commonly reported injection-site reactions were pain and redness (≥ 20%). The most common systemic adverse events were irritability (≥ 30%), diarrhea and loss of appetite (≥ 20%). (6.1)
- In children 36 through 59 months of age, the most commonly reported injection site reactions were pain (≥ 30%) and redness (≥ 20%). The most commonly reported systemic adverse events were malaise and fatigue, and diarrhea (≥ 10%). (6.1)

AFLURIA (trivalent formulation) administered by the PharmaJet Stratis Needle-Free Injection System:
- In adults 18 through 64 years of age, the most commonly reported injection-site adverse reactions were tenderness (≥ 80%), swelling, pain, redness (≥ 60%), itching (≥ 20%) and bruising (≥ 10%). The most common systemic adverse events were myalgia, malaise (≥ 30%), and headache (≥ 20%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Seqirus USA Inc. at 1-855-358-8966 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS----------
- The safety and effectiveness of AFLURIA QUADRIVALENT in persons less than 6 months of age have not been established. (8.4)
- Antibody responses were lower in geriatric subjects than in younger adults. (8.5)
- Pregnancy: There is a pregnancy exposure registry that monitors outcomes in women exposed to AFLURIA QUADRIVALENT during pregnancy. Enroll in the pregnancy registry by calling 1-855-358-8966 or sending an email to us.medicalinformation@seqirus.com. (8.1).

See 17 for PATIENT COUNSELING INFORMATION. **Revised: 07/2021**

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

AFLURIA® QUADRIVALENT is an inactivated influenza vaccine indicated for active immunization against influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine.

AFLURIA QUADRIVALENT is approved for use in persons 6 months of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular (IM) use only.

- By needle and syringe (6 months of age and older)
- By PharmaJet® Stratis® Needle-Free Injection System (18 through 64 years of age)

The dose and schedule for AFLURIA QUADRIVALENT are presented in Table 1.

Table 1: AFLURIA QUADRIVALENT Dosage and Schedule

Age	Dose	Schedule
6 months through 35 months	One or two doses[a], 0.25 mL each	If 2 doses, administer at least 1 month apart
36 months through 8 years	One or two doses[a], 0.5 mL each	If 2 doses, administer at least 1 month apart
9 years and older	One dose, 0.5mL	Not Applicable

[a]1 or 2 doses depends on vaccination history as per Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines.

Immediately before use, shake thoroughly and inspect visually. Parenteral drug products should be inspected visually for foreign particulate matter and discoloration prior to administration, whenever suspension and container permit. If either of these conditions exists, the vaccine should not be administered.

When using the single-dose pre-filled syringe, shake the syringe thoroughly and administer the dose immediately.

When using the multi-dose vial, shake the vial thoroughly before withdrawing each dose, and administer the dose immediately. The number of needle punctures should not exceed 20 per multi-dose vial.

- Needle and Syringe: Draw up the exact dose using a separate sterile needle and syringe for each individual patient. It is recommended that small syringes (0.5 mL or 1 mL) be used to minimize any product loss.
- PharmaJet Stratis Needle-Free Injection System: For instructions on withdrawal of a 0.5 mL dose and use of the PharmaJet Stratis Needle-Free Injection System, refer to the Instructions For Use for the PharmaJet Stratis Needle-Free Injection System.

The preferred sites for intramuscular injection are the anterolateral aspect of the thigh in infants 6 months through 11 months of age, the anterolateral aspect of the thigh (or the deltoid muscle of the upper arm if muscle mass is adequate) in persons 12 months through 35 months of age, or the deltoid muscle of the upper arm in persons ≥ 36 months of age.

3 DOSAGE FORMS AND STRENGTHS

AFLURIA QUADRIVALENT is a sterile suspension for intramuscular injection *(see Description [11])*.

AFLURIA QUADRIVALENT is supplied in three presentations:

- 0.25 mL pre-filled syringe (single dose, for persons 6 months through 35 months of age)
- 0.5 mL pre-filled syringe (single dose, for persons 36 months of age and older)
- 5 mL multi-dose vial (for persons 6 months of age and older)

4 CONTRAINDICATIONS

AFLURIA QUADRIVALENT is contraindicated in individuals with known severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine including egg protein, or to a previous dose of any influenza vaccine (*see Description [11]*).

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré Syndrome (GBS) has occurred within 6 weeks of previous influenza vaccination, the decision to give AFLURIA QUADRIVALENT should be based on careful consideration of the potential benefits and risks.

The 1976 swine influenza vaccine was associated with an increased frequency of GBS. Evidence for a causal relation of GBS with subsequent vaccines prepared from other influenza viruses is unclear. If influenza vaccine does pose a risk, it is probably slightly more than one additional case per 1 million persons vaccinated.

5.2 Preventing and Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

5.3 Altered Immunocompetence

If AFLURIA QUADRIVALENT is administered to immunocompromised persons, including those receiving immunosuppressive therapy, the immune response may be diminished.

5.4 Limitations of Vaccine Effectiveness

Vaccination with AFLURIA QUADRIVALENT may not protect all individuals.

6 ADVERSE REACTIONS

In adults 18 through 64 years of age, the most commonly reported injection-site adverse reaction observed in clinical studies with AFLURIA QUADRIVALENT administered by needle and syringe was pain (≥ 40%). The most common systemic adverse events observed were myalgia and headache (≥ 20%).

In adults 65 years of age and older, the most commonly reported injection-site adverse reaction observed in clinical studies with AFLURIA QUADRIVALENT administered by needle and syringe was pain (≥ 20%). The most common systemic adverse event observed was myalgia (≥ 10%).

The safety experience with AFLURIA (trivalent formulation) is relevant to AFLURIA QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions (*see Description [11]*).

In adults 18 through 64 years of age, the most commonly reported injection-site adverse reactions observed in a clinical study with AFLURIA (trivalent formulation) using the PharmaJet Stratis Needle-Free Injection System were tenderness (≥ 80%), swelling, pain, redness (≥ 60%), itching (≥ 20%) and bruising (≥ 10%). The most common systemic adverse events were myalgia, malaise (≥ 30%) and headache (≥ 20%).

In children 5 through 8 years, the most commonly reported injection-site adverse reactions when AFLURIA QUADRIVALENT was administered by needle and syringe were pain (≥ 50%) and redness and swelling (≥ 10%). The most common systemic adverse event was headache (≥ 10%).

In children 9 through 17 years, the most commonly reported injection-site adverse reactions when AFLURIA QUADRIVALENT was administered by needle and syringe were pain (≥ 50%) and redness and swelling (≥ 10%). The most common systemic adverse events were headache, myalgia, and malaise and fatigue (≥ 10%).

In children 6 months through 35 months of age, the most frequently reported injection site reactions in the clinical study with AFLURIA QUADRIVALENT administered by needle and syringe were pain and redness (≥ 20%). The most common systemic adverse events were irritability (≥ 30%), diarrhea and loss of appetite (≥ 20%).

In children 36 through 59 months of age, the most commonly reported injection site reactions were pain (≥ 30%) and redness (≥ 20%). The most commonly reported systemic adverse events were malaise and fatigue, and diarrhea (≥ 10%).

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a vaccine cannot be directly compared to rates in the clinical studies of another vaccine and may not reflect the rates observed in clinical practice.

Adults

Clinical safety data for AFLURIA QUADRIVALENT in adults have been collected in one clinical trial, Study 1, a randomized, double-blind, active-controlled trial conducted in the U.S. in 3449 subjects ages 18 years and older. Subjects in the safety population received one dose of either AFLURIA QUADRIVALENT (N=1721) or one of two formulations of comparator trivalent influenza vaccine (AFLURIA, TIV-1 N=864 or TIV-2 N=864) each containing an influenza type B virus that corresponded to one of the two B viruses in AFLURIA QUADRIVALENT (a type B virus of the Yamagata lineage or a type B virus of the Victoria lineage), respectively. The mean age of the population was 58 years, 57% were female, and racial groups consisted of 82% White, 16% Black, and 2% other; 5% of subjects were Hispanic/Latino. The age sub-groups were 18 through 64 years and 65 years and older with mean ages of 43 years and 73 years, respectively. In this study, AFLURIA QUADRIVALENT and comparator trivalent influenza vaccines were administered by needle and syringe (*see Clinical Studies [14]*).

Local (injection-site) adverse reactions and systemic adverse events were solicited for 7 days post-vaccination (Table 2). Injection site cellulitis, cellulitis-like reactions (defined as concurrent Grade 3 pain, redness, and swelling/lump), and Grade 3 swelling/lump were monitored for 28 days post-vaccination. Unsolicited adverse events were collected for 28 days post-vaccination. Serious adverse events (SAEs), including deaths, were collected for 180 days post-vaccination.

Table 2: Proportion of Subjects Per Age Cohort with Any Solicited Local Adverse Reactions or Systemic Adverse Events within 7 Days after Administration of AFLURIA QUADRIVALENT or Trivalent Influenza Vaccine (Study 1)[a]

	Percentage (%) [b] of Subjects in each Age Cohort Reporting an Event											
	Subjects 18 through 64 years						Subjects ≥ 65 years					
	AFLURIA Quadrivalent N= 854 [c]		TIV-1 N= 428 [c]		TIV-2 N= 430 [c]		AFLURIA Quadrivalent N= 867 [c]		TIV-1 N= 436 [c]		TIV-2 N= 434 [c]	
	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3
Local Adverse Reactions [d]												
Pain	47.9	0.7	43.7	1.4	50.7	1.2	24.6	0.1	22.7	0	21.0	0.2
Swelling/ Lump	3.7	0.1	2.3	0	3.5	0.2	3.2	0.5	1.8	0	1.6	0
Redness	2.9	0	2.8	0	2.8	0	4.2	0.3	2.1	0	2.5	0.2
Systemic Adverse Events [e]												
Myalgia (muscle ache)	25.5	1.9	23.4	1.4	24.2	1.2	12.7	0.3	14.0	0.7	12.2	0.5
Head-ache	21.7	1.7	15.2	0.9	19.1	1.2	8.4	0	7.1	0.2	7.8	0.7
Malaise	8.9	0.7	9.1	0	9.3	0.7	4.4	0.5	5.0	0.2	5.1	0.2
Nausea	6.9	0.6	7.7	0.5	6.3	1.2	1.6	0	1.8	0	2.1	0.2
Chills	4.8	0.6	4.4	0.2	4.7	0.5	2.0	0	2.1	0.5	1.4	0.2
Vomiting	1.5	0.4	0.9	0	2.3	0.7	0.5	0.1	0	0	0.7	0.2
Fever	1.1	0.4	0.9	0	0.5	0	0.2	0	0.9	0	0.5	0.2

Abbreviations: Gr 3, Grade 3.

[a] NCT02214225

[b] Proportion of subjects reporting each solicited local adverse reaction or systemic adverse event by study vaccine group based on the number of subjects contributing any follow up safety information for at least one data value of an individual sign/symptom.

[c] N = number of subjects in the Safety Population for each study vaccine group.

[d] Local adverse reactions: Grade 3 pain is that which prevents daily activity; Swelling/Lump and redness: any = ≥ 20mm diameter, Grade 3 = ≥ 100mm diameter.

[e] Systemic adverse events: Fever: any = ≥ 100.4°F (Oral), Grade 3 = ≥ 102.2°F (Oral); Grade 3 for all other adverse events is that which prevents daily activity.

In the 28 days following vaccination, no subject experienced cellulitis or a cellulitis-like reaction. All Grade 3 swelling/lump reactions began within 7 days of vaccination and are included in Table 2.

In the 28 days following vaccination, 20.5%, 20.1%, and 20.7% of adults 18 through 64 years and 20.3%, 24.1%, and 20.0% of adults ≥ 65 years who received AFLURIA QUADRIVALENT, TIV-1, and TIV-2, respectively, reported unsolicited adverse events. Rates of individual events were similar between treatment groups, and most events were mild to moderate in severity.

In the 180 days following vaccination, 2.3%, 1.6%, and 1.5% of all subjects who received AFLURIA QUADRIVALENT, TIV-1, and TIV-2, respectively, experienced SAEs, including six deaths, five in the AFLURIA QUADRIVALENT group and one in the TIV-2 group. The majority of SAEs occurred after Study Day 28 and in subjects ≥ 65 years of age who had co-morbid illnesses. No SAEs or deaths appeared related to the study vaccines.

Safety information has also been collected in a clinical study of AFLURIA (trivalent formulation) administered using the PharmaJet Stratis Needle-Free Injection System (Study 2). Study 2 included 1,247 subjects for safety analysis, ages 18 through 64 years, randomized to receive AFLURIA by either the PharmaJet Stratis Needle-Free Injection System (624 subjects) or needle and syringe (623 subjects). No deaths or vaccine-related serious adverse events were reported in Study 2. Local (injection-site) adverse reactions and systemic adverse events were solicited for 7 days post-vaccination (Table 3).

Table 3: Proportion of Subjects 18 through 64 Years of Age with Solicited Local Adverse Reactions or Systemic Adverse Events within 7 Days after Administration of AFLURIA (trivalent formulation) by PharmaJet Stratis Needle-Free Injection System or Needle and Syringe (Study 2)[a]

	Percentage [b] of Subjects Reporting Event			
	Subjects 18 through 64 years			
	AFLURIA (trivalent formulation)			
	PharmaJet Stratis Needle-Free Injection System N=540-616 [c]		Needle and Syringe N=599-606 [c]	
	Any	Grade 3	Any	Grade 3
Local Adverse Reactions [d]				
Tenderness	89.4	2.1	77.9	1.0
Swelling	64.8	1.7	19.7	0.2
Pain	64.4	0.8	49.3	0.7
Redness	60.1	1.3	19.2	0.3
Itching [f]	28.0	0.0	9.5	0.2
Bruising	17.6	0.2	5.3	0.0
Systemic Adverse Events [e]				
Myalgia	36.4	0.8	35.5	1.0
Malaise	31.2	0.7	28.4	0.5
Headache	24.7	1.3	22.1	1.3
Chills	7.0	0.2	7.2	0.2
Nausea	6.6	0.2	6.5	0.0
Vomiting	1.3	0.0	1.8	0.2
Fever	0.3	0.0	0.3	0.0

[a] NCT01688921

[b] Proportion of subjects reporting each local adverse reaction or systemic adverse event by treatment group based on the number of subjects contributing at least one data value for an individual sign/symptom (individual event denominators).

[c] N = number of subjects in the Safety Population for each treatment group. Denominators for the PharmaJet Stratis Needle-Free Injection System group were: N=540 for itching and N=605-616 for all other parameters. Denominators for the needle and syringe group were: N=527 for itching and N=599-606 for all other parameters.

[d] Local adverse reactions: Grade 3 is pain, tenderness or itching that prevents daily activity; Swelling, redness or bruising: any = ≥ 25mm diameter, Grade 3 = > 100mm diameter.

[e] Systemic adverse events: Fever: any = ≥ 100.4°F (Oral), Grade 3 = ≥ 102.2°F (Oral); Grade 3 for all other adverse events is that which prevents daily activity.

[f] A total of 155 subjects (approximately randomly distributed between PharmaJet Stratis Needle-Free Injection System and needle and syringe groups) received Diary Cards without itching listed as a solicited symptom.

In adults 18 through 64 years who received AFLURIA (trivalent formulation) administered by PharmaJet Stratis Needle-Free Injection System, commonly reported unsolicited adverse events were headache (4.2%), injection site hematoma (1.8%), injection site erythema (1.1%), myalgia (1.0%) and nausea (1.0%).

Children 5 Years Through 17 Years of Age

Clinical safety data for AFLURIA QUADRIVALENT in older children and adolescents have been collected in one clinical trial, Study 3, a randomized, observer-blinded, comparator-controlled trial conducted in the U.S. in 2278 subjects aged 5 through 17 years. Subjects were stratified into one of two age cohorts of 5 through 8 years or 9 through 17 years (51.2% and 48.8% of the study population, respectively). The mean age of the population was 9.5 years, 52.1% were male, and racial groups consisted of 73.3% White, 20.7% Black, 0.8% Asian, 0.3% American Indian/Native American, and 0.7% Native Hawaiian/Pacific Islander; 23.8% of subjects were Hispanic/Latino. The mean ages of subjects 5 through 8 years and 9 through 17 years were 6.7 years and 12.5 years, respectively. Subjects in the safety population (N=2252) received either AFLURIA QUADRIVALENT (N=1692) or a U.S.-licensed comparator quadrivalent influenza vaccine (N=560). Study subjects were scheduled to receive either a single vaccination or two vaccinations 28 days apart based on their previous vaccination history. In this study, AFLURIA QUADRIVALENT and comparator vaccine were administered by needle and syringe (*see Clinical Studies [14]*).

Local (injection site) adverse reactions and systemic adverse events were solicited for 7 days post-vaccination. Cellulitis-like reactions (defined as concurrent Grade 3 pain, redness, and swelling/lump) at the injection site were monitored for 28 days post-vaccination. Subjects were instructed to report and return to clinic within 24 hours in the event of a cellulitis-like reaction. Unsolicited adverse events were collected for 28 days post-vaccination. All solicited local adverse reactions and systemic adverse events following any vaccination (first or second dose) are presented in Table 4.

Table 4: Proportion of Subjects Per Age Cohort with Any Solicited Local Adverse Reactions or Systemic Adverse Events within 7 Days after Administration of AFLURIA QUADRIVALENT or Comparator (Study 3)[a]

	Percentage (%) [b] of Subjects in each Age Cohort Reporting an Event							
	Subjects 5 through 8 years				Subjects 9 through 17 years			
	AFLURIA Quadrivalent N= 828-829 [c]		Comparator N= 273-274 [c]		AFLURIA Quadrivalent N= 790-792 [c]		Comparator N= 261 [c]	
	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3
Local Adverse Reactions [d]								
Pain	51.3	0.8	49.6	0.7	51.5	0.3	45.2	0.4
Redness	19.4	3.5	18.6	1.8	14.8	1.9	16.1	1.9
Swelling/Lump	15.3	3.4	12.4	2.2	12.2	2.0	10.7	1.9
Systemic Adverse Events [e]								
Headache	12.3	0.1	10.6	0.4	18.8	0.4	14.6	0.4
Myalgia	9.8	0.1	11.3	0.4	16.7	0.3	11.1	0.4
Malaise and Fatigue	8.8	0.4	5.8	0	10.0	0.4	7.7	0
Nausea	7.1	0.1	8.4	0	7.7	0	8.0	0
Diarrhea	5.2	0	3.6	0	5.4	0	4.2	0
Fever	4.5	1.2	3.6	0.7	2.1	0.5	0.8	0
Vomiting	2.4	0.2	4.4	0	1.8	0	2.3	0

Abbreviations: Gr 3, Grade 3 (severe); Comparator, Comparator quadrivalent influenza vaccine [Fluarix® Quadrivalent (GlaxoSmithKline Biologicals)]

[a] NCT02545543

[b] Percent (%) is derived from the number of subjects that reported the event divided by the number of subjects in the Solicited Safety Population with non-missing data for each age cohort, treatment group, and each solicited parameter.

[c] N = number of subjects in the Solicited Safety Population (subjects who were vaccinated and provided any solicited safety data) for each study vaccine group.

[d] Local adverse reactions: Grade 3 pain is that which prevents daily activity; swelling/lump and redness: any = > 0mm diameter, Grade 3 = > 30mm diameter.

[e] Systemic adverse events: Fever: any = ≥ 100.4°F (Oral), Grade 3 = ≥ 102.2°F (Oral); Grade 3 for all other adverse events is that which prevents daily activity or requires significant medical intervention.

In subjects 5 through 8 years of age, all solicited local adverse reactions and systemic adverse events were reported at lower frequencies after the second vaccination than after the first vaccination with AFLURIA QUADRIVALENT with the exception of vomiting (which occurred at the same rate of 2.2% after each vaccination).

One subject, 8 years of age, experienced a cellulitis-like reaction at the injection site after vaccination with AFLURIA QUADRIVALENT.

The most commonly reported unsolicited adverse events in the 28 days following the first or second dose of AFLURIA QUADRIVALENT in subjects 5 through 8 years of age were cough (2.4%), pyrexia (1.8%), rhinorrhea (1.2%), and headache (1.0%), and were similar to the comparator.

For subjects ages 9 through 17 years who received AFLURIA QUADRIVALENT, the most commonly reported unsolicited adverse events in the 28 days following vaccination were oropharyngeal pain (1.6%), cough (1.3%), and upper respiratory tract infection (1.0%), and were similar to the comparator.

No deaths were reported in Study 3. In the 180 days following vaccinations, AFLURIA QUADRIVALENT and comparator vaccine recipients experienced similar rates of serious adverse events (SAEs). None of the SAEs appeared related to the study vaccines except for one case of influenza B infection (considered a vaccine failure) in an AFLURIA QUADRIVALENT recipient.

Children 6 Months Through 59 Months of Age

Clinical safety data for AFLURIA QUADRIVALENT in infants and young children have been collected in one clinical trial, Study 4, a randomized, observer-blind, comparator-controlled trial conducted in the U.S. in 2247 subjects aged 6 through 59 months. Subjects were stratified into one of two age cohorts of 6 through 35 months or 36 through 59 months (41.6% and 58.4% of the study population, respectively). The mean age of the population was 36.6 months, 51.6% were male, and racial groups consisted of 71.0% White, 21.5% Black, 1.1% Asian, 0.7% Native Hawaiian/Pacific Islander, and 0.3% American Indian/Native American; 26.4% of subjects were Hispanic/Latino. The mean ages of subjects 6 through 35 months and 36 through 59 months were 21.7 months and 47.1 months, respectively. Subjects in the safety population (N=2232) received either AFLURIA QUADRIVALENT (N=1673) or a U.S.-licensed comparator quadrivalent influenza vaccine (N=559). Study subjects were scheduled to receive either a single vaccination or two vaccinations 28 days apart based on their previous vaccination history. In this study, AFLURIA QUADRIVALENT and comparator vaccine were administered by needle and syringe (see *Clinical Studies [14]*).

Local (injection site) adverse reactions and systemic adverse events were solicited for 7 days post-vaccination. Cellulitis-like reactions (defined as concurrent Grade 3 pain, redness, and swelling/lump) at the injection site were monitored for 28 days post-vaccination. Subjects were instructed to report and return to clinic within 24 hours in the event of a cellulitis-like reaction. Unsolicited adverse events were collected for 28 days post-vaccination, and SAEs for 6 months following the last vaccination. All solicited local adverse reactions and systemic adverse events following any vaccination (first or second dose) are presented in Table 5.

Table 5: Proportion of Subjects Per Age Cohort with Any Solicited Local Adverse Reactions or Systemic Adverse Events within 7 Days after Administration of AFLURIA QUADRIVALENT or Comparator QIV (Study 4) [a]

	Percentage (%) [b] of Subjects in each Age Cohort Reporting an Event							
	6 through 35 months				36 through 59 months			
	AFLURIA Quadrivalent N= 668-669 [c]		Comparator N= 226-227 [c]		AFLURIA Quadrivalent N= 947-949 [c]		Comparator N= 317-318 [c]	
	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3
Local Adverse Reactions [d]								
Pain	20.8	0.1	25.6	0.4	35.5	0	31.4	0.6
Redness	20.8	0.6	17.6	1.8	22.4	2.3	20.8	5.3
Swelling/Lump	6.1	0.4	6.2	0.9	10.1	1.7	12.9	2.5
Systemic Adverse Events [e]								
Irritability	32.9	0.7	28.2	0.4	-	-	-	-
Diarrhea	24.2	0.1	25.6	0.4	12.1	0.1	8.8	0.6
Loss of Appetite	20.0	0.3	19.4	0.4	-	-	-	-
Malaise and Fatigue	-	-	-	-	14.3	0.5	13.2	0.3
Myalgia	-	-	-	-	9.9	0.1	9.4	0
Nausea and/or vomiting	9.4	0.7	11.0	0	9.2	0.4	6.6	0.3
Headache	-	-	-	-	6.2	0.4	5.0	0
Fever [f]	7.2	2.5	11.9	2.6	4.8	1.2	6.0	0.9

Abbreviations: Gr 3, Grade 3 (severe); Comparator, Comparator quadrivalent influenza vaccine [Fluzone® Quadrivalent (Sanofi Pasteur)]

[a] NCT02914275

[b] Percent (%) is derived from the number of subjects that reported the event divided by the number of subjects in the Solicited Safety Population with non-missing data for each age cohort, treatment group, and each solicited parameter.

[c] N = number of subjects in the Solicited Safety Population (subjects who were vaccinated and provided any solicited safety data) for each study vaccine group.

[d] Local adverse reactions: Grade 3 pain is that which prevents daily activity (36 through 59 month subjects); or cried when limb was moved or spontaneously painful (6 through 35 month subjects); Swelling/Lump and redness: any = ≥ 0mm diameter, Grade 3 = ≥ 30mm diameter.

[e] Systemic adverse events: Fever: any = ≥ 99.5°F (Axillary), Grade 3 = ≥ 101.3°F (Axillary); Grade 3 for all other adverse events is that which prevents daily activity; Irritability, Loss of Appetite, Malaise and Fatigue, Myalgia and Headache are age specific systemic adverse events, where "-" denotes event was not applicable to that age cohort.

[f] Prophylactic antipyretics (acetaminophen or ibuprophen-containing medications) were not permitted. Antipyretics used to treat fever were permitted and rates of use were as follows: 6 through 35 months (Afluria QIV 5.9%, Comparator QIV 9.0%); 36 through 59 months (Afluria QIV 3.7%, Comparator QIV 2.5%).

In subjects 6 through 35 months of age, all solicited local adverse reactions and systemic adverse events were reported at lower frequencies after the second vaccination than after the first vaccination with AFLURIA QUADRIVALENT.

In subjects 36 through 59 months of age, all solicited local adverse reactions and systemic adverse events were reported at lower frequencies after the second vaccination than after the first vaccination with AFLURIA QUADRIVALENT.

The most commonly reported unsolicited adverse events in the 28 days following the first or second dose of AFLURIA QUADRIVALENT in subjects 6 through 35 months of age were rhinorrhea (11.2%), cough (10.4%), pyrexia (6.3%), upper respiratory tract infection (4.8%), diarrhea (3.7%), otitis media (2.4%), vomiting (2.4%), nasal congestion (2.4%), nasopharyngitis (1.9%), irritability (1.7%), ear infection (1.6%), croup infectious (1.4%), teething (1.3%), rash (1.2%), influenza like illness (1.0%) and fatigue (1.0%), and were similar to comparator.

The most commonly reported unsolicited adverse events in the 28 days following the first or second dose of AFLURIA QUADRIVALENT in subjects 36 through 59 months of age were cough (7.7%), rhinorrhea (4.9%), pyrexia (3.7%), upper respiratory tract infection (2.5%), vomiting (2.1%), nasal congestion (1.6%), nasopharyngitis (1.7%), ororpharyngeal pain (1.2%), diarrhea (1.1%) and fatigue (1.1%), and were similar to the comparator.

No deaths were reported in Study 4. In the 180 days following vaccinations, AFLURIA QUADRIVALENT and comparator vaccine recipients experienced similar rates of serious adverse events (SAEs), none of which were related to study vaccines. No vaccine-related febrile seizures occurred in Study 4. Unrelated SAEs of febrile seizures occurred in two AFLURIA QUADRIVALENT recipients (6 through 35 months age group) at 43 and 104 days post-vaccinations.

6.2 Postmarketing Experience

Because postmarketing reporting of adverse events is voluntary and from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure. The adverse events described have been included in this section because they: 1) represent reactions that are known to occur following immunizations generally or influenza immunizations specifically; 2) are potentially serious; or 3) have been reported frequently. The adverse events listed below reflect experience in both children and adults and include those identified during post-approval use of AFLURIA (trivalent formulation) and AFLURIA QUADRIVALENT.

The post-marketing experience with AFLURIA (trivalent formulation) and AFLURIA QUADRIVALENT included the following:

Blood and lymphatic system disorders
Thrombocytopenia

Immune system disorders
Allergic or immediate hypersensitivity reactions including anaphylactic shock and serum sickness

Nervous system disorders
Neuralgia, paresthesia, convulsions (including febrile seizures), dizziness, encephalomyelitis, encephalopathy, neuritis or neuropathy, transverse myelitis, and GBS

Vascular disorders
Vasculitis which may be associated with renal involvement

Musculoskeletal and Connective Tissue Disorders
Musculoskeletal pain and pain in the extremity

Skin and subcutaneous tissue disorders
Pruritus, urticaria, and rash

General disorders and administration site conditions
Cellulitis and large injection site swelling
Influenza-like illness, injected limb mobility decreased, pyrexia, injection site erythema and injection site reaction

7 DRUG INTERACTIONS

No interaction studies have been performed on interaction between influenza vaccines in general and other vaccines or medications.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to AFLURIA QUADRIVALENT during pregnancy. Women who are vaccinated with AFLURIA QUADRIVALENT during pregnancy are encouraged to enroll in the registry by calling 1-855-358-8966 or sending an email to Seqirus at *us.medicalinformation@seqirus.com*.

Risk summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. Data for AFLURIA (trivalent formulation) administered to pregnant women are relevant to AFLURIA QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions (see *Description [11]*). There are limited data for AFLURIA QUADRIVALENT administered to pregnant women, and available data for AFLURIA (trivalent formulation) administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

There were no developmental toxicity studies of AFLURIA QUADRIVALENT performed in animals. A developmental toxicity study of AFLURIA (trivalent formulation) has been performed in female rats administered a single human dose [0.5 mL (divided)] of AFLURIA (trivalent formulation) prior to mating and during gestation. This study revealed no evidence of harm to the fetus due to AFLURIA (trivalent formulation) (*see 8.1 Data*).

Clinical Considerations

Disease-associated Maternal and/or Embryo-Fetal Risk

Pregnant women are at increased risk for severe illness due to influenza compared to non-pregnant women. Pregnant women with influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

Data

Animal Data

In a developmental toxicity study, female rats were administered a single human dose [0.5 mL (divided)] of AFLURIA (trivalent formulation) by intramuscular injection 21 days and 7 days prior to mating, and on gestation day 6. Some rats were administered an additional dose on gestation day 20. No vaccine-related fetal malformations or variations and no adverse effects on pre-weaning development were observed in the study.

8.2 Lactation

Risk Summary

It is not known whether AFLURIA QUADRIVALENT is excreted in human milk. Data are not available to assess the effects of AFLURIA QUADRIVALENT on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for AFLURIA QUADRIVALENT and any potential adverse effects on the breastfed child from AFLURIA QUADRIVALENT or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

The safety and effectiveness of AFLURIA QUADRIVALENT in persons less than 6 months of age have not been established.

The PharmaJet Stratis Needle-Free Injection System is not approved as a method of administering AFLURIA QUADRIVALENT to children and adolescents less than 18 years of age due to lack of adequate data supporting safety and effectiveness in this population.

8.5 Geriatric Use

In clinical studies, AFLURIA QUADRIVALENT has been administered to, and safety information collected for, 867 subjects aged 65 years and older *(see Adverse Reactions [6])*. The 65 years and older age group included 539 subjects 65 through 74 years and 328 subjects 75 years and older. After administration of AFLURIA QUADRIVALENT, hemagglutination-inhibiting antibody responses were non-inferior to comparator trivalent influenza (TIV-1 and TIV-2) in persons 65 years of age and older, but were lower than younger adult subjects (*see Clinical Studies [14]*).

The PharmaJet Stratis Needle-Free Injection System is not approved as a method of administering AFLURIA QUADRIVALENT to adults 65 years of age and older due to lack of adequate data supporting safety and effectiveness in this population.

11 DESCRIPTION

AFLURIA QUADRIVALENT, Influenza Vaccine for intramuscular injection, is a sterile, clear, colorless to slightly opalescent suspension with some sediment that resuspends upon shaking to form a homogeneous suspension. AFLURIA QUADRIVALENT is prepared from influenza virus propagated in the allantoic fluid of embryonated chicken eggs. Following harvest, the virus is purified in a sucrose density gradient using continuous flow zonal centrifugation. The purified virus is inactivated with beta-propiolactone, and the virus particles are disrupted using sodium taurodeoxycholate to produce a "split virion". The disrupted virus is further purified and suspended in a phosphate buffered isotonic solution.

AFLURIA QUADRIVALENT is standardized according to USPHS requirements for the 2021-2022 influenza season and is formulated to contain 60 mcg hemagglutinin (HA) per 0.5 mL dose in the recommended ratio of 15 mcg HA for each of the four influenza strains recommended for the 2021-2022 Northern Hemisphere influenza season:

A/Victoria/2570/2019 IVR-215 (an A/Victoria/2570/2019 (H1N1)pdm09-like virus), A/Cambodia/e0826360/2020 IVR-224 (an A/Cambodia/e0826360/2020 (H3N2)-like virus), B/Victoria/705/2018 BVR-11 (a B/Washington/02/2019-like virus) and B/Phuket/3073/2013 BVR-1B (a B/Phuket/3073/2013-like virus). A 0.25 mL dose contains 7.5 mcg HA of each of the same four influenza strains.

Thimerosal, a mercury derivative, is not used in the manufacturing process for the single dose presentation. This presentation does not contain preservative. The multi-dose presentation contains thimerosal added as a preservative; each 0.5 mL dose contains 24.5 mcg of mercury and each 0.25 mL dose contains 12.25 mcg of mercury.

A single 0.5 mL dose of AFLURIA QUADRIVALENT contains sodium chloride (4.1 mg), monobasic sodium phosphate (80 mcg), dibasic sodium phosphate (300 mcg), monobasic potassium phosphate (20 mcg), potassium chloride (20 mcg), and calcium chloride (0.5 mcg). From the manufacturing process, each 0.5 mL dose may also contain residual amounts of sodium taurodeoxycholate (≤ 10 ppm), ovalbumin (< 1 mcg), sucrose (< 10 mcg), neomycin sulfate (≤ 81.8 nanograms [ng]), polymyxin B (≤ 14 ng), beta-propiolactone (≤ 1.5 ng) and hydrocortisone (≤ 0.56 ng). A single 0.25 mL dose of AFLURIA QUADRIVALENT contains half of these quantities.

The rubber tip cap and plunger used for the preservative-free, single-dose syringes and the rubber stoppers used for the multi-dose vial are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Influenza illness and its complications follow infection with influenza viruses. Global surveillance of influenza identifies yearly antigenic variants. For example, since 1977 antigenic variants of influenza A (H1N1 and H3N2) and influenza B viruses have been in global circulation. Since 2001, two distinct lineages of influenza B (Victoria and Yamagata lineages) have co-circulated worldwide. Specific levels of hemagglutination inhibition (HI) antibody titers post-vaccination with inactivated influenza vaccine have not been correlated with protection from influenza virus. In some human studies, antibody titers of 1:40 or greater have been associated with protection from influenza illness in up to 50% of subjects.[2,3]

Antibody against one influenza virus type or subtype confers limited or no protection against another. Furthermore, antibody to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual change to one or more new strains in each year's influenza vaccine. Therefore, inactivated influenza vaccines are standardized to contain the HA of four strains (i.e., typically two type A and two type B) representing the influenza viruses likely to be circulating in the U.S. during the upcoming winter.

Annual revaccination with the current vaccine is recommended because immunity declines during the year after vaccination and circulating strains of influenza virus change from year to year.[1]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

AFLURIA QUADRIVALENT has not been evaluated for carcinogenic or mutagenic potential, or male infertility in animals. A developmental toxicity study conducted in rats vaccinated with AFLURIA (trivalent formulation) revealed no impact on female fertility (see *Pregnancy [8.1]*).

14 CLINICAL STUDIES

14.1 Efficacy Against Laboratory-Confirmed Influenza

The efficacy of AFLURIA (trivalent formulation) is relevant to AFLURIA QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions (*see Description [11]*).

The efficacy of AFLURIA (trivalent formulation) was demonstrated in Study 5, a randomized, observer-blind, placebo-controlled study conducted in 15,044 subjects. Healthy subjects 18 through 64 years of age were randomized in a 2:1 ratio to receive a single dose of AFLURIA (trivalent formulation) (enrolled subjects: 10,033; evaluable subjects: 9,889) or placebo (enrolled subjects: 5,011; evaluable subjects: 4,960). The mean age of all randomized subjects was 35.5 years. 54.4% were female and 90.2% were White. Laboratory-confirmed influenza was assessed by active and passive surveillance of influenza-like illness (ILI) beginning 2 weeks post-vaccination until the end of the influenza season, approximately 6 months post-vaccination. ILI was defined as at least one respiratory symptom (e.g., cough, sore throat, nasal congestion) and at least one systemic symptom (e.g., oral temperature of 100.0°F or higher, feverishness, chills, body aches). Nasal and throat swabs were collected from subjects who presented with an ILI for laboratory confirmation by viral culture and real-time reverse transcription polymerase chain reaction. Influenza virus strain was further characterized using gene sequencing and pyrosequencing.

Attack rates and vaccine efficacy, defined as the relative reduction in the influenza infection rate for AFLURIA (trivalent formulation) compared to placebo, were calculated using the Per Protocol Population. Vaccine efficacy against laboratory-confirmed influenza infection due to influenza A or B virus strains contained in the vaccine was 60% with a lower limit of the 95% CI of 41% (Table 6).

Table 6: AFLURIA (trivalent formulation): Laboratory-Confirmed Influenza Infection Rate and Vaccine Efficacy in Adults 18 through 64 Years of Age (Study 5)[a]

	Subjects[b]	Laboratory-Confirmed Influenza Cases	Influenza Infection Rate	Vaccine Efficacy[c]	
	N	N	n/N %	%	Lower Limit of the 95% CI
Vaccine-matched Strains					
AFLURIA	9889	58	0.59	60	41
Placebo	4960	73	1.47		
Any Influenza Virus Strain					
AFLURIA	9889	222	2.24	42	28
Placebo	4960	192	3.87		

Abbreviations: CI, confidence interval.

[a] NCT00562484

[b] The Per Protocol Population was identical to the Evaluable Population in this study.

[c] Vaccine efficacy = 1 minus the ratio of AFLURIA (trivalent formulation) /placebo infection rates. The objective of the study was to demonstrate that the lower limit of the CI for vaccine efficacy was greater than 40%.

14.2 Immunogenicity of AFLURIA QUADRIVALENT in Adults and Older Adults Administered by Needle and Syringe

Study 1 was a randomized, double-blind, active-controlled trial conducted in the U.S. in adults aged 18 years of age and older. Subjects received one dose of either AFLURIA QUADRIVALENT (N=1691) or one of two formulations of comparator trivalent influenza vaccine (AFLURIA, TIV-1 N=854 or TIV-2 N=850) each containing an influenza type B virus that corresponded to one of the two B viruses in AFLURIA QUADRIVALENT (a type B virus of the Yamagata lineage or a type B virus of the Victoria lineage, respectively).

Post-vaccination immunogenicity was evaluated on sera obtained 21 days after administration of a single dose of AFLURIA QUADRIVALENT or TIV comparator. The co-primary endpoints were HI Geometric Mean Titer (GMT) ratios (adjusted for baseline HI titers) and the difference in seroconversion rates for each vaccine strain, 21 days after vaccination. Pre-specified non-inferiority criteria required that the upper bound of the 2-sided 95% CI of the GMT ratio (TIV/AFLURIA QUADRIVALENT) did not exceed 1.5 and the upper bound of the 2-sided 95% CI of the seroconversion rate difference (TIV minus AFLURIA QUADRIVALENT) did not exceed 10.0% for each strain.

Serum HI antibody responses to AFLURIA QUADRIVALENT were non-inferior to both TIVs for all influenza strains for subjects 18 years of age and older. Additionally, non-inferiority was demonstrated for both endpoints in both age sub-groups, adults aged 18 through 64 years and 65 years and older, for all strains (Table 7). Superiority of the immune response to each of the influenza B strains contained in AFLURIA QUADRIVALENT was shown relative to the antibody response after vaccination with TIV formulations not containing that B lineage strain for subjects 18 years of age and older. Superiority against the alternate B strain was also demonstrated for each of the influenza B strains in both age sub-groups; 18 through 64 years and 65 years and older. Post-hoc analyses of immunogenicity endpoints by gender did not demonstrate meaningful differences between males and females. The study population was not sufficiently diverse to assess differences between races or ethnicities.

Table 7: Post-Vaccination HI Antibody GMTs, Seroconversion Rates, and Analyses of Non-Inferiority of AFLURIA QUADRIVALENT Relative to Trivalent Influenza Vaccine (TIV) by Age Cohort (Study 1)[a]

	Post-vaccination GMT		GMT Ratio[b]	Seroconversion %[c]		Difference	Met both pre-defined non-inferiority criteria?[d]
Strain	AFLURIA Quadrivalent	Pooled TIV or TIV-1 (B Yamagata) or TIV-2 (B Victoria)	Pooled TIV or TIV-1 or TIV-2 over AFLURIA Quadrivalent (95% CI)	AFLURIA Quadrivalent N=1691	Pooled TIV or TIV-1 (B Yamagata) or TIV-2 (B Victoria)	Pooled TIV or TIV-1 or TIV-2 minus AFLURIA Quadrivalent (95% CI)	
18 through 64 years	**AFLURIA Quadrivalent N=835, Pooled TIV N=845, TIV-1 N=424, TIV-2 N=421**						
A(H1N1)	432.7	402.8	0.93[e] (0.85, 1.02)	51.3	49.1	-2.1[h] (-6.9, 2.7)	Yes
A(H3N2)	569.1	515.1	0.91[e] (0.83, 0.99)	56.3	51.7	-4.6[h] (-9.4, 0.2)	Yes
B/Massachusetts/2/2012 (B Yamagata)	92.3	79.3	0.86[f] (0.76, 0.97)	45.7	41.3	-4.5[i] (-10.3, 1.4)	Yes
B/Brisbane/60/2008 (B Victoria)	110.7	95.2	0.86[g] (0.76, 0.98)	57.6	53.0	-4.6[j] (-10.5, 1.2)	Yes
≥ 65 years	**AFLURIA Quadrivalent N=856, Pooled TIV N=859, TIV-1 N=430, TIV-2 N=429**						
A(H1N1)	211.4	199.8	0.95[e] (0.88, 1.02)	26.6	26.4	-0.2[h] (-5.0, 4.5)	Yes
A(H3N2)	419.5	400.0	0.95[e] (0.89, 1.02)	25.9	27.0	1.1[h] (-3.7, 5.8)	Yes
B/Massachusetts/2/2012 (B Yamagata)	43.3	39.1	0.90[f] (0.84, 0.97)	16.6	14.4	-2.2[i] (-8.0, 3.6)	Yes
B/Brisbane/60/2008 (B Victoria)	66.1	68.4	1.03[g] (0.94, 1.14)	23.5	24.7	1.2[j] (-4.6, 7.0)	Yes

Abbreviations: CI, confidence interval; GMT, geometric mean titer.

[a] NCT02214225

[b] GMT ratio was computed after fitting a multi-variable model on the post-vaccination titers including sex, vaccination history, pre-vaccination HI titers and other factors.

[c] Seroconversion rate is defined as a 4-fold increase in post-vaccination HI antibody titer from pre-vaccination titer ≥ 1:10 or an increase in titer from < 1:10 to ≥ 1:40.

[d] Non-inferiority (NI) criterion for the GMT ratio: upper bound of 2-sided 95% CI on the GMT ratio of Pooled TIV or TIV-1 (B Yamagata) or TIV-2 (B Victoria)/AFLURIA Quadrivalent should not exceed 1.5. NI criterion for the SCR difference: upper bound of 2-sided 95% CI on the difference between SCR Pooled TIV or TIV-1 (B Yamagata) or TIV-2 (B Victoria) minus AFLURIA Quadrivalent should not exceed 10%.

[e] Pooled TIV/AFLURIA Quadrivalent

[f] TIV-1 (B Yamagata)/AFLURIA Quadrivalent

[g] TIV-2 (B Victoria)/AFLURIA Quadrivalent

[h] Pooled TIV – AFLURIA Quadrivalent

[i] TIV-1 (B Yamagata) - AFLURIA Quadrivalent

[j] TIV-2 (B Victoria) - AFLURIA Quadrivalent

14.3 Immunogenicity of AFLURIA (trivalent formulation) Administered by PharmaJet Stratis Needle-Free Injection System

Study 2 was a randomized, comparator-controlled, non-inferiority study that enrolled 1,250 subjects 18 through 64 years of age. This study compared the immune response following administration of AFLURIA (trivalent formulation) when delivered intramuscularly using either the PharmaJet Stratis Needle-Free Injection System or needle and syringe. Immunogenicity assessments were performed prior to vaccination and at 28 days after vaccination in the immunogenicity population (1130 subjects, 562 PharmaJet Stratis Needle-Free Injection System group, 568 needle and syringe group). The co-primary endpoints were HI GMT ratios for each vaccine strain and the absolute difference in seroconversion rates for each vaccine strain 28 days after vaccination. As shown in Table 8, non-inferiority of administration of AFLURIA (trivalent formulation) by the PharmaJet Stratis Needle-Free Injection System compared to administration of AFLURIA (trivalent formulation) by needle and syringe was demonstrated in the immunogenicity population for all strains. Post-hoc analyses of immunogenicity by age showed that younger subjects (18 through 49 years) elicited higher immunological responses than older subjects (50 through 64 years). Post-hoc analyses of immunogenicity according to sex and body mass

index did not reveal significant influences of these variables on immune responses. The study population was not sufficiently diverse to assess immunogenicity by race or ethnicity.

Table 8: Baseline and Post-Vaccination HI Antibody GMTs, Seroconversion Rates, and Analyses of Non-Inferiority of AFLURIA (trivalent formulation) Administered by PharmaJet Stratis Needle-Free Injection System or Needle and Syringe, Adults 18 through 64 Years of Age (Study 2)[a]

	Baseline GMT		Post-vaccination GMT		GMT Ratio [b]	Seroconversion % [c]		Difference	
Strain	Needle and Syringe N=568	PharmaJet Stratis Needle-Free Injection System N=562	Needle and Syringe N=568	PharmaJet Stratis Needle-Free Injection System N=562	Needle and Syringe over PharmaJet Stratis Needle-Free Injection System (95% CI)	Needle and Syringe N=568	PharmaJet Stratis Needle-Free Injection System N=562	Needle and Syringe minus PharmaJet Stratis Needle-Free Injection System (95% CI)	Met both pre-defined non-inferiority criteria? [d]
A(H1N1)	79.5	83.7	280.6	282.9	0.99 (0.88, 1.12)	38.4	37.5	0.8 (-4.8, 6.5)	Yes
A(H3N2)	75.4	68.1	265.9	247.3	1.08 (0.96, 1.21)	45.1	43.8	1.3 (-4.5, 7.1)	Yes
B	12.6	13.5	39.7	42.5	0.94 (0.83, 1.06)	35.2	34.9	0.3 (-5.2, 5.9)	Yes

Abbreviations: CI, confidence interval; GMT, geometric mean titer.
[a] NCT01688921
[b] GMT ratio is defined as post-vaccination GMT for Needle and Syringe/PharmaJet Stratis Needle-Free Injection System.
[c] Seroconversion rate is defined as a 4-fold increase in post-vaccination HI antibody titer from pre-vaccination titer ≥ 1:10 or an increase in titer from < 1:10 to ≥ 1:40.
[d] Non-inferiority (NI) criterion for the GMT ratio: upper bound of 2-sided 95% CI on the GMT ratio of Needle and Syringe/PharmaJet Stratis Needle-Free Injection System should not exceed 1.5. NI criterion for the seroconversion rate (SCR) difference: upper bound of 2-sided 95% CI on the difference between SCR Needle and Syringe – SCR PharmaJet Stratis Needle-Free Injection System should not exceed 10%.

14.4 Immunogenicity of AFLURIA QUADRIVALENT in Children 5 through 17 Years Administered via Needle and Syringe

Study 3 was a randomized, observer-blinded, comparator-controlled trial conducted in the U.S. in children 5 through 17 years of age. A total of 2278 subjects were randomized 3:1 to receive one or two doses of AFLURIA QUADRIVALENT (N=1709) or a U.S.-licensed comparator quadrivalent influenza vaccine (N=569). Subjects 5 through 8 years of age were eligible to receive a second dose at least 28 days after the first dose depending on their influenza vaccination history, consistent with the 2015-2016 recommendations of the Advisory Committee on Immunization Practices (ACIP) for Prevention and Control of Seasonal Influenza with Vaccines. Approximately 25% of subjects in each treatment group in the 5 through 8 years of age sub-group received two vaccine doses.

Baseline serology for HI assessment was collected prior to vaccination. Post-vaccination immunogenicity was evaluated by HI assay on sera obtained 28 days after the last vaccination dose.

The primary objective was to demonstrate that vaccination with AFLURIA QUADRIVALENT elicits an immune response that is not inferior to that of a comparator vaccine containing the same recommended virus strains. The Per Protocol Population (AFLURIA QUADRIVALENT n=1605, Comparator n=528) was used for the primary endpoint analyses. The co-primary endpoints were HI Geometric Mean Titer (GMT) ratios (adjusted for baseline HI titers and other covariates) and seroconversion rates for each vaccine strain, 28 days after the last vaccination. Pre-specified non-inferiority criteria required that the upper bound of the 2-sided 95% CI of the GMT ratio (Comparator/AFLURIA QUADRIVALENT) did not exceed 1.5 and the upper bound of the 2-sided 95% CI of the seroconversion rate difference (Comparator minus AFLURIA QUADRIVALENT) did not exceed 10.0% for each strain. Serum HI antibody responses to AFLURIA QUADRIVALENT were non-inferior for both GMT ratio and seroconversion rates relative to the comparator vaccine for all influenza strains (Table 9). Analyses of immunogenicity endpoints by gender did not demonstrate meaningful differences between males and females. The study population was not sufficiently diverse to assess differences among races or ethnicities.

Table 9: Post-Vaccination HI Antibody GMTs, SCRs, and Analyses of Non-Inferiority of AFLURIA QUADRIVALENT Relative to a U.S.-Licensed Comparator Quadrivalent Influenza Vaccine for each Strain 28 Days after Last Vaccination Among a Pediatric Population 5 through 17 Years of Age (Per Protocol Population) (Study 3)[a,b]

	Post-vaccination GMT		GMT Ratio [c]	Seroconversion % [d]		SCR Difference [e]	
Strain	AFLURIA Quadrivalent N=1605	Comparator N=528	Comparator over AFLURIA Quadrivalent (95% CI)	AFLURIA Quadrivalent N=1605 (95% CI)	Comparator N=528 (95% CI)	Comparator minus AFLURIA Quadrivalent (95% CI)	Met both pre-defined non-inferiority criteria? [f]
A(H1N1)	952.6 (n=1604 [g])	958.8	1.01 (0.93, 1.09)	66.4 (64.0, 68.7)	63.3 (59.0, 67.4)	-3.1 (-8.0, 1.8)	Yes
A(H3N2)	886.4 (n=1604 [g])	930.6	1.05 (0.96, 1.15)	82.9 (81.0, 84.7)	83.3 (79.9, 86.4)	0.4 (-4.5, 5.3)	Yes
B/Phuket/3073/2013 (B Yamagata)	60.9 (n=1604 [g])	54.3	0.89 (0.81, 0.98)	58.5 (56.0, 60.9)	55.1 (50.8, 59.4)	-3.4 (-8.3, 1.5)	Yes
B/Brisbane/60/2008 (B Victoria)	145.0 (n=1604 [g])	133.4	0.92 (0.83, 1.02)	72.1 (69.8, 74.3)	70.1 (66.0, 74.0)	-2.0 (-6.9, 2.9)	Yes

Abbreviations: CI, confidence interval; Comparator, Comparator quadrivalent influenza vaccine (Fluarix® Quadrivalent [GlaxoSmithKline Biologicals]); GMT (adjusted), geometric mean titer; SCR, seroconversion rate.
[a] NCT02545543
[b] The Per-Protocol Population comprised all subjects in the Evaluable Population who did not have any protocol deviations that were medically assessed as potentially impacting on immunogenicity results.
[c] GMT Ratio = Comparator /AFLURIA QUADRIVALENT. Adjusted analysis model: Log-transformed Post-Vaccination HI Titer=Vaccine + Age Strata [5-8, 9-17] + Gender + Vaccination History [y/n] + Log-transformed Pre-Vaccination HI Titer + Site + Number of Doses (1 vs 2) + Age Strata*Vaccine. The Age Strata*Vaccine interaction term was excluded from the model fit for the strains B/Yamagata and B/Victoria as the interaction result was non-significant (p>0.05). Least square means were back transformed.
[d] Seroconversion rate was defined as the percentage of subjects with either a prevaccination HI titer < 1:10 and a postvaccination HI titer ≥ 1:40 or a prevaccination HI titer ≥ 1:10 and a 4-fold increase in postvaccination HI titer.
[e] Seroconversion rate difference = Comparator SCR percentage minus AFLURIA QUADRIVALENT SCR percentage.
[f] Non-inferiority (NI) criterion for the GMT ratio: upper bound of two-sided 95% CI on the GMT ratio of Comparator / AFLURIA QUADRIVALENT should not exceed 1.5. NI criterion for the SCR difference: upper bound of two-sided 95% CI on the difference between SCR Comparator – AFLURIA QUADRIVALENT should not exceed 10%.
[g] Subject 8400394-0046 was excluded from the Per-Protocol Population for the adjusted GMT analysis for the GMT ratio since the subject did not have information on all covariates (unknown prevaccination history).

14.5 Immunogenicity of AFLURIA QUADRIVALENT in Children 6 Months through 59 Months Administered by Needle and Syringe

Study 4 was a randomized, observer-blind, comparator-controlled trial conducted in the U.S. in children 6 months through 59 months of age. A total of 2247 subjects were randomized 3:1 to receive AFLURIA QUADRIVALENT (N=1684) or a U.S.-licensed comparator quadrivalent influenza vaccine (N=563). Children 6 months through 35 months received one or two 0.25 mL doses and children 36 months through 59 months received one or two 0.5 mL doses. Subjects were eligible to receive a second dose at least 28 days after the first dose depending on their influenza vaccination history, consistent with the 2016-2017 recommendations of the Advisory Committee on Immunization Practices (ACIP) for Prevention and Control of Seasonal Influenza with Vaccines. Approximately 40% of subjects in each treatment group received two vaccine doses.

Baseline serology for HI assessment was collected prior to vaccination. Postvaccination immunogenicity was evaluated by HI assay on sera obtained 28 days after the last vaccination dose.

The primary objective was to demonstrate that vaccination with AFLURIA QUADRIVALENT elicits an immune response that is not inferior to that of a comparator vaccine containing the same recommended virus strains. The Per Protocol Population (AFLURIA QUADRIVALENT n=1456, Comparator QIV n=484) was used for the primary endpoint analyses. The co-primary endpoints were HI Geometric Mean Titer (GMT) ratios (adjusted for baseline HI titers and other covariates) and seroconversion rates for each vaccine strain, 28 days after the last vaccination. Pre-specified non-inferiority criteria required that the upper bound of the 2-sided 95% CI of the GMT ratio (Comparator QIV/AFLURIA QUADRIVALENT) did not exceed 1.5 and the upper bound of the 2-sided 95% CI of the seroconversion rate difference (Comparator QIV minus AFLURIA QUADRIVALENT) did not exceed 10.0% for each strain. Serum HI antibody responses to AFLURIA QUADRIVALENT were non-inferior for both GMT ratio and seroconversion rates relative to the comparator vaccine for all influenza strains (Table 10). Analyses of immunogenicity endpoints by gender did not demonstrate meaningful differences between males and females. The study population was not sufficiently diverse to assess differences among races or ethnicities.

Table 10: Post-Vaccination HI Antibody GMTs, SCRs, and Analyses of Non-Inferiority of AFLURIA QUADRIVALENT Relative to a U.S.-Licensed Comparator Quadrivalent Influenza Vaccine for each Strain 28 Days after Last Vaccination Among a Pediatric Population 6 through 59 Months of Age (Per Protocol Population) (Study 4)[a, b]

	Post-vaccination GMT		GMT Ratio [c]	Seroconversion % [d]		SCR Difference [e]	Met both pre-defined non-inferiority criteria? [f]
Strain	AFLURIA Quadrivalent N=1456	Comparator N=484	Comparator over AFLURIA Quadrivalent (95% CI)	AFLURIA Quadrivalent N=1456 (95% CI)	Comparator N=484 (95% CI)	Comparator minus AFLURIA Quadrivalent (95% CI)	
A(H1N1)	353.5 (n=1455 [g])	281.0 (n=484)	0.79 (0.72, 0.88)	79.1 (76.9, 81.1) (n=1456)	68.8 (64.5, 72.9) (n=484)	-10.3 (-15.4, -5.1)	Yes
A(H3N2)	393.0 (n=1454 [gi])	500.5 (n=484)	1.27 (1.15, 1.42)	82.3 (80.2, 84.2) (n=1455[i])	84.9 (81.4, 88.0) (n=484)	2.6 (-2.5, 7.8)	Yes
B/Phuket/ 3073/2013 (B Yamagata)	23.7 (n=1455 [g])	26.5 (n=484)	1.12 (1.01, 1.24)	38.9 (36.4, 41.4) (n=1456)	41.9 (37.5, 46.5) (n=484)	3.1 (-2.1, 8.2)	Yes
B/Brisbane/ 60/2008 (B Victoria)	54.6 (n=1455 [g])	52.9 (n=483[h])	0.97 (0.86, 1.09)	60.2 (57.6, 62.7) (n=1456)	61.1 (56.6, 65.4) (n=483[h])	0.9 (-4.2, 6.1)	Yes

Abbreviations: CI, confidence interval; Comparator, Comparator quadrivalent influenza vaccine (Fluzone Quadrivalent [Sanofi Aventis]); GMT (adjusted), geometric mean titer; SCR, seroconversion rate.

[a] NCT02914275

[b] The Per-Protocol Population comprised all subjects (6 through 35 months of age receiving one or two 0.25 mL doses and 36 through 59 months of age receiving one or two 0.5 mL doses) in the Evaluable Population who did not have any protocol deviations that were medically assessed as potentially impacting on immunogenicity results.

[c] GMT Ratio = Comparator / AFLURIA QUADRIVALENT. Adjusted analysis model: Log-transformed Post-Vaccination HI Titer=Vaccine + Age Cohort [6 through 35 months or 36 through 59 months] + Gender + Vaccination History [y/n] + Log-transformed Pre-Vaccination HI Titer + Site + Number of Doses (1 vs 2) + Age Cohort*Vaccine. The Age Cohort*Vaccine interaction term was excluded from the model fit for the strains A(H1N1), A(H3N2) and B/Yamagata as the interaction result was non-significant (p>0.05). Least square means were back transformed.

[d] Seroconversion rate was defined as the percentage of subjects with either a prevaccination HI titer < 1:10 and a postvaccination HI titer ≥ 1:40 or a prevaccination HI titer ≥ 1:10 and a 4-fold increase in postvaccination HI titer.

[e] Seroconversion rate difference = Comparator SCR percentage minus AFLURIA QUADRIVALENT SCR percentage.

[f] Noninferiority (NI) criterion for the GMT ratio: upper bound of two-sided 95% CI on the GMT ratio of Comparator / AFLURIA QUADRIVALENT should not exceed 1.5. NI criterion for the SCR difference: upper bound of two-sided 95% CI on the difference between SCR Comparator – AFLURIA QUADRIVALENT should not exceed 10%.

[g] Subject 8400402-0073 was excluded from the Per-Protocol Population for the adjusted GMT analysis for the GMT ratio because the subject did not have information on all covariates (unknown prevaccination history).

[h] Subject 8400427-0070 had missing B/Victoria Antigen pre-vaccination titer.

[i] Subject 8400402-0074 had missing A/H3N2 post-vaccination titer.

15 REFERENCES

1. Centers for Disease Control and Prevention. Prevention and Control of Influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;59 (RR-8):1-62.
2. Hannoun C, Megas F, Piercy J. Immunogenicity and Protective Efficacy of Influenza Vaccination. *Virus Res* 2004;103:133-138.
3. Hobson D, Curry RL, Beare AS, et al. The Role of Serum Hemagglutination-Inhibiting Antibody in Protection against Challenge Infection with Influenza A2 and B Viruses. *J Hyg Camb* 1972;70:767-777.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 HOW SUPPLIED

Each product presentation includes a package insert and the following components:

Presentation	Carton NDC Number	Components
Pre-Filled Syringe	33332-221-20	• Ten 0.25 mL single-dose syringes fitted with a Luer-Lok™ attachment without needles [NDC 33332-221-21]
Pre-Filled Syringe	33332-321-01	• Ten 0.5 mL single-dose syringes fitted with a Luer-Lok™ attachment without needles [NDC 33332-321-02]
Multi-Dose Vial	33332-421-10	• One 5 mL vial [NDC 33332-421-11]

16.2 Storage and Handling

- Store refrigerated at 2–8°C (36–46°F).
- Do not freeze. Discard if product has been frozen.
- Protect from light.
- Do not use AFLURIA QUADRIVALENT beyond the expiration date printed on the label.
- Between uses, return the multi-dose vial to the recommended storage conditions.
- Once the stopper of the multi-dose vial has been pierced the vial must be discarded within 28 days.
- The number of needle punctures should not exceed 20 per multi-dose vial.

17 PATIENT COUNSELING INFORMATION

- Inform the vaccine recipient or guardian of the potential benefits and risks of immunization with AFLURIA QUADRIVALENT.
- Inform the vaccine recipient or guardian that AFLURIA QUADRIVALENT is an inactivated vaccine that cannot cause influenza but stimulates the immune system to produce antibodies that protect against influenza, and that the full effect of the vaccine is generally achieved approximately 3 weeks after vaccination.
- Instruct the vaccine recipient or guardian to report any severe or unusual adverse reactions to their healthcare provider.
- Encourage women who receive AFLURIA QUADRIVALENT while pregnant to enroll in the pregnancy registry. Pregnant women can enroll in the pregnancy registry by calling 1-855-358-8966 or sending an email to Seqirus at us.medicalinformation@seqirus.com.
- Provide the vaccine recipient Vaccine Information Statements prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).
- Instruct the vaccine recipient that annual revaccination is recommended.

Seqirus™
A CSL COMPANY

Manufactured by:
Seqirus Pty Ltd. Parkville, Victoria, 3052, Australia
U.S. License No. 2044

Distributed by:
Seqirus USA Inc. 25 Deforest Avenue, Summit, NJ 07901, USA
1-855-358-8966

-------HIGHLIGHTS OF PRESCRIBING INFORMATION--------
These highlights do not include all the information needed to use FLUAD® QUADRIVALENT safely and effectively. See full prescribing information for FLUAD QUADRIVALENT.

FLUAD QUADRIVALENT (Influenza Vaccine, Adjuvanted)
Injectable Emulsion for Intramuscular Use
2022-2023 Formula
Initial U.S. Approval: 2020

----------**INDICATIONS AND USAGE**----------
FLUAD QUADRIVALENT is an inactivated influenza vaccine indicated for active immunization against influenza disease caused by influenza virus subtypes A and types B contained in the vaccine. FLUAD QUADRIVALENT is approved for use in persons 65 years of age and older. (1)

This indication is approved under accelerated approval based on the immune response elicited by FLUAD QUADRIVALENT (1). Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

----------**DOSAGE AND ADMINISTRATION**----------
A single 0.5 mL dose for intramuscular injection. (2.1)

----------**DOSAGE FORMS AND STRENGTHS**----------
Injectable emulsion supplied in 0.5 mL single-dose pre-filled syringes. (3)

----------**CONTRAINDICATIONS**----------
Severe allergic reaction to any component of the vaccine, including egg protein, or after a previous dose of any influenza vaccine. (4, 11)

----------**WARNINGS AND PRECAUTIONS**----------
If Guillain-Barré Syndrome (GBS) has occurred within six weeks of previous influenza vaccination, the decision to give FLUAD QUADRIVALENT should be based on careful consideration of the potential benefits and risks. (5.1)

----------**ADVERSE REACTIONS**----------
The most common (≥ 10%) local and systemic reactions in elderly subjects 65 years of age and older were injection site pain (16.3%), headache (10.8%) and fatigue (10.5%). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Seqirus at 1-855-358-8966 or VAERS at 1-800-822-7967 and www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION

Revised: 03/2022

FULL PRESCRIBING INFORMATION: CONTENTS

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

FLUAD QUADRIVALENT is an inactivated influenza vaccine indicated for active immunization against influenza disease caused by influenza virus subtypes A and types B contained in the vaccine. FLUAD QUADRIVALENT is approved for use in persons 65 years of age and older. This indication is approved under accelerated approval based on the immune response elicited by FLUAD QUADRIVALENT *[see Clinical Studies (14.1)]*. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only

2.1. Dosage and Schedule

Administer FLUAD QUADRIVALENT as a single 0.5 mL intramuscular injection in adults 65 years of age and older.

2.2. Administration

- Gently shake each syringe. FLUAD QUADRIVALENT has a milky-white appearance. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit *[see Description (11)]*. If either condition exists, FLUAD QUADRIVALENT should not be administered.
- To use a pre-filled syringe fitted with a Luer Lok system, remove the tip cap by unscrewing it in a counter-clockwise direction. Once the tip cap is removed, attach a needle to the syringe by screwing it on in a clockwise direction until it locks. Once the needle is locked in place, remove the needle protector and administer the vaccine.
- The vaccine should be administered by intramuscular injection, preferably in the region of the deltoid muscle of the upper arm. Do not inject the vaccine in the gluteal region or areas where there may be a major nerve trunk.

3 DOSAGE FORMS AND STRENGTHS

FLUAD QUADRIVALENT is a sterile injectable emulsion supplied in 0.5 mL single-dose pre-filled syringes.

4 CONTRAINDICATIONS

Do not administer FLUAD QUADRIVALENT to anyone with a history of severe allergic reaction (e.g. anaphylaxis) to any component of the vaccine, including egg protein *[see Description (11)]*, or to a previous influenza vaccine.

5 WARNINGS AND PRECAUTIONS

5.1. Guillain-Barré Syndrome

If Guillain-Barré syndrome (GBS) has occurred within 6 weeks of receipt of prior influenza vaccine, the decision to give FLUAD QUADRIVALENT should be based on careful consideration of the potential benefits and risks. The 1976 swine influenza vaccine was associated with an elevated risk of GBS. *[see References (1)]* Evidence for a causal relationship of GBS with other influenza vaccines is inconclusive; if an excess risk exists, it is probably slightly more than 1 additional case per 1 million persons vaccinated.

5.2. Preventing and Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

5.3 Altered Immunocompetence

The immune response to FLUAD QUADRIVALENT in immunocompromised persons, including individuals receiving immunosuppressive therapy, may be lower than in immunocompetent individuals. *[see Concurrent Use With Immunosuppressive Therapies (7.2)]*

5.4 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines including FLUAD QUADRIVALENT. Ensure procedures are in place to avoid injury from falling associated with syncope.

5.5 Limitations of Vaccine Effectiveness

Vaccination with FLUAD QUADRIVALENT may not protect all vaccine recipients against influenza disease.

6 ADVERSE REACTIONS

The most common (≥10%) local and systemic reactions in elderly subjects 65 years of age and older were injection site pain (16.3%), headache (10.8%) and fatigue (10.5%).

6.1. Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect rates observed in clinical practice.

The safety of FLUAD QUADRIVALENT was evaluated in two clinical studies in 4269 elderly subjects 65 years of age and older. Study 1 (NCT02587221) was a multi-center, randomized, observer-blind, non-influenza comparator-controlled efficacy and safety study conducted in 12 countries during the 2016-2017 Northern Hemisphere and 2017 Southern Hemisphere seasons. In this study, 3381 subjects received FLUAD QUADRIVALENT and 3380 subjects received a US-licensed non-influenza comparator vaccine (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Boostrix® [GlaxoSmithKline Biologicals]).

The mean age of subjects at enrollment was 72 years, 62% were female, 48% White, 34% Asian, 16% Other, 2% American Indian/Alaska Native, and 18% of Hispanic/Latino ethnicity.

Solicited local and systemic adverse reactions were collected for 7 days after vaccination in a subset of 665 subjects who received FLUAD QUADRIVALENT and 667 subjects who received the comparator vaccine. The percentages of subjects reporting solicited local adverse reactions are presented in Table 1a and systemic adverse reactions are presented in Table 1b. Onset usually occurred within the first 2 days after vaccination. The majority of solicited reactions resolved within 3 days.

Table 1a. Percentages of Subjects Reporting Solicited Local Adverse Reactions[a] in the Solicited Safety Population[b] within 7 Days of Vaccination (Study 1)

Local (Injection site) Reactions[c]	FLUAD QUADRIVALENT N=595-659	Non-Influenza Comparator Vaccine N=607-664
Injection site pain	16.3	11.2
Erythema ≥25mm	3.8	1.8
Induration ≥25mm	4.0	2.6
Ecchymosis ≥25mm	0.5	0.7

Study 1: NCT02587221

Abbreviation: N=number of subjects with solicited safety data

Non-Influenza Comparator Vaccine = combined Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Boostrix® (GlaxoSmithKline Biologicals)

[a] All solicited local adverse events reported within 7 days of vaccination are included

[b] Solicited Safety Population: all subjects in the exposed population who received a study vaccine and provided post-vaccination solicited safety data

[c] Severe reactions of each type were reported in 1.1% or fewer subjects receiving FLUAD QUADRIVALENT; severe reactions of each type were also reported in the comparator group at similar percentages. Severe definitions: Erythema, Induration and Ecchymosis = >100 mm diameter; Injection site pain, = prevents daily activity.

Table 1b. Percentages of Subjects Reporting Solicited Systemic Adverse Reactions[a] in the Solicited Safety Population[b] within 7 Days of Vaccination (Study 1)

Systemic Reactions[c]	FLUAD QUADRIVALENT N=595-659	Non-Influenza Comparator Vaccine N=607-664
Headache	10.8	8.3
Fatigue	10.5	8.8
Myalgia	7.7	6.1
Arthralgia	7.3	6.6
Chills	5.0	3.9
Diarrhea	4.1	3.0
Nausea	3.8	2.3
Loss of appetite	3.6	3.6
Fever ≥100.4°F (38°C)	1.7	1.2
Vomiting	0.8	1.1

Study 1: NCT02587221

Abbreviation: N=number of subjects with solicited safety data

Non-Influenza Comparator Vaccine = combined Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Boostrix® (GlaxoSmithKline Biologicals)

[a] All solicited systemic adverse events reported within 7 days of vaccination are included

[b] Solicited Safety Population: all subjects in the exposed population who received a study vaccine and provided post-vaccination solicited safety data

[c] Severe reactions of each type were reported in 1.1% or fewer subjects receiving FLUAD QUADRIVALENT; severe reactions of each type were also reported in the comparator group at similar percentages. Severe definitions: Nausea, Fatigue, Myalgia, Arthralgia, Headache, and Chills = prevents daily activity; Loss of appetite = not eating at all; Vomiting = 6 or more times in 24 hours or requires intravenous hydration; Diarrhea = 6 or more loose stools in 24 hours or requires intravenous hydration; Fever = ≥102.2°F (39°C).

Unsolicited adverse events (AEs) were collected for all subjects for 21 days after vaccination. Related unsolicited AEs were reported by 303 (9.0%) and by 261 (7.7%) of the subjects for FLUAD QUADRIVALENT and Boostrix, respectively. For FLUAD QUADRIVALENT, injection site pain and influenza-like illness were the only unsolicited adverse reactions reported in ≥ 1% of subjects (1.7% and 1.5%, respectively).

Serious adverse events (SAEs) and potentially immune-mediated adverse events of special interest (AESIs) were collected up to 366 days after vaccination. SAEs were reported by 238 (7.0%) FLUAD QUADRIVALENT recipients and 234 (6.9%) comparator recipients. There were no SAEs, AESIs or deaths in this study that were related to FLUAD QUADRIVALENT.

Study 2 (NCT03314662) was a multicenter, randomized, double-blind, comparator-controlled study conducted during the 2017-18 Northern Hemisphere influenza season. In this study, 888 subjects received FLUAD QUADRIVALENT, 444 subjects received the licensed adjuvanted trivalent vaccine (aTIV-1 - FLUAD® (trivalent formulation)) and 444 subjects received an adjuvanted trivalent influenza vaccine with an alternate B strain (aTIV-2).

The mean age of subjects at enrollment who received FLUAD QUADRIVALENT was 72.5 years. Female subjects represented 56.6% of the study population and the racial distribution of subjects was 91.6% Caucasian, 7.0% Black or African American, and ≤ 1% each for Asian, Native Hawaiian or Pacific Islander, American Indian or Alaska Native or Other.

Solicited local and systemic adverse reactions reported within 7 days after vaccination were similar to those reported for Study 1. Unsolicited AEs were collected for 21 days after vaccination. Related unsolicited AEs were reported by 39 (4.4%) and by 17-19 (3.8%-4.3%) of subjects administered FLUAD QUADRIVALENT or aTIV, respectively. For FLUAD QUADRIVALENT, injection site bruising (1.0%) was the only unsolicited adverse reaction reported in ≥ 1% of subjects.

Serious AEs and AESIs were collected up to 181 days after vaccination. Within 6 months after vaccination, 37 (4.2%) FLUAD QUADRIVALENT recipients and 18-28 (4.1%-6.3%) aTIV recipients experienced an SAE. There were no SAEs, AESIs or deaths in this study that were related to the study vaccine. There were no AEs leading to withdrawal from the study.

6.2. Postmarketing Experience

There are no postmarketing data available for FLUAD QUADRIVALENT. However, the post-marketing experience with FLUAD (trivalent formulation) is relevant to FLUAD QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions.

Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Blood and lymphatic system disorders:

Thrombocytopenia (some cases were severe with platelet counts less than 5,000 per mm^3), lymphadenopathy

General disorders and administration site conditions:

Extensive swelling of injected limb lasting more than one week, injection site cellulitis-like reactions (some cases of swelling, pain, and redness extending more than 10 cm and lasting more than 1 week)

Immune system disorders:

Allergic reactions including anaphylactic shock, anaphylaxis, and angioedema

Musculoskeletal and connective tissue disorders:

Muscular weakness

Nervous system disorders:

Encephalomyelitis, Guillain-Barré Syndrome, convulsions, neuritis, neuralgia, parasthesia, syncope, presyncope

Skin and subcutaneous tissue disorders:

Generalized skin reactions including erythema multiforme, urticaria, pruritis or non-specific rash

Vascular disorders:

Vasculitis, renal vasculitis

7 DRUG INTERACTIONS

7.1. Concomitant Use With Other Vaccines

No clinical data on concomitant administration of FLUAD QUADRIVALENT with other vaccines is available.

If FLUAD QUADRIVALENT is given at the same time as other injectable vaccine(s), the vaccine(s) should be administered at different injection sites.

Do not mix FLUAD QUADRIVALENT with any other vaccine in the same syringe.

7.2. Concurrent Use With Immunosuppressive Therapies

Immunosuppressive or corticosteroid therapies may reduce the immune response to FLUAD QUADRIVALENT.

8 USE IN SPECIFIC POPULATIONS

8.1. Pregnancy

Risk Summary

FLUAD QUADRIVALENT is not approved for use in persons < 65 years of age. There are insufficient human data to establish whether there is a vaccine-associated risk with use of FLUAD QUADRIVALENT in pregnancy.

There were no developmental toxicity studies of FLUAD QUADRIVALENT performed in animals. A developmental toxicity study has been performed in female rabbits administered FLUAD (trivalent formulation) prior to mating and during gestation. A 0.5 mL dose was injected on each occasion (a single human dose is 0.5 mL). (see 8.1 Animal Data).

Animal Data

In a developmental toxicity study, the effect of FLUAD (trivalent formulation) was evaluated in pregnant rabbits. Animals were administered FLUAD (trivalent formulation) by intramuscular injection twice prior to gestation, during the period of organogenesis (gestation day 7) and later in pregnancy (gestation day 20), 0.5 mL (45 mcg)/rabbit/occasion. No vaccine-related fetal malformations or variations and no adverse effects on pre-weaning development were observed in the study.

8.2. Lactation

FLUAD QUADRIVALENT is not approved for use in persons < 65 years of age. No human or animal data are available to assess the effects of FLUAD QUADRIVALENT on the breastfed infant or on milk production/excretion.

8.4. Pediatric Use

Safety and effectiveness of FLUAD and FLUAD QUADRIVALENT (same manufacturing process and overlapping composition with FLUAD) were evaluated in clinical trials conducted in children 6 months to <72 months of age. Data from these trials are inconclusive to demonstrate the safety and effectiveness of FLUAD QUADRIVALENT in children 6 months to <72 months of age. The safety and effectiveness of FLUAD QUADRIVALENT in infants less than 6 months of age and in children older than 72 months of age have not been evaluated.

8.5. Geriatric Use

Safety and immunogenicity of FLUAD QUADRIVALENT have been evaluated in adults 65 years of age and older. *[See Adverse Reactions (6.1) and Clinical Studies (14)]*

11 DESCRIPTION

FLUAD QUADRIVALENT (Influenza Vaccine, Adjuvanted), a sterile injectable emulsion for intramuscular use, is a quadrivalent, inactivated influenza vaccine prepared from virus propagated in the allantoic cavity of embryonated hens' eggs inoculated with a specific type of influenza virus.

FLUAD QUADRIVALENT is standardized according to United States Public Health Service requirements and each 0.5 mL dose is formulated to contain 15 mcg of hemagglutinin (HA) from each of the following four influenza strains recommended for the 2022-2023 influenza season: A/Victoria/2570/2019 IVR-215 (an A/Victoria/2570/2019 (H1N1)pdm09-like virus), A/Darwin/6/2021 IVR-227 (an A/Darwin/9/2021 (H3N2)-like virus), B/Austria/1359417/2021 BVR-26 (a B/Austria/1359417/2021-like virus), B/Phuket/3073/2013 BVR-1B (a B/Phuket/3073/2013-like virus). FLUAD QUADRIVALENT also contains MF59C.1 adjuvant (MF59®), a squalene based oil-in-water emulsion. Each of the strains is harvested and clarified separately by centrifugation and filtration prior to inactivation with formaldehyde. The inactivated virus is concentrated and purified by zonal centrifugation. The surface antigens, hemagglutinin and neuraminidase, are obtained from the influenza virus particle by further centrifugation in the presence of cetyltrimethylammonium bromide (CTAB). The antigen preparation is further purified.

FLUAD QUADRIVALENT is prepared by combining the four virus antigens with the MF59C.1 adjuvant. After combining, FLUAD QUADRIVALENT is a sterile, milky-white injectable emulsion supplied in single-dose pre-filled syringes containing 0.5 mL dose. Each 0.5 mL dose contains 15 mcg of hemagglutinin (HA) from each of the four recommended influenza strains and MF59C.1 adjuvant (9.75 mg squalene, 1.175 mg of polysorbate 80, 1.175 mg of sorbitan trioleate, 0.66 mg of sodium citrate dihydrate and 0.04 mg of citric acid monohydrate) at pH 6.9-7.7.

FLUAD QUADRIVALENT may contain trace amounts of neomycin (≤ 0.02 mcg by calculation), kanamycin (≤ 0.03 mcg by calculation) and hydrocortisone (≤ 0.005 ng by calculation) which are used during the initial stages of manufacture, as well as residual egg protein (ovalbumin) (≤ 1.0 mcg), formaldehyde (≤ 10 mcg) or CTAB (≤ 18 mcg).

FLUAD QUADRIVALENT does not contain a preservative. The syringe, syringe plunger stopper and tip caps are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1. Mechanism of Action

Influenza illness and its complications follow infection with influenza viruses. Global surveillance of influenza identifies yearly antigenic variants. For example, since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation. Specific levels of hemagglutination inhibition (HI) antibody titers induced by vaccination with inactivated influenza virus vaccine have not been correlated with protection from influenza illness. In some human studies, HI antibody titers of 1:40 or greater have been associated with protection from influenza illness in up to 50% of subjects. *[see References (2,3)]*

Antibody against one influenza virus type or subtype confers limited or no protection against another. Furthermore, antibody to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual change of one or more new strains in each year's influenza vaccine. Therefore, inactivated quadrivalent influenza vaccines are standardized to contain the hemagglutinin of influenza virus strains (two subtypes A and two types B), representing the influenza viruses likely to be circulating in the United States in the upcoming influenza season.

Annual influenza vaccination is recommended because immunity declines during the year after vaccination, and because circulating strains of influenza virus change from year to year.

13 NONCLINICAL TOXICOLOGY

13.1. Carcinogenesis, Mutagenesis, Impairment of Fertility

FLUAD QUADRIVALENT has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. FLUAD (trivalent formulation) did not affect female fertility in a rabbit developmental toxicity study *[see Pregnancy (8.1)]*.

14 CLINICAL STUDIES

14.1 Immunogenicity of FLUAD QUADRIVALENT

Immunogenicity of FLUAD QUADRIVALENT was evaluated in Study 1 (NCT02587221), a randomized, observer-blind, non-influenza comparator-controlled multicenter efficacy study conducted in 12 countries during the 2016-2017 Northern Hemisphere and 2017 Southern Hemisphere seasons. In this study, elderly subjects 65 years of age and older received one dose of either FLUAD QUADRIVALENT (N=3379) or a US-licensed non-influenza comparator vaccine (Boostrix; N=3382). Immunogenicity was evaluated 21 days after vaccination in a subgroup of subjects in a 4:1 ratio: FLUAD QUADRIVALENT (N=1324) and non-influenza control vaccines (N=332). In the immunogenicity set, the mean age across both vaccination groups was 72 years and females represented 59% of subjects. The racial distribution of subjects consisted of 89% Caucasian, 11% Asian and <1% American Indian or Alaska Native.

Immunogenicity endpoints measured 3 weeks after vaccination included percentage of subjects with HI titer ≥1:40 and percentage of subjects who achieved seroconversion. Success criteria required the lower bound of the 2-sided 95% CI for the proportion of subjects with an HI titer ≥1:40 to be ≥60% and for the lower bound of the 2-sided 95% CI for the proportion of subjects with seroconversion to be ≥30%. Antibody responses for all 4 strains are presented in Table 2.

Table 2: Immune Responses 21 Days After Vaccination with FLUAD QUADRIVALENT or a Non-Influenza Comparator Vaccine in Elderly Subjects 65 years of Age and Older (Study 1)

Strain	Proportion of subjects with HI titer ≥1:40[a] (95% CI) FLUAD QUADRIVALENT N=1324	Proportion of subjects with HI titer ≥1:40[a] (95% CI) Non-Influenza Comparator Vaccine N=332	Seroconversion[b] (95% CI) FLUAD QUADRIVALENT N=1324	Seroconversion[b] (95% CI) Non-Influenza Comparator Vaccine N=332
A/H1N1	96.2% (95.1%, 97.2%)	46.7% (41.2%, 52.2%)	78.0% (75.7%, 80.2%)	2.1% (0.9%, 4.3%)
A/H3N2	95.6% (94.4%, 96.7%)	41.7% (36.3%, 47.2%)	84.6% (82.5%, 86.5%)	3.9% (2.1%, 6.6%)
B/Yamagata	79.2% (77.0%, 81.4%)	21.5% (17.2%, 26.4%)	60.8% (58.1%, 63.4%)	3.6% (1.9%, 6.3%)
B/Victoria	81.6% (79.4%, 83.7%)	18.4% (14.4%, 23.0%)	65.5% (62.9%, 68.1%)	2.1% (0.9%, 4.3%)

Abbreviations: CI=Confidence Interval, N=number of subjects in full analysis immunogenicity set.
Non-Influenza Comparator Vaccine = combined Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Boostrix® (GlaxoSmithKline Biologicals)
aSuccess criteria: LB of the 95% CI for the % of subjects with HI titer ≥1:40 must be ≥60%
b Seroconversion is defined as a pre-vaccination HI titer <1:10 and post-vaccination HI titer ≥ 1:40 or at least a 4-fold increase in HI from pre-vaccination HI titer ≥ 1:10. Success criteria: the LB of the 95% CI for the SCR must be ≥30%.

15 REFERENCES

1. Lasky T, Terracciano GJ, Magder L, et al. The Guillain-Barre syndrome and the 1992-1993 and 1993-1994 influenza vaccines. N Engl J Med 1998; 339(25): 1797-1802.

2. Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. Virus Res 2004; 103:133-138.

3. Hobson D, Curry RL, Beare A, et. al. The role of serum hemagglutinin-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg Camb 1972; 767-777.

16 HOW SUPPLIED/STORAGE AND HANDLING

FLUAD QUADRIVALENT is supplied in the product presentation listed below:

Presentation	Carton NDC Number	Components
Pre-Filled Syringe	70461-122-03	0.5 mL dose in a pre-filled syringe (needle not supplied), package of 10 syringes per carton [NDC 70461-122-04]

Store FLUAD QUADRIVALENT refrigerated at 2°C to 8°C (36°F to 46°F). Protect from light. Do not freeze. Discard if the vaccine has been frozen. Do not use after expiration date.

The syringe, syringe plunger stopper and tip cap are not made with natural rubber latex.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipients of the potential benefits and risks of immunization with FLUAD QUADRIVALENT.
- Educate vaccine recipients regarding the potential side effects. Clinicians should emphasize that (1) FLUAD QUADRIVALENT contains non-infectious particles and cannot cause influenza and (2) FLUAD QUADRIVALENT is intended to help provide protection against illness due to influenza viruses only.
- Instruct vaccine recipients to report adverse reactions to their healthcare provider and/or to Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967 and www.vaers.hhs.gov. Provide vaccine recipients with the Vaccine Information Statements which are required by the National Childhood Vaccine Injury Act of 1986. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).
- Inform vaccine recipients that annual vaccination is recommended.

Manufactured by: **Seqirus Inc.,** 475 Green Oaks Parkway, Holly Springs, NC 27540, USA

Distributed by: **Seqirus USA Inc.,** 25 Deforest Avenue, Summit, NJ 07901, USA

Tel: 1-855-358-8966

US License No. 2049

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use FLUARIX QUADRIVALENT safely and effectively. See full prescribing information for FLUARIX QUADRIVALENT.

FLUARIX QUADRIVALENT (Influenza Vaccine) injectable suspension, for intramuscular use
2022-2023 Formula
Initial U.S. Approval: 2012

INDICATIONS AND USAGE

FLUARIX QUADRIVALENT is a vaccine indicated for active immunization for the prevention of disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. FLUARIX QUADRIVALENT is approved for use in persons aged 6 months and older. (1)

DOSAGE AND ADMINISTRATION

For intramuscular injection only. (2)

Age	Vaccination Status	Dose and Schedule
6 months through 8 years	Not previously vaccinated with influenza vaccine	Two doses (0.5-mL each) at least 4 weeks apart (2.1)
	Vaccinated with influenza vaccine in a previous season	One or 2 doses[a] (0.5-mL each) (2.1)
9 years and older	Not applicable	One 0.5-mL dose (2.1)

[a] One dose or 2 doses (0.5-mL each) depending on vaccination history as per the annual Advisory Committee on Immunization Practices (ACIP) recommendation on prevention and control of seasonal influenza with vaccines. If 2 doses, administer each 0.5-mL dose at least 4 weeks apart. (2.1)

DOSAGE FORMS AND STRENGTHS

Suspension for injection supplied in 0.5-mL single-dose prefilled syringes. (3)

CONTRAINDICATIONS

History of severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine, including egg protein, or following a previous dose of any influenza vaccine. (4, 11)

WARNINGS AND PRECAUTIONS

- If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give FLUARIX QUADRIVALENT should be based on careful consideration of potential benefits and risks. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including FLUARIX QUADRIVALENT. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)

ADVERSE REACTIONS

- In adults, the most common (≥10%) solicited local adverse reaction was pain (36%); the most common systemic adverse reactions were muscle aches (16%), headache (16%), and fatigue (16%). (6.1)
- In children aged 6 through 35 months, the most common (≥10%) solicited local adverse reactions were pain (17%) and redness (13%); the most common systemic adverse reactions were irritability (16%), loss of appetite (14%), and drowsiness (13%). (6.1)
- In children aged 3 through 17 years, the solicited local adverse reactions were pain (44%), redness (23%), and swelling (19%). (6.1)
- In children aged 3 through 5 years, the most common (≥10%) systemic adverse reactions were drowsiness (17%), irritability (17%), and loss of appetite (16%); in children aged 6 through 17 years, the most common systemic adverse reactions were fatigue (20%), muscle aches (18%), headache (16%), arthralgia (10%), and gastrointestinal symptoms (10%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

USE IN SPECIFIC POPULATIONS

Geriatric Use: Antibody responses were lower in geriatric subjects who received FLUARIX QUADRIVALENT than in younger subjects. (8.5)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 07/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

FLUARIX QUADRIVALENT is indicated for active immunization for the prevention of disease caused by influenza A subtype viruses and type B viruses contained in the vaccine *[see Description (11)]*. FLUARIX QUADRIVALENT is approved for use in persons aged 6 months and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Dosage and Schedule

The dose and schedule for FLUARIX QUADRIVALENT are presented in Table 1.

Table 1. FLUARIX QUADRIVALENT: Dosing

Age	Vaccination Status	Dose and Schedule
6 months through 8 years	Not previously vaccinated with influenza vaccine	Two doses (0.5-mL each) at least 4 weeks apart
	Vaccinated with influenza vaccine in a previous season	One or 2 doses[a] (0.5-mL each)
9 years and older	Not applicable	One 0.5-mL dose

[a] One dose or 2 doses (0.5-mL each) depending on vaccination history as per the annual Advisory Committee on Immunization Practices (ACIP) recommendation on prevention and control of seasonal influenza with vaccines. If 2 doses, administer each 0.5-mL dose at least 4 weeks apart.

2.2 Administration Instructions

Shake well before administration. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Attach a sterile needle to the prefilled syringe and administer intramuscularly.

The preferred sites for intramuscular injection are the anterolateral thigh for children aged 6 through 11 months and the deltoid muscle of the upper arm for persons aged 12 months and older if muscle mass is adequate. Do not inject in the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously, intradermally, or subcutaneously.

3 DOSAGE FORMS AND STRENGTHS

FLUARIX QUADRIVALENT is a suspension for injection. Each 0.5-mL dose is supplied in single-dose prefilled TIP-LOK syringes.

4 CONTRAINDICATIONS

Do not administer FLUARIX QUADRIVALENT to anyone with a history of severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine, including egg protein, or following a previous administration of any influenza vaccine *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome (GBS) has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give FLUARIX QUADRIVALENT should be based on careful consideration of the potential benefits and risks.

The 1976 swine influenza vaccine was associated with an increased frequency of GBS. Evidence for a causal relation of GBS with subsequent vaccines prepared from other influenza viruses is inconclusive. If influenza vaccine does pose a risk, it is probably slightly more than 1 additional case/1 million persons vaccinated.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including FLUARIX QUADRIVALENT. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions. Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of FLUARIX QUADRIVALENT.

5.4 Altered Immunocompetence

If FLUARIX QUADRIVALENT is administered to immunosuppressed persons, including individuals receiving immunosuppressive therapy, the immune response may be lower than in immunocompetent persons.

5.5 Limitations of Vaccine Effectiveness

Vaccination with FLUARIX QUADRIVALENT may not protect all susceptible individuals.

5.6 Persons at Risk of Bleeding

As with other intramuscular injections, FLUARIX QUADRIVALENT should be given with caution in individuals with bleeding disorders, such as hemophilia or on anticoagulant therapy, to avoid the risk of hematoma following the injection.

6 ADVERSE REACTIONS

The safety experience with FLUARIX (trivalent influenza vaccine) is relevant to FLUARIX QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*.

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. There is the possibility that broad use of FLUARIX QUADRIVALENT could reveal adverse reactions not observed in clinical trials.

In adults who received FLUARIX QUADRIVALENT, the most common (≥10%) solicited local adverse reaction was pain (36%). The most common (≥10%) systemic adverse reactions were muscle aches (16%), headache (16%), and fatigue (16%).

In children aged 6 through 35 months who received FLUARIX QUADRIVALENT, the most common (≥10%) solicited local adverse reactions were pain (17%) and redness (13%). The most common (≥10%) systemic adverse reactions were irritability (16%), loss of appetite (14%), and drowsiness (13%). In children aged 3 through 17 years who received FLUARIX QUADRIVALENT, solicited local adverse reactions were pain (44%), redness (23%), and swelling (19%). In children aged 3 through 5 years, the most common (≥10%) systemic adverse reactions were drowsiness (17%), irritability (17%), and loss of appetite (16%); in children aged 6 through 17 years, the most common systemic adverse reactions were fatigue (20%), muscle aches (18%), headache (16%), arthralgia (10%), and gastrointestinal symptoms (10%).

FLUARIX QUADRIVALENT in Adults

Trial 1 (NCT01204671) was a randomized, double-blind (2 arms) and open-label (one arm), active-controlled, safety, and immunogenicity trial. In this trial, subjects received FLUARIX QUADRIVALENT (n = 3,036) or one of 2 formulations of comparator trivalent influenza vaccine (FLUARIX; TIV-1, n = 1,010; or TIV-2, n = 610), each containing an influenza type B virus that corresponded to one of the 2 type B viruses in FLUARIX QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). The population was aged 18 years and older (mean age: 58 years) and 57% were female; 69% were white, 27% were Asian, and 4% were of other racial/ethnic groups. Solicited events were collected for 7 days (day of vaccination and the next 6 days). The frequencies of solicited adverse reactions are shown in Table 2.

Table 2. FLUARIX QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] of Vaccination in Adults[b] (Total Vaccinated Cohort)

	FLUARIX QUADRIVALENT[c] n = 3,011-3,015 %		Trivalent Influenza Vaccine (TIV)			
			TIV-1 (B Victoria)[d] n = 1,003 %		TIV-2 (B Yamagata)[e] n = 607 %	
Adverse Reaction	**Any**	**Grade 3[f]**	**Any**	**Grade 3[f]**	**Any**	**Grade 3[f]**
Local						
Pain	36	0.8	37	1	31	0.5
Redness	2	0	2	0	2	0
Swelling	2	0	2	0	1	0
Systemic						
Muscle aches	16	0.5	19	0.8	16	0.5
Headache	16	0.9	16	0.8	13	0.7
Fatigue	16	0.7	18	0.6	15	0.5
Arthralgia	8	0.5	10	0.7	9	0.3
Gastrointestinal symptoms[g]	7	0.4	7	0.2	6	0.3
Shivering	4	0.4	5	0.3	4	0.2
Fever[h]	2	0	1	0	2	0

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
n = Number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 1: NCT01204671.

[c] Contained the same composition as FLUARIX (trivalent formulation) manufactured for the 2010-2011 season and an additional influenza type B virus of Yamagata lineage.

[d] Contained the same composition as FLUARIX manufactured for the 2010-2011 season (2 influenza A subtype viruses and an influenza type B virus of Victoria lineage).

[e] Contained the same 2 influenza A subtype viruses as FLUARIX manufactured for the 2010-2011 season and an influenza type B virus of Yamagata lineage.

[f] Grade 3 pain: Defined as significant pain at rest; prevented normal everyday activities.
Grade 3 redness, swelling: Defined as >100 mm.
Grade 3 muscle aches, headache, fatigue, arthralgia, gastrointestinal symptoms, shivering: Defined as prevented normal activity.
Grade 3 fever: Defined as >102.2°F (39.0°C).

[g] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.

[h] Fever: Defined as ≥99.5°F (37.5°C).

Unsolicited events occurring within 21 days of vaccination (Day 0 to 20) were reported in 13%, 14%, and 15% of subjects who received FLUARIX QUADRIVALENT, TIV-1, or TIV-2, respectively. The unsolicited adverse reactions that occurred most frequently (≥0.1% for FLUARIX QUADRIVALENT) included dizziness, injection site hematoma, injection site pruritus, and rash. Serious adverse events occurring within 21 days of vaccination were reported in 0.5%, 0.6%, and 0.2% of subjects who received FLUARIX QUADRIVALENT, TIV-1, or TIV-2, respectively.

FLUARIX QUADRIVALENT in Children

Trial 7 (NCT01439360) was a randomized, observer-blind, non-influenza vaccine-controlled trial evaluating the efficacy of FLUARIX QUADRIVALENT. In this trial, subjects aged 6 through 35 months received FLUARIX QUADRIVALENT (n = 6,006) or a control vaccine (n = 6,012). The comparator was pneumococcal 13-valent conjugate vaccine [Diphtheria CRM197 Protein] (Wyeth Pharmaceuticals, Inc.) in children younger than 12 months, HAVRIX (Hepatitis A Vaccine) in children 12 months and older with a history of influenza vaccination, or HAVRIX (Dose 1) and a varicella vaccine (U.S. Licensed Manufactured by Merck & Co., Inc. or Non-U.S. Licensed Manufactured by GlaxoSmithKline Biologicals) (Dose 2) in those with no history of influenza vaccination. Subjects were aged 6 through 35 months, and one child aged 43 months (mean age: 22 months); 51% were male; 27% were white, 45% were Asian, and 28% were of other racial/ethnic groups. Children aged 12 months and older with no history of influenza vaccination and children younger than 12 months received 2 doses of FLUARIX QUADRIVALENT or the control vaccine approximately 28 days apart. Children aged 12 months and older with a history of influenza vaccination received one dose. Solicited local adverse reactions and systemic adverse events were collected using diary cards for 7 days (day of vaccination and the next 6 days). The incidences of solicited adverse reactions are shown in Table 3.

Table 3. FLUARIX QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] after First Vaccination in Children Aged 6 through 35 Months[b] (Total Vaccinated Cohort)

Adverse Reaction	FLUARIX QUADRIVALENT %		Non-Influenza Active Comparator[c,d] %	
	Any	Grade 3[e]	Any	Grade 3[e]
Local	**n = 5,899**		**n = 5,896**	
Pain	17	0.4	18	0.5
Redness	13	0	14	0
Swelling	8	0	9	0
Systemic	**n = 5,898**		**n = 5,896**	
Irritability	16	0.7	18	1
Loss of appetite	14	1	15	1
Drowsiness	13	0.7	14	0.9
Fever[f]	6	1	7	1

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
n = Number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 7: NCT01439360.

[c] Children younger than 12 months: pneumococcal 13-valent conjugate vaccine [Diphtheria CRM197 Protein] (Wyeth Pharmaceuticals, Inc.).

[d] Children 12 months and older: HAVRIX (Hepatitis A Vaccine) for those with a history of influenza vaccination; or HAVRIX (Dose 1) and a varicella vaccine (U.S. Licensed Manufactured by Merck & Co., Inc. or Non-U.S. Licensed Manufactured by GlaxoSmithKline Biologicals) (Dose 2) for those with no history of influenza vaccination.

[e] Grade 3 pain: Defined as cried when limb was moved/spontaneously painful.
Grade 3 swelling, redness: Defined as >50 mm.
Grade 3 irritability: Defined as crying that could not be comforted/prevented normal activity.
Grade 3 loss of appetite: Defined as not eating at all.
Grade 3 drowsiness: Defined as prevented normal activity.
Grade 3 fever: Defined as >102.2°F (39.0°C).

[f] Fever: Defined as ≥100.4°F (38.0°C).

In children who received a second dose of FLUARIX QUADRIVALENT or the Non-Influenza Active Comparator vaccine, the incidences of solicited adverse reactions following the second dose were generally lower than those observed after the first dose.

Unsolicited adverse events occurring within 28 days of vaccination were reported in 44% and 45% of subjects who received FLUARIX QUADRIVALENT (n = 6,006) and the comparator vaccine (n = 6,012), respectively. Serious adverse events (SAEs) occurring during the study period (6 to 8

months) were reported in 3.6% of subjects who received FLUARIX QUADRIVALENT and in 3.3% of subjects who received the comparator vaccine.

Trial 2 (NCT01196988) was a randomized, double-blind, active-controlled, safety, and immunogenicity trial. In this trial, subjects received FLUARIX QUADRIVALENT (n = 915) or one of 2 formulations of comparator trivalent influenza vaccine (FLUARIX; TIV-1, n = 912; or TIV-2, n = 911), each containing an influenza type B virus that corresponded to one of the 2 type B viruses in FLUARIX QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). Subjects were aged 3 through 17 years and 52% were male; 56% were white, 29% were Asian, 12% were black, and 3% were of other racial/ethnic groups. Children aged 3 through 8 years with no history of influenza vaccination received 2 doses approximately 28 days apart. Children aged 3 through 8 years with a history of influenza vaccination and children aged 9 years and older received one dose. Solicited local adverse reactions and systemic adverse events were collected using diary cards for 7 days (day of vaccination and the next 6 days). The frequencies of solicited adverse reactions are shown in Table 4.

Table 4. FLUARIX QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] after First Vaccination in Children Aged 3 through 17 Years[b] (Total Vaccinated Cohort)

	FLUARIX QUADRIVALENT[c] %		Trivalent Influenza Vaccine (TIV)			
			TIV-1 (B Victoria)[d] %		TIV-2 (B Yamagata)[e] %	
	Any	Grade 3[f]	Any	Grade 3[f]	Any	Grade 3[f]
Adverse Reaction	**Aged 3 through 17 Years**					
Local	**n = 903**		**n = 901**		**n = 905**	
Pain[g]	44	2	42	2	40	0.8
Redness	23	1	21	0.2	21	0.7
Swelling	19	0.8	17	1	15	0.2
	Aged 3 through 5 Years					
Systemic	**n = 291**		**n = 314**		**n = 279**	
Drowsiness	17	1	12	0.3	14	0.7
Irritability	17	0.7	13	0.3	14	0.7
Loss of appetite	16	0.3	8	0	10	0.7
Fever[h]	9	0.3	9	0.3	8	1
	Aged 6 through 17 Years					
Systemic	**n = 613**		**n = 588**		**n = 626**	
Fatigue	20	2	19	1	16	0.5
Muscle aches	18	0.7	16	1	16	0.5
Headache	16	1	19	0.7	15	0.6
Arthralgia	10	0.3	9	0.7	7	0.2
Gastrointestinal symptoms[i]	10	1	10	0.7	7	0.3
Shivering	6	0.5	4	0.5	5	0
Fever[h]	6	1	9	0.5	6	0.3

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
n = Number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 2: NCT01196988.

[c] Contained the same composition as FLUARIX (trivalent formulation) manufactured for the 2010-2011 season and an additional influenza type B virus of Yamagata lineage.

[d] Contained the same composition as FLUARIX manufactured for the 2010-2011 season (2 influenza A subtype viruses and an influenza type B virus of Victoria lineage).

[e] Contained the same 2 influenza A subtype viruses as FLUARIX manufactured for the 2010-2011 season and an influenza type B virus of Yamagata lineage.

[f] Grade 3 pain: Defined as cried when limb was moved/spontaneously painful (children <6 years), or significant pain at rest, prevented normal everyday activities (children ≥6 years).
Grade 3 redness, swelling: Defined as >50 mm.
Grade 3 drowsiness: Defined as prevented normal activity.
Grade 3 irritability: Defined as crying that could not be comforted/prevented normal activity.
Grade 3 loss of appetite: Defined as not eating at all.
Grade 3 fever: Defined as >102.2°F (39.0°C).
Grade 3 fatigue, muscle aches, headache, arthralgia, gastrointestinal symptoms, shivering: Defined as prevented normal activity.

[g] Percentage of subjects with any pain by age subgroup: 39%, 38%, and 37% for FLUARIX QUADRIVALENT, TIV-1, and TIV-2, respectively, in children aged 3 through 8 years and 52%, 50%, and 46% for FLUARIX QUADRIVALENT, TIV-1, and TIV-2, respectively, in children aged 9 through 17 years.

[h] Fever: Defined as ≥99.5°F (37.5°C).

[i] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.

In children who received a second dose of FLUARIX QUADRIVALENT, TIV-1, or TIV-2, the incidences of adverse reactions following the second dose were generally lower than those observed after the first dose.

Unsolicited adverse events occurring within 28 days of any vaccination were reported in 31%, 33%, and 34% of subjects who received FLUARIX QUADRIVALENT, TIV-1, or TIV-2, respectively. The unsolicited adverse reactions that occurred most frequently (≥0.1% for FLUARIX QUADRIVALENT) included injection site pruritus and rash. Serious adverse events occurring within 28 days of any vaccination were reported in 0.1%, 0.1%, and 0.1% of subjects who received FLUARIX QUADRIVALENT, TIV-1, or TIV-2, respectively.

FLUARIX (Trivalent Formulation)

FLUARIX has been administered to 10,317 adults aged 18 through 64 years, 606 subjects aged 65 years and older, and 2,115 children aged 6 months through 17 years in clinical trials. The incidence of solicited adverse reactions in each age-group is shown in Tables 5 and 6.

Table 5. FLUARIX (Trivalent Formulation): Incidence of Solicited Local and Systemic Adverse Reactions within 4 Days[a] of Vaccination in Adults (Total Vaccinated Cohort)

Adverse Reaction	Trial 3[b] Aged 18 through 64 Years				Trial 4[c] Aged 65 Years and Older			
	FLUARIX n = 760 %		Placebo n = 192 %		FLUARIX n = 601-602 %		Comparator n = 596 %	
	Any	Grade 3[d]	Any	Grade 3[d]	Any	Grade 3[d]	Any	Grade 3[d]
Local								
Pain	55	0.1	12	0	19	0	18	0
Redness	18	0	10	0	11	0.2	13	0.7
Swelling	9	0.1	6	0	6	0	9	0.7
Systemic								
Muscle aches	23	0.4	12	0.5	7	0.3	7	0
Fatigue	20	0.4	18	1	9	0.3	10	0.7
Headache	19	0.1	21	1	8	0.3	8	0.3
Arthralgia	6	0.1	6	0.5	6	0.5	5	0.2
Shivering	3	0.1	3	0	2	0.2	2	0
Fever[e]	2	0	2	0	2	0	0.5	0

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
n = Number of subjects with diary card completed.

[a] Four days included day of vaccination and the subsequent 3 days.

[b] Trial 3 was a randomized, double-blind, placebo-controlled, safety, and immunogenicity trial (NCT00100399).

[c] Trial 4 was a randomized, single-blind, active-controlled, safety, and immunogenicity trial (NCT00197288). The active control was FLUZONE, a U.S.-licensed trivalent, inactivated influenza vaccine (Sanofi Pasteur Inc.).

[d] Grade 3 pain, muscle aches, fatigue, headache, arthralgia, shivering: Defined as prevented normal activity.
Grade 3 redness, swelling: Defined as >50 mm.
Grade 3 fever: Defined as >102.2°F (39.0°C).

[e] Fever: Defined as ≥100.4°F (38.0°C) in Trial 3, and ≥99.5°F (37.5°C) in Trial 4.

Table 6. FLUARIX (Trivalent Formulation): Incidence of Solicited Local and Systemic Adverse Reactions within 4 Days[a] of First Vaccination in Children Aged 3 through 17 Years[b] (Total Vaccinated Cohort)

Adverse Reaction	Aged 3 through 4 Years				Aged 5 through 17 Years			
	FLUARIX n = 350 %		Comparator n = 341 %		FLUARIX n = 1,348 %		Comparator n = 451 %	
	Any	Grade 3[c]	Any	Grade 3[c]	Any	Grade 3[c]	Any	Grade 3[c]
Local								
Pain	35	2	38	1	56	0.8	56	0.7
Redness	23	0.3	20	0	18	1	16	0.7
Swelling	14	0	13	0	14	2	13	0.7
Systemic								
Irritability	21	0.9	22	0	–	–	–	–
Loss of appetite	13	0.9	15	0.9	–	–	–	–
Drowsiness	13	0.6	20	0.9	–	–	–	–
Fever[d]	7	1	8	2	4	0.3	3	0.2
Muscle aches	–	–	–	–	29	0.4	29	0.4
Fatigue	–	–	–	–	20	1	19	1
Headache	–	–	–	–	15	0.5	16	0.9
Arthralgia	–	–	–	–	6	0.1	6	0.2
Shivering	–	–	–	–	3	0.1	4	0.2

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
n = Number of subjects with diary card completed.

[a] Four days included day of vaccination and the subsequent 3 days.

[b] Trial 6 was a single-blind, active-controlled, safety, and immunogenicity U.S. trial (NCT00383123). The active control was FLUZONE, a U.S.-licensed trivalent, inactivated influenza vaccine (Sanofi Pasteur Inc.).

[c] Grade 3 pain, irritability, loss of appetite, drowsiness, muscle aches, fatigue, headache, arthralgia, shivering: Defined as prevented normal activity.
Grade 3 swelling, redness: Defined as >50 mm.
Grade 3 fever: Defined as >102.2°F (39.0°C).

[d] Fever: Defined as ≥99.5°F (37.5°C).

In children who received a second dose of FLUARIX or the comparator vaccine, the incidences of adverse reactions following the second dose were similar to those observed after the first dose.

Serious Adverse Reactions: In the 4 clinical trials in adults (N = 10,923), there was a single case of anaphylaxis within one day following administration of FLUARIX (<0.01%).

FLUARIX QUADRIVALENT Concomitant Administration with Zoster Vaccine Recombinant, Adjuvanted (SHINGRIX)

In an open-label, randomized trial (NCT 01954251), adults aged 50 years and older (median 63 years, range 50 to 92 years) received FLUARIX QUADRIVALENT and SHINGRIX at Month 0 and SHINGRIX at Month 2 (n = 413), or FLUARIX QUADRIVALENT at Month 0 and SHINGRIX at Months 2 and 4 (n = 415). Information about solicited local and systemic adverse reactions was collected using diary cards for 7 days (day of vaccination and the next 6 days). The rates of the solicited, systemic adverse reactions of fatigue, headache, myalgia, shivering, and fever (≥37.5°C) reported in subjects receiving FLUARIX QUADRIVALENT and SHINGRIX concomitantly were similar to those observed with SHINGRIX alone, and higher than when FLUARIX QUADRIVALENT was given alone.

6.2 Postmarketing Experience

Beyond those events reported above in the clinical trials for FLUARIX QUADRIVALENT or FLUARIX, the following adverse reactions have been identified during post-approval use of FLUARIX QUADRIVALENT or FLUARIX (trivalent influenza vaccine). Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Blood and Lymphatic System Disorders

Lymphadenopathy.

Cardiac Disorders

Tachycardia.

Ear and Labyrinth Disorders

Vertigo.

Eye Disorders

Conjunctivitis, eye irritation, eye pain, eye redness, eye swelling, eyelid swelling.

Gastrointestinal Disorders

Abdominal pain or discomfort, swelling of the mouth, throat, and/or tongue.

General Disorders and Administration Site Conditions

Asthenia, chest pain, influenza-like illness, feeling hot, injection site mass, injection site reaction, injection site warmth, body aches.

Immune System Disorders

Anaphylactic reaction including shock, anaphylactoid reaction, hypersensitivity, serum sickness.

Infections and Infestations

Injection site abscess, injection site cellulitis, pharyngitis, rhinitis, tonsillitis.

Nervous System Disorders

Convulsion, encephalomyelitis, facial palsy, facial paresis, Guillain-Barré syndrome, hypoesthesia, myelitis, neuritis, neuropathy, paresthesia, syncope.

Respiratory, Thoracic, and Mediastinal Disorders

Asthma, bronchospasm, dyspnea, respiratory distress, stridor.

Skin and Subcutaneous Tissue Disorders

Angioedema, erythema, erythema multiforme, facial swelling, pruritus, Stevens-Johnson syndrome, sweating, urticaria.

Vascular Disorders

Henoch-Schönlein purpura, vasculitis.

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

In an open-label trial (NCT 01954251), FLUARIX QUADRIVALENT was administered concomitantly with Zoster Vaccine Recombinant, Adjuvanted (SHINGRIX) *[see Adverse Reactions (6.1), Clinical Studies (14.4)]*.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater-than-physiologic doses), may reduce the immune response to FLUARIX QUADRIVALENT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are insufficient data on FLUARIX QUADRIVALENT in pregnant women to inform vaccine-associated risks.

A developmental toxicity study was performed in female rats administered FLUARIX QUADRIVALENT prior to mating and during gestation and lactation periods. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). This study revealed no adverse effects on fetal or pre-weaning development due to FLUARIX QUADRIVALENT *(see Data)*.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk: Pregnant women infected with seasonal influenza are at increased risk of severe illness associated with influenza infection compared with non-pregnant women. Pregnant women with influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

Data

Animal Data: In a developmental toxicity study, female rats were administered FLUARIX QUADRIVALENT by intramuscular injection 4 and 2 weeks prior to mating, on Gestation Days 3, 8, 11, and 15, and on Lactation Day 7. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to Postnatal Day 25 were observed. There were no vaccine-related fetal malformations or variations.

8.2 Lactation

Risk Summary

It is not known whether FLUARIX QUADRIVALENT is excreted in human milk. Data are not available to assess the effects of FLUARIX QUADRIVALENT on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for FLUARIX QUADRIVALENT and any potential adverse effects on the breastfed child from FLUARIX QUADRIVALENT or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of FLUARIX QUADRIVALENT in children younger than 6 months have not been established.

Safety and effectiveness of FLUARIX QUADRIVALENT in individuals aged 6 months through 17 years have been established *[see Adverse Reactions (6.1), Clinical Studies (14.3)]*.

8.5 Geriatric Use

In a randomized, double-blind (2 arms) and open-label (one arm), active-controlled trial, immunogenicity and safety were evaluated in a cohort of subjects aged 65 years and older who received FLUARIX QUADRIVALENT (n = 1,517); 469 of these subjects were aged 75 years and older. In subjects aged 65 years and older, the geometric mean antibody titers (GMTs) post-vaccination and seroconversion rates were lower than in younger subjects (aged 18 through 64 years) and the frequencies of solicited and unsolicited adverse reactions were generally lower than in younger subjects.

11 DESCRIPTION

FLUARIX QUADRIVALENT, Influenza Vaccine, for intramuscular injection, is a sterile, colorless, and slightly opalescent suspension. FLUARIX QUADRIVALENT is prepared from influenza viruses

propagated in embryonated chicken eggs. Each of the influenza viruses is produced and purified separately. After harvesting the virus-containing fluids, each influenza virus is concentrated and purified by zonal centrifugation using a linear sucrose density gradient solution containing detergent to disrupt the viruses. Following dilution, the vaccine is further purified by diafiltration. Each influenza virus solution is inactivated by the consecutive effects of sodium deoxycholate and formaldehyde leading to the production of a "split virus." Each split inactivated virus is then suspended in sodium phosphate-buffered isotonic sodium chloride solution. Each vaccine is formulated from the split inactivated virus solutions.

FLUARIX QUADRIVALENT has been standardized according to U.S. Public Health Service (USPHS) requirements for the 2022-2023 influenza season and is formulated to contain 60 micrograms (mcg) hemagglutinin (HA) per 0.5-mL dose, in the recommended ratio of 15 mcg HA of each of the following 4 influenza virus strains (2 A strains and 2 B strains): A/Victoria/2570/2019 (H1N1) IVR-215, A/Darwin/6/2021 (H3N2) IVR-227 (an A/Darwin/9/2021 (H3N2)-like virus), B/Austria/1359417/2021 BVR-26 (B-Victoria lineage), and B/Phuket/3073/2013 (B-Yamagata lineage).

FLUARIX QUADRIVALENT is formulated without preservatives. FLUARIX QUADRIVALENT does not contain thimerosal. Each 0.5-mL dose also contains octoxynol-10 (TRITON X-100) ≤0.115 mg, α-tocopheryl hydrogen succinate ≤0.135 mg, and polysorbate 80 (Tween 80) ≤0.550 mg. Each dose may also contain residual amounts of hydrocortisone ≤0.0015 mcg, gentamicin sulfate ≤0.15 mcg, ovalbumin ≤0.050 mcg, formaldehyde ≤5 mcg, and sodium deoxycholate ≤65 mcg from the manufacturing process.

The tip caps and plungers of the prefilled syringes of FLUARIX QUADRIVALENT are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Influenza illness and its complications follow infection with influenza viruses. Global surveillance of influenza identifies yearly antigenic variants. Since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation.

Public health authorities give annual influenza vaccine composition recommendations. Inactivated influenza vaccines are standardized to contain the hemagglutinins of influenza viruses representing the virus types or subtypes likely to circulate in the United States during the influenza season. Two influenza type B virus lineages (Victoria and Yamagata) are of public health importance because they have co-circulated since 2001. FLUARIX (trivalent influenza vaccine) contains 2 influenza A subtype viruses and one influenza type B virus.

Specific levels of hemagglutination-inhibition (HI) antibody titer post-vaccination with inactivated influenza virus vaccines have not been correlated with protection from influenza illness but the HI antibody titers have been used as a measure of vaccine activity. In some human challenge studies, HI antibody titers of ≥1:40 have been associated with protection from influenza illness in up to 50% of subjects.[1,2] Antibody against one influenza virus type or subtype confers little or no protection against another virus. Furthermore, antibody to one antigenic variant of influenza virus might not protect against

a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virological basis for seasonal epidemics and the reason for the usual replacement of one or more influenza viruses in each year's influenza vaccine.

Annual revaccination is recommended because immunity declines during the year after vaccination, and because circulating strains of influenza virus change from year to year.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

FLUARIX QUADRIVALENT has not been evaluated for carcinogenic or mutagenic potential or male infertility in animals. Vaccination of female rats with FLUARIX QUADRIVALENT had no effect on fertility *[see Use in Specific Populations (8.1)]*.

14 CLINICAL STUDIES

14.1 Efficacy against Influenza

The efficacy experience with FLUARIX is relevant to FLUARIX QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*.

The efficacy of FLUARIX was evaluated in a randomized, double-blind, placebo-controlled trial conducted in 2 European countries during the 2006-2007 influenza season. Efficacy of FLUARIX, containing A/New Caledonia/20/1999 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 influenza virus strains, was defined as the prevention of culture-confirmed influenza A and/or B cases, for vaccine antigenically matched strains, compared with placebo. Healthy subjects aged 18 through 64 years (mean age: 40 years) were randomized (2:1) to receive FLUARIX (n = 5,103) or placebo (n = 2,549) and monitored for influenza-like illnesses (ILI) starting 2 weeks post-vaccination and lasting for approximately 7 months. In the overall population, 60% of subjects were female and 99.9% were white. Culture-confirmed influenza was assessed by active and passive surveillance of ILI. Influenza-like illness was defined as at least one general symptom (fever ≥100°F and/or myalgia) and at least one respiratory symptom (cough and/or sore throat). After an episode of ILI, nose and throat swab samples were collected for analysis; attack rates and vaccine efficacy were calculated (Table 7).

Table 7. FLUARIX (Trivalent Formulation): Attack Rates and Vaccine Efficacy against Culture-Confirmed Influenza A and/or B in Adults (Total Vaccinated Cohort)

	N	n	Attack Rates (n/N)	Vaccine Efficacy		
			%	%	Lower Limit	Upper Limit
Antigenically Matched Strains[a]						
FLUARIX	5,103	49	1.0	66.9[b]	51.9	77.4
Placebo	2,549	74	2.9	–	–	–
All Culture-Confirmed Influenza (Matched, Unmatched, and Untyped)[c]						
FLUARIX	5,103	63	1.2	61.6[b]	46.0	72.8
Placebo	2,549	82	3.2	–	–	–

[a] There were no vaccine matched culture-confirmed cases of A/New Caledonia/20/1999 (H1N1) or B/Malaysia/2506/2004 influenza virus strains with FLUARIX or placebo.

[b] Vaccine efficacy for FLUARIX exceeded a pre-defined threshold of 35% for the lower limit of the 2-sided 95% Confidence Interval (CI).

[c] Of the 22 additional cases, 18 were unmatched and 4 were untyped; 15 of the 22 cases were A (H3N2) (11 cases with FLUARIX and 4 cases with placebo).

In a post-hoc exploratory analysis by age, vaccine efficacy (against culture-confirmed influenza A and/or B cases, for vaccine antigenically matched strains) in subjects aged 18 through 49 years was 73.4% (95% CI: 59.3, 82.8) (number of influenza cases: FLUARIX [n = 35/3,602] and placebo [n = 66/1,810]). In subjects aged 50 through 64 years, vaccine efficacy was 13.8% (95% CI: -137.0, 66.3) (number of influenza cases: FLUARIX [n = 14/1,501] and placebo [n = 8/739]). As the trial lacked statistical power to evaluate efficacy within age subgroups, the clinical significance of these results is unknown.

The efficacy of FLUARIX QUADRIVALENT was evaluated in Trial 7, a randomized, observer-blind, non-influenza vaccine-controlled trial conducted in 13 countries in Asia, Europe, and Central America during the 2011-2012 and 2012-2013 Northern Hemisphere influenza seasons, and from 2012 to 2014 during influenza seasons in subtropical countries. Healthy subjects aged 6 through 35 months (mean age: 22 months) were randomized (1:1) to receive FLUARIX QUADRIVALENT (n = 6,006) or a non-influenza control vaccine (n = 6,012). In the overall population, 51% were male; 27% were white, 45% were Asian, and 28% were of other racial/ethnic groups. Children aged 12 months and older with no history of influenza vaccination and children younger than 12 months received 2 doses of FLUARIX QUADRIVALENT or the Non-Influenza Active Comparator vaccine approximately 28 days apart. Children aged 12 months and older with a history of influenza vaccination received one dose.

The influenza virus strain composition of FLUARIX QUADRIVALENT administered in each of the 5 study cohorts followed the World Health Organization (WHO) recommendations (which included 2nd B strain from 2012 onwards) for each influenza season associated with a particular cohort.

Efficacy of FLUARIX QUADRIVALENT was assessed for the prevention of reverse transcriptase polymerase chain reaction (RT-PCR)-confirmed influenza°A and/or B°disease, due to any seasonal

influenza strain, compared with non-influenza control vaccines. Influenza disease included episodes of influenza-like illness (ILI, i.e., fever ≥100.4°F with any of the following: cough, runny nose, nasal congestion, or breathing difficulty) or a consequence of influenza virus infection (acute otitis media or lower respiratory illnesses). Among subjects with RT-PCR-positive influenza A and/or B disease, subjects were further prospectively classified based on the presence of adverse outcomes associated with influenza infection: fever >102.2°F, physician-diagnosed acute otitis media, physician-diagnosed lower respiratory tract illness, physician-diagnosed serious extra-pulmonary complications, hospitalization in the intensive care unit, or supplemental oxygen required for more than 8 hours. Subjects were monitored for influenza disease by passive and active surveillance starting 2 weeks post-vaccination and lasting for approximately 6 months. After an episode of ILI, lower respiratory illness, or acute otitis media, nasal swabs were collected and tested for influenza°A and/or°B by RT-PCR. All RT-PCR-positive specimens were further tested in cell culture and by antigenic characterization to determine whether the viral strains matched those in the vaccine. Vaccine efficacy for subjects with RT-PCR confirmed and culture-confirmed vaccine matching strains (According-to-Protocol (ATP) cohort for efficacy – time to event) is presented in Table 8.

Table 8. Attack Rates and Vaccine Efficacy against Influenza A and/or B in Children Aged 6 through 35 Months[a] (ATP Cohort for Efficacy – Time to Event)

	N[b]	n[c]	Attack Rates (n/N)	Vaccine Efficacy		
			%	%	Lower Limit	Upper Limit
All RT-PCR-Confirmed Influenza						
FLUARIX QUADRIVALENT	5,707	344	6.03	49.8	41.8[d]	56.8
Non-Influenza Comparator[e,f]	5,697	662	11.62	-	-	-
All Culture-Confirmed Influenza						
FLUARIX QUADRIVALENT	5,707	303	5.31	51.2	44.1[g]	57.6
Non-Influenza Comparator[e,f]	5,697	602	10.57	-	-	-
All Antigenically Matched Culture-Confirmed Influenza						
FLUARIX QUADRIVALENT	5,707	88	1.54	60.1	49.1[h]	69.0
Non-Influenza Comparator[e,f]	5,697	216	3.79	-	-	-

ATP = According-to-Protocol; RT-PCR = Reverse Transcriptase Polymerase Chain Reaction.

[a] Trial 7: NCT01439360.

[b] Number of subjects in the ATP cohort for efficacy – time to event, which included subjects who met all eligibility criteria, who were followed for efficacy and complied with the study protocol until the influenza-like episode.

[c] Number of subjects who reported at least one case in the reporting period.

[d] Vaccine efficacy for FLUARIX QUADRIVALENT met the pre-defined criterion for the lower limit of the 2-sided 97.5% CI (>15% for all influenza).

[e] Children younger than 12 months: pneumococcal 13-valent conjugate vaccine [Diphtheria CRM197 Protein] (Wyeth Pharmaceuticals, Inc.).

[f] Children 12 months and older: HAVRIX (Hepatitis A Vaccine) for those with a history of influenza vaccination; or HAVRIX (Dose 1) and a varicella vaccine (U.S. Licensed Manufactured by Merck & Co., Inc. or Non-U.S. Licensed Manufactured by GlaxoSmithKline Biologicals) (Dose 2) for those with no history of influenza vaccination.

[g] Vaccine efficacy for FLUARIX QUADRIVALENT met the pre-defined criterion of >10% for the lower limit of the 2-sided 95% CI.

[h] Vaccine efficacy for FLUARIX QUADRIVALENT met the pre-defined criterion of >15% for the lower limit of the 2-sided 95% CI.

The vaccine efficacy against RT-PCR-confirmed influenza associated with adverse outcomes was 64.6% (97.5% CI 53.2%, 73.5%). The vaccine efficacy against RT-PCR-confirmed influenza associated with adverse outcomes due to A/H1N1, A/H3N2, B/Victoria, and B/Yamagata was 71.4% (95% CI 48.5%, 85.2%), 51.3% (95% CI 32.7%, 65.2%), 86.7% (95% CI 52.8%, 97.9%), and 68.9% (95% CI 50.6%, 81.2%), respectively.

For RT-PCR-confirmed influenza cases associated with adverse outcomes, the incidence of the specified adverse outcomes is presented in Table 9.

Table 9. Incidence of Adverse Outcomes Associated with RT-PCR-Positive Influenza in Children Aged 6 through 35 Months[a] (ATP Cohort for Efficacy- Time to Event)[b]

Influenza-Associated Symptom[e]	FLUARIX QUADRIVALENT n = 5,707			Non-Influenza Active Comparator[c,d] n = 5,697		
	Number of Events	Number of Subjects[f]	%	Number of Events	Number of Subjects[f]	%
Fever >102.2^{0}F/39^{0}C	62	61	1.1	184	183	3.2
Acute otitis media (AOM)[g]	5	5	0.1	15	15	0.3
Physician-diagnosed lower respiratory tract illness[h]	28	28	0.5	62	61	1.1
Physician-diagnosed serious extra-pulmonary complications[i]	2	2	0	3	3	0.1
Hospitalization in the intensive care unit	0	0	0	0	0	0
Supplemental oxygen required for more than 8 hours	0	0	0	0	0	0

ATP = According-to-Protocol; RT-PCR = Reverse transcriptase polymerase chain reaction.

[a] Trial 7: NCT01439360.

[b] Number of subjects in the ATP cohort for efficacy – time to event, which included subjects who met all eligibility criteria, who were followed for efficacy and complied with the study protocol until the influenza-like episode.

[c] Children younger than 12 months: pneumococcal 13-valent conjugate vaccine [Diphtheria CRM197 Protein] (Wyeth Pharmaceuticals, Inc.).

[d] Children 12 months and older: HAVRIX (Hepatitis A Vaccine) for those with a history of influenza vaccination; or HAVRIX (Dose 1) and a varicella vaccine (U.S. Licensed Manufactured by Merck & Co., Inc. or Non-U.S. Licensed Manufactured by GlaxoSmithKline Biologicals) (Dose 2) for those with no history of influenza vaccination.

[e] Subjects who experienced more than one adverse outcome, each outcome was counted in the respective category.

[f] Number of subjects with at least one event in a given category.

[g] Analyses considered AOM cases confirmed by otoscopy.

[h] Pneumonia, lower respiratory tract infection, bronchiolitis, bronchitis, or croup infection as per final diagnosis by physician.

[i] Includes myositis, encephalitis or other neurologic condition including seizure, myocarditis/pericarditis or other serious medical condition as per final diagnosis by physician.

14.2 Immunological Evaluation of FLUARIX QUADRIVALENT in Adults

Trial 1 was a randomized, double-blind (2 arms) and open-label (one arm), active-controlled, safety, immunogenicity, and non-inferiority trial. In this trial, subjects received FLUARIX QUADRIVALENT (n = 1,809) or one of 2 formulations of comparator trivalent influenza vaccine (FLUARIX, TIV-1, n = 608 or TIV-2, n = 534), each containing an influenza type B virus that corresponded to one of the 2 type B viruses in FLUARIX QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). Subjects aged 18 years and older (mean age: 58 years) were evaluated for immune responses to each of the vaccine antigens 21 days following vaccination. In the overall population, 57% of subjects were female; 69% were white, 27% were Asian, and 4% were of other racial/ethnic groups.

The immunogenicity endpoints were GMTs of serum HI antibodies adjusted for baseline, and the percentage of subjects who achieved seroconversion, defined as a pre-vaccination HI titer of <1:10 with a post-vaccination titer ≥1:40 or at least a 4-fold increase in serum HI antibody titer over baseline to ≥1:40 following vaccination, performed on the According-to-Protocol (ATP) cohort for whom immunogenicity assay results were available after vaccination. FLUARIX QUADRIVALENT was non-inferior to both TIVs based on adjusted GMTs (upper limit of the 2-sided 95% CI for the GMT ratio [TIV/FLUARIX QUADRIVALENT] ≤1.5) and seroconversion rates (upper limit of the 2-sided 95% CI on difference of the TIV minus FLUARIX QUADRIVALENT ≤10%). The antibody response to influenza B strains contained in FLUARIX QUADRIVALENT was higher than the antibody response after vaccination with a TIV containing an influenza B strain from a different lineage. There was no evidence that the addition of the second B strain resulted in immune interference to other strains included in the vaccine (Table 10).

Table 10. FLUARIX QUADRIVALENT: Immune Responses to Each Antigen 21 Days after Vaccination in Adults (ATP Cohort for Immunogenicity)

		Trivalent Influenza Vaccine (TIV)	
	FLUARIX QUADRIVALENT[a]	**TIV-1 (B Victoria)[b]**	**TIV-2 (B Yamagata)[c]**
Geometric Mean Antibody Titer	**n = 1,809 (95% CI)**	**n = 608 (95% CI)**	**n = 534 (95% CI)**
A/California/7/2009 (H1N1)	201.1 (188.1, 215.1)	218.4 (194.2, 245.6)	213.0 (187.6, 241.9)
A/Victoria/210/2009 (H3N2)	314.7 (296.8, 333.6)	298.2 (268.4, 331.3)	340.4 (304.3, 380.9)
B/Brisbane/60/2008 (Victoria lineage)	404.6 (386.6, 423.4)	393.8 (362.7, 427.6)	258.5 (234.6, 284.8)
B/Brisbane/3/2007 (Yamagata lineage)	601.8 (573.3, 631.6)	386.6 (351.5, 425.3)	582.5 (534.6, 634.7)
Seroconversion[d]	**n = 1,801 % (95% CI)**	**n = 605 % (95% CI)**	**n = 530 % (95% CI)**
A/California/7/2009 (H1N1)	77.5 (75.5, 79.4)	77.2 (73.6, 80.5)	80.2 (76.5, 83.5)
A/Victoria/210/2009 (H3N2)	71.5 (69.3, 73.5)	65.8 (61.9, 69.6)	70.0 (65.9, 73.9)
B/Brisbane/60/2008 (Victoria lineage)	58.1 (55.8, 60.4)	55.4 (51.3, 59.4)	47.5 (43.2, 51.9)
B/Brisbane/3/2007 (Yamagata lineage)	61.7 (59.5, 64.0)	45.6 (41.6, 49.7)	59.1 (54.7, 63.3)

ATP = According-to-protocol; CI = Confidence Interval.

ATP cohort for immunogenicity included subjects for whom assay results were available after vaccination for at least one trial vaccine antigen.

[a] Contained the same composition as FLUARIX (trivalent formulation) manufactured for the 2010-2011 season and an additional influenza type B virus of Yamagata lineage.

[b] Contained the same composition as FLUARIX manufactured for the 2010-2011 season (2 influenza A subtype viruses and an influenza type B virus of Victoria lineage).

[c] Contained the same 2 influenza A subtype viruses as FLUARIX manufactured for the 2010-2011 season and an influenza type B virus of Yamagata lineage.

[d] Seroconversion defined as a pre-vaccination HI titer of <1:10 with a post-vaccination titer ≥1:40 or at least a 4-fold increase in serum titers of HI antibodies to ≥1:40.

14.3 Immunological Evaluation of FLUARIX QUADRIVALENT in Children

Trial 7 was a randomized, observer-blind, non-influenza vaccine-controlled trial evaluating the efficacy of FLUARIX QUADRIVALENT. In this trial, subjects aged 6 through 35 months received FLUARIX QUADRIVALENT (n = 6,006) or a non-influenza control vaccine (n = 6,012). Immune responses to each of the vaccine antigens were evaluated in sera 28 days following 1 or 2 doses in a subgroup of subjects (n = 753 for FLUARIX QUADRIVALENT, n = 579 for control in the ATP cohort for immunogenicity).

Immunogenicity endpoints (GMTs and the percentage of subjects who achieved seroconversion) were analyzed based on the ATP cohort for whom immunogenicity assay results were available after vaccination. Antibody responses for all 4 influenza strains are presented in Table 11.

Table 11. FLUARIX QUADRIVALENT: Immune Responses to Each Antigen 28 Days after Last Vaccination in Children Aged 6 through 35 Months[a] (ATP Cohort for Immunogenicity)

	FLUARIX QUADRIVALENT	**Non-Influenza Active Comparator[b,c]**
Geometric Mean Antibody Titer	**n = 750-753 (95% CI)**	**n = 578-579 (95% CI)**
A (H1N1)	165.3 (148.6, 183.8)	12.6 (11.1, 14.3)
A (H3N2)	132.1 (119.1, 146.5)	14.7 (12.9, 16.7)
B (Victoria lineage)	92.6 (82.3, 104.1)	9.2 (8.4, 10.1)
B (Yamagata lineage)	121.4 (110.1, 133.8)	7.6 (7.0, 8.3)
Seroconversion[d]	**n = 742-746 % (95% CI)**	**n = 566-568 % (95% CI)**
A (H1N1)	80.2 (77.2, 83.0)	3.5 (2.2, 5.4)
A (H3N2)	68.8 (65.3, 72.1)	4.2 (2.7, 6.2)
B (Victoria lineage)	69.3 (65.8, 72.6)	0.9 (0.3, 2.0)
B (Yamagata lineage)	81.2 (78.2, 84.0)	2.3 (1.2, 3.9)

ATP = According-to-protocol; CI = Confidence Interval.

ATP cohort for immunogenicity included subjects for whom assay results were available after vaccination for at least one trial vaccine antigen.

[a] Trial 7: NCT01439360.

[b] Children younger than 12 months: pneumococcal 13-valent conjugate vaccine [Diphtheria CRM197 Protein] (Wyeth Pharmaceuticals, Inc.).

[c] Children 12 months and older: HAVRIX (Hepatitis A Vaccine) for those with a history of influenza vaccination; or HAVRIX (Dose 1) and a varicella vaccine (U.S. Licensed Manufactured by Merck & Co., Inc. or Non-U.S. Licensed Manufactured by GlaxoSmithKline Biologicals) (Dose 2) for those with no history of influenza vaccination.

[d] Seroconversion defined as a pre-vaccination HI titer of <1:10 with a post-vaccination titer ≥1:40 or at least a 4-fold increase in serum titers of HI antibodies to ≥1:40.

Trial 2 was a randomized, double-blind, active-controlled, safety, immunogenicity, and non-inferiority trial. In this trial, subjects received FLUARIX QUADRIVALENT (n = 791) or one of 2 formulations of

comparator trivalent influenza vaccine (FLUARIX; TIV-1, n = 819; or TIV-2, n = 801), each containing an influenza type B virus that corresponded to one of the 2 type B viruses in FLUARIX QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). In children aged 3 through 17 years, immune responses to each of the vaccine antigens were evaluated in sera 28 days following 1 or 2 doses. In the overall population, 52% of subjects were male; 56% were white, 29% were Asian, 12% were black, and 3% were of other racial/ethnic groups.

The immunogenicity endpoints were GMTs adjusted for baseline, and the percentage of subjects who achieved seroconversion, defined as a pre-vaccination HI titer of <1:10 with a post-vaccination titer ≥1:40 or at least a 4-fold increase in serum HI titer over baseline to ≥1:40, following vaccination, performed on the ATP cohort for whom immunogenicity assay results were available after vaccination. FLUARIX QUADRIVALENT was non-inferior to both TIVs based on adjusted GMTs (upper limit of the 2-sided 95% CI for the GMT ratio [TIV/FLUARIX QUADRIVALENT] ≤1.5) and seroconversion rates (upper limit of the 2-sided 95% CI on difference of the TIV minus FLUARIX QUADRIVALENT ≤10%). The antibody response to influenza B strains contained in FLUARIX QUADRIVALENT was higher than the antibody response after vaccination with a TIV containing an influenza B strain from a different lineage. There was no evidence that the addition of the second B strain resulted in immune interference to other strains included in the vaccine (Table 12).

Table 12. FLUARIX QUADRIVALENT: Immune Responses to Each Antigen 28 Days after Last Vaccination in Children Aged 3 through 17 Years (ATP Cohort for Immunogenicity)

		Trivalent Influenza Vaccine (TIV)	
	FLUARIX QUADRIVALENT[a]	TIV-1 (B Victoria)[b]	TIV-2 (B Yamagata)[c]
Geometric Mean Antibody Titer	**n = 791 (95% CI)**	**n = 818 (95% CI)**	**n = 801 (95% CI)**
A/California/7/2009 (H1N1)	386.2 (357.3, 417.4)	433.2 (401.0, 468.0)	422.3 (390.5, 456.5)
A/Victoria/210/2009 (H3N2)	228.8 (215.0, 243.4)	227.3 (213.3, 242.3)	234.0 (219.1, 249.9)
B/Brisbane/60/2008 (Victoria lineage)	244.2 (227.5, 262.1)	245.6 (229.2, 263.2)	88.4 (81.5, 95.8)
B/Brisbane/3/2007 (Yamagata lineage)	569.6 (533.6, 608.1)	224.7 (207.9, 242.9)	643.3 (603.2, 686.1)
Seroconversion[d]	**n = 790 % (95% CI)**	**n = 818 % (95% CI)**	**n = 800 % (95% CI)**
A/California/7/2009 (H1N1)	91.4 (89.2, 93.3)	89.9 (87.6, 91.8)	91.6 (89.5, 93.5)
A/Victoria/210/2009 (H3N2)	72.3 (69.0, 75.4)	70.7 (67.4, 73.8)	71.9 (68.6, 75.0)
B/Brisbane/60/2008 (Victoria lineage)	70.0 (66.7, 73.2)	68.5 (65.2, 71.6)	29.6 (26.5, 32.9)
B/Brisbane/3/2007 (Yamagata lineage)	72.5 (69.3, 75.6)	37.0 (33.7, 40.5)	70.8 (67.5, 73.9)

ATP = According-to-protocol; CI = Confidence Interval.

ATP cohort for immunogenicity included subjects for whom assay results were available after vaccination for at least one trial vaccine antigen.

[a] Contained the same composition as FLUARIX (trivalent formulation) manufactured for the 2010-2011 season and an additional influenza type B virus of Yamagata lineage.

[b] Contained the same composition as FLUARIX manufactured for the 2010-2011 season (2 influenza A subtype viruses and an influenza type B virus of Victoria lineage).

[c] Contained the same 2 influenza A subtype viruses as FLUARIX manufactured for the 2010-2011 season and an influenza B virus of Yamagata lineage.

[d] Seroconversion defined as a pre-vaccination HI titer of <1:10 with a post-vaccination titer ≥1:40 or at least a 4-fold increase in serum titers of HI antibodies to ≥1:40.

14.4 FLUARIX QUADRIVALENT Concomitant Administration with Zoster Vaccine Recombinant, Adjuvanted (SHINGRIX)

In an open-label, randomized clinical trial (NCT 01954251) in adults aged 50 years and older, there was no evidence for interference in antibody responses (HI antibodies and anti-gE antibodies) to FLUARIX QUADRIVALENT or the coadministered vaccine, SHINGRIX *[see Adverse Reactions (6.1)]*.

15 REFERENCES

1. Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. *Virus Res.* 2004;103:133-138.
2. Hobson D, Curry RL, Beare AS, et al. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J Hyg Camb.* 1972;70:767-777.

16 HOW SUPPLIED/STORAGE AND HANDLING

FLUARIX QUADRIVALENT is available in 0.5-mL single-dose, disposable, prefilled TIP-LOK syringes (packaged without needles).

NDC 58160-890-41 Syringe in Package of 10: NDC 58160-890-52

Store refrigerated between 2º and 8ºC (36º and 46ºF). Do not freeze. Discard if the vaccine has been frozen. Store in the original package to protect from light.

17 PATIENT COUNSELING INFORMATION

Provide the following information to the vaccine recipient or guardian:

- Inform of the potential benefits and risks of immunization with FLUARIX QUADRIVALENT.
- Educate regarding potential side effects, emphasizing that: (1) FLUARIX QUADRIVALENT contains non-infectious killed viruses and cannot cause influenza and (2) FLUARIX QUADRIVALENT is intended to provide protection against illness due to influenza viruses only and cannot provide protection against all respiratory illness.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 prior to each immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).
- Instruct that annual revaccination is recommended.

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FLQ:17PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use Flublok® Quadrivalent safely and effectively. See full prescribing information for Flublok Quadrivalent.

Flublok Quadrivalent (Influenza Vaccine), Sterile Solution for Intramuscular Injection
2022-2023 Formula
Initial U.S. Approval: 2013

----------INDICATIONS AND USAGE----------
- Flublok Quadrivalent is a vaccine indicated for active immunization against disease caused by influenza A subtype viruses and influenza type B viruses contained in the vaccine. Flublok Quadrivalent is approved for use in persons 18 years of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------
For intramuscular (IM) injection only (0.5 mL). (2)

----------DOSAGE FORMS AND STRENGTHS----------
A sterile solution for injection supplied in 0.5 mL single dose prefilled syringes. (3)

----------CONTRAINDICATIONS----------
- Severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine. (4, 6.2, 11)

----------WARNINGS AND PRECAUTIONS----------
- Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of Flublok Quadrivalent. (5.1)
- If Guillain Barré syndrome has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give Flublok Quadrivalent should be based on careful consideration of potential benefits and risks. (5.2)

----------ADVERSE REACTIONS----------
- In adults 18 through 49 years of age, the most common (≥10%) injection-site reactions were tenderness (48%) and pain (37%); the most common (≥10%) solicited systemic adverse reactions were headache (20%), fatigue (17%), myalgia (13%) and arthralgia (10%). (6.1)
- In adults 50 years of age and older, the most common (≥10%) injection site reactions were tenderness (34%) and pain (19%); the most common (≥10%) solicited systemic adverse reactions were headache (13%) and fatigue (12%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc., at 1-800-822-2463 (1-800-Vaccine) or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS----------
- Pregnancy: Pregnancy outcomes in women exposed to Flublok Quadrivalent during pregnancy are being monitored. Contact: Sanofi Pasteur Inc. by calling 1-800-822-2463. (8.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: xx/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Flublok Quadrivalent is a vaccine indicated for active immunization against disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. Flublok Quadrivalent is approved for use in persons 18 years of age and older *[see Clinical Studies (14)]*.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Dosage

Administer Flublok Quadrivalent as a single 0.5 mL dose.

2.2 Administration

Invert the prefilled syringe containing Flublok Quadrivalent gently prior to affixing the appropriate size needle for intramuscular administration.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

The preferred site for injection is the deltoid muscle. Flublok Quadrivalent should not be mixed in the same syringe with any other vaccine.

3 DOSAGE FORMS AND STRENGTHS

Flublok Quadrivalent is a sterile solution supplied in prefilled, single-dose syringes, 0.5 mL.

4 CONTRAINDICATIONS

Flublok Quadrivalent is contraindicated in individuals with known severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine *[see Postmarketing Experience (6.2) and Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

5.2 Guillain Barré Syndrome

The 1976 swine influenza vaccine was associated with an increased frequency of Guillain-Barré Syndrome (GBS). Evidence for a causal relation of GBS with other influenza vaccines is inconclusive; if an excess risk exists, it is probably slightly more than one additional case per 1 million persons vaccinated. If GBS has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give Flublok should be based on careful consideration of the potential benefits and risks.

5.3 Altered Immunocompetence

If Flublok Quadrivalent is administered to immunocompromised individuals, including persons receiving immunosuppressive therapy, the immune response may be diminished.

5.4 Limitations of Vaccine Effectiveness

Vaccination with Flublok Quadrivalent may not protect all vaccine recipients.

6 ADVERSE REACTIONS

In adults 18 through 49 years of age, the most common (≥10%) injection-site reactions were tenderness (48%) and pain (37%); the most common (≥10%) solicited systemic adverse reactions were headache (20%), fatigue (17%), myalgia (13%), and arthralgia (10%) *[see Clinical Trials Experience (6.1)]*.

In adults 50 years of age and older, the most common (≥10%) injection site reactions were tenderness (34%) and pain (19%); the most common (≥10%) solicited systemic adverse reactions were headache (13%) and fatigue (12%) *[see Clinical Trials Experience (6.1)]*.

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a vaccine cannot be directly compared to rates in the clinical studies of another vaccine and may not reflect the rates observed in clinical practice.

Flublok Quadrivalent

Flublok Quadrivalent has been administered to and safety data collected from 998 adults 18-49 years of age (Study 1) and 4328 adults 50 years of age and older (Study 2).

In Studies 1 and 2, local (injection site) and systemic adverse reactions were solicited with the use of a memory aid for 7 days following vaccination, unsolicited adverse events were collected for ~28 days post-vaccination, and serious adverse events (SAEs) were collected for 6 months post-vaccination via clinic visit or remote contact.

Study 1 included 1330 subjects 18 through 49 years of age for safety analysis, randomized to receive Flublok Quadrivalent (n=998) or a comparator inactivated influenza vaccine (Fluarix Quadrivalent, manufactured by GlaxoSmithKline) (n=332) *[see Clinical Studies (14)]*. The mean age of participants was 33.5 years. Overall, 65% of subjects were female, 59% white/Caucasian, 37% black/African American, 1.0% Native Hawaiian/Pacific Islander, 0.8% American Indian/Alaskan Native, 0.5% Asian, 1.4% other racial groups, and 16% of Hispanic/Latino ethnicity. Table 1 summarizes the incidence of solicited local and systemic adverse reactions reported within seven days of vaccination with Flublok Quadrivalent or the comparator vaccine.

Table 1: Frequency of Solicited Local Injection Site Reactions and Systemic Adverse Reactions within 7 Days of Administration of Flublok Quadrivalent or Comparator[1] in Adults 18-49 Years of Age, Study 1 (Reactogenicity Populations)[1,2]

Reactogenicity Term	Flublok Quadrivalent N=996 %			Comparator N=332 %		
	Any Grade[6]	Grade 3	Grade 4	Any Grade[6]	Grade 3	Grade 4
Subjects with ≥1 injection site reaction[3,4]	51	1	0	52	2	0
Local Tenderness	48	1	0	47	1	0
Local Pain	37	1	0	36	1	0
Firmness / Swelling	5	0	0	3	0	0
Redness	4	0	0	1	0	0
Subjects with ≥1 systemic reaction[3,5]	34	2	<1	36	3	<1

Reactogenicity Term	Flublok Quadrivalent N=996 %			Comparator N=332 %		
	Any Grade[6]	Grade 3	Grade 4	Any Grade[6]	Grade 3	Grade 4
Headache	20	1	0	21	2	<1
Fatigue	17	1	0	17	1	0
Muscle Pain	13	1	0	12	1	0
Joint Pain	10	1	0	10	1	0
Nausea	9	1	<1	9	1	0
Shivering / Chills	7	1	0	6	1	0
Fever[6,7]	2	<1	0	1	<1	0

NOTE: Data based on the most severe response reported by subjects. Results ≥1% reported to nearest whole percent; results >0 but <1% reported as <1%.

[1] Comparator = U.S.–licensed comparator quadrivalent inactivated influenza vaccine manufactured by GlaxoSmithKline.

[2] Study 1 is registered as NCT02290509 under the National Clinical Trials registry.

[3] Reactogenicity Populations were defined as all randomized subjects who received study vaccine according to the treatment actually received and who had at least one non-missing data point for injection site, systemic or body temperature reactogenicity categories. For local pain, tenderness and systemic reactions: Grade 1 = No interference with activities. Grade 2 = Prevented some activities, and headache may have required non-narcotic pain reliever. Grade 3 = Prevented most or all normal activities or required prescription medications. Grade 4 = Required visit to ER or hospitalization. For injection site redness and firmness/swelling: Grade 1=25 to ≤50 mm (small). Grade 2=51 to ≤100 mm (medium). Grade 3=>100 mm (large). Grade 4=necrosis or exfoliative dermatitis.

[4] Denominators for injection site reactions: Flublok Quadrivalent n = 996, Comparator n =332.

[5] Denominators for systemic reactions: Flublok Quadrivalent n = 994, Comparator n = 332.

[6] Denominators for fever: Flublok Quadrivalent n = 990, Comparator n = 327.

[7] Fever defined as ≥100.4°F (38°C). Grade 1 (≥100.4°F to ≤101.1°F); Grade 2 (101.2°F to ≤102.0°F); Grade 3 (102.1°F to ≤104°F). Grade 4 >104°F.

Study 2 included 8672 subjects 50 years of age and older for safety analysis, randomized to receive Flublok Quadrivalent (n=4328) or Comparator (Fluarix® Quadrivalent, manufactured by GlaxoSmithKline) as an active control (n=4344) *[see Clinical Studies (14)]*. The mean age of participants was 62.7 years. Overall, 58% of subjects were female, 80% white/Caucasian, 18% black/African American, 0.9% American Indian/Alaskan Native, 0.4% Asian, 0.2% Native Hawaiian/Pacific Islander, 0.7% other racial groups, and 5% of Hispanic/Latino ethnicity. Table 2 summarizes the incidence of solicited local and systemic adverse reactions reported within seven days of vaccination with Flublok Quadrivalent or Comparator.

Table 2: Frequency of Solicited Local Injection Site Reactions and Systemic Adverse Reactions within 7 Days of Administration of Flublok Quadrivalent or Comparator[1] in Adults 50 Years of Age and Older, Study 2 (Reactogenicity Populations)[2, 3]

	Flublok Quadrivalent N=4312 %			Comparator N=4327 %		
Reactogenicity Term	**Any Grade**	**Grade 3**	**Grade 4**	**Any Grade**	**Grade 3**	**Grade 4**
Subjects with ≥1 injection site reaction[3,4]	38	<1	<1	40	<1	<1
Local Tenderness	34	<1	<1	37	<1	<1
Local Pain	19	<1	0	22	<1	<1
Firmness / Swelling	3	<1	0	3	<1	0
Redness	3	<1	0	2	<1	0
Subjects with ≥1 systemic reactogenicity event[3,5]	25	1	<1	26	1	<1
Headache	13	<1	<1	14	1	<1
Fatigue	12	<1	0	12	<1	<1
Muscle Pain	9	<1	<1	9	<1	<1
Joint Pain	8	<1	0	8	<1	<1
Nausea	5	<1	0	5	<1	<1
Shivering / Chills	5	<1	0	4	<1	<1
Fever[6,7]	<1	<1	0	1	<1	0

NOTE: Data based on the most severe response reported by subjects. Results ≥1% reported to nearest whole percent; results >0 but <1% reported as <1%.

[1] Comparator = U.S.–licensed comparator quadrivalent inactivated influenza vaccine, Fluarix Quadrivalent, manufactured by GlaxoSmithKline.

[2] Study 2 is registered as NCT02285998 under the National Clinical Trials registry.

[3] Reactogenicity Populations were defined as all randomized subjects who received study vaccine according to the treatment actually received and who had at least one non-missing data point for injection site, systemic or body temperature reactogenicity categories. For local pain, tenderness, and systemic reactions: Grade 1=No interference with activity. Grade 2=Some interference with activity. Grade 3=Prevents daily activity. Grade 4=Required ER visit or hospitalization. For injection site redness and firmness/swelling: Grade 1=25 to ≤50 mm (small). Grade 2=51 to ≤100 mm (medium). Grade 3=>100 mm (large). Grade 4=necrosis or exfoliative dermatitis.

[4] Denominators for injection site reactions: Flublok Quadrivalent n = 4307, Comparator n = 4319.

[5] Denominators for systemic reactions: Flublok Quadrivalent n = 4306, Comparator n = 4318.

[6] Denominators for fever: Flublok Quadrivalent n = 4262, Comparator n = 4282.

[7] Fever defined as ≥100.4°F (38°C). Grade 1 (≥100.4°F to ≤101.1°F); Grade 2 (101.2°F to ≤102.0°F); Grade 3 (102.1°F to ≤104°F). Grade 4 >104°F.

Among adults 18-49 years of age (Study 1), through 6 months post-vaccination, no deaths were reported. SAEs were reported by 12 subjects, 10 (1%) Flublok Quadrivalent recipients and 2 (0.6%) Comparator recipients. No SAEs were considered related to study vaccine.

Among adults 50 years of age and older (Study 2), 20 deaths occurred in the 6 months post-vaccination, including 8 Flublok Quadrivalent and 12 Comparator recipients. No deaths were considered related to study vaccine. SAEs were reported by 145 (3.4%) Flublok Quadrivalent recipients and 132 (3%) Comparator recipients. No SAEs were considered related to study vaccine.

In the 28 days following vaccination, one or more unsolicited treatment emergent adverse events occurred in 10.3% of Flublok Quadrivalent and 10.5% of Comparator recipients in Study 1 (adults 18-49 years of age) and in 13.9% of Flublok Quadrivalent and 14.1% of Comparator recipients in Study 2 (adults ≥50 years of age). In both studies, rates of individual events were similar between treatment groups, and most events were mild to moderate in severity.

Flublok (Trivalent Formulation)

The safety experience with Flublok is relevant to Flublok Quadrivalent because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*.

Flublok (trivalent formulation) has been administered to and safety data collected from a total of 4547 subjects in five clinical trials (Studies 3-7): 2497 adults 18 through 49 years, 972 adults 50 through 64 years, and 1078 adults 65 years and older. In Studies 3 - 5 and 7, SAEs were collected for 6 months post-vaccination. Study 6 collected SAEs through 30 days following receipt of vaccine. Study 6 also actively solicited pre-specified common hypersensitivity-type reactions through 30 days following receipt of vaccine as a primary endpoint.

Study 3 included 4648 subjects 18 through 49 years of age for safety analysis, randomized to receive Flublok (n=2344) or placebo (n=2304) *[see Clinical Studies (14)]*.

Study 4 included 602 subjects 50 through 64 years of age for safety analysis, randomized to receive Flublok (n=300) or another U.S.–licensed trivalent influenza vaccine (Fluzone®, manufactured by Sanofi Pasteur, Inc.) as an active control (n=302).

Study 5 included 869 subjects aged 65 years and older for safety analysis, randomized to receive Flublok (n=436) or another U.S.–licensed trivalent influenza vaccine (Fluzone) as an active control (n=433).

Study 6 included 2627 subjects aged 50 years and older for safety analysis, randomized to receive Flublok (n=1314) or another U.S.–licensed trivalent influenza vaccine (Afluria, manufactured by Seqirus Pty Ltd.) as an active control (n=1313). Among subjects 50 through 64 years of age, 672 received Flublok and 665 received Afluria. Among subjects aged 65 years and older, 642 received Flublok and 648 received Afluria.

Study 7 was a Phase 2 dose-finding trial conducted in adults 18 through 49 years of age, 153 of whom received Flublok 135 mcg, the licensed trivalent formulation.

Serious Adverse Events

Among 2497 adults 18-49 years of age (Studies 3 and 7 pooled), through 6 months post-vaccination, two deaths were reported, one in a Flublok recipient and one in a placebo recipient. Both deaths occurred more than 28 days following vaccination and neither was considered vaccine-related. SAEs were reported by 32 Flublok recipients and 35 placebo recipients. One SAE (pleuropericarditis) in a Flublok recipient was assessed as possibly related to the vaccine.

Among 972 adults 50-64 years of age (Studies 4 and 6 pooled), through up to 6 months post-vaccination, no deaths occurred, and SAEs were reported by 10 subjects, 6 Flublok recipients and 4 Comparator recipients. One of the SAEs, vasovagal syncope following injection of Flublok, was considered related to administration of study vaccine.

Among 1078 adults 65 years of age and older (Studies 5 and 6 pooled), through up to 6 months post-vaccination, 4 deaths occurred, 2 in Flublok recipients and 2 in Comparator recipients. None were

considered related to the study vaccines. SAEs were reported by 80 subjects (37 Flublok recipients, 43 Comparator recipients). None were considered related to the study vaccines.

Among 1314 adults 50 years of age and older (Study 7) for whom the incidence of rash, urticaria, swelling, non-pitting edema, or other potential hypersensitivity reactions were actively solicited for 30 days following vaccination, a total of 2.4% of Flublok recipients and 1.6% of Comparator recipients reported such events over the 30 day follow-up period. A total of 1.9% and 0.9% of Flublok and Comparator recipients, respectively, reported these events in the 7 days following vaccination. Of these solicited events, rash was most frequently reported (Flublok 1.3%, Comparator 0.8%) over the 30 day follow-up period.

6.2 Postmarketing Experience

The following events have been spontaneously reported during post-approval use of Flublok Quadrivalent. They are described because of the temporal relationship, the biologic plausibility of a causal relationship to Flublok Quadrivalent, and their potential seriousness. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Immune system disorders: anaphylaxis, allergic reactions, and other forms of hypersensitivity (including urticaria).

7 DRUG INTERACTIONS

Data evaluating the concomitant administration of Flublok Quadrivalent with other vaccines are not available.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure

Pregnancy outcomes in women who have been exposed to Flublok Quadrivalent during pregnancy are being monitored. Sanofi Pasteur Inc. is maintaining a prospective pregnancy exposure registry to collect data on pregnancy outcomes and newborn health status following vaccination with Flublok Quadrivalent during pregnancy. Healthcare providers are encouraged to enroll women who receive Flublok Quadrivalent during pregnancy in Sanofi Pasteur Inc.'s vaccination pregnancy registry by calling 1-800-822-2463.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risks of major birth defects and miscarriage in clinically recognized pregnancies are 2% to 4% and 15% to 20%, respectively. Available data on Flublok Quadrivalent and Flublok (trivalent formulation) administered to pregnant women are insufficient to inform vaccine-associated risks in pregnant women.

There were no developmental studies of Flublok Quadrivalent formulation performed in animals. The developmental effects of Flublok (trivalent formulation) are relevant to Flublok Quadrivalent because both vaccines are manufactured using the same process and have overlapping compositions. A developmental study of Flublok (trivalent formulation) has been performed in rats administered 0.5 mL divided of Flublok (trivalent formulation) prior to mating and during gestation. This study revealed no evidence of harm to the fetus due to Flublok (trivalent formulation) *[see Data]*.

Clinical Considerations

Disease-associated Maternal and/or Embryo/Fetal Risk

Pregnant women are at increased risk of complications associated with influenza infection compared to non-pregnant women. Pregnant women with influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

Data

Animal

In a developmental toxicity study, female rats were administered 0.5 mL divided of Flublok (trivalent formulation) by intramuscular injection twice prior to mating (35 days and 14 days prior to mating) and on gestation Day 6. No vaccine-related fetal malformations or variations and no adverse effects on pre-weaning development were observed in the study.

8.2 Lactation

Risk Summary

It is not known whether Flublok Quadrivalent is excreted in human milk. Data are not available to assess the effects of Flublok (trivalent formulation) or Flublok Quadrivalent on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Flublok Quadrivalent and any potential adverse effects on the breastfed child from Flublok Quadrivalent or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Data from a randomized, controlled trial demonstrated that children 6 months to less than 3 years of age had diminished hemagglutinin inhibition (HI) responses to Flublok (trivalent formulation) as compared to a U.S.–licensed influenza vaccine approved for use in this population, strongly suggesting that Flublok (trivalent formulation) would not be effective in children younger than 3 years of age. Safety and effectiveness of Flublok Quadrivalent have not been established in children 3 years to less than 18 years of age.

8.5 Geriatric Use

Data from an efficacy study (Study 2), which included 1759 subjects ≥65 years and 525 subjects ≥75 years who received Flublok Quadrivalent, are insufficient to determine whether elderly subjects respond differently from younger subjects *[see Clinical Trials Experience (6.1) and Clinical Studies (14)]*.

11 DESCRIPTION

Flublok Quadrivalent [Quadrivalent Influenza Vaccine] is a sterile, clear, colorless solution of recombinant hemagglutinin (HA) proteins from four influenza viruses for intramuscular injection. It contains purified HA proteins produced in a continuous insect cell line (*expres*SF+®) that is derived from Sf9 cells of the fall armyworm, *Spodoptera frugiperda* (which is related to moths, caterpillars and butterflies), and grown in serum-free medium composed of chemically-defined lipids, vitamins, amino acids, and mineral salts. Each of the four HAs is expressed in this cell line using a baculovirus vector (*Autographa californica* nuclear polyhedrosis virus), extracted from the cells with Triton X-100 and further purified by column chromatography. The purified HAs are then blended and filled into single-dose syringes.

Flublok Quadrivalent is standardized according to United States Public Health Service (USPHS) requirements. For the 2022-2023 influenza season it is formulated to contain 180 mcg HA per 0.5 mL dose, with 45 mcg HA of each of the following 4 influenza virus strains: A/Wisconsin/588/2019 (H1N1), A/Darwin/6/2021 (H3N2), B/Austria/1359417/2021 and B/Phuket/3073/2013.

A single 0.5 mL dose of Flublok Quadrivalent contains sodium chloride (4.4 mg), monobasic sodium phosphate (0.2 mg), dibasic sodium phosphate (0.5 mg), and polysorbate 20 (Tween®20) (27.5 mcg). Each 0.5 mL dose of Flublok Quadrivalent may also contain residual amounts of baculovirus and *Spodoptera frugiperda* cell proteins (≤19 mcg), baculovirus and cellular DNA (≤10 ng), and Triton X-100 (≤100 mcg).

Flublok Quadrivalent contains no egg proteins, antibiotics, or preservatives. The single-dose, prefilled syringes contain no natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Flublok Quadrivalent contains recombinant HA proteins of the four strains of influenza virus specified by health authorities for inclusion in the annual seasonal vaccine. These proteins function as antigens which induce a humoral immune response, measured by hemagglutination inhibition (HI) antibody.

Antibodies against one influenza virus type or subtype confer limited or no protection against another. Furthermore, antibodies to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent (usually annual) development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual replacement of one or more influenza virus strains in each year's influenza vaccine. Therefore, influenza vaccines are standardized to contain the hemagglutinins of influenza virus strains (i.e., typically two type A and, in quadrivalent formulations, two type B), representing the influenza viruses likely to be circulating in the U.S. in the upcoming winter.

13 NONCLINICAL TOXICOLOGY

Flublok Quadrivalent has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. A developmental toxicity study conducted in rats vaccinated with Flublok (trivalent formulation) revealed no evidence of impaired female fertility *[see Pregnancy (8.1)]*.

14 CLINICAL STUDIES

14.1 Efficacy against Laboratory-Confirmed Influenza

The efficacy of Flublok (trivalent formulation) is relevant to Flublok Quadrivalent because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*.

The efficacy of Flublok (trivalent formulation) in protecting against influenza illness was evaluated in a randomized, observer-blind, placebo-controlled multicenter trial conducted in the U.S. during the 2007-2008 influenza season in adults 18-49 years of age (Study 3) .

Study 3 enrolled and vaccinated 4648 healthy adults (mean age 32.5 years) randomized in a 1:1 ratio to receive a single dose of Flublok (n=2344) or saline placebo (n=2304). Among enrolled subjects, 59% were female, 67% were white, 19% African-American, 2% Asian, <1% other races, and 11% of Latino/Hispanic ethnicity. Culture-confirmed influenza was assessed by active and passive surveillance for influenza-like illness (ILI) beginning 2 weeks post-vaccination until the end of the influenza season, approximately 7 months post-vaccination. ILI was defined as having at least 2 of 3 symptoms (no specified duration) in the following categories: 1) fever ≥100°F; 2) respiratory symptoms (cough, sore throat, or runny nose/stuffy nose); or 3) systemic symptoms (myalgias, arthralgias, headache, chills/sweats, or tiredness/malaise). For subjects with an episode of ILI, nasal and throat swab samples were collected for viral culture.

The primary efficacy endpoint of Study 3 was Centers for Disease Control-defined influenza-like illness (CDC-ILI) with a positive culture for an influenza virus strain antigenically resembling a strain represented

in Flublok. CDC-ILI is defined as fever of ≥100°F oral accompanied by cough, sore throat, or both on the same day or on consecutive days. Attack rates and vaccine efficacy (VE), defined as the reduction in the influenza rate for Flublok relative to placebo, were calculated for the total vaccinated cohort (n=4648).

The pre-defined success criterion for the primary efficacy analysis was that the lower bound of the 95% confidence interval (CI) of VE should be at least 40%. Vaccine efficacy against antigenically matched culture-confirmed CDC-ILI could not be determined reliably because 96% of the influenza isolates obtained from subjects in Study 3 were not antigenically matched to the strains represented in the vaccine. An exploratory analysis of VE of Flublok against all strains, regardless of antigenic match, isolated from any subject with an ILI, not necessarily meeting CDC- ILI criteria, demonstrated an efficacy estimate of 44.8% (95% CI 24.4, 60.0). See Table 3 for a presentation of VE by case definition and antigenic similarity.

Table 3: Vaccine Efficacy against Culture-Confirmed Influenza in Healthy Adults 18-49 Years of Age, Study 3*

Case definition	Flublok (trivalent) (N=2344)		Saline Placebo (N=2304)		Flublok Vaccine Efficacy[1], %	95% Confidence Interval
	Cases, n	Rate, %	Cases, n	Rate, %		
Positive culture with a strain represented in the vaccine						
CDC-ILI, all matched strains[2,3]	1	0.04	4	0.2	75.4	(-148.0, 99.5)
Any ILI, all matched strains[4,5]	2	0.1	6	0.3	67.2	(-83.2, 96.8)
Positive culture with any strain, regardless of match to the vaccine						
CDC-ILI, all strains[2,6]	44	1.9	78	3.4	44.6	(18.8, 62.6)
Sub-Type A	26	1.1	56	2.4	54.4	(26.1, 72.5)
Type B	18	0.8	23	1.0	23.1	(-49.0, 60.9)
Any ILI, all strains[4]	64	2.7	114	4.9	44.8	(24.4, 60.0)
Sub-Type A	41	1.7	79	3.4	49.0	(24.7, 65.9)
Type B	23	1.0	36	1.6	37.2	(-8.9, 64.5)

* In Study 3 (NCT00539981) vaccine efficacy analyses were conducted on the Total Vaccinated Cohort (all randomized subjects who received study vaccine according to the treatment actually received and who provided data). Vaccine efficacy (VE) = 1 minus the ratio of Flublok/placebo infection rates.

[1] Determined under the assumption of Poisson event rates, according to Breslow and Day, 1987.

[2] Meets CDC influenza-like illness (CDC-ILI) defined as fever of ≥100°F oral accompanied by cough and/or sore throat, on the same day or on consecutive days.

[3] Primary endpoint of trial.

[4] All culture-confirmed cases are considered, regardless of whether they qualified as CDC-ILI.

[5] Secondary endpoint of trial.

[6] Exploratory (prespecified) endpoint of trial.

Study 2 evaluated the efficacy of Flublok Quadrivalent in a randomized, observer-blind, active-controlled, multicenter trial conducted during the 2014-2015 influenza season in adults 50 years of age and older. A total of 8963 healthy, medically stable adults (mean age 62.5 years) were randomized in a 1:1 ratio to receive a single dose of Flublok Quadrivalent (n=4474) or a U.S.–licensed quadrivalent inactivated influenza vaccine (Comparator, Fluarix Quadrivalent, manufactured by Glaxo SmithKline) (n=4489). Among randomized subjects, 58% were female, 80% white, 18% black/African-American, 2% other races, and 5% of Hispanic/Latino ethnicity. A total of 5186 (60%) subjects were 50-64 years of age and 3486 (40%) were ≥65 years of age. Real-time polymerase chain reaction (rtPCR)–confirmed influenza was assessed by active and passive surveillance for influenza-like illness (ILI) beginning 2 weeks post-

vaccination until the end of the influenza season, approximately 6 months post- vaccination. ILI was defined as having at least one symptom (no specified duration) in each of two categories of respiratory and systemic symptoms. Respiratory symptoms included sore throat, cough, sputum production, wheezing and difficulty breathing. Systemic symptoms included fever >99°F (>37°C) oral, chills, fatigue, headache and myalgia. For subjects with an episode of ILI, a nasopharyngeal swab sample was collected for rtPCR testing and reflex viral culture of rtPCR-positive samples.

The primary efficacy endpoint of Study 2 was rtPCR-positive, protocol-defined ILI due to any strain of influenza. Attack rates and relative vaccine efficacy (rVE), defined as 1 – (Attack rate Flublok Quadrivalent/ Attack Rate Comparator), were calculated for the total efficacy population (n=8604) for the primary efficacy endpoint and for several alternative efficacy endpoints (Table 4). Antigenic and phylogenetic evaluations of the similarity ("matching") of clinical isolates to vaccine antigens were not performed. CDC epidemiological data for the 2014-2015 influenza season indicated that Influenza A (H3N2) viruses predominated and that most influenza A/H3N2 viruses were antigenically dissimilar while A/H1N1 and B viruses were antigenically similar to vaccine antigens.

Table 4: Relative Vaccine Efficacy (rVE) of Flublok Quadrivalent versus Comparator against Laboratory-Confirmed Influenza, Regardless of Antigenic Similarity to Vaccine Antigens, Adults 50 Years of Age and Older, Study 2 (Efficacy Population)[1,2]

	Flublok Quadrivalent (N=4303)		Comparator (N=4301)		RR	rVE % (95% CI)
	n	Attack Rate % (n/N)	n	Attack Rate % (n/N)		
All rtPCR-positive Influenza[3]	96	2.2	138	3.2	0.70	30 (10, 47)
All rtPCR-positive Influenza A[4]	73	1.7	114	2.7	0.64	36 (14, 53)
All rtPCR-positive Influenza B[4]	23	0.5	24	0.6	0.96	4 (-72, 46)
All Culture-confirmed Protocol-defined ILI[4,5]	58	1.3	101	2.3	0.57	43 (21, 59)

Abbreviations: rtPCR=reverse transcriptase polymerase chain reaction; Comparator=U.S.–licensed quadrivalent inactivated influenza vaccine, Fluarix Quadrivalent, manufactured by GlaxoSmithKline; n=number of influenza cases; N=number of subjects in treatment group; RR=relative risk (Attack Rate Flublok/Attack Rate IIV4); rVE = ([1-RR] × 100).

[1] Study 2 is registered as NCT02285998.

[2] Efficacy Population included all randomized subjects who received study vaccine and provided any follow-up documentation for influenza-like illness beginning at least 14 days post vaccination. Excluded subjects with protocol deviations that could adversely affect efficacy.

[3] Primary Analysis. All cases of rtPCR-confirmed influenza are included. Antigenic characterization and genetic sequencing to determine similarity of isolates to vaccine antigens were not performed. CDC surveillance data indicated that the majority of influenza A/H3N2 wild type viruses were antigenically distinct whereas influenza A/H1N1 and type B viruses were antigenically similar to vaccine antigens during the 2014-2015 season. Study 2 met the pre-specified success criterion for the primary endpoint (lower limit of the 2-sided 95% CI of vaccine efficacy for Flublok Quadrivalent relative to Comparator should be not less than -20%).

[4] *Post hoc* analyses. All cases of influenza A were A/H3N2. Cases of influenza B were not distinguished by lineage.

[5] Culture of rtPCR-positive samples was performed in MDCK cells.

14.2 Immunogenicity of Flublok Quadrivalent

Study 1 evaluated the immunogenicity of Flublok Quadrivalent as compared to a U.S.–licensed quadrivalent inactivated influenza vaccine (Comparator) (Fluarix Quadrivalent, manufactured by GlaxoSmithKline) in a randomized, observer-blind, active-controlled, multicenter trial conducted during the 2014-2015 influenza season in healthy adults 18-49 years of age. A total of 1350 subjects were enrolled, randomized 3:1, and vaccinated with Flublok Quadrivalent (998 subjects) or Comparator (332

subjects). Subjects were predominantly female (65%), white (60%), black/African American (37%), and of non-Hispanic/Latino ethnicity (84%), with a mean age of 33.5 years. Of the total vaccinated population, 1292 subjects (969 Flublok Quadrivalent and 323 IIV4 recipients, respectively) were evaluable for immune responses (Immunogenicity Population).

Post-vaccination immunogenicity was evaluated on sera obtained 28 days after administration of a single dose of study vaccine. Hemagglutination inhibition (HI) geometric mean titers (GMTs) were determined for the two vaccine groups for each vaccine antigen. Immunogenicity was compared by calculating the difference in seroconversion rates (SCR) and the ratios of GMTs of Comparator to Flublok Quadrivalent. Seroconversion was defined as either a pre-vaccination HI titer of <1:10 and a postvaccination HI titer of ≥1:40, or a pre-vaccination HI titer of ≥1:10 and a minimum 4-fold rise in postvaccination HI titer, at Day 28.

Study 1 had eight co-primary endpoints: Day 28 HI seroconversion rates and GMTs for each of the four antigens contained in the study vaccines. GMTs were compared based on the upper bound of the two-sided 95% CI of the GMT ratio of Comparator to Flublok Quadrivalent. Success in meeting this endpoint was pre-defined as an upper bound (UB) of the two-sided 95% CI of $GMT_{Comparator}$ / $GMT_{Flublok\ Quadrivalent}$ ≤1.5. Flublok Quadrivalent met the success criterion for GMTs for three of the four antigens but not for the B/Victoria lineage antigen (Table 5).

Table 5: Comparison of Day 28 Post-Vaccination Geometric Mean Titers (GMT) for Flublok Quadrivalent and Comparator in Adults 18-49 Years of Age, Study 1 (Immunogenicity Population)[1,2,3,4]

Antigen	Post-vaccination GMT Flublok Quadrivalent N=969	Post-vaccination GMT Comparator N=323	GMT Ratio Comparator/ Flublok Quadrivalent [95% CI]
A/H1N1	493	397	0.81 (0.71, 0.92)
A/H3N2	748	377	0.50 (0.44, 0.57)
B/Yamagata	156	134	0.86 (0.74, 0.99)
B/Victoria	43	64	1.49 (1.29, 1.71)

Abbreviations: CI, confidence interval; GMT, geometric mean titer.
[1] Study 1 is registered as NCT02290509.
[2] The Immunogenicity Population included all randomized subjects who received a dose of study vaccine, provided serum samples for Day 0 and Day 28 within specified windows, and had no major protocol deviations that might adversely affect the immune response. The pre-defined success criterion for the GMT ratio of Comparator to Flublok Quadrivalent was that the upper bound of the 2-sided 95% CI of the GMT ratio, $GMT_{Comparator}$ / $GMT_{Flublok\ Quadrivalent}$ at 28 days post-vaccination, must not exceed 1.5.
[3] HI titers were assayed using egg-derived antigens.
[4] Comparator: U.S.–licensed quadrivalent inactivated influenza vaccine, Fluarix Quadrivalent, manufactured by GlaxoSmithKline.

Success in meeting the seroconversion rate (SCR) endpoint was pre-defined as an upper bound (UB) of the two-sided 95% CI of $SCR_{Comparator} - SCR_{Flublok\ Quadrivalent}$ ≤10%. Flublok Quadrivalent met the success criterion for SCRs for three of the four antigens but not for the B/Victoria lineage antigen (Table 6). Sub-population analyses of immunogenicity did not reveal significant differences between genders. Sub-analyses according to race and ethnicity were not informative because the sizes of the subsets were insufficient to reach meaningful conclusions. The HI response to the B/Victoria lineage antigen was low in both vaccine groups.

Table 6: Comparison of Day 28 Seroconversion Rates for Flublok Quadrivalent and Comparator in Adults 18-49 Years of Age, Study 1 (Immunogenicity Population)[1,2,3,4]

Antigen	SCR (%, 95% CI) Flublok Quadrivalent N=969	SCR (%, 95% CI) Comparator N=323	SCR Difference (%) Comparator - Flublok Quadrivalent [95% CI]
A/H1N1	66.7 (63.6, 69.6)	63.5 (58.0, 68.7)	-3.2 (-9.2, 2.8)
A/H3N2	72.1 (69.2, 74.9)	57.0 (51.4, 62.4)	-15.2 (-21.3, -9.1)
B/Yamagata	59.6 (56.5, 62.8)	60.4 (54.8, 65.7)	0.7 (-5.4, 6.9)
B/Victoria	40.6 (37.4, 43.7)	58.2 (52.6, 63.6)	17.6 (11.4, 23.9)

Abbreviations: CI, confidence interval; SCR, seroconversion rate

Seroconversion was defined as a pre-vaccination HI titer <1:10 and a post-vaccination HI titer ≥1:40 or a pre-vaccination HI titer ≥1:10 and a minimum four-fold rise in post-vaccination HI antibody titer.

[1] Study 1 is registered as NCT02290509.

[2] The Immunogenicity Population included all randomized subjects who received a dose of study vaccine, provided serum samples for Day 0 and Day 28 within specified windows, and had no major protocol deviations that might adversely affect the immune response. The pre-defined success criterion for the SCR difference between Comparator and Flublok Quadrivalent was that the upper bound of the 2-sided 95% CI of the SCR difference IIV4 – Flublok Quadrivalent at 28 days post-vaccination, must not exceed 10%.

[3] HI titers were assayed using egg-derived antigens.

[4] Comparator was a U.S.–licensed quadrivalent inactivated influenza vaccine, Fluarix Quadrivalent, manufactured by GlaxoSmithKline.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Flublok Quadrivalent is supplied as a single-dose, 0.5 mL syringe in a 5 or 10 syringe carton:

Presentation	Carton NDC Number	Components and NDC Number
Single-Dose Prefilled Syringe	49281-722-10	Ten 0.5 mL single-dose prefilled syringes [NDC 49281-722-88]

16.2 Storage and Handling

- Store refrigerated between 2°C and 8°C (36°F and 46°F).
- Do not freeze. Discard if product has been frozen.
- Protect syringes from light.
- Do not use after expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

Inform the vaccine recipient of the potential benefits and risks of vaccination with Flublok Quadrivalent.

Inform the vaccine recipient that:

- Flublok Quadrivalent contains non-infectious proteins that cannot cause influenza.

- Flublok Quadrivalent stimulates the immune system to produce antibodies that help protect against the influenza viruses carrying the proteins contained in the vaccine, but does not prevent other respiratory infections.

Instruct the vaccine recipient to report any adverse events to their healthcare provider and/or to the Vaccine Adverse Event Reporting System (VAERS).

Provide the vaccine recipient with the Vaccine Information Statements which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to vaccination. These materials are available free of charge at the Centers for Disease Control (CDC) website (www.cdc.gov/vaccines).

Encourage women who receive Flublok or Flublok Quadrivalent while pregnant to notify Sanofi Pasteur Inc. by calling 1-800-822-2463.

Instruct the vaccine recipient that annual vaccination to prevent influenza is recommended.

Manufactured by Protein Sciences Corporation (Meriden, CT).
U.S. license No. 1795.

Distributed by Sanofi Pasteur Inc.

Flublok is a registered trademark of Protein Sciences Corporation.

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use FLUCELVAX® QUADRIVALENT safely and effectively. See full prescribing information for FLUCELVAX QUADRIVALENT.

FLUCELVAX QUADRIVALENT (Influenza Vaccine)
Suspension for Intramuscular Injection
2022-2023 Formula
Initial U.S. Approval: 2016

----------RECENT MAJOR CHANGES----------

Indications and Usage (1)	10/2021
Dosage and Administration (2.1)	10/2021

----------INDICATIONS AND USAGE----------

FLUCELVAX QUADRIVALENT is an inactivated vaccine indicated for active immunization for the prevention of influenza disease caused by influenza virus subtypes A and types B contained in the vaccine. (1)

FLUCELVAX QUADRIVALENT is approved for use in persons 6 months of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------

For intramuscular use only

Age	Dose	Schedule
6 months through 8 years of age	One or two doses[a], 0.5 mL each	If 2 doses, administer at least 4 weeks apart
9 years of age and older	One dose, 0.5 mL	Not Applicable

[a] 1 or 2 doses depends on vaccination history as per Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines.

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection supplied in two presentations:

- 0.5 mL single-dose pre-filled syringes. (3, 11)
- 5 mL multi-dose vial containing 10 doses (each dose is 0.5 mL). (3, 11)

----------CONTRAINDICATIONS----------

History of severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine. (4, 11)

----------WARNINGS AND PRECAUTIONS----------

- If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give FLUCELVAX QUADRIVALENT should be based on careful consideration of the potential benefits and risks. (5.1)

----------ADVERSE REACTIONS----------

- In children 6 months through 3 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were tenderness (27.9%), erythema (25.8%), induration (17.3%) and ecchymosis (10.7%). The most common systemic adverse reactions were irritability (27.9%), sleepiness (26.9%), diarrhea (17.9%) and change of eating habits (17.4%). (6)
- In children 2 through 8 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were tenderness (28.7%), pain (27.9%) and erythema (21.3%), induration (14.9%) and ecchymosis (10.0%). The most common systemic adverse reactions were sleepiness (14.9%), headache (13.8%), fatigue (13.8%), irritability (13.8%) and loss of appetite (10.6%). (6)
- In children and adolescents 9 through 17 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were injection site pain (21.7%), erythema (17.2%) and induration (10.5%). The most common systemic adverse reactions were headache (18.1%) and fatigue (17.0%). (6)
- In adults 18 through 64 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were pain (45.4%), erythema (13.4%) and induration (11.6%). The most common systemic adverse reactions were headache (18.7%), fatigue (17.8%) and myalgia (15.4%). (6)
- In adults ≥65 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were pain (21.6%) and erythema (11.9%). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Seqirus at 1-855-358-8966 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS----------

- Geriatric Use: Antibody responses were lower in adults 65 years and older than in younger adults. (8.5)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 3/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

FLUCELVAX QUADRIVALENT is an inactivated vaccine indicated for active immunization for the prevention of influenza disease caused by influenza virus subtypes A and types B contained in the vaccine. FLUCELVAX QUADRIVALENT is approved for use in persons 6 months of age and older. *[see Clinical Studies (14)]*

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Dosage and Schedule

Administer FLUCELVAX QUADRIVALENT as a single 0.5 mL intramuscular injection.

Table 1: Dosage and Schedule

Age	Dose	Schedule
6 months through 8 years of age	One or two doses[1], 0.5 mL each	If 2 doses, administer at least 4 weeks apart
9 years of age and older	One dose, 0.5 mL	Not Applicable

[1] 1 or 2 doses depends on vaccination history as per Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines.

2.2 Administration

Shake the syringe vigorously before administering and shake the multi-dose vial preparation each time before withdrawing a dose of vaccine. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. *[see Description (11)]* If either condition exists, do not administer the vaccine. Between uses, return the multi-dose vial to the recommended storage conditions between 2º and 8ºC (36º and 46ºF). Do not freeze. Discard if the vaccine has been frozen.

Administer intramuscularly only, preferably in the region of the deltoid muscle of the upper arm. Younger children with insufficient deltoid mass should be vaccinated in the anterolateral aspect of the thigh.

3 DOSAGE FORMS AND STRENGTHS

FLUCELVAX QUADRIVALENT is a suspension for injection supplied in two presentations:

- a 0.5 mL single-dose pre-filled Luer Lock syringe
- a 5 mL multi-dose vial containing 10 doses (each dose is 0.5 mL)

4 CONTRAINDICATIONS

Do not administer FLUCELVAX QUADRIVALENT to anyone with a history of severe allergic reaction (e.g. anaphylaxis) to any component of the vaccine *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

The 1976 swine influenza vaccine was associated with an elevated risk of Guillain-Barré syndrome (GBS). Evidence for a causal relation of GBS with other influenza vaccines is inconclusive; if an excess risk exists, it is probably slightly more than 1 additional case per 1 million persons vaccinated.[1] If GBS has occurred after receipt of a prior influenza vaccine, the decision to give FLUCELVAX QUADRIVALENT should be based on careful consideration of the potential benefits and risks.

5.2 Preventing and Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

5.3 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including FLUCELVAX QUADRIVALENT. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope by maintaining a supine or Trendelenburg position.

5.4 Altered Immunocompetence

After vaccination with FLUCELVAX QUADRIVALENT, immunocompromised individuals, including those receiving immunosuppressive therapy, may have a reduced immune response.

5.5 Limitations of Vaccine Effectiveness

Vaccination with FLUCELVAX QUADRIVALENT may not protect all vaccine recipients against influenza disease.

6 ADVERSE REACTIONS

In children 6 months through 3 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were tenderness (27.9%), erythema (25.8%), induration (17.3%) and ecchymosis (10.7%). The most common systemic adverse reactions were irritability (27.9%), sleepiness (26.9%), diarrhea (17.9%) and change of eating habits (17.4%).

In children 2 through 8 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were tenderness (28.7%), pain (27.9%) and erythema (21.3%), induration (14.9%) and ecchymosis (10.0%). The most common systemic adverse reactions were sleepiness (14.9%), headache (13.8%), fatigue (13.8%), irritability (13.8%) and loss of appetite (10.6%).

In children and adolescents 9 through 17 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were injection

site pain (21.7%), erythema (17.2%) and induration (10.5%). The most common systemic adverse reactions were headache (18.1%) and fatigue (17.0%).

In adults 18 through 64 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported (≥ 10%) injection-site adverse reactions were pain (45.4%), erythema (13.4%) and induration (11.6%). The most common systemic adverse reactions were headache (18.7%), fatigue (17.8%) and myalgia (15.4%).

In adults ≥65 years of age who received FLUCELVAX QUADRIVALENT, the most commonly reported injection-site adverse reactions were pain (21.6%) and erythema (11.9%).

6.1 Clinical Trials Experience

Because clinical studies are conducted under widely varying conditions, adverse reaction rates observed in the clinical studies of a vaccine cannot be directly compared to rates in clinical studies of another vaccine and may not reflect rates observed in clinical practice.

Children and Adolescents 6 months through 17 years of age:

The safety of FLUCELVAX QUADRIVALENT was evaluated in children and adolescents in two clinical studies: Study 1 and 2.

Study 1 was a randomized, observer-blind, multicenter study in children 6 months through 3 years of age. The safety population included a total of 2402 children 6 months through 3 years of age who received FLUCELVAX QUADRIVALENT (N=1597) or a US-licensed quadrivalent influenza vaccine comparator, AFLURIA QUADRIVALENT (N=805). In the safety population, 894 subjects (37.2%) were 6 months through 23 months of age, and 1508 subjects (62.8%) were 24 months through 47 months of age. The solicited safety set consisted of 2348 subjects who received FLUCELVAX QUADRIVALENT (N=1564) or a US-licensed quadrivalent influenza vaccine comparator (N=784). Study subjects received one or two doses (separated by 4 weeks) of FLUCELVAX QUADRIVALENT or the comparator vaccine depending on the subject's prior influenza vaccination history.

In this study, solicited local injection site and systemic adverse reactions were collected on a symptom diary card for 7 days following vaccination.

In children 6 months through 3 years of age, the incidence of local and systemic solicited adverse reactions reported by children who received FLUCELVAX QUADRIVALENT and comparator are summarized in Table 2.

Table 2: Incidence of Solicited Adverse Reactions in the Safety Population[1] (6 months through 3 years of age) Reported Within 7 Days of Any Dose of Vaccination (Study 1)

	Percentage (%)[2] of participants Reporting a Reaction							
	Participants 6 through 23 months				Participants 24 through 47 months			
	FLUCELVAX QUADRIVALENT N=581		Comparator[3] N=292		FLUCELVAX QUADRIVALENT N=983		Comparator[3] N=492	
	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3
Local Adverse Reactions[4]								
Tenderness	25.5	2.1	23.3	1.4	29.3	2.2	33.9	1.4
Erythema	25.3	0	18.2	0	26.0	0.7	28.5	0
Induration	16.5	0.5	12.0	0	17.7	0.3	18.3	0
Ecchymosis	11.2	0.2	7.5	0	10.5	0.1	12.8	0
Systemic Adverse Reactions[5]								
Irritability	35.1	5.2	35.6	2.1	23.6	1.8	26.0	3.0
Sleepiness	35.5	2.4	30.5	1.7	21.8	1.9	22.6	1.2
Diarrhea	23.2	2.4	20.2	0.7	14.8	1.1	14.0	1.2
Change of eating habits	21.0	1.7	21.9	2.4	15.3	1.4	15.0	1.2
Fever	9.3	0.7	10.3	0	5.4	0.6	4.8	0.2
Vomiting	10.5	0.7	6.8	0.7	4.6	0.5	5.9	0.4
Shivering	3.1	0.2	3.1	0	3.3	0.2	3.7	0

Abbreviations: Gr 3, Grade 3.

N = number of participants in the Safety Population for each study vaccine group.

[1] Solicited Safety Population: participants who were vaccinated and provided any solicited local or systemic adverse reaction safety data on subject diary cards from Day 1 through Day 7 after vaccination.

[2] Proportion of participants reporting each solicited local adverse reaction or systemic adverse event by study vaccine group based on the number of participants contributing any follow up safety information for at least one data value of an individual sign/symptom

[3] Comparator: US-Licensed Quadrivalent Influenza vaccine

[4] Local adverse reactions: Grade 3 tenderness defined as, "Cried when limb was moved/spontaneously painful" in subjects 6 through 23 months, and "Prevents daily activity" in subjects 24 months and older; Erythema, induration and ecchymosis: any = ≥1mm diameter, Grade 3 => 50 mm diameter.

[5] Systemic adverse reactions: Fever: any = ≥ 100.4°F , Grade 3 = ≥ 102.2°F (either rectal, oral, axillary, or tympanic membrane); Grade 3 change of eating habits: Missed more than 2 feeds/meals; Grade 3 sleepiness: Sleeps most of the time and is hard to arouse him/her; Grade 3 vomiting: 6 or more times in 24 hours or requires intravenous hydration; Grade 3 diarrhea: 6 or more loose stools in 24 hours or requires intravenous hydration; Grade 3 irritability: unable to console. Grade 3 for all other adverse reactions is that which prevents daily activity.

The rates of antipyretic or analgesic use reported on the diary card for prophylaxis or treatment of high temperature or pain were as follows: 6 through 23 months of age FLUCELVAX QUADRIVALENT 20.3%, Comparator 23.6%; 24 through 47 months of age FLUCELVAX QUADRIVALENT 12.4%, Comparator 13.6%.

Study 1: NCT 04074928

In children who received two doses, the rates of solicited local and systemic adverse reactions were generally similar or lower after the second dose compared to the first dose.

All unsolicited adverse events were collected for 28 days after last vaccination. In children 6 months through 3 years of age, unsolicited adverse events were reported in 26.2% of subjects who received FLUCELVAX QUADRIVALENT and 25.7% of subjects who received the US-licensed quadrivalent influenza vaccine comparator within 28 days after last vaccination.

In children 6 months through 3 years of age, serious adverse events (SAEs) were collected throughout the study duration (until 6 months after last vaccination) and were reported by 0.9% of the subjects who received FLUCELVAX QUADRIVALENT and 0.9% of subjects who received the US-licensed quadrivalent influenza vaccine comparator. None of the SAEs were assessed as being related to study vaccine.

Study 2 was a multi-season, multi-national (Australia, Estonia, Finland, Lithuania, Philippines, Poland, Spain, Thailand), randomized, observer-blind study in children and adolescents 2 through 17 years of age. The solicited safety population included a total of 4509 children and adolescents 2 through 17 years of age who received FLUCELVAX QUADRIVALENT(N=2255) or a non-influenza (meningococcal (Groups A, C, Y, and W-135) oligosaccharide diphtheria CRM197 conjugate) comparator vaccine (N=2254).

Children 2 through 8 years of age received one or two doses (separated by 4 weeks) of FLUCELVAX QUADRIVALENT or comparator vaccine depending on the subject's prior influenza vaccination history. Children in the 2-dose comparator group received non-influenza comparator as the first dose and saline placebo as the second dose. Children and adolescents 9 through 17 years of age received a single dose of FLUCELVAX QUADRIVALENT or non-influenza comparator vaccine.

In this study, solicited local injection site and systemic adverse reactions were collected on a symptom diary card for 7 days following vaccination.

In children 2 through 8 and children and adolescents 9 through 17 years of age, the incidence of local and systemic solicited adverse reactions reported by children and adolescents who received FLUCELVAX QUADRIVALENT and comparator are summarized in Table 3.

Table 3: Incidence of Solicited Adverse Reactions in the Safety Population[1] (2 through 8 and 9 through 17 years of age) Reported Within 7 Days of Any Dose of Vaccination (Study 2)

	Percentage (%)[2] of participants in each Age Cohort Reporting a Reaction							
	Participants 2 through 8 years				**Participants 9 through 17 years**			
	FLUCELVAX QUADRIVALENT N=559-1143		**Comparator[3] N=562-1142**		**FLUCELVAX QUADRIVALENT N=1096-1109**		**Comparator[3] N=1100-1108**	
	Any	**Gr 3**	**Any**	**Gr 3**	**Any**	**Gr 3**	**Any**	**Gr 3**
Local Adverse Reactions[4]								
Tenderness[5]	28.7	1.0	25.4	1.4	-	-	-	-
Pain	27.9	1.2	20.3	1.6	21.7	0.5	18.3	1.0
Erythema	21.3	0.4	23.7	1.1	17.2	0	18.7	0.5
Induration	14.9	0.2	15.2	0.4	10.5	0.1	11.0	0.2
Ecchymosis	10.0	0	7.5	0.1	5.0	0	5.2	0
Systemic Adverse Reactions[6]								
Sleepiness[5]	14.9	0.9	17.6	1.8	-	-	-	-
Headache	13.8	0.4	11.8	0.5	18.1	1.4	17.4	0.6
Fatigue	13.8	0.9	12.7	0.7	17.0	1.1	18.2	1.2
Irritability[5]	13.8	0.2	10.8	0.5	-	-	-	-
Loss of Appetite	10.6	0.5	8.0	0.5	8.5	0.5	7.5	0.5

	Percentage (%)[2] of participants in each Age Cohort Reporting a Reaction							
	Participants 2 through 8 years				Participants 9 through 17 years			
	FLUCELVAX QUADRIVALENT N=559-1143		Comparator[3] N=562-1142		FLUCELVAX QUADRIVALENT N=1096-1109		Comparator[3] N=1100-1108	
	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3
Change of eating habits[5]	9.9	1.0	10.1	0.7	-	-	-	-
Fever	7.6	0.5	6.1	0.2	2.8	0.1	3.0	0.3
Diarrhea	6.5	0.4	6.8	0.6	7.4	0.5	8.1	0.3
Arthralgia	5.2	0.4	6.2	0.3	7.1	0.4	8.4	0.5
Nausea	5.2	0	4.5	0.7	6.0	0.2	6.1	0.6
Vomiting	4.9	0.6	4.1	0.6	3.0	0.3	3.0	0.4
Shivering/Chills	4.7	0.7	3.9	0.4	7.6	0.4	7.6	0.3
Myalgia	2.9	0.2	4.0	0.3	6.1	0.5	5.5	0.5

Abbreviations: Gr 3, Grade 3.
N = number of participants in the Safety Population for each study vaccine group.
[1] Solicited Safety Population: participants who were vaccinated and provided any solicited local or systemic adverse reaction safety data on subject diary cards from Day 1 through Day 7 after vaccination.
[2] Proportion of participants reporting each solicited local adverse reaction or systemic adverse event by study vaccine group based on the number of participants contributing any follow up safety information for at least one data value of an individual sign/symptom
[3] Non-influenza vaccine comparator: MENVEO, meningococcal (Groups A, C, Y, and W-135) oligosaccharide diphtheria CRM197 conjugate vaccine (GlaxoSmithKline Biologicals SA); children assigned to 2 doses received saline placebo as the second dose.
[4] Local adverse reactions: Grade 3 pain is that which prevents daily activity; Erythema, induration and ecchymosis: any = ≥ 1mm diameter, Grade 3 = > 50 mm diameter for 2 through 5 years and > 100 mm diameter for 6 through 17 years.
[5] Tenderness, change in eating habits, sleepiness, and irritability were collected for participants 2 through 6 years of age only.
[6] Systemic adverse reactions: Fever: any = ≥ 100.4°F (Oral), Grade 3 = ≥ 102.2°F (Oral); Grade 3 change of eating habits: Missed more than 2 feeds/meals; Grade 3 sleepiness: Sleeps most of the time and is hard to arouse him/her; Grade 3 vomiting: 6 or more times in 24 hours or requires intravenous hydration; Grade 3 diarrhea: 6 or more loose stools in 24 hours or requires intravenous hydration; Grade 3 irritability: unable to console. Grade 3 for all other adverse events is that which prevents daily activity.
The rates of antipyretic or analgesic use reported on the diary card for prophylaxis or treatment of high temperature or pain were as follows: 2 through 8 years of age FLUCELVAX QUADRIVALENT 11.0%, Comparator 7.7%; 9 through 18 years of age FLUCELVAX QUADRIVALENT 6.7%, Comparator 7.1%.
Study 2: NCT03165617

In children who received a second dose (N=762) of FLUCELVAX QUADRIVALENT, the rates of solicited local and systemic adverse reactions were generally lower after the second dose compared to the first dose.

Serious adverse events (SAEs) were collected throughout the study duration (until 6 months after last vaccination) and were reported by 1.1% of the children and adolescents who received FLUCELVAX QUADRIVALENT. None of the SAEs were assessed as being related to study vaccine.

Adults 18 years of age and older:

The safety of FLUCELVAX QUADRIVALENT in adults was evaluated in a randomized, double-blind, controlled study conducted in the US (Study 3). The safety population included a total of 2680 adults 18 years of age and older; 1340 adults 18 through 64 years of age and 1340 adults 65 years of age and older.

In this study, adults received FLUCELVAX QUADRIVALENT or one of the two formulations of comparator trivalent influenza vaccine (TIV1c and TIV2c) (FLUCELVAX QUADRIVALENT (N=1335), TIV1c, N=676 or TIV2c, N= 669). The mean age of adults who received FLUCELVAX QUADRIVALENT was 57.4 years of age; 54.8% of adults were female and 75.6% were Caucasian, 13.4% were Black, 9.1% were Hispanics, 0.7% were American Indian and 0.3%, 0.1% and 0.7% were Asian, Native Hawaiian and others, respectively. The safety data observed are summarized in Table 2.

In this study, solicited local injection site and systemic adverse reactions were collected from adults who completed a symptom diary card for 7 days following vaccination.

Solicited adverse reactions for FLUCELVAX QUADRIVALENT and comparator are summarized in Table 4.

Table 4: Incidence of Solicited Adverse Reactions in the Adult Safety Population[1] Reported Within 7 Days of Vaccination (Study 3)

	Percentage (%)[2] of participants in each Age Cohort Reporting a Reaction											
	Participants 18 through 64 years						Participants ≥ 65 years					
	FLUCELVAX Quadrivalent N=663		TIV1c N=330		TIV2c N=327		FLUCELVAX Quadrivalent N=656		TIV1c N=340		TIV2c N=336	
	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3	Any	Gr 3
Local Adverse Reactions[3]												
Pain	45.4	0.5	37.0	0.3	40.7	0	21.6	0	18.8	0	18.5	0
Erythema	13.4	0	13.3	0	10.1	0	11.9	0	10.6	0	10.4	0
Induration	11.6	0	9.7	0.3	10.4	0	8.7	0	6.8	0	7.7	0
Ecchymosis	3.8	0	3.3	0.3	5.2	0	4.7	0	4.4	0	5.4	0
Systemic Adverse Reactions[4]												
Headache	18.7	0.9	18.5	0.9	18.7	0.6	9.3	0.3	8.5	0.6	8.3	0.6
Fatigue	17.8	0.6	22.1	0.3	15.6	1.5	9.1	0.8	10.6	0.3	8.9	0.6
Myalgia	15.4	0.8	14.5	0.9	15.0	1.2	8.2	0.2	9.4	0.3	8.3	0.6
Nausea	9.7	0.3	7.3	0.9	8.9	1.2	3.8	0.2	4.1	0	4.2	0.3
Loss of appetite	8.3	0.3	8.5	0.3	8.3	0.9	4.0	0.2	5.0	0	3.6	0.3
Arthralgia	8.1	0.5	8.2	0	9.5	0.9	5.5	0.5	5.0	0.3	6.8	0.9
Diarrhea	7.4	0.6	7.6	0	7.6	0.6	4.3	0.5	5.0	0.9	5.1	0.3
Chills	6.2	0.2	6.4	0.6	6.4	0	4.4	0.3	4.1	0.3	4.5	0.6
Vomiting	2.6	0	1.5	0.3	0.9	0	0.9	0.2	0.3	0	0.6	0
Fever	0.8	0	0.6	0	0.3	0	0.3	0	0.9	0	0.6	0

Abbreviations: Gr 3, Grade 3.
N = number of participants in the Safety Population for each study vaccine group.
[1] Safety population: all participants in the exposed population who provided post-vaccination safety data
[2] Proportion of participants reporting each solicited local adverse reaction or systemic adverse reaction by study vaccine group based on the number of participants contributing any follow up safety information for at least one data value of an individual sign/symptom
[3] Local Adverse reactions: Grade 3 pain is that which prevents daily activity; Erythema, induration and ecchymosis: any = ≥ 1 mm diameter, Grade 3 = > 100 mm diameter.

[4] Systemic adverse reactions: Fever: any = ≥ 100.4°F (Oral), Grade 3 = ≥ 102.2°F (Oral); Grade 3 vomiting: requires outpatient hydration; Grade 3 diarrhea: 6 or more stools or requires outpatient IV hydration; Grade 3 for all other adverse reactions is that which prevents daily activity.

Study 3: NCT01992094

Unsolicited adverse events were collected for 21 days after vaccination. In adults 18 years of age and older, unsolicited adverse events were reported in 16.1% of adults who received FLUCELVAX QUADRIVALENT, within 21 days after vaccination.

In adults 18 years of age and older, serious adverse events (SAEs) were collected throughout the study duration (until 6 months after vaccination) and were reported by 3.9%, of the adults who received FLUCELVAX QUADRIVALENT. None of the SAEs were assessed as being related to study vaccine.

6.2 Postmarketing Experience

The following additional adverse events have been identified during post-approval use of FLUCELVAX QUADRIVALENT. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Immune system disorders: Allergic or immediate hypersensitivity reactions, including anaphylactic shock.

Nervous systems disorders: Syncope, presyncope, paresthesia.

Skin and subcutaneous tissue disorders: Generalized skin reactions including pruritus, urticaria or non-specific rash.

General disorders and administration site conditions: Extensive swelling of injected limb.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are insufficient data for FLUCELVAX QUADRIVALENT in pregnant women to inform vaccine-associated risks in pregnancy.

There were no developmental toxicity studies of FLUCELVAX QUADRIVALENT performed in animals. A developmental toxicity study has been performed in female rabbits administered FLUCELVAX (trivalent formulation) prior to mating and during gestation. The dose was 0.5 mL on each occasion (a single human dose is 0.5 mL). This study revealed no evidence of harm to the fetus due to FLUCELVAX (trivalent formulation).

Clinical Considerations

Disease-associated Maternal and/or Embryo-Fetal Risk

Pregnant women are at increased risk for severe illness due to influenza compared to non-pregnant women. Pregnant women with influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

Data

Animal Data

In a developmental toxicity study, female rabbits were administered FLUCELVAX (trivalent formulation) by intramuscular injection 1, 3, and 5 weeks prior to mating, and on gestation days 7 and 20. The dose was 0.5 mL on each occasion (a single human dose is 0.5 mL). No vaccine-related fetal malformations or variations and no adverse effects on pre-weaning development were observed in the study.

8.2 Lactation

Risk Summary

It is not known whether FLUCELVAX QUADRIVALENT is excreted in human milk. Data are not available to assess the effects of FLUCELVAX QUADRIVALENT on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for FLUCELVAX QUADRIVALENT and any potential adverse effects on the breastfed child from FLUCELVAX QUADRIVALENT or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine or the effects on milk production.

8.4 Pediatric Use

Safety and effectiveness have not been established in children less than 6 months of age.

8.5 Geriatric Use

Of the total number of adults who received one dose of FLUCELVAX QUADRIVALENT in clinical studies and included in the safety population (2493), 26% (660) were 65 years of age and older and 8% (194) were 75 years of age or older.

Antibody responses to FLUCELVAX QUADRIVALENT were lower in the geriatric (adults 65 years and older) population than in younger adults. *[see Clinical Studies (14.3)]*

11 DESCRIPTION

FLUCELVAX QUADRIVALENT (Influenza Vaccine) is a subunit influenza vaccine manufactured using cell derived candidate vaccine viruses (CVV) that are propagated in Madin Darby Canine Kidney (MDCK) cells, a continuous cell line. These cells were adapted to grow freely in suspension in culture medium. The virus is inactivated with β-propiolactone, disrupted by the detergent cetyltrimethylammonium bromide and purified through several process steps. Each of the 4 virus strains is produced and purified separately then pooled to formulate the quadrivalent vaccine.

FLUCELVAX QUADRIVALENT is a sterile, slightly opalescent suspension in phosphate buffered saline. FLUCELVAX QUADRIVALENT is standardized according to United States Public Health Service requirements for the 2022-2023 influenza season and is formulated to contain a total of 60 micrograms (mcg) hemagglutinin (HA) per 0.5 mL dose in the recommended ratio of 15 mcg HA of each of the following four influenza strains:

A/Delaware/55/2019 CVR-45 (an A/Wisconsin/588/2019 (H1N1)pdm09-like virus);

A/Darwin/11/2021 (an A/Darwin/6/2021 (H3N2)-like virus);

B/Singapore/WUH4618/2021 (a B/Austria/1359417/2021-like virus);

B/Singapore/INFTT-16-0610/2016 (a B/Phuket/3073/2013-like virus).

Each dose of FLUCELVAX QUADRIVALENT may contain residual amounts of MDCK cell protein (≤ 25.2 mcg), protein other than HA (≤ 240 mcg), MDCK cell DNA (≤ 10 ng), polysorbate 80 (≤ 1500 mcg), cetyltrimethylammonium bromide (≤ 18 mcg), and β-propiolactone (< 0.5 mcg), which are used in the manufacturing process.

FLUCELVAX QUADRIVALENT contains no egg protein or antibiotics.

FLUCELVAX QUADRIVALENT 0.5 mL pre-filled syringes contain no preservative.

FLUCELVAX QUADRIVALENT 5 mL multi-dose vials contain thimerosal, a mercury derivative, added as a preservative. Each 0.5 mL dose from the multi-dose vial contains 25 mcg mercury.

The tip caps and plungers of the pre-filled syringes and the multi-dose vial stopper are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Influenza illness and its complications follow infection with influenza viruses. Global surveillance and analysis of influenza virus isolates permits identification of yearly antigenic variants. Since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation. Specific levels of hemagglutination inhibition (HI) antibody titers induced by vaccination with inactivated influenza virus vaccine have not been correlated with protection from influenza illness. In some studies, HI antibody titers of ≥ 1:40 have been associated with protection from influenza illness in up to 50% of adults.[2,3]

Antibody against one influenza virus type or subtype confers little or no protection against another. Furthermore, antibody to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual change of one or more strains in each year's influenza vaccine. Therefore, inactivated influenza vaccines are standardized to contain the hemagglutinin of influenza virus strains representing the influenza viruses likely to circulate in the United States in the upcoming winter.

Annual influenza vaccination is recommended by the Advisory Committee on Immunization Practices because immunity declines during the year after vaccination, and because circulating strains of influenza virus change from year to year.[4]

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

FLUCELVAX QUADRIVALENT has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals.

FLUCELVAX (trivalent formulation) administered to female rabbits had no effect on fertility. *[see Use in Specific Population (8.1)]*

14 CLINICAL STUDIES

14.1 Efficacy against Culture-Confirmed Influenza

The efficacy experience with FLUCELVAX is relevant to FLUCELVAX QUADRIVALENT because both vaccines are manufactured using the same process and have overlapping compositions.

A multinational (US, Finland, and Poland), randomized, observer-blind, placebo-controlled trial was performed to assess clinical efficacy and safety of FLUCELVAX during the 2007-2008 influenza season in adults aged 18 through 49 years (Study 4). A total of 11,404 adults were enrolled to receive FLUCELVAX (N=3828), AGRIFLU (N=3676) or placebo (N=3900) in a 1:1:1 ratio. Among the overall study population enrolled, the mean age was 33 years, 55% were female, 84% were Caucasian, 7% were Black, 7% were Hispanic, and 2% were of other ethnic origin.

FLUCELVAX efficacy was assessed by the prevention of culture-confirmed symptomatic influenza illness caused by viruses antigenically matched to those in the vaccine and prevention of influenza illness caused by all influenza viruses compared to placebo. Influenza cases were identified by active and passive surveillance of influenza-like illness (ILI). ILI was defined as a fever (oral temperature ≥ 100.0°F / 38°C) and cough or sore throat. Nose and throat swab samples were collected for analysis within 120 hours of onset of an influenza-like illness in the period from 21 days to 6 months after vaccination. Overall vaccine efficacy against all influenza viral subtypes and vaccine efficacy against individual influenza viral subtypes were calculated (Tables 5 and 6, respectively).

Table 5: Vaccine Efficacy against Culture-Confirmed Influenza in Participants aged 18 through 49 years (Study 4)

	Number of participants per protocol	Number of participants with influenza	Attack Rate (%)	Vaccine Efficacy (VE)[1,2]	
				%	Lower Limit of One-Sided 97.5% CI of VE[2,3]
Antigenically Matched Strains					
FLUCELVAX	3776	7	0.19	83.8	61.0
Placebo	3843	44	1.14	--	--
All Culture-Confirmed Influenza					
FLUCELVAX	3776	42	1.11	69.5	55.0
Placebo	3843	140	3.64	--	--

[1] Efficacy against influenza was evaluated over a 9-month period in 2007/2008

[2] Simultaneous one-sided 97.5% confidence intervals for the vaccine efficacy (VE) of FLUCELVAX relative to placebo based on the Sidak-corrected score confidence intervals for the relative risk. Vaccine Efficacy = (1 - Relative Risk) x 100 %

[3] VE success criterion: the lower limit of the one-sided 97.5% CI for the estimate of the VE relative to placebo is > 40%

Study 4: NCT00630331

Table 6: Efficacy of FLUCELVAX against Culture-Confirmed Influenza by Influenza Viral Subtype in Participants aged 18 through 49 years (Study 4)

	FLUCELVAX (N=3776)		Placebo (N=3843)		Vaccine Efficacy (VE)[2]	
	Attack Rate (%)	Number of Participants with Influenza	Attack Rate (%)	Number of Participants with Influenza	%	Lower Limit of One-Sided 97.5% CI of VE[1,2]
Antigenically Matched Strains						
A/H3N2[3]	0. 05	2	0	0	--	--
A/H1N1	0.13	5	1.12	43	88.2	67.4
B[3]	0	0	0.03	1	--	--
All Culture-Confirmed Influenza						
A/H3N2	0.16	6	0.65	25	75.6	35.1
A/H1N1	0.16	6	1.48	57	89.3	73.0
B	0.79	30	1.59	61	49.9	18.2

[1] No VE success criterion was prespecified in the protocol for each individual influenza virus subtype.

[2] Simultaneous one-sided 97.5% confidence intervals for the vaccine efficacy (VE) of FLUCELVAX relative to placebo based on the Sidak-corrected score confidence intervals for the relative risk. Vaccine Efficacy = (1 - Relative Risk) x 100 %;

[3] There were too few cases of influenza due to vaccine-matched influenza A/H3N2 or B to adequately assess vaccine efficacy.

Study 4: NCT00630331

14.2 Efficacy of FLUCELVAX QUADRIVALENT in Children and Adolescents 2 through 17 Years of Age

Absolute efficacy of FLUCELVAX QUADRIVALENT was evaluated in children and adolescents 2 through 17 years of age in Study 2. This was a multinational, randomized, non-influenza vaccine comparator-controlled efficacy, immunogenicity and safety study conducted in

8 countries during the following 3 influenza seasons: Southern Hemisphere 2017, Northern Hemisphere 2017/2018 and Northern Hemisphere 2018/2019. The study enrolled 4514 children and adolescents. Out of the 4514 enrolled, 4513 received either FLUCELVAX QUADRIVALENT (N=2258) or a non-influenza (meningococcal (Groups A, C, Y, and W-135) oligosaccharide diphtheria CRM197 conjugate) comparator vaccine (N=2255). The full analysis set (FAS) for efficacy consisted of 4509 children and adolescents.

Children 2 through 8 years of age received either one or two doses (separated by 4 weeks) of FLUCELVAX QUADRIVALENT or comparator vaccine depending on the subject's prior influenza vaccination history. Children in the 2-dose comparator group received non-influenza comparator as the first dose and saline placebo as the second dose. Children and adolescents 9 through 17 years of age received a single dose of FLUCELVAX QUADRIVALENT or non-influenza comparator vaccine. Among all enrolled children and adolescents (N=4514), the mean age was 8.8 years, 48% were female, 51% were 2 through 8 years of age, 50% were Caucasian and 49% were Asian. There were no notable differences in the distribution of demographic and baseline characteristics between the two treatment groups.

FLUCELVAX QUADRIVALENT efficacy was assessed by the prevention of confirmed influenza illness caused by any influenza Type A or B strain. Influenza cases were identified by active and passive surveillance of influenza-like illness (ILI) and confirmed by cell culture and/or real-time polymerase chain reaction (RT-PCR). ILI was defined as a fever (oral temperature ≥ 100.0°F / 37.8°C) along with any of the following: cough, sore throat, nasal congestion, or rhinorrhea. The overall vaccine efficacy for the entire study population (2 through 17 years) was 54.6% (95% CI 45.7 – 62.1), which met predefined success criteria. In addition, vaccine efficacy was 50.5% (95% CI 38.4 – 60.2) in children 2 through 8 years of age and 61.9% (95% CI 47.4 – 72.3) in those 9 through 17 years of age. Vaccine efficacy against all influenza viral subtypes and against individual influenza viral subtypes antigenically similar to the subtypes in the vaccine were calculated (Table 7).

Table 7: Efficacy of FLUCELVAX QUADRIVALENT Against First Occurrence RT-PCR Confirmed or Culture Confirmed Influenza in Participants 2 through 17 years of age – FAS Efficacy[1] (Study 2).

	Number of participants per protocol[1]	Number of cases of influenza	Attack Rate (%)	Vaccine Efficacy (VE)[2]	
				VE %	95% Confidence Interval[3]
RT-PCR or Culture Confirmed Influenza					
FLUCELVAX QUADRIVALENT	2257	175	7.8	54.6	45.7 - 62.1
Non-Influenza Comparator[4]	2252	364	16.2	-	-
Culture Confirmed Influenza					
FLUCELVAX QUADRIVALENT	2257	115	5.1	60.8	51.3 - 68.5
Non-Influenza Comparator[4]	2252	279	12.4	-	-
Antigenically Matched Culture-Confirmed Influenza					
FLUCELVAX QUADRIVALENT	2257	90	4.0	63.6	53.6 - 71.5
Non-Influenza Comparator[4]	2252	236	10.5	-	-

[1] Number of participants in the Full-Analysis Set (FAS) – Efficacy, which included all participants randomized, received a study vaccination and provided efficacy data

[2] Efficacy against influenza was evaluated over three influenza seasons, SH 2017, NH 2017-18 and NH 2018-19

[3] FLUCELVAX QUADRIVALENT met the pre-defined success criterion defined as the lower limit of the two-sided 95% CI of absolute vaccine efficacy greater than 20%

[4] Non-Influenza Comparator: (MENVEO, meningococcal (Groups A, C, Y, and W-135) oligosaccharide diphtheria CRM197 conjugate vaccine, GlaxoSmithKline Biologicals SA); children assigned to 2 doses received saline placebo as the second dose.

Study 2: NCT03165617

14.3 Immunogenicity of FLUCELVAX QUADRIVALENT in Adults 18 years of age and above

Immunogenicity of FLUCELVAX QUADRIVALENT was evaluated in adults 18 years of age and older in a randomized, double-blind, controlled study conducted in the US (Study 3). In this study, adults received FLUCELVAX QUADRIVALENT or one of the two formulations of comparator trivalent influenza vaccine (FLUCELVAX QUADRIVALENT (N=1334), TIV1c, N=677 or TIV2c, N=669). In the per protocol set, the mean age of adults who received FLUCELVAX QUADRIVALENT was 57.5 years; 55.1% of adults were female and 76.1% of adults were Caucasian, 13% were black and 9% were Hispanics. The immune response to each of the vaccine antigens was assessed, 21 days after vaccination.

The immunogenicity endpoints were geometric mean antibody titers (GMTs) of hemagglutination inhibition (HI) antibodies response and percentage of adults who achieved seroconversions, defined as a pre-vaccination HI titer of $< 1:10$ with a post-vaccination titer $\geq 1:40$ or a pre-vaccination HI titer $> 1:10$ and at least 4-fold increase in serum HI antibody titer.

FLUCELVAX QUADRIVALENT was noninferior to TIVc. Noninferiority was established for all 4 influenza strains included in FLUCELVAX QUADRIVALENT, as assessed by ratios of GMTs and the differences in the percentages of adults achieving seroconversion at 3 weeks

following vaccination. The antibody response to influenza B strains contained in FLUCELVAX QUADRIVALENT was superior to the antibody response after vaccination with TIVc containing an influenza B strain from the alternate lineage. There was no evidence that the addition of the second influenza B strain resulted in immune interference to other strains included in the vaccine. (See Table 8)

Table 8: Noninferiority of FLUCELVAX QUADRIVALENT relative to TIVc in adults 18 Years of Age and Above – Per Protocol Analysis Set[1] (Study 3)

		FLUCELVAX Quadrivalent N=1250	TIV1c/TIV2c[2] N=635 / N=639	Vaccine Group Ratio (95% CI)	Vaccine Group Difference (95% CI)
A/H1N1	GMT (95% CI)	302.8 (281.8-325.5)	298.9 (270.3-330.5)	1.0 (0.9-**1.1**)	-
	Seroconversion Rate[3] (95% CI)	49.2% (46.4-52.0)	48.7% (44.7-52.6)	-	-0.5% (-5.3-**4.2**)
A/H3N2	GMT (95% CI)	372.3 (349.2-396.9)	378.4 (345.1-414.8)	1.0 (0.9-**1.1**)	-
	Seroconversion Rate[3] (95% CI)	38.3% (35.6-41.1)	35.6% (31.9-39.5)	-	-2.7% (-7.2-**1.9**)
B1	GMT (95% CI)	133.2 (125.3-141.7)	115.6 (106.4-125.6)	0.9 (0.8-**1.0**)	-
	Seroconversion Rate[3] (95% CI)	36.6% (33.9-39.3)	34.8% (31.1-38.7)	-	-1.8% (-6.2-**2.8**)
B2	GMT (95% CI)	177.2 (167.6-187.5)	164.0 (151.4-177.7)	0.9 (0.9-**1.0**)	-
	Seroconversion Rate[3] (95% CI)	39.8% (37.0-42.5)	35.4% (31.7-39.2)	-	-4.4% (-8.9-**0.2**)

Abbreviations: HI = hemagglutination inhibition. PPS = per protocol set. GMT = geometric mean titer. CI = confidence interval.

[1] Per protocol set: All participants in Full Analysis Set, immunogenicity population, who has correctly received the assigned vaccine, have no major protocol deviations leading to exclusion as defined prior to unblinding/ analysis and are not excluded due to other reasons defined prior to unblinding or analysis.

[2] The comparator vaccine for noninferiority comparisons for A/H1N1, A/H3N2 and B1 is TIV1c, for B2 it is TIV2c.

[3] Seroconversion rate = percentage of participants with either a pre-vaccination HI titer < 1:10 and post-vaccination HI titer ≥ 1:40 or with a pre-vaccination HI titer ≥ 1:10 and a minimum 4-fold increase in post-vaccination HI antibody titer

Study 3: NCT01992094

14.4 Immunogenicity in Children and Adolescents 6 months through 17 years of age

Immunogenicity of FLUCELVAX QUADRIVALENT was evaluated in two clinical studies in children 6 months through 3 years of age (Study 1) and 4 through 17 years of age (Study 5).

Study 1 was a randomized, observer-blind, multicenter study in children 6 months through 3 years of age conducted in the US. In this study, subjects received FLUCELVAX QUADRIVALENT or a US-licensed comparator quadrivalent influenza vaccine (FLUCELVAX QUADRIVALENT N=1597, Comparator QUADRIVALENT (QIV) N=805). In the per protocol set, the mean age of subjects who received FLUCELVAX QUADRIVALENT was 29 months; 49% of subjects were female and 67% of subjects were Caucasian, 27% were Black and < 1% were Asian, Hawaiian or other Pacific Islander and American Indian or Alaska Native. Twenty

six percent of subjects were of Hispanic origin. The immune response to each of the vaccine antigens was assessed 28 days after last vaccination.

The immunogenicity endpoints were geometric mean antibody titers (GMTs) and percentage of subjects who achieved seroconversion, defined as a pre-vaccination HI or microneutralization (MN) titer of < 1:10 with a post-vaccination titer ≥ 1:40 or with a pre-vaccination HI or MN titer ≥ 1:10 and a minimum 4-fold increase in serum antibody titer. GMTs and seroconversion rates were measured by hemagglutination inhibition (HI) assay for A/H1N1, B/Yamagata and B/Victoria strains and by microneutralization (MN) assay for the A/H3N2 strain.

FLUCELVAX QUADRIVALENT was noninferior to the Comparator QIV. Noninferiority was established for all 4 influenza strains as assessed by ratios of GMTs and the differences in the percentages of subjects achieving seroconversion at 4 weeks following vaccination.

The noninferiority data observed are summarized in Table 9.

Table 9: Noninferiority[1] of FLUCELVAX QUADRIVALENT Relative to Comparator QIV in Children 6 Months through 3 Years of Age – Per-Protocol Analysis Set[2] (Study 1)

		FLUCELVAX QUADRIVALENT	Comparator QIV	Vaccine Group Ratio	Vaccine Group Difference
A/H1N1*		N=1092	N=575		
	GMT (95% CI)	78.0 (70.75, 86.03)	57.3 (50.76, 64.63)	0.73 (0.65, **0.84**)	-
	Seroconversion Rate[3] (95% CI)	58.24% (55.25, 61.19)	46.78% (42.64, 50.96)	-	-11.46 (-16.45, **-6.42**)
A/H3N2#		N = 1078	N = 572		
	GMT (95% CI)	23.1 (21.21, 25.12)	23.9 (21.57, 26.57)	1.04 (0.93, **1.16**)	-
	Seroconversion Rate[3] (95% CI)	27.64% (24.99, 30.42)	30.77% (27.01, 34.73)	-	3.13 (-1.44, **7.81**)
B/Yamagata*		N = 1092	N = 575		
	GMT (95% CI)	35.6 (32.93, 38.58)	26.0 (23.54, 28.63)	0.73 (0.66, **0.81**)	-
	Seroconversion Rate[3] (95% CI)	46.52% (43.53, 49.53)	31.65% (27.87, 35.63)	-	-14.87 (-19.61, **-9.98**)
B/Victoria*		N = 1092	N = 575		
	GMT (95% CI)	22.4 (20.70, 24.19)	19.6 (17.81, 21.58)	0.88 (0.79, **0.97**)	-
	Seroconversion Rate[3] (95% CI)	30.31% (27.60, 33.13)	24.35% (20.89, 28.07)	-	-5.96 (-10.33, **-1.44**)

Abbreviations: GMT = geometric mean titer. CI = confidence interval.

Assays: GMTs and seroconversion rates were measured by hemagglutination inhibition (HI)* assay for A/H1N1, B/Yamagata and B/Victoria strains and by microneutralization (MN)# assay for the A/H3N2 strain, using cell-derived target viruses. The MN assay was used for A/H3N2 as circulating strains indicated a reduced ability to agglutinate red blood cells. FLUCELVAX QUADRIVALENT was noninferior to the Comparator QIV irrespective of the assay used. HI assay data for A/H3N2: GMT (95%CI) for FLUCELVAX QUADRIVALENT (N=1089) = 288.1 (261.46, 317.54), Comparator QIV (N=575) = 227.6 (201.87, 256.58), Vaccine group ratio (95%CI) = 0.79 (0.69, **0.90**), Seroconversion rate (95%CI) for FLUCELVAX QUADRIVALENT (N=1089) =

72.27% (69.51,74.91), Comparator QIV (N=575) = 64.52% (60.46, 68.44), Vaccine Group Difference (95%CI) = -7.75% (-12.51, **-3.06**).

Success criteria: The upper bound of the two-sided 95% confidence interval (CI) on the ratio of the GMTs (calculated as GMT US-licensed comparator QIV divided by GMT FLUCELVAX QUADRIVALENT) does not exceed 1.5. The upper bound of the two-sided 95% CI on the difference between the seroconversion rates (calculated as Seroconversion rate US-licensed comparator QIV minus Seroconversion rate FLUCELVAX QUADRIVALENT) does not exceed 10%.

[1] Analyses are performed on data for Day 29 for previously vaccinated subjects and Day 57 for not previously vaccinated subjects

[2] Per protocol set: All participants in Full Analysis Set, immunogenicity population, who have correctly received the assigned vaccine, have no major protocol deviations leading to exclusion as defined prior to unblinding/ analysis and are not excluded due to other reasons defined prior to unblinding or analysis.

[3] Seroconversion rate = percentage of subjects with either a pre-vaccination titer < 1:10 and post-vaccination titer ≥ 1:40 or with a pre-vaccination titer ≥ 1:10 and a minimum 4-fold increase in post-vaccination antibody titer

Study 1: NCT 04074928

Study 5 was a randomized, double-blind, controlled study in children and adolescents 4 through 17 years of age conducted in the US. In this study, 1159 children and adolescents received FLUCELVAX QUADRIVALENT. In the per protocol set, the mean age of children and adolescents who received FLUCELVAX QUADRIVALENT was 9.8 years; 47% of children and adolescents were female and 54% of children and adolescents were Caucasian, 22% were black and 19% were Hispanics. The immune response to each of the vaccine antigens was assessed, 21 days after vaccination.

The immunogenicity endpoints were the percentage of children and adolescents who achieved seroconversion, defined as a pre-vaccination hemagglutination inhibition (HI) titer of < 1:10 with a post-vaccination HI titer ≥ 1:40 or at least a 4-fold increase in serum HI titer; and percentage of children and adolescents with a post-vaccination HI titer ≥ 1:40.

In children and adolescents receiving FLUCELVAX QUADRIVALENT, for all four influenza strains, the 95% LBCI seroconversion rates were ≥ 40% and the percentage of children and adolescents who achieved HI titer ≥ 1:40 post vaccination were ≥ 70% (95% LBCI). (See Table 10)

Table 10: The Percentage of Children and Adolescents 4 through 17 years of Age with Seroconversion[1] and HI Titers ≥ 1:40 post vaccination with FLUCELVAX QUADRIVALENT– Per-Protocol Analysis Set[2] (Study 5)

	A/H1N1 N=1014	**A/H3N2 N=1013**	**B1 N=1013**	**B2 N=1009**
Seroconversion Rate[1] (95% CI)	72% (69-75)	47% (44-50)	66% (63-69)	73% (70-76)
HI titer ≥ 1:40	99% (98-100)	100% (99-100)	93% (91-94)	92% (90-93)

Abbreviations: HI = hemagglutinin inhibition. CI = confidence interval.

Analyses are performed on data for day 22 for previously vaccinated participants and day 50 for not previously vaccinated participants.

[1] Seroconversion rate = percentage of participants with either a pre-vaccination HI titer < 1:10 and post-vaccination HI titer ≥ 1:40 or with a pre-vaccination HI titer ≥ 1:10 and a minimum 4-fold increase in post-vaccination HI titer. Immunogenicity success criteria were met if the lower limit of the 95% confidence interval (CI) of the

percentage of participants with HI titer ≥ 1:40 is ≥ 70%; and the lower limit of the 95% CI of the percentage of participants with seroconversion is ≥ 40%.

[2] Per protocol set: All participants in Full Analysis Set, immunogenicity population, who has correctly received the assigned vaccine, have no major protocol deviations leading to exclusion as defined prior to unblinding/ analysis and are not excluded due to other reasons defined prior to unblinding or analysis.

Study 5: NCT 01992107

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3. Hobson D, Curry RL, Beare A, et al. The role of serum hemagglutinin-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg Camb 1972; 767-777.
4. Centers for Disease Control and Prevention. Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 2011; 60(33): 1128-1132

16 HOW SUPPLIED/STORAGE AND HANDLING

FLUCELVAX QUADRIVALENT product presentations are listed in Table 11 below:

Table 11: Flucelvax Product Presentations

Presentation	Carton NDC Number	Components
Pre-filled Syringe	70461-322-03	0.5 mL single dose pre-filled syringe, package of 10 syringes per carton [NDC 70461-322-04]
Multi-dose Vial	70461-422-10	5 mL multi-dose vial, individually packaged in a carton [NDC 70461-422-11]

Store this product refrigerated at 2°C to 8°C (36ºF to 46ºF). Between uses, return the multi-dose vial to the recommended storage conditions. Do not freeze. Protect from light. Do not use after the expiration date.

17 PATIENT COUNSELING INFORMATION

Inform vaccine recipients of the potential benefits and risks of immunization with FLUCELVAX QUADRIVALENT.

Educate vaccine recipients regarding the potential side effects; clinicians should emphasize that (1) FLUCELVAX QUADRIVALENT contains non-infectious particles and cannot cause influenza and (2) FLUCELVAX QUADRIVALENT is intended to provide protection against illness due to influenza viruses only and cannot provide protection against other respiratory illnesses.

Instruct vaccine recipients to report adverse reactions to their healthcare provider.

Provide vaccine recipients with the Vaccine Information Statements which are required by the National Childhood Vaccine Injury Act of 1986. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (*www.cdc.gov/vaccines*).

Inform vaccine recipients that annual vaccination is recommended.

Manufactured by: **Seqirus Inc.** Holly Springs, NC 27540, USA

US License No. 2049

Distributed by: **Seqirus USA Inc.** 25 Deforest Avenue, Summit, NJ 07901, USA

1-855-358-8966

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use FLULAVAL QUADRIVALENT safely and effectively. See full prescribing information for FLULAVAL QUADRIVALENT.

FLULAVAL QUADRIVALENT (Influenza Vaccine) injectable suspension, for intramuscular use
2022-2023 Formula
Initial U.S. Approval: 2013

INDICATIONS AND USAGE

FLULAVAL QUADRIVALENT is a vaccine indicated for active immunization for the prevention of disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. FLULAVAL QUADRIVALENT is approved for use in persons aged 6 months and older. (1)

DOSAGE AND ADMINISTRATION

For intramuscular injection only. (2)

Age	Vaccination Status	Dose and Schedule
6 months through 8 years	Not previously vaccinated with influenza vaccine	Two doses (0.5-mL each) at least 4 weeks apart (2.1)
	Vaccinated with influenza vaccine in a previous season	One or 2 doses[a] (0.5-mL each) (2.1)
9 years and older	Not applicable	One 0.5-mL dose (2.1)

[a] One dose or 2 doses (0.5-mL each) depending on vaccination history as per the annual Advisory Committee on Immunization Practices (ACIP) recommendation on prevention and control of seasonal influenza with vaccines. If 2 doses, administer each 0.5-mL dose at least 4 weeks apart. (2.1)

DOSAGE FORMS AND STRENGTHS

Suspension for injection supplied in 0.5-mL single-dose prefilled syringes. (3)

CONTRAINDICATIONS

History of severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine, including egg protein, or following a previous dose of any influenza vaccine. (4, 11)

WARNINGS AND PRECAUTIONS

- If Guillain-Barré syndrome has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give FLULAVAL QUADRIVALENT should be based on careful consideration of the potential benefits and risks. (5.1)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including FLULAVAL QUADRIVALENT. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.2)

ADVERSE REACTIONS

- In adults, the most common (≥10%) solicited local adverse reaction was pain (60%); most common solicited systemic adverse reactions were muscle aches (26%), headache (22%), fatigue (22%), and arthralgia (15%). (6.1)
- In children aged 6 through 35 months, the most common (≥10%) solicited local adverse reaction was pain (40%); most common solicited systemic adverse reactions were irritability (49%), drowsiness (37%), and loss of appetite (29%). (6.1)
- In children aged 3 through 17 years, the most common (≥10%) solicited local adverse reaction was pain (65%). (6.1)
- In children aged 3 through 4 years, the most common (≥10%) solicited systemic adverse reactions were irritability (26%), drowsiness (21%), and loss of appetite (17%). (6.1)
- In children aged 5 through 17 years, the most common (≥10%) solicited systemic adverse reactions were muscle aches (29%), fatigue (22%), headache (22%), arthralgia (13%), and gastrointestinal symptoms (10%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

USE IN SPECIFIC POPULATIONS

Geriatric Use: Antibody responses were lower in geriatric subjects who received FLULAVAL QUADRIVALENT than in younger subjects. (8.5)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 07/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

FLULAVAL QUADRIVALENT is indicated for active immunization for the prevention of disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. FLULAVAL QUADRIVALENT is approved for use in persons aged 6 months and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Dosage and Schedule

The dose and schedule for FLULAVAL QUADRIVALENT are presented in Table 1.

Table 1. FLULAVAL QUADRIVALENT: Dosing

Age	Vaccination Status	Dose and Schedule
6 months through 8 years	Not previously vaccinated with influenza vaccine	Two doses (0.5-mL each) at least 4 weeks apart
	Vaccinated with influenza vaccine in a previous season	One or 2 doses[a] (0.5-mL each)
9 years and older	Not applicable	One 0.5-mL dose

[a] One dose or 2 doses (0.5-mL each) depending on vaccination history as per the annual Advisory Committee on Immunization Practices (ACIP) recommendation on prevention and control of seasonal influenza with vaccines. If 2 doses, administer each 0.5-mL dose at least 4 weeks apart.

2.2 Administration Instructions

Shake well before administration. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Attach a sterile needle to the prefilled syringe and administer intramuscularly.

The preferred sites for intramuscular injection are the anterolateral thigh for children aged 6 through 11 months and the deltoid muscle of the upper arm for persons aged 12 months and older. Do not inject in the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously, intradermally, or subcutaneously.

3 DOSAGE FORMS AND STRENGTHS

FLULAVAL QUADRIVALENT is a suspension for injection available in 0.5-mL prefilled TIP-LOK syringes.

4 CONTRAINDICATIONS

Do not administer FLULAVAL QUADRIVALENT to anyone with a history of severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine, including egg protein, or following a previous dose of any influenza vaccine *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome (GBS) has occurred within 6 weeks of receipt of a prior influenza vaccine, the decision to give FLULAVAL QUADRIVALENT should be based on careful consideration of the potential benefits and risks.

The 1976 swine influenza vaccine was associated with an elevated risk of GBS. Evidence for a causal relation of GBS with other influenza vaccines is inconclusive; if an excess risk exists, it is probably slightly more than 1 additional case/1 million persons vaccinated.

5.2 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including FLULAVAL QUADRIVALENT. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

5.3 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions. Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of FLULAVAL QUADRIVALENT.

5.4 Altered Immunocompetence

If FLULAVAL QUADRIVALENT is administered to immunosuppressed persons, including individuals receiving immunosuppressive therapy, the immune response may be lower than in immunocompetent persons.

5.5 Limitations of Vaccine Effectiveness

Vaccination with FLULAVAL QUADRIVALENT may not protect all susceptible individuals.

5.6 Persons at Risk of Bleeding

As with other intramuscular injections, FLULAVAL QUADRIVALENT should be given with caution in individuals with bleeding disorders such as hemophilia or on anticoagulant therapy to avoid the risk of hematoma following the injection.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. There is the possibility that broad use of FLULAVAL QUADRIVALENT could reveal adverse reactions not observed in clinical trials.

In adults who received FLULAVAL QUADRIVALENT, the most common (≥10%) solicited local adverse reaction was pain (60%); the most common (≥10%) solicited systemic adverse reactions were muscle aches (26%), headache (22%), fatigue (22%), and arthralgia (15%).

In children aged 6 through 35 months who received FLULAVAL QUADRIVALENT, the most common (≥10%) solicited local adverse reaction was pain (40%); the most common (≥10%) solicited systemic adverse reactions were irritability (49%), drowsiness (37%), and loss of appetite (29%).

In children aged 3 through 17 years who received FLULAVAL QUADRIVALENT, the most common (≥10%) solicited local adverse reaction was pain (65%). In children aged 3 through 4 years, the most common (≥10%) solicited systemic adverse reactions were irritability (26%), drowsiness (21%), and loss of appetite (17%). In children aged 5 through 17 years, the most common (≥10%) systemic adverse reactions were muscle aches (29%), fatigue (22%), headache (22%), arthralgia (13%), and gastrointestinal symptoms (10%).

FLULAVAL QUADRIVALENT has been administered in 8 clinical trials to 1,384 adults aged 18 years and older, 1,965 children aged 6 through 35 months, and 3,516 children aged 3 through 17 years.

FLULAVAL QUADRIVALENT in Adults

Trial 1 (NCT01196975) was a randomized, double-blind, active-controlled, safety and immunogenicity trial. In this trial, subjects received FLULAVAL QUADRIVALENT (n = 1,272), or one of 2 formulations of a comparator trivalent influenza vaccine (FLULAVAL, TIV-1, n = 213 or TIV-2, n = 218), each containing an influenza type B virus that corresponded to one of the 2 B viruses in FLULAVAL QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). The population was aged 18 years and older (mean age: 50 years) and 61% were female; 61% of subjects were white, 3% were black, 1% were Asian, and 35% were of other racial/ethnic groups. Solicited adverse events were collected for 7 days (day of vaccination and the next 6 days). The incidence of solicited adverse reactions occurring within 7 days of vaccination in adults are shown in Table 2.

Table 2. FLULAVAL QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] of Vaccination in Adults Aged 18 Years and Older[b] (Total Vaccinated Cohort)

			Trivalent Influenza Vaccine (TIV)			
	FLULAVAL QUADRIVALENT[c] n = 1,260 %		TIV-1 (B Victoria)[d] n = 208 %		TIV-2 (B Yamagata)[e] n = 216 %	
Adverse Reaction	Any	Grade 3[f]	Any	Grade 3[f]	Any	Grade 3[f]
Local						
Pain	60	2	45	1	41	1
Swelling	3	0	1	0	4	0
Redness	2	0	3	0	1	0
Systemic						
Muscle aches	26	1	25	1	19	1
Headache	22	1	20	1	23	0
Fatigue	22	1	22	1	17	2
Arthralgia	15	1	17	1	15	3
Gastrointestinal symptoms[g]	9	1	10	2	7	1
Shivering	9	1	8	1	6	1
Fever[h]	1	0	1	0	1	1

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
n = Number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 1: NCT01196975.

[c] Contained 2 A strains and 2 B strains, one of Victoria lineage and one of Yamagata lineage.

[d] Contained the same 2 A strains as FLULAVAL QUADRIVALENT and a B strain of Victoria lineage.

[e] Contained the same 2 A strains as FLULAVAL QUADRIVALENT and a B strain of Yamagata lineage.

[f] Grade 3 pain: Defined as significant pain at rest; prevented normal everyday activities.
Grade 3 swelling, redness: Defined as >100 mm.
Grade 3 muscle aches, headache, fatigue, arthralgia, gastrointestinal symptoms, shivering: Defined as prevented normal activity.
Grade 3 (or higher) fever: Defined as ≥102.2°F (39.0°C).

[g] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.

[h] Fever: Defined as ≥100.4°F (38.0°C)

Unsolicited adverse events occurring within 21 days of vaccination were reported in 19%, 23%, and 23% of subjects who received FLULAVAL QUADRIVALENT (n = 1,272), TIV-1 (B Victoria) (n = 213), or TIV-2 (B Yamagata) (n = 218), respectively. The unsolicited adverse reactions that occurred most frequently (≥1% for FLULAVAL QUADRIVALENT) included nasopharyngitis, upper

respiratory tract infection, headache, cough, and oropharyngeal pain. Serious adverse events occurring within 21 days of vaccination were reported in 0.4%, 0%, and 0% of subjects who received FLULAVAL QUADRIVALENT, TIV-1 (B Victoria), or TIV-2 (B Yamagata), respectively.

FLULAVAL QUADRIVALENT in Children

Trial 4 (NCT02242643) was a randomized, observer-blind, active-controlled immunogenicity and safety trial. The trial included subjects aged 6 through 35 months who received FLULAVAL QUADRIVALENT (n = 1,207) or FLUZONE QUADRIVALENT, a U.S.-licensed inactivated influenza vaccine (n = 1,217) used as comparator, manufactured by Sanofi Pasteur Inc. Children with no history of influenza vaccination received 2 doses of FLULAVAL QUADRIVALENT or the comparator vaccine approximately 28 days apart. Children with a history of influenza vaccination received one dose of FLULAVAL QUADRIVALENT or the comparator vaccine. In the overall population, 53% were male; 64% were white, 16% were black, 3% were Asian, and 17% were of other racial/ethnic groups. The mean age of subjects was 20 months. Subjects were followed for safety for 6 months; solicited local adverse reactions and systemic adverse events were collected for 7 days (day of vaccination and the next 6 days) post vaccination. The incidence of solicited adverse reactions occurring within 7 days of vaccination in children are shown in Table 3.

Table 3. FLULAVAL QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] of First Vaccination in Children Aged 6 through 35 Months[b] (Total Vaccinated Cohort)

Adverse Reaction	FLULAVAL QUADRIVALENT %		Active Comparator[c] %	
	Any	Grade 3[d]	Any	Grade 3[d]
Local	**n = 1,151**		**n = 1,146**	
Pain	40	2	37	1
Swelling	1	0	0	0
Redness	1	0	1	0
Systemic	**n = 1,155**		**n = 1,148**	
Irritability	49	4	46	3
Drowsiness	37	3	37	3
Loss of appetite	29	2	29	1
Fever[e]	6	1	6	1

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available (i.e., diary card completed for solicited symptoms).

n = Number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 4: NCT02242643.

[c] U.S.-licensed quadrivalent, inactivated influenza vaccine (manufactured by Sanofi Pasteur Inc).

[d] Grade 3 pain: Defined as cried when limb was moved/spontaneously painful.
Grade 3 swelling, redness: Defined as >100 mm.
Grade 3 irritability: Defined as crying that could not be comforted/prevented normal activity.
Grade 3 drowsiness: Defined as prevented normal activity.
Grade 3 loss of appetite: Defined as not eating at all.
Grade 3 (or higher) fever: Defined as >102.2°F (39.0°C).
[e] Fever: Defined as ≥100.4°F (38.0°C).

In children who received a second dose of FLULAVAL QUADRIVALENT or the comparator vaccine, the incidences of solicited adverse reactions following the second dose were generally similar or lower than those observed after the first dose.

Unsolicited adverse events occurring within 28 days of vaccination were reported in 46% and 44% of subjects who received FLULAVAL QUADRIVALENT (n = 1,207) and the comparator vaccine (n = 1,217), respectively. The unsolicited adverse reactions that occurred most frequently (≥1%) for FLULAVAL QUADRIVALENT included upper respiratory tract infection, cough, diarrhea, pyrexia, vomiting, and rash. Serious adverse events occurring during the study period (approximately 6 months) were reported in 2% of subjects who received FLULAVAL QUADRIVALENT and in 2% of subjects who received the comparator vaccine. There were no deaths reported during the study period.

Trial 2 (NCT01198756) was a randomized, double-blind, active-controlled trial. In this trial, subjects received FLULAVAL QUADRIVALENT (n = 932) or one of 2 formulations of a comparator trivalent influenza vaccine [FLUARIX (Influenza Vaccine), TIV-1 (B Victoria), n = 929 or TIV-2 (B Yamagata), n = 932], each containing an influenza type B virus that corresponded to one of the 2 B viruses in FLULAVAL QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). The population was aged 3 through 17 years (mean age: 9 years) and 53% were male; 65% were white, 13% were Asian, 9% were black, and 13% were of other racial/ethnic groups. Children aged 3 through 8 years with no history of influenza vaccination received 2 doses approximately 28 days apart. Children aged 3 through 8 years with a history of influenza vaccination and children aged 9 years and older received one dose. Solicited local adverse reactions and systemic adverse events were collected for 7 days (day of vaccination and the next 6 days). The incidence of solicited adverse reactions occurring within 7 days of vaccination in children are shown in Table 4.

Table 4. FLULAVAL QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] of First Vaccination in Children Aged 3 through 17 Years[b] (Total Vaccinated Cohort)

	FLULAVAL QUADRIVALENT[c] %		Trivalent Influenza Vaccine (TIV) TIV-1 (B Victoria)[d] %		TIV-2 (B Yamagata)[e] %	
	Any	Grade 3[f]	Any	Grade 3[f]	Any	Grade 3[f]
Adverse Reaction	**Aged 3 through 17 Years**					
Local	**n = 913**		**n = 911**		**n = 915**	
Pain	65	3	55	2	56	2
Swelling	6	0	3	0	4	0
Redness	5	0	3	0	4	0
	Aged 3 through 4 Years					
Systemic	**n = 185**		**n = 187**		**n = 189**	
Irritability	26	1	17	0	22	2
Drowsiness	21	0	20	2	23	1
Loss of appetite	17	0	16	2	13	1
Fever[g]	5	1	6	1	4	2
	Aged 5 through 17 Years					
Systemic	**n = 727**		**n = 724**		**n = 725**	
Muscle aches	29	1	25	1	25	1
Fatigue	22	1	24	2	23	1
Headache	22	1	22	1	20	1
Arthralgia	13	0	12	1	11	0
Gastrointestinal symptoms[h]	10	1	10	1	9	1
Shivering	7	0	7	1	7	1
Fever[g]	2	1	4	1	3	0

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
N = number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 2: NCT01198756.

[c] Contained 2 A strains and 2 B strains, one of Victoria lineage and one of Yamagata lineage.

[d] Contained the same 2 A strains as FLULAVAL QUADRIVALENT and a B strain of Victoria lineage.

[e] Contained the same 2 A strains as FLULAVAL QUADRIVALENT and a B strain of Yamagata lineage.

[f] Grade 3 pain: Defined as cried when limb was moved/spontaneously painful (children <5 years), or significant pain at rest, prevented normal everyday activities (children ≥5 years).
Grade 3 swelling, redness: Defined as >100 mm.
Grade 3 irritability: Defined as crying that could not be comforted/prevented normal activity.
Grade 3 drowsiness: Defined as prevented normal activity.

Grade 3 loss of appetite: Defined as not eating at all.
Grade 3 (or higher) fever: Defined as ≥102.2°F (39.0°C).
Grade 3 muscle aches, fatigue, headache, arthralgia, gastrointestinal symptoms, shivering: Defined as prevented normal activity.

[g] Fever: Defined as ≥100.4°F (38.0°C).

[h] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.

In children who received a second dose of FLULAVAL QUADRIVALENT, FLUARIX TIV-1 (B Victoria), or TIV-2 (B Yamagata), the incidences of adverse reactions following the second dose were generally lower than those observed after the first dose.

Unsolicited adverse events occurring within 28 days of vaccination were reported in 30%, 31%, and 30% of subjects who received FLULAVAL QUADRIVALENT (n = 932), FLUARIX TIV-1 (B Victoria) (n = 929), or TIV-2 (B Yamagata) (n = 932), respectively. The unsolicited adverse reactions that occurred most frequently (≥1% for FLULAVAL QUADRIVALENT) included vomiting, pyrexia, bronchitis, nasopharyngitis, pharyngitis, upper respiratory tract infection, headache, cough, oropharyngeal pain, and rhinorrhea. Serious adverse events occurring within 28 days of any vaccination were reported in 0.1%, 0.2%, and 0.2% of subjects who received FLULAVAL QUADRIVALENT, FLUARIX TIV-1 (B Victoria), or TIV-2 (B Yamagata), respectively.

Trial 3 (NCT01218308) was a randomized, observer-blind, non-influenza vaccine-controlled trial evaluating the efficacy of FLULAVAL QUADRIVALENT. The trial included subjects aged 3 through 8 years who received FLULAVAL QUADRIVALENT (n = 2,584) or HAVRIX (Hepatitis A Vaccine) (n = 2,584) as a control vaccine. Children with no history of influenza vaccination received 2 doses of FLULAVAL QUADRIVALENT or HAVRIX approximately 28 days apart (this dosing regimen for HAVRIX is not a U.S.-licensed schedule). Children with a history of influenza vaccination received one dose of FLULAVAL QUADRIVALENT or HAVRIX. In the overall population, 52% were male; 60% were Asian, 5% were white, and 35% were of other racial/ethnic groups. The mean age of subjects was 5 years. Solicited local adverse reactions and systemic adverse events were collected for 7 days (day of vaccination and the next 6 days). The incidence of solicited adverse reactions occurring within 7 days of vaccination in children are shown in Table 5.

Table 5. FLULAVAL QUADRIVALENT: Incidence of Solicited Local and Systemic Adverse Reactions within 7 Days[a] of First Vaccination in Children Aged 3 through 8 Years[b] (Total Vaccinated Cohort)

	FLULAVAL QUADRIVALENT %		HAVRIX[c] %	
	Any	Grade 3[d]	Any	Grade 3[d]
Adverse Reaction	**Aged 3 through 8 Years**			
Local	**n = 2,546**		**n = 2,551**	
Pain	39	1	28	1
Swelling	1	0	0	0
Redness	0	0	0	0
	Aged 3 through 4 Years			
Systemic	**n = 898**		**n = 895**	
Loss of appetite	9	0	8	0
Irritability	8	0	8	0
Drowsiness	8	0	7	0
Fever[e]	4	1	4	1
	Aged 5 through 8 Years			
Systemic	**n = 1,648**		**n = 1,654**	
Muscle aches	12	0	10	0
Headache	11	0	11	1
Fatigue	8	0	7	0
Arthralgia	6	0	5	0
Gastrointestinal symptoms[f]	6	0	6	0
Shivering	3	0	3	0
Fever[e]	3	1	3	1

Total vaccinated cohort for safety included all vaccinated subjects for whom safety data were available.
N = number of subjects with diary card completed.

[a] Seven days included day of vaccination and the subsequent 6 days.

[b] Trial 3: NCT01218308.

[c] Hepatitis A Vaccine used as a control vaccine.

[d] Grade 3 pain: Defined as cried when limb was moved/spontaneously painful (children <5 years), or significant pain at rest, prevented normal everyday activities (children ≥5 years).
Grade 3 swelling, redness: Defined as >100 mm.
Grade 3 loss of appetite: Defined as not eating at all.
Grade 3 irritability: Defined as crying that could not be comforted/prevented normal activity.
Grade 3 drowsiness: Defined as prevented normal activity.
Grade 3 (or higher) fever: Defined as ≥102.2°F (39.0°C).
Grade 3 muscle aches, headache, fatigue, arthralgia, gastrointestinal symptoms, shivering: Defined as prevented normal activity.

[e] Fever: Defined as ≥100.4°F (38.0°C).
[f] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.

In children who received a second dose of FLULAVAL QUADRIVALENT or HAVRIX, the incidences of adverse reactions following the second dose were generally lower than those observed after the first dose.

The frequency of unsolicited adverse events occurring within 28 days of vaccination was similar in both groups (33% for both FLULAVAL QUADRIVALENT and HAVRIX). The unsolicited adverse reactions that occurred most frequently (≥1% for FLULAVAL QUADRIVALENT) included diarrhea, pyrexia, gastroenteritis, nasopharyngitis, upper respiratory tract infection, varicella, cough, and rhinorrhea. Serious adverse events occurring within 28 days of any vaccination were reported in 0.7% of subjects who received FLULAVAL QUADRIVALENT and in 0.2% of subjects who received HAVRIX.

6.2 Postmarketing Experience

Beyond those events reported in the clinical trials for FLULAVAL QUADRIVALENT or FLULAVAL, the following adverse reactions have been identified during postapproval use of FLULAVAL QUADRIVALENT or FLULAVAL (trivalent influenza vaccine). Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Blood and Lymphatic System Disorders

Lymphadenopathy.

Eye Disorders

Eye pain, photophobia.

Gastrointestinal Disorders

Dysphagia, vomiting.

General Disorders and Administration Site Conditions

Chest pain, injection site inflammation, asthenia, injection site rash, influenza-like symptoms, abnormal gait, injection site bruising, injection site sterile abscess.

Immune System Disorders

Allergic reactions including anaphylaxis, angioedema.

Infections and Infestations

Rhinitis, laryngitis, cellulitis.

Musculoskeletal and Connective Tissue Disorders

Muscle weakness, arthritis.

Nervous System Disorders

Dizziness, paresthesia, hypoesthesia, hypokinesia, tremor, somnolence, syncope, Guillain-Barré syndrome, convulsions/seizures, facial or cranial nerve paralysis, encephalopathy, limb paralysis.

Psychiatric Disorders

Insomnia.

Respiratory, Thoracic, and Mediastinal Disorders

Dyspnea, dysphonia, bronchospasm, throat tightness.

Skin and Subcutaneous Tissue Disorders

Urticaria, localized or generalized rash, pruritus, sweating.

Vascular Disorders

Flushing, pallor.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

FLULAVAL QUADRIVALENT should not be mixed with any other vaccine in the same syringe or vial.

There are insufficient data to assess the concomitant administration of FLULAVAL QUADRIVALENT with other vaccines. When concomitant administration of other vaccines is required, the vaccines should be administered at different injection sites.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses) may reduce the immune response to FLULAVAL QUADRIVALENT.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are insufficient data on FLULAVAL QUADRIVALENT in pregnant women to inform vaccine-associated risks.

A developmental toxicity study was performed in female rats administered FLULAVAL

QUADRIVALENT prior to mating and during gestation and lactation periods. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). This study revealed no adverse effects on fetal or pre-weaning development due to FLULAVAL QUADRIVALENT *(see Data)*.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk: Pregnant women infected with seasonal influenza are at increased risk of severe illness associated with influenza infection compared with non-pregnant women. Pregnant women with influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

Data

Animal Data: In a developmental toxicity study, female rats were administered FLULAVAL QUADRIVALENT by intramuscular injection 4 and 2 weeks prior to mating, on Gestation Days 3, 8, 11, and 15, and on Lactation Day 7. The total dose was 0.2 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to Postnatal Day 25 were observed. There were no vaccine-related fetal malformations or variations.

8.2 Lactation

Risk Summary

It is not known whether FLULAVAL QUADRIVALENT is excreted in human milk. Data are not available to assess the effects of FLULAVAL QUADRIVALENT on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for FLULAVAL QUADRIVALENT and any potential adverse effects on the breastfed child from FLULAVAL QUADRIVALENT or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of FLULAVAL QUADRIVALENT in children younger than 6 months have not been established.

8.5 Geriatric Use

In a randomized, double-blind, active-controlled trial, immunogenicity and safety were evaluated in a cohort of subjects aged 65 years and older who received FLULAVAL QUADRIVALENT (n = 397); approximately one-third of these subjects were aged 75 years and older. In subjects aged 65 years and older, the geometric mean antibody titers (GMTs) post-vaccination and seroconversion rates were lower than in younger subjects (aged 18 to 64 years) and the frequencies of solicited and unsolicited adverse reactions were generally lower than in younger subjects *[see Adverse Reactions (6.1), Clinical Studies (14.2)]*.

11 DESCRIPTION

FLULAVAL QUADRIVALENT, Influenza Vaccine, for intramuscular injection, is a quadrivalent, split-virion, inactivated influenza virus vaccine prepared from virus propagated in the allantoic cavity of embryonated hens' eggs. Each of the influenza viruses is produced and purified separately. The virus is inactivated with ultraviolet light treatment followed by formaldehyde treatment, purified by centrifugation, and disrupted with sodium deoxycholate.

FLULAVAL QUADRIVALENT is a sterile, opalescent, translucent to off-white suspension in a phosphate-buffered saline solution that may sediment slightly. The sediment resuspends upon shaking to form a homogeneous suspension.

FLULAVAL QUADRIVALENT has been standardized according to U.S. Public Health Service (USPHS) requirements for the 2022-2023 influenza season and is formulated to contain 60 micrograms (mcg) hemagglutinin (HA) per 0.5-mL dose in the recommended ratio of 15 mcg HA of each of the following 4 influenza virus strains (2 A strains and 2 B strains): A/Victoria/2570/2019 (H1N1) IVR-215, A/Darwin/9/2021 (H3N2) IVR-228, B/Austria/1359417/2021 BVR-26 (B-Victoria lineage), and B/Phuket/3073/2013 (B-Yamagata lineage).

FLULAVAL QUADRIVALENT is formulated without preservatives and does not contain thimerosal. Each 0.5-mL dose may also contain residual amounts of ovalbumin (≤0.3 mcg), formaldehyde (≤25 mcg), sodium deoxycholate (≤50 mcg), α-tocopheryl hydrogen succinate (≤320 mcg), and polysorbate 80 (≤887 mcg) from the manufacturing process. Antibiotics are not used in the manufacture of this vaccine.

The tip caps and plungers of the prefilled syringes of FLULAVAL QUADRIVALENT are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Influenza illness and its complications follow infection with influenza viruses. Global surveillance of influenza identifies yearly antigenic variants. Since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation.

Public health authorities recommend influenza vaccine strains annually. Inactivated influenza vaccines are standardized to contain the hemagglutinins of strains representing the influenza viruses likely to circulate in the United States during the influenza season.

Specific levels of hemagglutination inhibition (HI) antibody titer post-vaccination with inactivated influenza virus vaccines have not been correlated with protection from influenza illness but the antibody titers have been used as a measure of vaccine activity. In some human challenge studies, antibody titers of ≥1:40 have been associated with protection from influenza illness in up to 50% of subjects.[1,2] Antibody against one influenza virus type or subtype confers little or no protection against another virus. Furthermore, antibody to one antigenic variant of influenza virus might not protect

against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virological basis for seasonal epidemics and the reason for the usual change of one or more new strains in each year's influenza vaccine.

Annual revaccination is recommended because immunity declines during the year after vaccination and because circulating strains of influenza virus change from year to year.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

FLULAVAL QUADRIVALENT has not been evaluated for carcinogenic, mutagenic potential, or male infertility in animals. Vaccination of female rats with FLULAVAL QUADRIVALENT had no effect on fertility *[see Use in Specific Populations (8.1)]*.

14 CLINICAL STUDIES

14.1 Efficacy against Influenza

The efficacy of FLULAVAL QUADRIVALENT was evaluated in Trial 3, a randomized, observer-blind, non-influenza vaccine-controlled trial conducted in 3 countries in Asia, 3 in Latin America, and 2 in the Middle East/Europe during the 2010-2011 influenza season. Healthy subjects aged 3 through 8 years were randomized (1:1) to receive FLULAVAL QUADRIVALENT (n = 2,584), containing A/California/7/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/4/2006 (Yamagata lineage) influenza strains, or HAVRIX (n = 2,584), as a control vaccine. Children with no history of influenza vaccination received 2 doses of FLULAVAL QUADRIVALENT or HAVRIX approximately 28 days apart. Children with a history of influenza vaccination received one dose of FLULAVAL QUADRIVALENT or HAVRIX *[see Adverse Reactions (6.1)]*. In the overall population, 52% were male; 60% were Asian, 5% were white, and 35% were of other racial/ethnic groups. The mean age of subjects was 5 years.

Efficacy of FLULAVAL QUADRIVALENT was assessed for the prevention of reverse transcriptase polymerase chain reaction (RT-PCR)-positive influenza A and/or B disease presenting as influenza-like illness (ILI). ILI was defined as a temperature ≥100°F in the presence of at least one of the following symptoms on the same day: cough, sore throat, runny nose, or nasal congestion. Subjects with ILI (monitored by passive and active surveillance for approximately 6 months) had nasal and throat swabs collected and tested for influenza A and/or B by RT-PCR. All RT-PCR-positive specimens were further tested in cell culture. Vaccine efficacy was calculated based on the ATP cohort for efficacy (Table 6).

Table 6. FLULAVAL QUADRIVALENT: Influenza Attack Rates and Vaccine Efficacy against Influenza A and/or B in Children Aged 3 through 8 Years[a] (According-to-Protocol Cohort for Efficacy)

	N[b]	n[c]	Influenza Attack Rate % (n/N)	Vaccine Efficacy % (CI)
All RT-PCR-Positive Influenza				
FLULAVAL QUADRIVALENT	2,379	58	2.4	55.4[d] (95% CI: 39.1, 67.3)
HAVRIX[e]	2,398	128	5.3	–
All Culture-Confirmed Influenza[f]				
FLULAVAL QUADRIVALENT	2,379	50	2.1	55.9 (97.5% CI: 35.4, 69.9)
HAVRIX[e]	2,398	112	4.7	–
Antigenically Matched Culture-Confirmed Influenza				
FLULAVAL QUADRIVALENT	2,379	31	1.3	45.1[g] (97.5% CI: 9.3, 66.8)
HAVRIX[e]	2,398	56	2.3	–

CI = Confidence Interval; RT-PCR = Reverse transcriptase polymerase chain reaction.

[a] Trial 3: NCT01218308.

[b] According-to-protocol cohort for efficacy included subjects who met all eligibility criteria, were successfully contacted at least once post-vaccination, and complied with the protocol-specified efficacy criteria.

[c] Number of influenza cases.

[d] Vaccine efficacy for FLULAVAL QUADRIVALENT met the pre-defined criterion of >30% for the lower limit of the 2-sided 95% CI.

[e] Hepatitis A Vaccine used as a control vaccine.

[f] Of 162 culture-confirmed influenza cases, 108 (67%) were antigenically typed (87 matched; 21 unmatched); 54 (33%) could not be antigenically typed [but were typed by RT-PCR and nucleic acid sequence analysis: 5 cases A (H1N1) (5 with HAVRIX), 47 cases A (H3N2) (10 with FLULAVAL QUADRIVALENT; 37 with HAVRIX), and 2 cases B Victoria (2 with HAVRIX)].

[g] Since only 67% of cases could be typed, the clinical significance of this result is unknown.

In an exploratory analysis by age, vaccine efficacy against RT-PCR-positive influenza A and/or B disease presenting as ILI was evaluated in subjects aged 3 through 4 years and 5 through 8 years; vaccine efficacy was 35.3% (95% CI: -1.3, 58.6) and 67.7% (95% CI: 49.7, 79.2), respectively. As the trial lacked statistical power to evaluate efficacy within age subgroups, the clinical significance of these results is unknown.

As a secondary objective in the trial, subjects with RT-PCR-positive influenza A and/or B were prospectively classified based on the presence of adverse outcomes that have been associated with

influenza infection (defined as fever >102.2°F/39.0°C, physician-verified shortness of breath, pneumonia, wheezing, bronchitis, bronchiolitis, pulmonary congestion, croup, and/or acute otitis media, and/or physician-diagnosed serious extra-pulmonary complications, including myositis, encephalitis, seizure and/or myocarditis).

The risk reduction of fever >102.2°F/39.0°C associated with RT-PCR-positive influenza was 71.0% (95% CI: 44.8, 84.8) based on the ATP cohort for efficacy [FLULAVAL QUADRIVALENT (n = 12/2,379); HAVRIX (n = 41/2,398)]. The other pre-specified adverse outcomes had too few cases to calculate a risk reduction. The incidence of these adverse outcomes is presented in Table 7.

Table 7. FLULAVAL QUADRIVALENT: Incidence of Adverse Outcomes Associated with RT-PCR-Positive Influenza in Children Aged 3 through 8 Years[a] (Total Vaccinated Cohort)[b]

	FLULAVAL QUADRIVALENT n = 2,584			HAVRIX[c] n = 2,584		
Adverse Outcome[d]	**Number of Events**	**Number of Subjects[e]**	**%**	**Number of Events**	**Number of Subjects[e]**	**%**
Fever >102.2°F/39.0°C	16[f]	15	0.6	51[f]	50	1.9
Shortness of breath	0	0	0	5	5	0.2
Pneumonia	0	0	0	3	3	0.1
Wheezing	1	1	0	1	1	0
Bronchitis	1	1	0	1	1	0
Pulmonary congestion	0	0	0	1	1	0
Acute otitis media	0	0	0	1	1	0
Bronchiolitis	0	0	0	0	0	0
Croup	0	0	0	0	0	0
Encephalitis	0	0	0	0	0	0
Myocarditis	0	0	0	0	0	0
Myositis	0	0	0	0	0	0
Seizure	0	0	0	0	0	0

[a] Trial 3: NCT01218308.

[b] Total vaccinated cohort included all vaccinated subjects for whom data were available.

[c] Hepatitis A Vaccine used as a control vaccine.

[d] In subjects who presented with more than one adverse outcome, each outcome was counted in the respective category.

[e] Number of subjects presenting with at least one event in each group.

[f] One subject in each group had sequential influenza due to influenza type A and type B viruses.

14.2 Immunological Evaluation

Adults

Trial 1 was a randomized, double-blind, active-controlled, safety and immunogenicity trial conducted in subjects aged 18 years and older. In this trial, subjects received FLULAVAL QUADRIVALENT (n = 1,246) or one of 2 formulations of a comparator trivalent influenza vaccine (FLULAVAL, TIV-1, n = 204 or TIV-2, n = 211), each containing an influenza type B virus that corresponded to one of the 2 B viruses in FLULAVAL QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage) *[see Adverse Reactions (6.1)]*.

Immune responses, specifically hemagglutination inhibition (HI) antibody titers to each virus strain in the vaccine, were evaluated in sera obtained 21 days after administration of FLULAVAL QUADRIVALENT or the comparators. The immunogenicity endpoint was GMTs adjusted for baseline, performed on the According-to-Protocol (ATP) cohort for whom immunogenicity assay results were available after vaccination. FLULAVAL QUADRIVALENT was non-inferior to both TIVs based on adjusted GMTs (Table 8). The antibody response to influenza B strains contained in FLULAVAL QUADRIVALENT was higher than the antibody response after vaccination with a TIV containing an influenza B strain from a different lineage. There was no evidence that the addition of the second B strain resulted in immune interference to other strains included in the vaccine (Table 8).

Table 8. Non-inferiority of FLULAVAL QUADRIVALENT Relative to Trivalent Influenza Vaccine (TIV) 21 Days Post-vaccination in Adults Aged 18 Years and Older[a] (According-to-Protocol Cohort for Immunogenicity)[b]

Geometric Mean Titers Against	FLULAVAL QUADRIVALENT[c] n = 1,245-1,246 (95% CI)	TIV-1 (B Victoria)[d] n = 204 (95% CI)	TIV-2 (B Yamagata)[e] n = 210-211 (95% CI)
A/California/7/2009 (H1N1)	204.6[f] (190.4, 219.9)	176.0 (149.1, 207.7)	149.0 (122.9, 180.7)
A/Victoria/210/2009 (H3N2)	125.4[f] (117.4, 133.9)	147.5 (124.1, 175.2)	141.0 (118.1, 168.3)
B/Brisbane/60/2008 (Victoria lineage)	177.7[f] (167.8, 188.1)	135.9 (118.1, 156.5)	71.9 (61.3, 84.2)
B/Florida/4/2006 (Yamagata lineage)	399.7[f] (378.1, 422.6)	176.9 (153.8, 203.5)	306.6 (266.2, 353.3)

CI = Confidence Interval.

[a] Trial 1: NCT01196975.

[b] According-to-protocol cohort for immunogenicity included all evaluable subjects for whom assay results were available after vaccination for at least one trial vaccine antigen.

[c] Containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Florida/04/2006 (Yamagata lineage), and B/Brisbane/60/2008 (Victoria lineage).

[d] Containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage).

[e] Containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage).

[f] Non-inferior to both TIVs based on adjusted GMTs [upper limit of the 2-sided 95% CI for the GMT ratio (TIV/FLULAVAL QUADRIVALENT) ≤1.5]; superior to TIV-1 (B Victoria) with respect to the B strain of Yamagata lineage and to TIV-2 (B Yamagata) with respect to the B strain of Victoria lineage based on adjusted GMTs [lower limit of the 2-sided 95% CI for the GMT ratio (FLULAVAL QUADRIVALENT/TIV) >1.5].

Children

Trial 4 was a randomized, observer-blind, active-controlled trial in children aged 6 through 35 months which was conducted in the United States and Mexico. In this trial, subjects received 0.5 mL of FLULAVAL QUADRIVALENT containing 15 mcg HA of each of the 4 influenza strains included in the vaccine (n = 1,207); or 0.25 mL of control vaccine FLUZONE QUADRIVALENT (Influenza Vaccine) containing 7.5 mcg HA of each of the 4 influenza strains included in the vaccine (n = 1,217) *[see Adverse Reactions (6.1)]*.

Immune responses, specifically HI antibody titers to each virus strain in the vaccine, were evaluated in sera obtained 28 days following completion of vaccination regimen. Previously vaccinated children received one dose and previously unvaccinated children (i.e., unprimed individuals) received 2 doses 4 weeks apart of FLULAVAL QUADRIVALENT or the comparator. The immunogenicity endpoints were GMTs adjusted for baseline, and the percentage of subjects who achieved seroconversion, defined as a pre-vaccination HI titer of <1:10 with a post-vaccination titer ≥1:40 or at least a 4-fold increase in serum HI titer over baseline to ≥1:40, following vaccination, performed on the ATP cohort. FLULAVAL QUADRIVALENT was non-inferior to the comparator for all 4 vaccine strains based on adjusted GMTs and seroconversion rates (Table 9).

Table 9. Non-inferiority of FLULAVAL QUADRIVALENT Relative to Comparator Quadrivalent Influenza Vaccine at 28 Days Post-vaccination in Children Aged 6 through 35 Months[a] (According-to-Protocol Cohort for Immunogenicity)[b]

Adjusted Geometric Mean Titers Against	FLULAVAL QUADRIVALENT[c] n = 972-974	Active Comparator[d] n = 980
A/California/07/2009 (H1N1)	99.6[e]	85.1
A/Texas/50/2012 (H3N2)	99.8[e]	84.6
B/Massachusetts/02/2012 (Yamagata lineage)	258.1[e]	167.3
B/Brisbane/60/2008 (Victoria lineage)	54.5[e]	33.7
Seroconversion[f] to:	**n = 972-974 % (95% CI)**	**n = 980 % (95% CI)**
A/California/07/2009 (H1N1)	73.7[e] (70.8, 76.4)	67.3 (64.3, 70.3)
A/Texas/50/2012 (H3N2)	76.1[e] (73.3, 78.8)	69.4 (66.4, 72.3)
B/Massachusetts/02/2012 (Yamagata lineage)	85.5[e] (83.2, 87.7)	73.8 (70.9, 76.5)
B/Brisbane/60/2008 (Victoria lineage)	64.9[e] (61.8, 67.9)	48.5 (45.3, 51.6)

CI = Confidence Interval.

[a] Trial 4: NCT02242643.

[b] According-to-protocol cohort for immunogenicity included all evaluable subjects for whom assay results were available after vaccination for at least one trial vaccine antigen.

[c] A 0.5-mL dose containing 15 mcg each of A/California/07/2009 (H1N1), A/Texas/50/2012 (H3N2), B/Massachusetts/02/2012 (Yamagata lineage), and B/Brisbane/60/2008 (Victoria lineage).

[d] A 0.25-mL dose of U.S.-licensed quadrivalent, inactivated influenza vaccine (manufactured by Sanofi Pasteur Inc.) containing 7.5 mcg each of A/California/07/2009 (H1N1), A/Texas/50/2012 (H3N2), B/Massachusetts/02/2012 (Yamagata lineage), and B/Brisbane/60/2008 (Victoria lineage).

[e] Non-inferior to the comparator vaccine based on adjusted GMTs [upper limit of the 2-sided 95% CI for the GMT ratio (comparator/FLULAVAL QUADRIVALENT) ≤1.5] and seroconversion rates (upper limit of the 2-sided 95% CI on difference of comparator vaccine minus FLULAVAL QUADRIVALENT ≤10%).

[f] Seroconversion defined as a 4-fold increase in post-vaccination antibody titer from pre-vaccination titer ≥1:10, or an increase in titer from <1:10 to ≥1:40.

Trial 2 was a randomized, double-blind, active-controlled trial conducted in children aged 3 through 17 years. In this trial, subjects received FLULAVAL QUADRIVALENT (n = 878), or one of 2 formulations of a comparator trivalent influenza vaccine (FLUARIX, TIV-1, n = 871 or TIV-2 n = 878), each containing an influenza type B virus that corresponded to one of the 2 B viruses in FLULAVAL QUADRIVALENT (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage) *[see Adverse Reactions (6.1)]*.

Immune responses, specifically HI antibody titers to each virus strain in the vaccine, were evaluated in sera obtained 28 days following one or 2 doses of FLULAVAL QUADRIVALENT or the comparators. The immunogenicity endpoints were GMTs adjusted for baseline, and the percentage of subjects who achieved seroconversion, defined as at least a 4-fold increase in serum HI titer over baseline to ≥1:40, following vaccination, performed on the ATP cohort. FLULAVAL QUADRIVALENT was non-inferior to both TIVs based on adjusted GMTs and seroconversion rates (Table 10). The antibody response to influenza B strains contained in FLULAVAL QUADRIVALENT was higher than the antibody response after vaccination with a TIV containing an influenza B strain from a different lineage. There was no evidence that the addition of the second B strain resulted in immune interference to other strains included in the vaccine (Table 10).

Table 10. Non-inferiority of FLULAVAL QUADRIVALENT Relative to Trivalent Influenza Vaccine (TIV) at 28 Days Post-vaccination in Children Aged 3 through 17 Years[a] (According-to-Protocol Cohort for Immunogenicity)[b]

	FLULAVAL QUADRIVALENT[c]	TIV-1 (B Victoria)[d]	TIV-2 (B Yamagata)[e]
Geometric Mean Titers Against	**n = 878 (95% CI)**	**n = 871 (95% CI)**	**n = 877-878 (95% CI)**
A/California/7/2009 (H1N1)	362.7[f] (335.3, 392.3)	429.1 (396.5, 464.3)	420.2 (388.8, 454.0)
A/Victoria/210/2009 (H3N2)	143.7[f] (134.2, 153.9)	139.6 (130.5, 149.3)	151.0 (141.0, 161.6)
B/Brisbane/60/2008 (Victoria lineage)	250.5[f] (230.8, 272.0)	245.4 (226.9, 265.4)	68.1 (61.9, 74.9)
B/Florida/4/2006 (Yamagata lineage)	512.5[f] (477.6, 549.9)	197.0 (180.7, 214.8)	579.0 (541.2, 619.3)
Seroconversion[g] to:	**n = 876 % (95% CI)**	**n = 870 % (95% CI)**	**n = 876-877 % (95% CI)**
A/California/7/2009 (H1N1)	84.4[f] (81.8, 86.7)	86.8 (84.3, 89.0)	85.5 (83.0, 87.8)
A/Victoria/210/2009 (H3N2)	70.1[f] (66.9, 73.1)	67.8 (64.6, 70.9)	69.6 (66.5, 72.7)
B/Brisbane/60/2008 (Victoria lineage)	74.5[f] (71.5, 77.4)	71.5 (68.4, 74.5)	29.9 (26.9, 33.1)
B/Florida/4/2006 (Yamagata lineage)	75.2[f] (72.2, 78.1)	41.3 (38.0, 44.6)	73.4 (70.4, 76.3)

CI = Confidence Interval.

[a] Trial 2: NCT01198756.

[b] According-to-protocol cohort for immunogenicity included all evaluable subjects for whom assay results were available after vaccination for at least one trial vaccine antigen.

[c] Containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Florida/04/2006 (Yamagata lineage), and B/Brisbane/60/2008 (Victoria lineage).

[d] Containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage).

[e] Containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage).

[f] Non-inferior to both TIVs based on adjusted GMTs [upper limit of the 2-sided 95% CI for the GMT ratio (TIV/FLULAVAL QUADRIVALENT) ≤1.5] and seroconversion rates (upper limit of the 2-sided 95% CI on difference of the TIV minus FLULAVAL QUADRIVALENT ≤10%); superior to TIV-1 (B Victoria) with respect to the B strain of Yamagata lineage and to TIV-2 (B Yamagata) with respect to the B strain of Victoria lineage based on adjusted GMTs [lower limit of the 2-sided 95% CI

for the GMT ratio (FLULAVAL QUADRIVALENT/TIV) >1.5] and seroconversion rates (lower limit of the 2-sided 95% CI on difference of FLULAVAL QUADRIVALENT minus the TIV >10%).

[g] Seroconversion defined as a 4-fold increase in post-vaccination antibody titer from pre-vaccination titer ≥1:10, or an increase in titer from <1:10 to ≥1:40.

15 REFERENCES

1. Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. *Virus Res.* 2004;103:133-138.
2. Hobson D, Curry RL, Beare AS, et al. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. *J Hyg Camb.* 1972;70:767-777.

16 HOW SUPPLIED/STORAGE AND HANDLING

FLULAVAL QUADRIVALENT is available in 0.5-mL single-dose, disposable, prefilled TIP-LOK syringes (packaged without needles).

NDC 19515-808-41 Syringe in Package of 10: NDC 19515-808-52

Store refrigerated between 2º and 8ºC (36º and 46ºF). Do not freeze. Discard if the vaccine has been frozen. Store in the original package to protect from light.

17 PATIENT COUNSELING INFORMATION

Provide the following information to the vaccine recipient or guardian:

- Inform of the potential benefits and risks of immunization with FLULAVAL QUADRIVALENT.
- Educate regarding potential side effects, emphasizing that (1) FLULAVAL QUADRIVALENT contains non-infectious killed viruses and cannot cause influenza, and (2) FLULAVAL QUADRIVALENT is intended to provide protection against illness due to influenza viruses only, and cannot provide protection against all respiratory illness.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 prior to each immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).
- Instruct that annual revaccination is recommended.

Manufactured by **ID Biomedical Corporation of Quebec**
Quebec City, QC, Canada, U.S. License 1739
Distributed by **GlaxoSmithKline**
Research Triangle Park, NC 27709

FVQ:12PI

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use FLUMIST® QUADRIVALENT safely and effectively. See full prescribing information for FLUMIST® QUADRIVALENT.

FluMist® Quadrivalent (Influenza Vaccine Live, Intranasal)
Intranasal Spray
2022-2023 Formula
Initial U.S. Approval: 2003

----------INDICATIONS AND USAGE----------

FluMist Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. (1, 11)
FluMist Quadrivalent is approved for use in persons 2 through 49 years of age. (1)

----------DOSAGE AND ADMINISTRATION----------

For intranasal administration by a healthcare provider. (2)

Age	Dose	Schedule
2 years through 8 years	1 or 2 doses[a], 0.2 mL[b] each	If 2 doses, administer at least 1 month apart
9 years through 49 years	1 dose, 0.2 mL[b]	-

[a] 1 or 2 doses depends on vaccination history as per Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines.
[b] Administer as 0.1 mL per nostril.
"-" indicates information is not applicable.

----------DOSAGE FORMS AND STRENGTHS----------

Each 0.2 mL dose is a suspension supplied in a single-dose pre-filled intranasal sprayer. (3)

----------CONTRAINDICATIONS----------

- Severe allergic reaction (e.g., anaphylaxis) to any component of FluMist Quadrivalent, including egg protein, or after a previous dose of any influenza vaccine. (4.1, 11)
- Concomitant aspirin therapy in children and adolescents. (4.2)

----------WARNINGS AND PRECAUTIONS----------

- In clinical trials, risks of hospitalization and wheezing were increased in children younger than 2 years of age who received FluMist (trivalent Influenza Vaccine Live, Intranasal). (5.1)
- Children younger than 5 years of age with recurrent wheezing and persons of any age with asthma may be at increased risk of wheezing following the administration of FluMist Quadrivalent. (5.2)
- If Guillain-Barré syndrome has occurred within 6 weeks of any prior influenza vaccination, the decision to give FluMist Quadrivalent should be based on careful consideration of the potential benefits and risks. (5.3)
- FluMist Quadrivalent has not been studied in immunocompromised persons. (5.4)

----------ADVERSE REACTIONS----------

The most common solicited adverse reactions (≥ 10% in vaccine recipients and at least 5% greater than in placebo recipients) reported after FluMist were runny nose or nasal congestion (ages 2 years through 49 years), fever over 100°F (children ages 2 years through 6 years), and sore throat (adults ages 18 years through 49 years). Among children and adolescents 2 through 17 years of age who received FluMist Quadrivalent, 32% reported runny nose or nasal congestion and 7% reported fever over 100°F. Among adults 18 through 49 years of age who received FluMist Quadrivalent, 44% reported runny nose or nasal congestion and 19% reported sore throat. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact MedImmune at 1-877-633-4411 or VAERS at 1-800-822-7967 or *http://vaers.hhs.gov.*

----------DRUG INTERACTIONS----------

- Antiviral drugs that are active against influenza A and/or B may reduce the effectiveness of FluMist Quadrivalent if administered within 48 hours before, or within 2 weeks after, receipt of the vaccine. (7.2)

----------USE IN SPECIFIC POPULATIONS----------

- In clinical trials, in children 6 through 23 months of age, FluMist was associated with an increased risk of hospitalization and wheezing. (8.4)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 8/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

FluMist® Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine *[see Description (11)]*.

FluMist Quadrivalent is approved for use in persons 2 through 49 years of age.

2 DOSAGE AND ADMINISTRATION

FOR INTRANASAL ADMINISTRATION BY A HEALTHCARE PROVIDER.

2.1 Dosing Information

Administer FluMist Quadrivalent according to the following schedule:

Age	Dose	Schedule
2 years through 8 years	1 or 2 doses[a], 0.2 mL[b] each	If 2 doses, administer at least 1 month apart
9 years through 49 years	1 dose, 0.2 mL[b]	-

[a] 1 or 2 doses depends on vaccination history as per Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines.

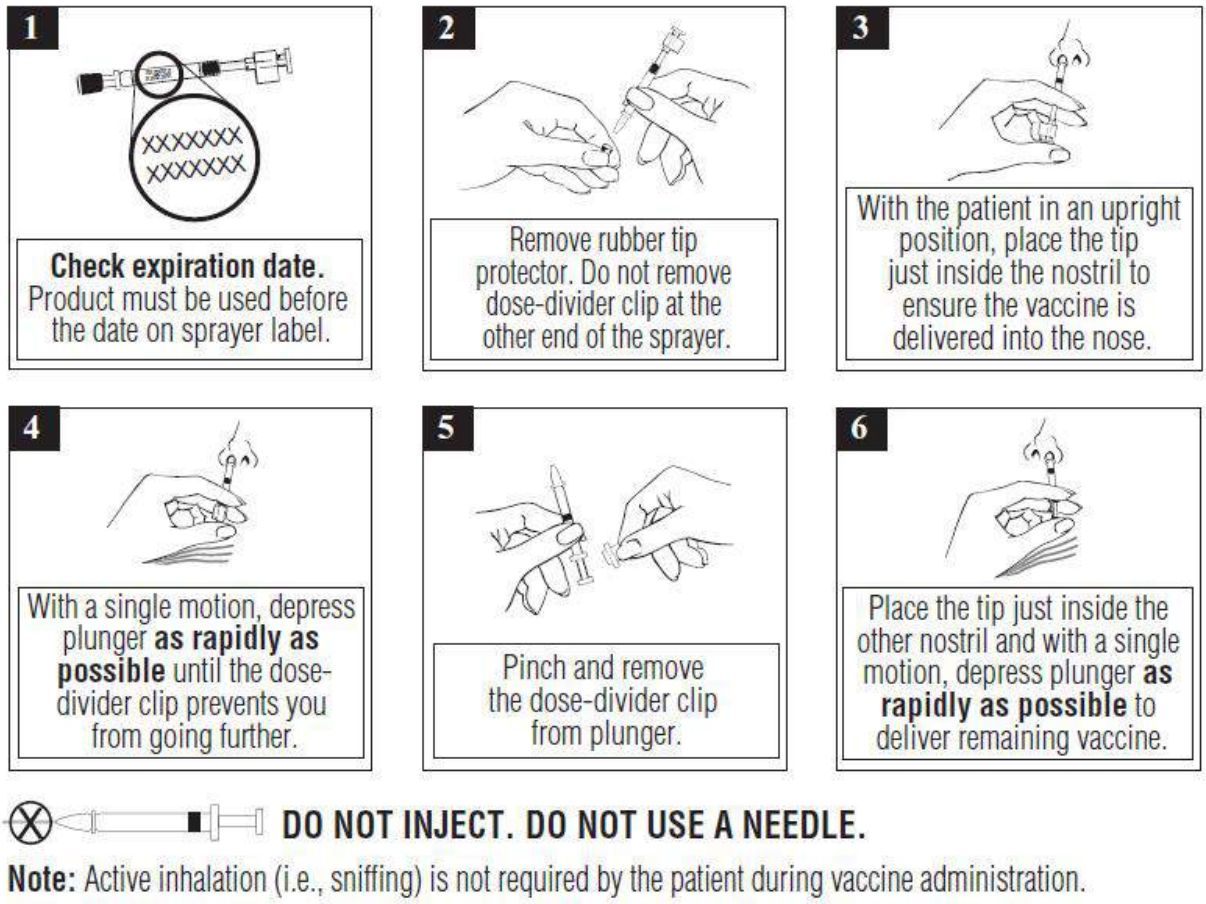
[b] Administer as 0.1 mL per nostril.

"-" indicates information is not applicable.

2.2 Administration Instructions

Each sprayer contains a single dose (0.2 mL) of FluMist Quadrivalent; administer approximately one half of the contents of the single-dose intranasal sprayer into each nostril (each sprayer contains 0.2 mL of vaccine). Refer to Figure 1 for step-by-step administration instructions. Following administration, dispose of the sprayer according to the standard procedures for medical waste (e.g., sharps container or biohazard container).

Figure 1



3 DOSAGE FORMS AND STRENGTHS

Each 0.2 mL dose is a suspension supplied in a single-dose pre-filled intranasal sprayer.

4 CONTRAINDICATIONS

4.1 Severe Allergic Reactions

Do not administer FluMist Quadrivalent to persons who have had a severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine *[see Description (11)]* including egg protein, or after a previous dose of any influenza vaccine.

4.2 Concomitant Aspirin Therapy and Reye's Syndrome in Children and Adolescents

Do not administer FluMist Quadrivalent to children and adolescents through 17 years of age who are receiving aspirin therapy or aspirin-containing therapy because of the association of Reye's syndrome with aspirin and wild-type influenza infection *[see Drug Interactions (7.1)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Risks of Hospitalization and Wheezing in Children Younger than 24 Months of Age

In clinical trials, risks of hospitalization and wheezing were increased in children younger than 2 years of age who received FluMist (trivalent Influenza Vaccine Live, Intranasal) *[see Adverse Reactions (6.1)]*. This observation with FluMist is relevant to FluMist Quadrivalent because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*.

5.2 Asthma, Recurrent Wheezing, and Active Wheezing

Children younger than 5 years of age with recurrent wheezing and persons of any age with asthma may be at increased risk of wheezing following administration of FluMist Quadrivalent. FluMist Quadrivalent has not been studied in persons with severe asthma or active wheezing.

5.3 Guillain-Barré Syndrome

The 1976 swine influenza vaccine (inactivated) was associated with an elevated risk of Guillain-Barré syndrome (GBS). Evidence for causal relation of GBS with other influenza vaccines is inconclusive; if an excess risk exists, based on data for inactivated influenza vaccines, it is probably slightly more than 1 additional case per 1 million persons vaccinated[1]. If GBS has occurred within 6 weeks of any prior influenza vaccination, the decision to give FluMist Quadrivalent should be based on careful consideration of the potential benefits and potential risks.

5.4 Altered Immunocompetence

FluMist Quadrivalent has not been studied in immunocompromised persons. The effectiveness of FluMist has not been studied in immunocompromised persons. Data on safety and shedding of vaccine virus after administration of FluMist in immunocompromised persons are limited to 173 persons with HIV infection and 10 mild to moderately immunocompromised children and adolescents with cancer *[see Clinical Pharmacology (12.2)]*.

5.5 Medical Conditions Predisposing to Influenza Complications

The safety of FluMist Quadrivalent in individuals with underlying medical conditions that may predispose them to complications following wild-type influenza infection has not been established.

5.6 Management of Acute Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine *[see Contraindications (4.1)]*.

5.7 Limitations of Vaccine Effectiveness

FluMist Quadrivalent may not protect all individuals receiving the vaccine.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

This safety experience with FluMist is relevant to FluMist Quadrivalent because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*. A total of 9537 children and adolescents 1 through 17 years of age and 3041 adults 18 through 64 years of age received FluMist in randomized, placebo-controlled Studies D153-P501, AV006, D153-P526, AV019, and AV009 [3 used Allantoic Fluid containing Sucrose-Phosphate-Glutamate (AF-SPG) placebo, and 2 used saline placebo] described below. In addition, 4179 children 6 through 59 months of age received FluMist in Study MI-CP111, a randomized, active-controlled trial. Among pediatric FluMist recipients 6 months through 17 years of age, 50% were female; in the study of adults, 55% were female. In MI-CP111, AV006, D153-P526, AV019, and AV009, subjects were White (71%), Hispanic (11%), Asian (7%), Black (6%), and Other (5%), while in D153-P501, 99% of subjects were Asian.

A total of 1382 children and adolescents 2 through 17 years of age and 1198 adults 18 through 49 years of age received FluMist Quadrivalent in randomized, active-controlled Studies MI-CP208 and MI-CP185. Among pediatric FluMist Quadrivalent recipients 2 through 17 years of age, 51% were female; in the study of adults, 55% were female. In Studies MI-CP208 and MI-CP185, subjects were White (73%), Asian (1%), Black or African-American (19%), and Other (7%); overall, 22% were Hispanic or Latino.

FluMist in Children and Adolescents

The safety of FluMist was evaluated in an AF-SPG placebo-controlled Study (AV019) conducted in a Health Maintenance Organization (HMO) in children 1 through 17 years of age (FluMist = 6473, placebo = 3216). An increase in asthma events, captured by review of diagnostic codes, was observed in children younger than 5 years of age who received FluMist compared to those who received placebo (Relative Risk 3.53, 90% CI: 1.1, 15.7).

In Study MI-CP111, children 6 through 59 months of age were randomized to receive FluMist or inactivated Influenza Virus Vaccine manufactured by Sanofi Pasteur Inc. Wheezing requiring bronchodilator therapy or accompanied by respiratory distress or hypoxia was prospectively monitored from randomization through 42 days post last vaccination. Hospitalization due to all causes was prospectively monitored from randomization through 180 days post last vaccination. Increases in wheezing and hospitalization (for any cause) were observed in children 6 months through 23 months of age who received FluMist compared to those who received inactivated Influenza Virus Vaccine, as shown in Table 1.

Table 1: Percentages of Children with Hospitalizations and Wheezing from Study MI-CP111[a]

Adverse Reaction	Age Group	FluMist (n/N)	Active Control[b] (n/N)
Hospitalizations[c]	6-23 months	4.2% (84/1992)	3.2% (63/1975)
	24-59 months	2.1% (46/2187)	2.5% (56/2198)
Wheezing[d]	6-23 months	5.9% (117/1992)	3.8% (75/1975)
	24-59 months	2.1% (47/2187)	2.5% (56/2198)

[a] NCT00128167; see www.clinicaltrials.gov
[b] Inactivated Influenza Virus Vaccine manufactured by Sanofi Pasteur Inc., administered intramuscularly.
[c] Hospitalization due to any cause from randomization through 180 days post last vaccination.
[d] Wheezing requiring bronchodilator therapy or accompanied by respiratory distress or hypoxia evaluated from randomization through 42 days post last vaccination.

Most hospitalizations observed were due to gastrointestinal and respiratory tract infections and occurred more than 6 weeks post vaccination. In post-hoc analysis, rates of hospitalization in children 6 through 11 months of age were 6.1% (42/684) in FluMist recipients and 2.6% (18/683) in inactivated Influenza Virus Vaccine recipients.

Table 2 shows pooled solicited adverse reactions occurring in at least 1% of FluMist recipients and at a higher rate (≥ 1% rate difference after rounding) compared to placebo post Dose 1 for Studies D153-P501 and AV006, and solicited adverse reactions post Dose 1 for Study MI-CP111. Solicited adverse reactions were those about which parents/guardians were specifically queried after receipt of FluMist, placebo, or control vaccine. In these studies, solicited reactions were documented for 10 days post vaccination. Solicited reactions following the second dose of FluMist were similar to those following the first dose and were generally observed at a lower frequency.

Table 2: Summary of Solicited Adverse Reactions Observed Within 10 Days after Dose 1 for FluMist and Either Placebo or Active Control Recipients in Children 2 through 6 Years of Age

	Studies D153-P501[a] & AV006		Study MI-CP111[b]	
	FluMist N = 876-1759[e]	Placebo[c] N = 424-1034[e]	FluMist N = 2170[e]	Active Control[d] N = 2165[e]
Event	%	%	%	%
Runny Nose/ Nasal Congestion	58	50	51	42
Decreased Appetite	21	17	13	12
Irritability	21	19	12	11
Decreased Activity (Lethargy)	14	11	7	6
Sore Throat	11	9	5	6
Headache	9	7	3	3
Muscle Aches	6	3	2	2
Chills	4	3	2	2
Fever				
> 100°F Oral	16	11	13	11
> 100 - ≤ 101°F Oral	9	6	6	4
> 101 - ≤ 102°F Oral	4	3	4	3

[a] NCT00192244; see www.clinicaltrials.gov
[b] NCT00128167; see www.clinicaltrials.gov
[c] Study D153-P501 used saline placebo; Study AV006 used AF-SPG placebo.
[d] Inactivated Influenza Virus Vaccine manufactured by Sanofi Pasteur Inc., administered intramuscularly.
[e] Number of evaluable subjects (those who returned diary cards) for each reaction. Range reflects differences in data collection between the 2 pooled studies.

In clinical studies D153-P501 and AV006, unsolicited adverse reactions in children occurring in at least 1% of FluMist recipients and at a higher rate (≥ 1% rate difference after rounding) compared to placebo were abdominal pain (2% FluMist vs. 0% placebo) and otitis media (3% FluMist vs. 1% placebo). An additional adverse reaction identified in the active-controlled trial MI-CP111 occurring in at least 1% of FluMist recipients and at a higher rate (≥ 1% rate difference after rounding) compared to active control was sneezing (2% FluMist vs.1% active control).

In a separate saline placebo-controlled trial (D153-P526) in a subset of older children and adolescents 9 through 17 years of age who received one dose of FluMist, the solicited adverse reactions as well as unsolicited adverse reactions reported were generally consistent with observations from the trials in Table 2. Abdominal pain was reported in 12% of FluMist recipients compared to 4% of placebo recipients and decreased activity was reported in 6% of FluMist recipients compared to 0% of placebo recipients.

In Study AV018, in which FluMist was concomitantly administered with Measles, Mumps, and Rubella Virus Vaccine Live (MMR, manufactured by Merck & Co., Inc.) and Varicella Virus Vaccine Live (manufactured by Merck & Co., Inc.) to children 12 through 15 months of age, adverse reactions were similar to those seen in other clinical trials of FluMist.

FluMist Quadrivalent in Children and Adolescents

In the randomized, active-controlled Study MI-CP208 that compared FluMist Quadrivalent and FluMist in children and adolescents 2 through 17 years of age, the rates of solicited adverse reactions reported were similar between subjects who received FluMist Quadrivalent and FluMist. Table 3 includes solicited adverse reactions post Dose 1 from Study MI-CP208 that either occurred at a higher rate (≥ 1% rate difference after rounding) in FluMist Quadrivalent recipients compared to FluMist recipients or were identified in previous FluMist clinical studies *(see Table 2)*. In this study, solicited adverse reactions were documented for 14 days post vaccination. Solicited adverse reactions post Dose 2 were observed at a lower frequency compared to those post Dose 1 for FluMist Quadrivalent and were similar between subjects who received FluMist Quadrivalent and FluMist.

Table 3: Summary of Solicited Adverse Reactions[a] Observed Within 14 Days after Dose 1 for FluMist Quadrivalent and FluMist Recipients in Study MI-CP208[b] in Children and Adolescents 2 through 17 Years of Age

	FluMist Quadrivalent N = 1341-1377[d]	FluMist[c] N = 901-920[d]
Event	%	%
Runny Nose/Nasal Congestion	32	32
Headache	13	12
Decreased Activity (Lethargy)	10	10
Sore Throat	9	10
Decreased Appetite	6	7
Muscle Aches	4	5
Fever		
> 100°F by any route	7	5
> 100 - ≤ 101°F by any route	3	2
> 101 - ≤ 102°F by any route	2	2

[a] Solicited adverse reactions that occurred at a higher rate (≥ 1% rate difference after rounding) in FluMist Quadrivalent recipients compared to FluMist recipients or were identified in previous FluMist trials (*see Table 2)*.
[b] NCT01091246; see www.clinicaltrials.gov
[c] Represents pooled data from the two FluMist study arms *[see Clinical Studies (14.2)]*.
[d] Number of evaluable subjects for each event.

In Study MI-CP208, no unsolicited adverse reactions occurred at a higher rate (1% or greater) in FluMist Quadrivalent recipients compared to FluMist recipients.

FluMist in Adults

In adults 18 through 49 years of age in Study AV009, solicited adverse reactions occurring in at least 1% of FluMist recipients and at a higher rate (≥ 1% rate difference after rounding) compared to AF-SPG placebo include runny nose (44% FluMist vs. 27% placebo), headache (40% FluMist vs. 38% placebo), sore throat (28% FluMist vs. 17% placebo), tiredness/weakness (26% FluMist vs. 22% placebo), muscle aches (17% FluMist vs. 15% placebo), cough (14% FluMist vs. 11% placebo), and chills (9% FluMist vs. 6% placebo).

In Study AV009, unsolicited adverse reactions occurring in at least 1% of FluMist recipients and at a higher rate (≥ 1% rate difference after rounding) compared to placebo were nasal congestion (9% FluMist vs. 2% placebo) and sinusitis (4% FluMist vs. 2% placebo).

FluMist Quadrivalent in Adults

In the randomized, active-controlled Study MI-CP185 that compared FluMist Quadrivalent and FluMist in adults 18 through 49 years of age, the rates of solicited adverse reactions reported were generally similar between subjects who received FluMist Quadrivalent and FluMist. Table 4 presents solicited adverse reactions that either occurred at a higher rate (≥ 1% rate difference after rounding) in FluMist Quadrivalent recipients compared to FluMist recipients or were identified in Study AV009.

Table 4: Summary of Solicited Adverse Reactions[a] Observed Within 14 Days after Dose 1 for FluMist Quadrivalent and FluMist Recipients in Study MI-CP185[b] in Adults 18 through 49 Years of Age

	FluMist Quadrivalent N = 1197[d]	FluMist[c] N = 597[d]
Event	%	%
Runny Nose/Nasal Congestion	44	40
Headache	28	27
Sore Throat	19	20
Decreased Activity (Lethargy)	18	18
Cough	14	13
Muscle Aches	10	10
Decreased Appetite	6	5

[a] Solicited adverse reactions that occurred at a higher rate (≥ 1% rate difference after rounding) in FluMist Quadrivalent recipients compared to FluMist recipients or were identified in Study AV009.
[b] NCT00860067; see www.clinicaltrials.gov
[c] Represents pooled data from the two FluMist study arms *[see Clinical Studies (14.4)]*.
[d] Number of evaluable subjects for each event.

In Study MI-CP185, no unsolicited adverse reactions occurred at a higher rate (1% or greater) in FluMist Quadrivalent recipients compared to FluMist recipients.

6.2 Postmarketing Experience

The following events have been spontaneously reported during post approval use of FluMist. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Cardiac disorders: Pericarditis

Congenital, familial, and genetic disorders: Exacerbation of symptoms of mitochondrial encephalomyopathy (Leigh syndrome)

Gastrointestinal disorders: Nausea, vomiting, diarrhea

Immune system disorders: Hypersensitivity reactions (including anaphylactic reaction, facial edema, and urticaria)

Nervous system disorders: Guillain-Barré syndrome, Bell's Palsy, meningitis, eosinophilic meningitis, vaccine-associated encephalitis

Respiratory, thoracic, and mediastinal disorders: Epistaxis

Skin and subcutaneous tissue disorders: Rash

7 DRUG INTERACTIONS

7.1 Aspirin Therapy

Do not administer FluMist Quadrivalent to children and adolescents through 17 years of age who are receiving aspirin therapy or aspirin-containing therapy because of the association of Reye's syndrome with aspirin and wild-type influenza *[see Contraindications (4.2)]*. Avoid aspirin-containing therapy in these age groups during the first 4 weeks after vaccination with FluMist Quadrivalent unless clearly needed.

7.2 Antiviral Agents Against Influenza A and/or B

Antiviral drugs that are active against influenza A and/or B viruses may reduce the effectiveness of FluMist Quadrivalent if administered within 48 hours before, or within 2 weeks after vaccination. The concurrent use of FluMist Quadrivalent with antiviral agents that are active against influenza A and/or B viruses has not been evaluated. If antiviral agents and FluMist Quadrivalent are administered concomitantly, revaccination should be considered when appropriate.

7.3 Concomitant Administration with Inactivated Vaccines

The safety and immunogenicity of FluMist Quadrivalent when administered concomitantly with inactivated vaccines have not been determined. Studies of FluMist and FluMist Quadrivalent excluded subjects who received any inactivated or subunit vaccine within two weeks of enrollment.

7.4 Concomitant Administration with Other Live Vaccines

Concomitant administration of the trivalent formulation of FluMist with Measles, Mumps, and Rubella Virus Vaccine Live (MMR, manufactured by Merck & Co., Inc.) and the Varicella Vaccine Live (manufactured by Merck & Co., Inc.) was studied in children 12 through 15 months of age *[see Clinical Studies (14.5)]*. Concomitant administration of the MMR and the varicella vaccine with the trivalent or quadrivalent FluMist formulations has not been studied in children older than 15 months of age.

7.5 Intranasal Products

There are no data regarding co-administration of FluMist Quadrivalent with other intranasal preparations.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

FluMist Quadrivalent is not absorbed systemically following intranasal administration and maternal use is not expected to result in fetal exposure to the drug.

Clinical Considerations

Disease-Associated Maternal and/or Embryo/Fetal Risk: Pregnant women infected with seasonal influenza are at increased risk of severe illness associated with influenza infection compared with non-pregnant women. Pregnant women with influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

Data

Animal Data: In a developmental and reproductive toxicity study, female rats were administered FluMist Quadrivalent either three times (during the period of organogenesis) or six times (prior to gestation and during the period of organogenesis), 200 microliter/rat/occasion (approximately 150 human dose equivalents), by intranasal instillation revealing no evidence of impaired fertility or harm to the fetus due to FluMist Quadrivalent.

8.2 Lactation

Risk Summary

FluMist is not absorbed systemically by the mother following intranasal administration and breastfeeding is not expected to result in exposure of the child to FluMist.

8.4 Pediatric Use

Safety and effectiveness of FluMist Quadrivalent in children 24 months of age and older is based on data from FluMist clinical studies and a comparison of post-vaccination antibody titers between persons who received FluMist Quadrivalent and those who received FluMist *[see Clinical Studies (14.1, 14.2)]*. FluMist Quadrivalent is not approved for use in children younger than 24 months of age because use of FluMist in

children 6 through 23 months has been associated with increased risks of hospitalization and wheezing in clinical trials *[see Warnings and Precautions (5.1) and Adverse Reactions (6.1)]*.

8.5 Geriatric Use

FluMist Quadrivalent is not approved for use in persons 65 years of age and older because in a clinical study (AV009), effectiveness of FluMist to prevent febrile illness was not demonstrated in adults 50 through 64 years of age *[see Clinical Studies (14.3)]*. In this study, solicited events among individuals 50 through 64 years of age were similar in type and frequency to those reported in younger adults. In a clinical study of FluMist in persons 65 years of age and older, subjects with underlying high-risk medical conditions (N = 200) were studied for safety. Compared to controls, FluMist recipients had a higher rate of sore throat.

11 DESCRIPTION

FluMist Quadrivalent (Influenza Vaccine Live, Intranasal) is a live quadrivalent vaccine for administration by intranasal spray. FluMist Quadrivalent contains four vaccine virus strains: an A/H1N1 strain, an A/H3N2 strain and two B strains. FluMist Quadrivalent contains B strains from both the B/Yamagata/16/88 and the B/Victoria/2/87 lineages. FluMist Quadrivalent is manufactured according to the same process as FluMist.

The influenza virus strains in FluMist Quadrivalent are (a) *cold-adapted* (*ca*) (i.e., they replicate efficiently at 25°C, a temperature that is restrictive for replication of many wild-type influenza viruses); (b) *temperature-sensitive* (*ts*) (i.e., they are restricted in replication at 37°C (Type B strains) or 39°C (Type A strains), temperatures at which many wild-type influenza viruses grow efficiently); and (c) *attenuated* (*att*) (i.e., they do not produce classic influenza-like illness in the ferret model of human influenza infection).

No evidence of reversion has been observed in the recovered vaccine strains that have been tested (135 of possible 250 recovered isolates) using FluMist *[see Clinical Pharmacology (12.2)]*. For each of the four reassortant strains in FluMist Quadrivalent, the six internal gene segments responsible for *ca*, *ts*, and *att* phenotypes are derived from a master donor virus (MDV), and the two segments that encode the two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), are derived from the corresponding antigenically relevant wild-type influenza viruses. Thus, the four viruses contained in FluMist Quadrivalent maintain the replication characteristics and phenotypic properties of the MDV and express the HA and NA of wild-type viruses. For the Type A MDV, at least five genetic loci in three different internal gene segments contribute to the *ts* and *att* phenotypes. For the Type B MDV, at least three genetic loci in two different internal gene segments contribute to both the *ts* and *att* properties; five genetic loci in three gene segments control the *ca* property.

Each of the reassortant strains in FluMist Quadrivalent express the HA and NA of wild- type viruses that are related to strains expected to circulate during the 2022-2023 influenza season. Three of the viruses

(A/H1N1, A/H3N2 and one B strain) have been recommended by the United States Public Health Service (USPHS) for inclusion in the annual trivalent and quadrivalent influenza vaccine formulations. An additional B strain has been recommended by the USPHS for inclusion in the quadrivalent influenza vaccine formulation.

Specific pathogen-free (SPF) eggs are inoculated with each of the reassortant strains and incubated to allow vaccine virus replication. The allantoic fluid of these eggs is harvested, pooled, and then clarified by filtration. The virus is concentrated by ultracentrifugation and diluted with stabilizing buffer to obtain the final sucrose and potassium phosphate concentrations. The viral harvests are then sterile filtered to produce the monovalent bulks. Each lot is tested for *ca*, *ts*, and *att* phenotypes and is also tested extensively by *in vitro* and *in vivo* methods to detect adventitious agents. Monovalent bulks from the four strains are subsequently blended and diluted as required to attain the desired potency with stabilizing buffers to produce the quadrivalent bulk vaccine. The bulk vaccine is then filled directly into individual sprayers for nasal administration.

Each pre-filled refrigerated FluMist Quadrivalent sprayer contains a single 0.2 mL dose. Each 0.2 mL dose contains $10^{6.5-7.5}$ FFU (fluorescent focus units) of live attenuated influenza virus reassortants of each of the four strains: A/Victoria/1/2020 (H1N1) (an A/Victoria/2570/2019 (H1N1)pdm09 - like virus), A/Norway/16606/2021 (H3N2) (an A/Darwin/9/2021 (H3N2) - like virus), B/Phuket/3073/2013 (B/Yamagata lineage), and B/Austria/1359417/2021 (B/Victoria lineage). Each 0.2 mL dose also contains 0.188 mg/dose monosodium glutamate, 2.00 mg/dose hydrolyzed porcine gelatin, 2.42 mg/dose arginine, 13.68 mg/dose sucrose, 2.26 mg/dose dibasic potassium phosphate, and 0.96 mg/dose monobasic potassium phosphate. Each dose contains residual amounts of ovalbumin (< 0.024 mcg/dose), and may also contain residual amounts of gentamicin sulfate (< 0.015 mcg/mL), and ethylenediaminetetraacetic acid (EDTA) (< 2.3 mcg/dose). FluMist Quadrivalent contains no preservatives.

The tip attached to the sprayer is equipped with a nozzle that produces a fine mist that is primarily deposited in the nose and nasopharynx. FluMist Quadrivalent is a colorless to pale yellow suspension and is clear to slightly cloudy.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Immune mechanisms conferring protection against influenza following receipt of FluMist Quadrivalent vaccine are not fully understood; serum antibodies, mucosal antibodies, and influenza-specific T cells may play a role.

FluMist and FluMist Quadrivalent contain live attenuated influenza viruses that must infect and replicate in cells lining the nasopharynx of the recipient to induce immunity. Vaccine viruses capable of infection and replication can be cultured from nasal secretions obtained from vaccine recipients (shedding) *[see Pharmacodynamics (12.2)]*.

12.2 Pharmacodynamics

Shedding Studies

Shedding of vaccine viruses within 28 days of vaccination with FluMist was evaluated in (1) multi-center Study MI-CP129 which enrolled healthy individuals 6 through 59 months of age (N = 200); and (2) multi-center Study FM026 which enrolled healthy individuals 5 through 49 years of age (N = 344). In each study, nasal secretions were obtained daily for the first 7 days and every other day through either Day 25 and on Day 28 or through Day 28. In Study MI-CP129, individuals with a positive shedding sample at Day 25 or Day 28 were to have additional shedding samples collected every 7 days until culture negative on 2 consecutive samples. Results of these studies are presented in Table 5.

Table 5: Characterization of Shedding with FluMist in Specified Age Groups by Frequency, Amount, and Duration (Study MI-CP129[a] and Study FM026[b])

Age	Number of Subjects	% Shedding[c]	Peak Titer ($TCID_{50}$/mL)[d]	% Shedding After Day 11	Day of Last Positive Culture
6-23 months[e]	99	89	< 5 log_{10}	7.0	Day 23[f]
24-59 months	100	69	< 5 log_{10}	1.0	Day 25[g]
5-8 years	102	50	< 5 log_{10}	2.9	Day 23[h]
9-17 years	126	29	< 4 log_{10}	1.6	Day 28[h]
18-49 years	115	20	< 3 log_{10}	0.9	Day 17[h]

[a] NCT00344305; see www.clinicaltrials.gov
[b] NCT00192140; see www.clinicaltrials.gov
[c] Proportion of subjects with detectable virus at any time point during the 28 days.
[d] Peak titer at any time point during the 28 days among samples positive for a single vaccine virus.
[e] FluMist and FluMist Quadrivalent are not approved for use in children younger than 24 months of age *[see Adverse Reactions (6.1)]*.
[f] A single subject who shed previously on Days 1-3; $TCID_{50}$/mL was less than 1.5 log_{10} on Day 23.
[g] A single subject who did not shed previously; $TCID_{50}$/mL was less than 1.5 log_{10}.
[h] A single subject who did not shed previously; $TCID_{50}$/mL was less than 1.0 log_{10}.

The highest proportion of subjects in each group shed one or more vaccine strains on Days 2-3 post vaccination. After Day 11 among individuals 2 through 49 years of age (n = 443), virus titers did not exceed 1.5 log_{10} $TCID_{50}$/mL.

Studies in Immunocompromised Individuals

Safety and shedding of vaccine virus following FluMist administration were evaluated in 28 HIV-infected adults [median CD4 cell count of 541 cells/mm^3] and 27 HIV-negative adults 18 through 58 years of age. No serious adverse events were reported during the one-month follow-up period. Vaccine strain (type B) virus was detected in 1 of 28 HIV-infected subjects on Day 5 only, and in none of the HIV-negative FluMist recipients.

Safety and shedding of vaccine virus following FluMist administration were also evaluated in children in a randomized (1:1), cross-over, double-blind, AF-SPG placebo-controlled trial in 24 HIV-infected children [median CD4 cell count of 1013 cells/mm^3] and 25 HIV-negative children 1 through 7 years of age, and in a randomized (1:1), open-label, inactivated influenza vaccine-controlled trial in 243 HIV-infected children

and adolescents 5 through 17 years of age receiving stable anti-retroviral therapy. Frequency and duration of vaccine virus shedding in HIV-infected individuals were comparable to that seen in healthy individuals. No adverse effects on HIV viral load or CD4 counts were identified following FluMist administration. In the 5 through 17 year old age group, one inactivated influenza vaccine recipient and one FluMist recipient experienced pneumonia within 28 days of vaccination (days 17 and 13, respectively). The effectiveness of FluMist and FluMist Quadrivalent in preventing influenza illness in HIV-infected individuals has not been evaluated.

Twenty mild to moderately immunocompromised children and adolescents 5 through 17 years of age (receiving chemotherapy and/or radiation therapy or who had received chemotherapy in the 12 weeks prior to enrollment) were randomized 1:1 to receive FluMist or AF-SPG placebo. Frequency and duration of vaccine virus shedding in these immunocompromised children and adolescents were comparable to that seen in healthy children and adolescents. The effectiveness of FluMist and FluMist Quadrivalent in preventing influenza illness in immunocompromised individuals has not been evaluated.

Transmission Study

A prospective, randomized, double-blind, placebo-controlled trial was performed in a daycare setting in children younger than 3 years of age to assess the transmission of vaccine viruses from a vaccinated individual to a non-vaccinated individual. A total of 197 children 8 through 36 months of age were randomized to receive one dose of FluMist (N = 98) or AF-SPG placebo (N = 99). Virus shedding was evaluated for 21 days by culture of nasal swab specimens. Wild-type A (A/H3N2) influenza virus was documented to have circulated in the community and in the study population during the trial, whereas Type A (A/H1N1) and Type B strains did not.

At least one vaccine strain was isolated from 80% of FluMist recipients; strains were recovered from 1-21 days post vaccination (mean duration of 7.6 days ± 3.4 days). The cold-adapted (*ca*) and temperature-sensitive (*ts*) phenotypes were preserved in 135 tested of 250 strains isolated at the local laboratory. Ten influenza isolates (9 influenza A, 1 influenza B) were cultured from a total of seven placebo subjects. One placebo subject had mild symptomatic Type B virus infection confirmed as a transmitted vaccine virus by a FluMist recipient in the same playgroup. This Type B isolate retained the *ca*, *ts*, and *att* phenotypes of the vaccine strain and had the same genetic sequence when compared to a Type B virus cultured from a vaccine recipient within the same playgroup. Four of the influenza Type A isolates were confirmed as wild-type A/Panama (H3N2). The remaining isolates could not be further characterized.

Assuming a single transmission event (isolation of the Type B vaccine strain), the probability of a young child acquiring vaccine virus following close contact with a single FluMist vaccinee in this daycare setting was 0.58% (95% CI: 0, 1.7) based on the Reed-Frost model. With documented transmission of one Type B in one placebo subject and possible transmission of Type A viruses in four placebo subjects, the probability of acquiring a transmitted vaccine virus was estimated to be 2.4% (95% CI: 0.13, 4.6) using the Reed-Frost model.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

FluMist Quadrivalent has not been evaluated for its carcinogenic or mutagenic potential or its potential to impair fertility.

14 CLINICAL STUDIES

The effectiveness of FluMist Quadrivalent is based on data demonstrating the clinical efficacy of FluMist in children and the effectiveness of FluMist in adults, and a comparison of post vaccination geometric mean titers (GMTs) of hemagglutination inhibition (HI) antibodies between individuals receiving FluMist and FluMist Quadrivalent. The clinical experience with FluMist is relevant to FluMist Quadrivalent because both vaccines are manufactured using the same process and have overlapping compositions *[see Description (11)]*.

14.1 Efficacy Studies of FluMist in Children and Adolescents

A multi-national, randomized, double-blind, active-controlled trial (MI-CP111) was performed to assess the efficacy of FluMist compared to an intramuscularly administered, inactivated Influenza Virus Vaccine manufactured by Sanofi Pasteur Inc. (active control) in children 6 months to less than 5 years of age during the 2004-2005 influenza season. A total number of 3916 children without severe asthma, without use of bronchodilator or steroids, and without wheezing within the prior 6 weeks were randomized to FluMist and 3936 were randomized to active control. Children who previously received any influenza vaccine received a single dose of study vaccine, while those who never previously received an influenza vaccination (or had an unknown history of influenza vaccination) received two doses. Participants were then followed through the influenza season to identify illness caused by influenza virus. As the primary endpoint, culture-confirmed modified CDC-ILI (CDC-defined influenza-like illness) was defined as a positive culture for a wild-type influenza virus associated within ±7 days of modified CDC-ILI. Modified CDC-ILI was defined as fever (temperature ≥ 100°F oral or equivalent) with cough, sore throat, or runny nose/nasal congestion on the same or consecutive days.

In the primary efficacy analysis, FluMist demonstrated a 44.5% (95% CI: 22.4, 60.6) reduction in influenza rate compared to active control as measured by culture-confirmed modified CDC-ILI caused by wild-type strains antigenically similar to those contained in the vaccine. See Table 6 for a description of the results by strain and antigenic similarity.

Table 6: Comparative Efficacy Against Culture-Confirmed Modified CDC-ILI[a] Caused by Wild-Type Strains (Study MI-CP111)[b,c]

	FluMist			Active Control[d]			% Reduction in Rate for FluMist[e]	95% CI
	N	# of Cases	Rate (cases/N)	N	# of Cases	Rate (cases/N)		
Matched Strains								
All strains	3916	53	1.4%	3936	93	2.4%	44.5%	22.4, 60.6
A/H1N1	3916	3	0.1%	3936	27	0.7%	89.2%	67.7, 97.4
A/H3N2	3916	0	0.0%	3936	0	0.0%	--	--
B	3916	50	1.3%	3936	67	1.7%	27.3%	-4.8, 49.9
Mismatched Strains								
All strains	3916	102	2.6%	3936	245	6.2%	58.2%	47.4, 67.0
A/H1N1	3916	0	0.0%	3936	0	0.0%	--	--
A/H3N2	3916	37	0.9%	3936	178	4.5%	79.2%	70.6, 85.7
B	3916	66	1.7%	3936	71	1.8%	6.3%	-31.6, 33.3
Regardless of Match								
All strains	3916	153	3.9%	3936	338	8.6%	54.9%	45.4, 62.9
A/H1N1	3916	3	0.1%	3936	27	0.7%	89.2%	67.7, 97.4
A/H3N2	3916	37	0.9%	3936	178	4.5%	79.2%	70.6, 85.7
B	3916	115	2.9%	3936	136	3.5%	16.1%	-7.7, 34.7

ATP Population.

[a] Modified CDC-ILI was defined as fever (temperature ≥ 100°F oral or equivalent) plus cough, sore throat, or runny nose/nasal congestion on the same or consecutive days.
[b] In children 6 months through 5 years of age
[c] NCT00128167; see www.clinicaltrials.gov
[d] Inactivated Influenza Virus Vaccine manufactured by Sanofi Pasteur Inc., administered intramuscularly.
[e] Reduction in rate was adjusted for country, age, prior influenza vaccination status, and wheezing history status.

A randomized, double-blind, saline placebo-controlled trial (D153-P501) was performed to evaluate the efficacy of FluMist in children 12 through 35 months of age without high-risk medical conditions against culture-confirmed influenza illness. This study was performed in Asia over two successive seasons (2000-2001 and 2001-2002). The primary endpoint of the trial was the prevention of culture-confirmed influenza illness due to antigenically matched wild-type influenza. Respiratory illness that prompted an influenza culture was defined as at least one of the following: fever (≥ 100.4°F rectal or ≥ 99.5°F axillary), wheezing, shortness of breath, pulmonary congestion, pneumonia, or otitis media; or two of the following: runny nose/nasal congestion, sore throat, cough, muscle aches, chills, headache, irritability, decreased activity, or vomiting. A total of 3174 children were randomized 3:2 (vaccine:placebo) to receive 2 doses of study vaccine or placebo at least 28 days apart in Year 1. See Table 7 for a description of the results.

During the second year of Study D153-P501, for children who received two doses in Year 1 and one dose in Year 2, FluMist demonstrated 84.3% (95% CI: 70.1, 92.4) efficacy against culture-confirmed influenza illness due to antigenically matched wild-type influenza.

Study AV006 was a second multi-center, randomized, double-blind, AF-SPG placebo-controlled trial performed in U.S. children without high-risk medical conditions to evaluate the efficacy of FluMist against culture-confirmed influenza over two successive seasons (1996-1997 and 1997-1998). The primary endpoint of the trial was the prevention of culture-confirmed influenza illness due to antigenically matched wild-type influenza in children who received two doses of vaccine in the first year and a single revaccination dose in the second year. Respiratory illness that prompted an influenza culture was defined as at least one of the following: fever (≥ 101°F rectal or oral; or ≥ 100.4°F axillary), wheezing, shortness of breath, pulmonary congestion, pneumonia, or otitis media; or two of the following: runny nose/nasal congestion, sore throat, cough, muscle aches, chills, headache, irritability, decreased activity, or vomiting. During the first year of the study, 1602 children 15 through 71 months of age were randomized 2:1 (vaccine:placebo). See Table 7 for a description of the results.

Table 7: Efficacy[a] of FluMist vs. Placebo Against Culture-Confirmed Influenza Illness Due to Antigenically Matched Wild-Type Strains (Studies D153-P501[b] & AV006[c], Year 1)

	D153-P501[d]			AV006[e]		
	FluMist n[f] (%)	**Placebo n[f] (%)**	**% Efficacy (95% CI)**	**FluMist n[f] (%)**	**Placebo n[f] (%)**	**% Efficacy (95% CI)**
	N[g] = 1653	**N[g] = 1111**		**N[g] = 849**	**N[g] = 410**	
Any strain	56 (3.4%)	139 (12.5%)	72.9%[h] (62.8, 80.5)	10 (1%)	73 (18%)	93.4% (87.5, 96.5)
A/H1N1	23 (1.4%)	81 (7.3%)	80.9% (69.4, 88.5)[i]	0	0	--
A/H3N2	4 (0.2%)	27 (2.4%)	90.0% (71.4, 97.5)	4 (0.5%)	48 (12%)	96.0% (89.4, 98.5)
B	29 (1.8%)	35 (3.2%)	44.3% (6.2, 67.2)	6 (0.7%)	31 (7%)	90.5% (78.0, 95.9)

[a] D153-P501 and AV006 data are for subjects who received two doses of study vaccine.
[b] In children 12 through 35 months of age
[c] In children 15 through 71 months of age
[d] NCT00192244; see www.clinicaltrials.gov
[e] NCT00192179; see www.clinicaltrials.gov
[f] Number and percent of subjects in per-protocol efficacy analysis population with culture-confirmed influenza illness.
[g] Number of subjects in per-protocol efficacy analysis population of each treatment group of each study for the "any strain" analysis.
[h] For D153-P501, influenza circulated through 12 months following vaccination.
[i] Estimate includes A/H1N1 and A/H1N2 strains. Both were considered antigenically similar to the vaccine.

During the second year of Study AV006, children remained in the same treatment group as in Year 1 and received a single dose of FluMist or placebo. During the second year, the primary circulating strain was the A/Sydney/05/97 H3N2 strain, which was antigenically dissimilar from the H3N2 strain represented in the vaccine, A/Wuhan/359/95; FluMist demonstrated 87.0% (95% CI: 77.0, 92.6) efficacy against culture-confirmed influenza illness.

14.2 Immune Response Study of FluMist Quadrivalent in Children and Adolescents

A multi-center, randomized, double-blind, active-controlled, non-inferiority study (MI-CP208) was performed to assess the immunogenicity of FluMist Quadrivalent compared to FluMist (active control) in children and adolescents 2 through 17 years of age. A total of 2312 subjects were randomized by site at a 3:1:1 ratio to receive either FluMist Quadrivalent or one of two formulations of comparator vaccine FluMist, each containing a B strain that corresponded to one of the two B strains in FluMist Quadrivalent (a B strain of the Yamagata lineage or a B strain of the Victoria lineage).

Children 2 through 8 years of age received 2 doses of vaccine approximately 30 days apart; children 9 years of age and older received 1 dose. For children 2 through 8 years of age with a history of influenza vaccination, immunogenicity assessments were performed prior to vaccination and at 28 days after the first dose. For children 2 through 8 years of age without a history of influenza vaccination, immunogenicity assessments were performed prior to vaccination and 28 days after the second dose. For children

9 years of age and older, immunogenicity assessments were performed prior to vaccination and at 28 days post vaccination.

Immunogenicity was evaluated by comparing the 4 strain-specific serum hemagglutination inhibition (HAI) antibody geometric mean titers (GMTs) post dosing and provided evidence that the addition of the second B strain did not result in immune interference to other strains included in the vaccine.

14.3 Effectiveness Study of FluMist in Adults

AV009 was a U.S. multi-center, randomized, double-blind, AF-SPG placebo-controlled trial to evaluate effectiveness of FluMist in adults 18 through 64 years of age without high-risk medical conditions over the 1997-1998 influenza season. Participants were randomized 2:1 (vaccine:placebo). Cultures for influenza virus were not obtained from subjects in the trial, thus efficacy against culture-confirmed influenza was not assessed. The A/Wuhan/359/95 (H3N2) strain, which was contained in FluMist, was antigenically distinct from the predominant circulating strain of influenza virus during the trial period, A/Sydney/05/97 (H3N2). Type A/Wuhan (H3N2) and Type B strains also circulated in the U.S. during the study period. The primary endpoint of the trial was the reduction in the proportion of participants with one or more episodes of any febrile illness, and prospective secondary endpoints were severe febrile illness and febrile upper respiratory illness. Effectiveness for any of the three endpoints was not demonstrated in a subgroup of adults 50 through 64 years of age. Primary and secondary effectiveness endpoints from the age group 18 through 49 years are presented in Table 8. Effectiveness was not demonstrated for the primary endpoint in adults 18 through 49 years of age.

Table 8: Effectiveness of FluMist to Prevent Febrile Illness in Adults 18 through 49 Years of Age During the 7-Week Site-Specific Outbreak Period (Study AV009)

Endpoint	FluMist N = 2411[a] n (%)	Placebo N = 1226[a] n (%)	Percent Reduction	(95% CI)
Participants with one or more events of:[b]				
Primary Endpoint:				
Any febrile illness	331 (13.73)	189 (15.42)	**10.9**	(-5.1, 24.4)
Secondary Endpoints:				
Severe febrile illness	250 (10.37)	158 (12.89)	**19.5**	(3.0, 33.2)
Febrile upper respiratory illness	213 (8.83)	142 (11.58)	**23.7**	(6.7, 37.5)

[a] Number of evaluable subjects (92.7% and 93.0% of FluMist and placebo recipients, respectively).
[b] The predominantly circulating virus during the trial period was A/Sydney/05/97 (H3N2), an antigenic variant not included in the vaccine.

Effectiveness was shown in a post-hoc analysis using an endpoint of CDC-ILI in the age group 18 through 49 years of age.

14.4 Immune Response Study of FluMist Quadrivalent in Adults

A multicenter, randomized, double-blind, active-controlled, and non-inferiority study (MI-CP185) was performed to assess the safety and immunogenicity of FluMist Quadrivalent compared to those of FluMist

(active control) in adults 18 through 49 years of age. A total of 1800 subjects were randomized by site at a 4:1:1 ratio to receive either 1 dose of FluMist Quadrivalent or 1 dose of one of two formulations of comparator vaccine, FluMist, each containing a B strain that corresponded to one of the two B strains in FluMist Quadrivalent (a B strain of the Yamagata lineage and a B strain of the Victoria lineage).

Immunogenicity in Study MI-CP185 was evaluated by comparing the 4 strain-specific serum hemagglutination inhibition (HAI) antibody geometric mean titers (GMTs) post dosing and provided evidence that the addition of the second B strain did not result in immune interference to other strains included in the vaccine.

14.5 Concomitantly Administered Live Virus Vaccines

In Study AV018, concomitant administration of FluMist, MMR (manufactured by Merck & Co., Inc.) and Varicella Virus Vaccine Live (manufactured by Merck & Co., Inc.) was studied in 1245 subjects 12 through 15 months of age. Subjects were randomized in a 1:1:1 ratio to MMR, Varicella vaccine and AF-SPG placebo (group 1); MMR, Varicella vaccine and FluMist (group 2); or FluMist alone (group 3). Immune responses to MMR and Varicella vaccines were evaluated 6 weeks post-vaccination while the immune responses to FluMist were evaluated 4 weeks after the second dose. No evidence of interference with immune response to measles, mumps, rubella, varicella and FluMist vaccines was observed.

15 REFERENCES

1. Lasky T, Terracciano GJ, Magder L, et al. The Guillain-Barré syndrome and the 1992 – 1993 and 1993 – 1994 influenza vaccines. N Engl J Med 1998;339(25):1797-802.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

FluMist Quadrivalent is supplied in a package of 10 pre-filled, single-dose (0.2 mL) intranasal sprayers. The single-use intranasal sprayer is not made with natural rubber latex.
Carton containing 10 intranasal sprayers: NDC 66019-309-10

Single intranasal sprayer: NDC 66019-309-01

16.2 Storage and Handling

The cold chain [2-8°C (35-46°F)] must be maintained when transporting FluMist Quadrivalent.

FLUMIST QUADRIVALENT SHOULD BE STORED IN A REFRIGERATOR BETWEEN 2-8°C (35-46°F) UPON RECEIPT. THE PRODUCT MUST BE USED BEFORE THE EXPIRATION DATE ON THE SPRAYER LABEL.

DO NOT FREEZE.

Keep FluMist Quadrivalent sprayer in outer carton in order to protect from light.

A single temperature excursion up to 25°C (77°F) for 12 hours has been shown to have no adverse impact on the vaccine. After a temperature excursion, the vaccine should be returned immediately to the recommended storage condition (2°C – 8°C) and used as soon as feasible. Subsequent excursions are not permitted.

Once FluMist Quadrivalent has been administered or has expired, the sprayer should be disposed of according to the standard procedures for medical waste (e.g., sharps container or biohazard container).

17 PATIENT COUNSELING INFORMATION

Advise the vaccine recipient or caregiver to read the FDA-approved patient labeling (Information for Patients and Their Caregivers).

Inform vaccine recipients or their parents/guardians of the need for two doses at least 1 month apart in children 2 through 8 years of age, depending on vaccination history. Provide the Vaccine Information Statements (VIS) which are required by the National Childhood Vaccine Injury Act of 1986 to be given with each immunization.

17.1 Asthma and Recurrent Wheezing

Ask the vaccinee or their parent/guardian if the vaccinee has asthma. For children younger than 5 years of age, also ask if the vaccinee has recurrent wheezing since this may be an asthma equivalent in this age group. Inform the vaccinee or their parent/guardian that there may be an increased risk of wheezing associated with FluMist Quadrivalent in persons younger than 5 years of age with recurrent wheezing and persons of any age with asthma *[see Warnings and Precautions (5.2)]*.

17.2 Vaccination with a Live Virus Vaccine

Inform vaccine recipients or their parents/guardians that FluMist Quadrivalent is an attenuated live virus vaccine and has the potential for transmission to immunocompromised household contacts.

17.3 Adverse Event Reporting

Instruct the vaccine recipient or their parent/guardian to report adverse reactions to their healthcare provider.

FluMist® is a registered trademark of MedImmune, LLC.

MedImmune

Manufactured by:

MedImmune, LLC

Gaithersburg, MD 20878

1-877-633-4411

U.S. Government License No. 1799

Information for Patients and Their Caregivers

FluMist® Quadrivalent (pronounced FLEW-mĭst Kwä-drə-VĀ-lənt)
(Influenza Vaccine Live, Intranasal)

Please read this Patient Information carefully before you or your child is vaccinated with FluMist Quadrivalent.

This is a summary of information about FluMist Quadrivalent. It does not take the place of talking with your healthcare provider about influenza vaccination. If you have questions or would like more information, please talk with your healthcare provider.

What is FluMist Quadrivalent?

FluMist Quadrivalent is a vaccine that is sprayed into the nose to help protect against influenza. It can be used in children, adolescents, and adults ages 2 through 49. FluMist Quadrivalent is similar to MedImmune's trivalent Influenza Vaccine Live, Intranasal (FluMist), except FluMist Quadrivalent provides protection against an additional influenza strain. FluMist Quadrivalent may not prevent influenza in everyone who gets vaccinated.

Who should not get FluMist Quadrivalent?

You should not get FluMist Quadrivalent if you:

- have a severe allergy to eggs or to any inactive ingredient in the vaccine (see "What are the ingredients in FluMist Quadrivalent?")
- have ever had a life-threatening reaction to influenza vaccinations
- are 2 through 17 years old and take aspirin or medicines containing aspirin. Children or adolescents should not be given aspirin for 4 weeks after getting FluMist or FluMist Quadrivalent unless your healthcare provider tells you otherwise.

Please talk to your healthcare provider if you are not sure if the items listed above apply to you or your child.

Children under 2 years old have an increased risk of wheezing (difficulty with breathing) after getting FluMist Quadrivalent.

Who may not be able to get FluMist Quadrivalent?

Tell your healthcare provider if you or your child:

- are currently wheezing
- have a history of wheezing if under 5 years old
- have had Guillain-Barré syndrome
- have a weakened immune system or live with someone who has a severely weakened immune system

- have problems with your heart, kidneys, or lungs
- have diabetes
- are pregnant or nursing
- are taking Tamiflu®, Relenza®, amantadine, or rimantadine

If you or your child cannot take FluMist Quadrivalent, you may still be able to get an influenza shot. Talk to your healthcare provider about this.

How is FluMist Quadrivalent given?

- FluMist Quadrivalent is a liquid that is sprayed into the nose.
- You can breathe normally while getting FluMist Quadrivalent. There is no need to inhale or "sniff" it.
- People 9 years of age and older need one dose of FluMist Quadrivalent each year.
- Children 2 through 8 years old may need 2 doses of FluMist Quadrivalent, depending on their history of previous influenza vaccination. Your healthcare provider will decide if your child needs to come back for a second dose.

What are the possible side effects of FluMist Quadrivalent?

The most common side effects are:

- runny or stuffy nose
- sore throat
- fever over 100°F

Other possible side effects include:

- decreased appetite
- irritability
- tiredness
- cough
- headache
- muscle ache
- chills

Call your healthcare provider or go to the emergency department right away if you or your child experience:

- hives or a bad rash
- trouble breathing
- swelling of the face, tongue, or throat

These are not all the possible side effects of FluMist Quadrivalent. You can ask your healthcare provider for a complete list of side effects that is available to healthcare professionals.

Call your healthcare provider for medical advice about side effects. You may report side effects to VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

What are the ingredients in FluMist Quadrivalent?

Active Ingredient: FluMist Quadrivalent contains 4 influenza virus strains that are weakened (A(H1N1), A(H3N2), B Yamagata lineage, and B Victoria lineage).

Inactive Ingredients: monosodium glutamate, gelatin, arginine, sucrose, dibasic potassium phosphate, monobasic potassium phosphate, and gentamicin.

FluMist Quadrivalent does not contain preservatives.

How is FluMist Quadrivalent Stored?

FluMist Quadrivalent is stored in a refrigerator (not the freezer) between 35-46°F (2-8°C) upon receipt. FluMist Quadrivalent sprayer must be kept in the carton until use in order to protect from light. FluMist Quadrivalent must be used before the expiration date on the sprayer label.

If you would like more information, talk to your healthcare provider or visit www.flumistquadrivalent.com or call 1-877-633-4411.

MedImmune

Manufactured by:

MedImmune, LLC

Gaithersburg, MD 20878

Issue date: August 2022
RAL-FLUQV11

Sanofi Pasteur
522 Fluzone® High-Dose Quadrivalent

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Fluzone® High-Dose Quadrivalent safely and effectively. See full prescribing information for Fluzone High-Dose Quadrivalent.

Fluzone High-Dose Quadrivalent (Influenza Vaccine), Suspension, for intramuscular injection
20XX-20XX Formula
Initial U.S. Approval: 20XX

----------INDICATIONS AND USAGE----------

Fluzone High-Dose Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. (1)

Fluzone High-Dose Quadrivalent is approved for use in persons 65 years of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------

For intramuscular use only

A single 0.7 mL dose for intramuscular injection in adults 65 years of age and older (2.1)

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection in prefilled syringe, 0.7 mL (3)

----------CONTRAINDICATIONS----------

Severe allergic reaction to any component of the vaccine, including egg protein, or after previous dose of any influenza vaccine (4)

----------WARNINGS AND PRECAUTIONS----------

If Guillain-Barré syndrome (GBS) has occurred within 6 weeks following previous influenza vaccination, the decision to give Fluzone High-Dose Quadrivalent should be based on careful consideration of the potential benefits and risks. (5.1)

----------ADVERSE REACTIONS----------

In adults ≥65 years of age, the most common injection-site reaction was pain (41.3%); the most common solicited systemic adverse event was myalgia (22.7%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or https://vaers.hhs.gov.

See **17 PATIENT COUNSELING INFORMATION and FDA-approved patient labeling**.

Revised: 1/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Fluzone® High-Dose Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza caused by influenza A subtype viruses and type B viruses contained in the vaccine.

Fluzone High-Dose Quadrivalent is indicated for use in persons 65 years of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only

2.1 Dose and Schedule

Fluzone High-Dose Quadrivalent should be administered as a single 0.7 mL injection by the intramuscular route in adults 65 years of age and older.

2.2 Administration

Inspect Fluzone High-Dose Quadrivalent visually for particulate matter and/or discoloration prior to administration. If either of these conditions exists the vaccine should not be administered.

Before administering a dose of vaccine, shake the prefilled syringe.

The preferred site for intramuscular injection is the deltoid muscle. The vaccine should not be injected into the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously.

Fluzone High-Dose Quadrivalent should not be combined through reconstitution or mixed with any other vaccine.

3 DOSAGE FORMS AND STRENGTHS

Fluzone High-Dose Quadrivalent is a suspension for injection.

Fluzone High-Dose Quadrivalent is supplied in prefilled syringes, 0.7 mL, for adults 65 years of age and older.

4 CONTRAINDICATIONS

A severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine *[see Description (11)]*, including egg protein, or to a previous dose of any influenza vaccine is a contraindication to administration of Fluzone High-Dose Quadrivalent.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

If Guillain-Barré syndrome (GBS) has occurred within 6 weeks following any previous influenza vaccination, the decision to give Fluzone High-Dose Quadrivalent should be based on careful consideration of the potential benefits and risks.

The 1976 swine influenza vaccine was associated with an elevated risk of GBS. Evidence for a causal relation of GBS with other influenza vaccines is inconclusive; if an excess risk exists, it is probably slightly more than 1 additional case per 1 million persons vaccinated. GBS has also been temporally associated with influenza disease. (See references 1 and 2.)

5.2 Preventing and Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

5.3 Altered Immunocompetence

If Fluzone High-Dose Quadrivalent is administered to immunocompromised persons, including those receiving immunosuppressive therapy, the immune response may be lower than expected.

5.4 Limitations of Vaccine Effectiveness

Vaccination with Fluzone High-Dose Quadrivalent may not protect all recipients.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse event rates observed in the clinical trial(s) of a vaccine cannot be directly compared to rates in the clinical trial(s) of another vaccine and may not reflect the rates observed in practice. One clinical study has evaluated the safety of Fluzone High-Dose Quadrivalent.

Study 1 (NCT03282240, see https://clinicaltrials.gov) was a randomized, active-controlled, modified double-blind pre-licensure trial conducted in the U.S. The study compared the safety and immunogenicity of Fluzone High-Dose Quadrivalent to those of Fluzone High-Dose (trivalent formulation). The safety analysis set included 1777 Fluzone High-Dose Quadrivalent recipients, 443 Fluzone High-Dose recipients, and 450 investigational Fluzone High-Dose containing the alternate B influenza strain recipients.

The most common reactions occurring after Fluzone High-Dose Quadrivalent administration were injection-site pain (41.3%), myalgia (22.7%), headache (14.4%), and malaise (13.2%). Onset usually occurred within the first 3 days after vaccination. The majority of solicited reactions resolved within three days of vaccination.

Table 1 displays solicited adverse reactions for Fluzone High-Dose Quadrivalent compared to Fluzone High-Dose reported within 7 days after vaccination and collected using standardized diary cards.

Table 1: Study 1[a]: Frequency of Solicited Injection-Site Reactions and Systemic Adverse Events within 7 Days after Vaccination with Fluzone High-Dose Quadrivalent or Fluzone High-Dose, Adults 65 Years of Age and Older

	Fluzone High-Dose Quadrivalent (N[b]=1761-1768) Percentage		Fluzone High-Dose[f] (N[b]=885-889) Percentage	
	Any	**Grade 3**	**Any**	**Grade 3**
Local Reactions				
Injection Site Pain[c]	41.3	0.7	36.4	0.2
Injection Site Erythema[d]	6.2	0.6	5.7	0.2
Injection Site Swelling[d]	4.9	0.3	4.7	0.1
Injection Site Induration[d]	3.7	0.2	3.5	0.1
Injection Site Bruising[d]	1.3	0.0	1.1	0.0
Systemic Reactions				
Myalgia[c]	22.7	0.9	18.9	0.7
Headache[c]	14.4	0.6	13.6	0.4
Malaise[c]	13.2	0.7	13.4	0.4
Shivering[c]	5.4	0.3	4.7	0.3
Fever[e]	0.4	0.2	0.9	0.2

[a] NCT03282240
[b] N is the number of vaccinated participants with available data for the events listed
[c] Grade 3: A type of AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.
[d] Grade 3: > 100 mm
[e] Grade 3: ≥ 102.1°F (39.0°C)
[f] Safety results for the Fluzone High-Dose and investigational Fluzone High-Dose containing the alternate B influenza strain recipients were pooled for the analysis.

Based on data from Fluzone High-Dose, solicited injection site reactions and systemic adverse reactions were slightly more frequent after vaccination with Fluzone High-Dose compared to a standard-dose vaccine.

Unsolicited non-serious adverse events were reported in 279 (15.7%) recipients in the Fluzone High-Dose Quadrivalent group and 140 (15.7%) recipients in the Fluzone High-Dose group. The most commonly reported unsolicited adverse event was cough.

Within 180 days post-vaccination, 80 (4.5%) Fluzone High-Dose Quadrivalent recipients and 48 (5.4%) Fluzone High-Dose recipients experienced a serious adverse event (SAE). None of the SAEs were assessed as related to the study vaccines.

6.2 Postmarketing Experience

The following additional adverse events have been spontaneously reported during the postmarketing use of Fluzone High-Dose, Fluzone, or Fluzone Quadrivalent and may occur in people receiving Fluzone High-Dose Quadrivalent. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure. Adverse events were included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Fluzone High-Dose, Fluzone, or Fluzone Quadrivalent.

- *Blood and Lymphatic System Disorders:* Thrombocytopenia, lymphadenopathy
- *Immune System Disorders:* Anaphylaxis, other allergic/hypersensitivity reactions (including urticaria, angioedema)
- *Eye Disorders:* Ocular hyperemia
- *Nervous System Disorders:* Guillain-Barré syndrome (GBS), convulsions, febrile convulsions, myelitis (including encephalomyelitis and transverse myelitis), facial palsy (Bell's palsy), optic neuritis/neuropathy, brachial neuritis, syncope (shortly after vaccination), dizziness, paresthesia
- *Vascular Disorders:* Vasculitis, vasodilatation
- *Respiratory, Thoracic and Mediastinal Disorders:* Dyspnea, cough, wheezing, throat tightness, oropharyngeal pain, and rhinorrhea
- *Gastrointestinal Disorders:* Vomiting
- *Skin and Subcutaneous Tissue Disorders:* Stevens-Johnson syndrome
- *General Disorders and Administration Site Conditions:* pruritus, asthenia/fatigue, chest pain, chills

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Fluzone High-Dose Quadrivalent is not approved for use in persons <65 years of age. There are limited human data on Fluzone High-Dose and no animal data available on Fluzone High-Dose Quadrivalent to establish whether there is a vaccine-associated risk with use of Fluzone High-Dose Quadrivalent in pregnancy.

8.2 Lactation

Fluzone High-Dose Quadrivalent is not approved for use in persons <65 years of age. No human or animal data are available to assess the effects of Fluzone High-Dose Quadrivalent on the breastfed infant or on milk production/excretion.

8.4 Pediatric Use

Safety and effectiveness of Fluzone High-Dose Quadrivalent in children younger than 18 years of age have not been established.

8.5 Geriatric Use

Safety, immunogenicity, and efficacy of Fluzone High-Dose Quadrivalent have been evaluated in adults 65 years of age and older *[see Adverse Reactions (6.1) and Clinical Studies (14)]*.

11 DESCRIPTION

Fluzone High-Dose Quadrivalent for intramuscular injection is an inactivated influenza vaccine, prepared from influenza viruses propagated in embryonated chicken eggs. The virus-containing allantoic fluid is harvested and inactivated with formaldehyde. Influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted using a non-ionic surfactant, octylphenol ethoxylate (Triton® X-100), producing a "split virus." The split virus is further purified and then suspended in sodium phosphate-buffered isotonic sodium chloride solution. The Fluzone High-Dose Quadrivalent process uses an additional concentration factor after the ultrafiltration step in order to obtain a higher hemagglutinin (HA) antigen concentration.

Fluzone High-Dose Quadrivalent suspension for injection is clear and slightly opalescent in color.

Neither antibiotics nor preservative are used in the manufacture of Fluzone High-Dose Quadrivalent.

The Fluzone High-Dose Quadrivalent prefilled syringe presentation is not made with natural rubber latex.

Fluzone High-Dose Quadrivalent is standardized according to United States Public Health Service requirements and is formulated to contain HA of each of the following four influenza strains recommended for the 2018-2019 influenza season: A/Michigan/45/2015 X-275 (H1N1), A/Singapore/INFIMH-16-0019/2016 IVR-186 (H3N2), B/Phuket/3073/2013 (B Yamagata lineage), and B/Maryland/15/2016 BX-69A (a B/Colorado/6/2017-like virus, B Victoria lineage). The amounts of HA and other ingredients per dose of vaccine are listed in Table 2.

Table 2: Fluzone High-Dose Quadrivalent Ingredients

Ingredient	**Quantity (per dose)**
	Fluzone High-Dose Quadrivalent 0.7 mL Dose
Active Substance: Split influenza virus, inactivated strains[a]:	240 mcg HA total
A (H1N1)	60 mcg HA
A (H3N2)	60 mcg HA
B (Victoria Lineage)	60 mcg HA
B (Yamagata Lineage)	60 mcg HA
Other:	
Sodium phosphate-buffered isotonic sodium chloride solution	QS[b] to appropriate volume
Formaldehyde	≤140 mcg
Octylphenol ethoxylate	≤350 mcg
Gelatin	None

Ingredient	Quantity (per dose)
	Fluzone High-Dose Quadrivalent 0.7 mL Dose
Preservative	None

[a] per United States Public Health Service (USPHS) requirement
[b] Quantity sufficient

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Influenza illness and its complications may follow influenza infection. Global surveillance of influenza viruses identifies yearly antigenic variants. Since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation. Specific levels of hemagglutination inhibition (HI) antibody titer post-vaccination with inactivated influenza virus vaccines have not been correlated with protection from influenza virus infection. In some human studies, antibody titers ≥1:40 have been associated with protection from influenza illness in up to 50% of participants. (See references 3 and 4.)

Antibodies against one influenza virus type or subtype confer limited or no protection against another. Furthermore, antibodies to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual change of one or more new strains in each year's influenza vaccine. Therefore, influenza vaccines are standardized to contain the hemagglutinins of influenza virus strains representing the influenza viruses likely to be circulating in the U.S. during the influenza season.

Fluzone High-Dose Quadrivalent stimulates the immune system to produce antibodies that help prevent influenza disease.

13 NONCLINICAL TOXICOLOGY

Carcinogenesis, Mutagenesis, Impairment of Fertility

Fluzone High-Dose Quadrivalent has not been evaluated for carcinogenic or mutagenic potential or for impairment of fertility.

14 CLINICAL STUDIES

14.1 Immunogenicity of Fluzone High-Dose Quadrivalent in Adults 65 Years of Age and Older

Study 1 (NCT03282240, see http://clinicaltrials.gov) was a randomized, active-controlled, modified double-blind trial in adults 65 years of age and older conducted in the US. The study compared the safety and immunogenicity of Fluzone High-Dose Quadrivalent to those of Fluzone High-Dose. The objective was to demonstrate immunologic non-inferiority of Fluzone High-Dose Quadrivalent to Fluzone High-Dose, as assessed by HAI geometric mean antibody

titers (GMTs) at Day 28 and seroconversion rates, to strains common to formulations of both vaccines, based on pre-specified criteria.

A total of 2670 adults from 65 years of age were randomized (4:1:1) to receive one dose of either Fluzone High-Dose Quadrivalent or one of two formulations of Fluzone High-Dose (one formulation contained a B strain of the Victoria lineage [TIV-HD1] while the other contained a B strain of the Yamagata lineage [TIV-HD2]).

Females accounted for 58.2% of participants in the Fluzone High-Dose Quadrivalent group and 57.4% of participants in the Fluzone High-Dose group (TIV-HD1 and TIV-HD2, pooled). The mean age was 72.9 years (range: 65 through 100 years) in the Fluzone High-Dose Quadrivalent group and the mean age was 73.0 (range: 65 through 95 years) in the Fluzone High-Dose group. The percentage of subjects 75 years of age or older was 35.4% in the Fluzone High-Dose Quadrivalent group and 35.8% in the Fluzone High-Dose group. Most participants were White (91.2% and 89.7%), followed by Black (6.8% and 8.0%), and Hispanic (2.8% and 2.6%) in the Fluzone High-Dose Quadrivalent and Fluzone High-Dose groups, respectively.

The immunogenicity results of Study 1 are summarized in Table 3 and Table 4 below.

Table 3: Study 1[a]: Post-vaccination HAI Antibody GMTs and Analyses of Non-inferiority of Fluzone High-Dose Quadrivalent Relative to Fluzone High-Dose, Adults 65 Years of Age and Older, Per-Protocol Analysis Set

Influenza Strain	GMT			GMT Ratio	Met Predefined Non-inferiority Criteria[e]
	QIV-HD N[b]=1679-1680	TIV-HD1[c] (B1 Victoria) N[b]=423	TIV-HD2[d] (B2 Yamagata) N[b]=430	QIV-HD over TIV-HD (95% CI)	
A (H1N1)[f]	312	374		0.83 (0.744; 0.932)	Yes
A (H3N2)[f]	563	594		0.95 (0.842; 1.066)	Yes
B1 (Victoria)	516	476	--	1.08 (0.958; 1.224)	Yes
B2 (Yamagata)	578	--	580	1.00 (0.881; 1.129)	Yes

[a] NCT03282240

[b] N is the number of vaccinated participants with available data for the immunologic endpoint listed

[c] TIV-HD1 contained A/Michigan/45/2015 (H1N1), A/Hong Kong/4801/2014 (H3N2), and B/Brisbane/60/2008 (B1, Victoria lineage)

[d] TIV-HD2 contained A/Michigan/45/2015 (H1N1), A/Hong Kong/4801/2014 (H3N2), and B/Phuket/3073/2013 (B2, Yamagata lineage)

[e] Predefined noninferiority criterion for the GMT ratio: the lower limit of the 95% CI of the GMT ratio (QIV-HD divided by TIV-HD) is >0.667

[f] Pooled TIV-HD group includes subjects vaccinated with either TIV-HD1 or TIV-HD2 for the A strain comparison

Table 4: Study 1[a]: Seroconversion Rates and Analyses of Non-inferiority of Fluzone High-Dose Quadrivalent Relative to Fluzone High-Dose, Adults 65 Years of Age and Older, Per-Protocol Analysis Set

Influenza Strain	Seroconversion Rates (Percentage)[b]			Difference of Seroconversion Rates	Met Predefined Non-inferiority Criteria[f]
	QIV-HD N[c]=1668-1669	TIV-HD1[d] (B1 Victoria) N[c]=420-421	TIV-HD2[e] (B2 Yamagata) N[c]=428	QIV-HD minus TIV-HD (95% CI)	
A (H1N1)[g]	50.4	53.7		-3.27 (-7.37; 0.86)	Yes
A (H3N2)[g]	49.8	50.5		-0.71 (-4.83; 3.42)	Yes
B1 (Victoria)	36.5	39.0	--	-2.41 (-7.66; 2.70)	Yes
B2 (Yamagata)	46.6	--	48.4	-1.75 (-7.04; 3.53)	Yes

[a] NCT03282240
[b] Seroconversion Rates: For subjects with a pre-vaccination titer <10 (1/dil), proportion of subjects with a post-vaccination titer ≥40 (1/dil) and for subjects with a pre-vaccination titer ≥10 (1/dil), proportion of subjects with a ≥four-fold increase from pre-vaccination to post-vaccination titer
[c] N is the number of vaccinated participants with available data for the immunologic endpoint listed
[d] TIV-HD1 contained A/Michigan/45/2015 (H1N1), A/Hong Kong/4801/2014 (H3N2), and B/Brisbane/60/2008 (B1, Victoria lineage)
[e] TIV-HD2 contained A/Michigan/45/2015 (H1N1), A/Hong Kong/4801/2014 (H3N2), and B/Phuket/3073/2013 (B2, Yamagata lineage)
[f] Predefined noninferiority criterion for seroconversion: the lower limit of the two-sided 95% CI of the difference of the seroconversion rates (QIV-HD minus TIV-HD) is >-10%
[g] Pooled TIV-HD group includes subjects vaccinated with either TIV-HD1 or TIV-HD2 for the A strain comparison

Fluzone High-Dose Quadrivalent was as immunogenic as Fluzone High-Dose for GMTs and seroconversion rates for the common influenza strains. Fluzone High-Dose Quadrivalent induced a superior immune response, based on a pre-specified superiority criterion, with respect to the additional B strain than the immune response induced by Fluzone High-Dose formulation that did not contain the additional B strain.

14.2 Efficacy of Fluzone High-Dose in Adults 65 Years of Age and Older

The efficacy of Fluzone High-Dose (trivalent formulation) is relevant to Fluzone High-Dose Quadrivalent since both vaccines are manufactured according to the same process and have overlapping compositions.

Study 2 (NCT01427309) was a multi-center, double-blind, post-licensure efficacy trial conducted in the U.S. and Canada in which adults 65 years of age and older were randomized (1:1) to receive either Fluzone High-Dose or Fluzone. The study was conducted over two influenza seasons (2011-2012 and 2012-2013); 53% of participants enrolled in the first year of the study were re-enrolled and re-randomized in the second year. The per-protocol analysis set for efficacy assessments included 15,892 Fluzone High-Dose recipients and 15,911 Fluzone

recipients. The majority (67%) of participants in the per-protocol analysis set for efficacy had one or more high-risk chronic comorbid conditions.

In the per-protocol analysis set, females accounted for 57.2% of participants in the Fluzone High-Dose group and 56.1% of participants in the Fluzone group. In both groups, the median age was 72.2 years (range 65 through 100 years). Overall, most participants in the study were White (95%); approximately 4% of study participants were Black, and approximately 6% reported Hispanic ethnicity.

The primary endpoint of the study was the occurrence of laboratory-confirmed influenza (as determined by culture or polymerase chain reaction) caused by any influenza viral type/subtype in association with influenza-like illness (ILI), defined as the occurrence of at least one of the following respiratory symptoms: sore throat, cough, sputum production, wheezing, or difficulty breathing; concurrent with at least one of the following systemic signs or symptoms: temperature >99.0°F, chills, tiredness, headaches or myalgia. Participants were monitored for the occurrence of a respiratory illness by both active and passive surveillance, starting 2 weeks post-vaccination for approximately 7 months. After an episode of respiratory illness, nasopharyngeal swab samples were collected for analysis; attack rates and vaccine efficacy were calculated (see Table 5).

Table 5: Study 2[a]: Relative Efficacy Against Laboratory-Confirmed Influenza[b] Regardless of Similarity to the Vaccine Components, Associated with Influenza-Like Illness[c], Adults 65 Years of Age and Older

	Fluzone High-Dose N[d]=15,892 n[e] (%)	Fluzone N[d]=15,911 n[e] (%)	Relative Efficacy % (95% CI)
Any type/subtype[f]	227 (1.43)	300 (1.89)	24.2 (9.7; 36.5)[g]
Influenza A	190 (1.20)	249 (1.56)	23.6 (7.4; 37.1)
A (H1N1)	8 (0.05)	9 (0.06)	11.0 (-159.9; 70.1)
A (H3N2)	171 (1.08)	222 (1.40)	22.9 (5.4; 37.2)
Influenza B[h]	37 (0.23)	51 (0.32)	27.4 (-13.1; 53.8)

[a] NCT01427309
[b] Laboratory-confirmed: culture or polymerase-chain-reaction–confirmed
[c] Occurrence of at least one of the following respiratory symptoms: sore throat, cough, sputum production, wheezing, or difficulty breathing; concurrent with at least one of the following systemic signs or symptoms: temperature >99.0°F, chills, tiredness, headaches or myalgia
[d] N is the number of vaccinated participants in the per-protocol analysis set for efficacy assessments
[e] n is the number of participants with protocol-defined influenza-like illness with laboratory confirmation
[f] Primary endpoint
[g] The prespecified statistical superiority criterion for the primary endpoint (lower limit of the 2-sided 95% CI of the vaccine efficacy of Fluzone High-Dose relative to Fluzone >9.1%) was met.
[h] In the first year of the study the influenza B component of the vaccine and the majority of influenza B cases were of the Victoria lineage; in the second year the influenza B component of the vaccine and the majority of influenza B cases were of the Yamagata lineage

A secondary endpoint of the study was the occurrence of culture-confirmed influenza caused by viral types/subtypes antigenically similar to those contained in the respective annual vaccine formulations in association with a modified CDC-defined ILI, defined as the occurrence of a temperature >99.0°F (>37.2°C) with cough or sore throat. The efficacy of Fluzone High-Dose relative to Fluzone for this endpoint was 51.1% (95% CI: 16.8; 72.0).

15 REFERENCES

1 Lasky T, Terracciano GJ, Magder L, et al. The Guillain-Barré syndrome and the 1992-1993 and 1993-1994 influenza vaccines. N Engl J Med 1998;339:1797-802.

2 Baxter, R, et al. Lack of Association of Guillain-Barré Syndrome with Vaccinations. Clin Infect Dis 2013;57(2):197-204.

3 Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. Virus Res 2004;103:133-138.

4 Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg Camb 1972;70:767-777.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Single-dose, prefilled syringe, without needle, 0.7 mL (NDC 49281-XXX-XX) (not made with natural rubber latex). Supplied as package of 10 (NDC 49281-XXX-XX).

16.2 Storage and Handling

Store Fluzone High-Dose Quadrivalent refrigerated at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Discard if vaccine has been frozen.

Do not use after the expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

See FDA-approved patient labeling (Patient Information).

- Inform the patient or caregiver that Fluzone High-Dose Quadrivalent contains killed viruses and cannot cause influenza.
- Fluzone High-Dose Quadrivalent stimulates the immune system to produce antibodies that help protect against influenza.
- Instruct that annual influenza vaccination is recommended.
- Instruct vaccine recipients and caregivers to report adverse reactions to their healthcare provider and/or to Vaccine Adverse Event Reporting System (VAERS).
- Give the Vaccine Information Statements to recipients or caregivers, which are required by the National Childhood Vaccine Injury Act of 1986 prior to each immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by:
Sanofi Pasteur Inc.
Swiftwater PA 18370 USA

Patient Information Sheet
Fluzone® High-Dose Quadrivalent
Influenza Vaccine

Please read this information sheet before getting Fluzone High-Dose Quadrivalent vaccine. This summary is not intended to take the place of talking with your healthcare provider. If you have questions or would like more information, please talk with your healthcare provider.

What is Fluzone High-Dose Quadrivalent vaccine?

Fluzone High-Dose Quadrivalent is a vaccine that helps protect against influenza illness (flu).

Fluzone High-Dose Quadrivalent vaccine is for people 65 years of age and older.

Vaccination with Fluzone High-Dose Quadrivalent vaccine may not protect all people who receive the vaccine.

Who should not get Fluzone High-Dose Quadrivalent vaccine?

You should not get Fluzone High-Dose Quadrivalent vaccine if you:

- ever had a severe allergic reaction to eggs or egg products.
- ever had a severe allergic reaction after getting any flu vaccine.
- are younger than 65 years of age.

Tell your healthcare provider if you have or have had:

- Guillain-Barré syndrome (severe muscle weakness) after getting a flu vaccine.
- problems with your immune system as the immune response may be diminished.

How is Fluzone High-Dose Quadrivalent vaccine given?

Fluzone High-Dose Quadrivalent vaccine is a shot given into the muscle of the arm.

What are the possible side effects of Fluzone High-Dose Quadrivalent vaccine?

The most common side effects of Fluzone High-Dose Quadrivalent vaccine are:

- pain, redness, and swelling where you got the shot
- muscle ache
- tiredness
- headache

These are not all of the possible side effects of Fluzone High-Dose Quadrivalent vaccine. You can ask your healthcare provider for a list of other side effects that is available to healthcare professionals.

Call your healthcare provider for advice about any side effects that concern you. You may report side effects to the Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967 or https://vaers.hhs.gov.

Why should I get Fluzone High-Dose Quadrivalent vaccine instead of a standard-dose quadrivalent influenza vaccine?

Among persons 65 years of age and older, Fluzone High-Dose Quadrivalent generated a similar immune response to Fluzone High-Dose and is expected to provide better protection against influenza compared to standard-dose quadrivalent influenza vaccines.

What are the ingredients in Fluzone High-Dose Quadrivalent vaccine?

Fluzone High-Dose Quadrivalent vaccine contains 4 killed flu virus strains. There is no live flu virus in Fluzone High-Dose Quadrivalent. Fluzone High-Dose Quadrivalent cannot cause the flu.

Inactive ingredients include formaldehyde and octylphenol ethoxylate.

Manufactured by: Sanofi Pasteur Inc.
Swiftwater, PA 18370 USA

Sanofi Pasteur
450/477 Fluzone® Quadrivalent

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Fluzone® Quadrivalent safely and effectively. See full prescribing information for Fluzone Quadrivalent.

Fluzone Quadrivalent (Influenza Vaccine)
Suspension for Intramuscular Injection
2022-2023 Formula
Initial US Approval (Fluzone Quadrivalent): 2013

----------INDICATIONS AND USAGE----------

Fluzone Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine. (1)
Fluzone Quadrivalent is approved for use in persons 6 months of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------

- **For intramuscular use only** (2)

Age	Vaccination Status	Dose	Schedule
6 months through 35 months	Not previously vaccinated with influenza vaccine or unknown vaccination history	Two doses, either 0.25 mL or 0.5 mL [a]	Administer at least 4 weeks apart
	Previously vaccinated with influenza vaccine	One or two doses[b], either 0.25 mL or 0.5 mL[a]	If two doses, administer at least 4 weeks apart
36 months through 8 years	Not previously vaccinated with influenza vaccine or unknown vaccination history	Two 0.5 mL doses	Administer at least 4 weeks apart
	Previously vaccinated with influenza vaccine	One or two 0.5 mL doses[b]	If two doses, administer at least 4 weeks apart
9 years and older	-	One 0.5 mL dose	-

[a]The schedule can be completed as two 0.25-mL doses ≥4 weeks apart, two 0.5-mL doses ≥ 4 weeks apart, or any combination of 2 doses (either 0.25 mL or 0.5 mL) administered ≥4 weeks apart.
[b]To determine if 1 or 2 doses are required, refer to Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines.
"-" Indicates information is not applicable

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection supplied in 3 presentations prefilled single-dose syringe (clear plunger rod), 0.5 mL; single-dose vial, 0.5 mL; multi-dose vial, 5 mL. (3)

----------CONTRAINDICATIONS----------

Severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine, including egg protein, or after previous dose of any influenza vaccine. (4)

----------WARNINGS AND PRECAUTIONS----------

- If Guillain-Barré syndrome (GBS) has occurred within 6 weeks following previous influenza vaccination, the decision to give Fluzone Quadrivalent should be based on careful consideration of the potential benefits and risks. (5.1)

----------ADVERSE REACTIONS----------

- In children 6 months through 35 months of age, the most common (≥10%) injection-site reactions were pain (57%) or tenderness (47%-54%), erythema (23%-37%), and swelling (13%-22%); the most common solicited systemic adverse reactions were irritability (47%-54%), abnormal crying (33%-41%), malaise (38%), drowsiness (31%-38%), appetite loss (27%-32%), myalgia (27%), vomiting (10%-15%), and fever (11%-14%). (6.1)
- In children 3 years through 8 years of age, the most common (≥10%) injection-site reactions were pain (67%), erythema (34%), and swelling (25%); the most common solicited systemic adverse reactions were myalgia (39%), malaise (32%), and headache (23%). (6.1)
- In adults 18 years and older, the most common (≥10%) injection-site reaction was pain (47%); the most common solicited systemic adverse reactions were myalgia (24%), headache (16%), and malaise (11%). (6.1)
- In adults 65 years of age and older, the most common (≥10%) injection-site reaction was pain (33%); the most common solicited systemic adverse reactions were myalgia (18%), headache (13%), and malaise (11%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc., at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS----------

- Pregnancy: Pregnancy exposure registry available. Call Sanofi Pasteur Inc. at 1-800-822-2463.
- Antibody responses to Fluzone Quadrivalent are lower in persons ≥65 years of age than in younger adults. (8.5)

See **17 for PATIENT COUNSELING INFORMATION and FDA - approved patient labeling**.

Revised:07/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION:

1 INDICATIONS AND USAGE

Fluzone® Quadrivalent is a vaccine indicated for active immunization for the prevention of influenza disease caused by influenza A subtype viruses and type B viruses contained in the vaccine.

Fluzone Quadrivalent is approved for use in persons 6 months of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only

2.1 Dose and Schedule

The dose and schedule for Fluzone Quadrivalent are presented in Table 1.

Prior to vaccination, always refer to the current Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza vaccines.

Table 1: Dose and Schedule for Fluzone Quadrivalent

Age	Vaccination Status	Dose	Schedule
6 months through 35 months	Not previously vaccinated with influenza vaccine or unknown vaccination history	Two doses, either 0.25 mL or 0.5 mL[a]	Administer at least 4 weeks apart
	Previously vaccinated with influenza vaccine	One or two doses[b], either 0.25 mL or 0.5 mL[a]	If two doses, administer at least 4 weeks apart
36 months through 8 years	Not previously vaccinated with influenza vaccine or unknown vaccination	Two 0.5 mL doses	Administer at least 4 weeks apart

	history		
	Previously vaccinated with influenza vaccine	One or two 0.5 mL doses[b]	If two doses, administer at least 4 weeks apart
9 years and older	-	One 0.5 mL dose	-

[a]The schedule can be completed as two 0.25-mL doses ≥4 weeks apart, two 0.5-mL doses ≥4 weeks apart, or any combination of 2 doses (either 0.25 mL or 0.5 mL) administered ≥4 weeks apart
[b]To determine if 1 or 2 doses are required, refer to Advisory Committee on Immunization Practices annual recommendations on prevention and control of influenza with vaccines
"-" Indicates information is not applicable

2.2 Administration

Parenteral drug products should be inspected visually for particulate matter and/or discoloration prior to administration, whenever solution and container permit. If any of these defects or conditions exist, Fluzone Quadrivalent should not be administered.

Before administering a dose of vaccine, shake the prefilled syringe or vial. Withdraw one dose of vaccine from the single-dose vial using a sterile needle and syringe. Discard unused portion. Use a separate sterile needle and syringe for each dose withdrawn from the multi-dose vial. A maximum of ten doses can be withdrawn from the multi-dose vial.

The preferred sites for intramuscular injection are the anterolateral aspect of the thigh in infants 6 months through 11 months of age, the anterolateral aspect of the thigh (or the deltoid muscle if muscle mass is adequate) in persons 12 months through 35 months of age, or the deltoid muscle in persons ≥36 months of age. The vaccine should not be injected into the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously, intradermally, or subcutaneously.

Fluzone Quadrivalent should not be combined through reconstitution or mixed with any other vaccine.

3 DOSAGE FORMS AND STRENGTHS

Fluzone Quadrivalent is a suspension for injection.

Fluzone Quadrivalent is supplied in 3 presentations:

1) Prefilled single-dose syringe (clear syringe plunger rod), 0.5 mL, for persons 6 months of age and older.

2) Single-dose vial, 0.5 mL, for persons 6 months of age and older.

3) Multi-dose vial, 5 mL, for persons 6 months of age and older.

4 CONTRAINDICATIONS

Do not administer Fluzone Quadrivalent to anyone with a history of a severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine [see *Description* (11)], including egg protein, or to a previous dose of any influenza vaccine.

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

The 1976 swine influenza vaccine was associated with an elevated risk of Guillain-Barré syndrome (GBS). Evidence for a causal relation of GBS with other influenza vaccines is inconclusive; if an excess risk exists, it is probably slightly more than 1 additional case per 1

million persons vaccinated. (See ref. 1) If GBS has occurred within 6 weeks following previous influenza vaccination, the decision to give Fluzone Quadrivalent should be based on careful consideration of the potential benefits and risks.

5.2 Preventing and Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of Fluzone Quadrivalent.

5.3 Altered Immunocompetence

If Fluzone Quadrivalent is administered to immunocompromised persons, including those receiving immunosuppressive therapy, the expected immune response may not be obtained.

5.4 Limitations of Vaccine Effectiveness

Vaccination with Fluzone Quadrivalent may not protect all recipients.

6 ADVERSE REACTIONS

In children 6 months through 35 months of age receiving a 0.25 mL dose of Fluzone Quadrivalent in Study 1 (NCT01240746, see http://clinicaltrials.gov), the most common (≥10%) injection-site reactions were pain (57%)[a] or tenderness (54%)[b], erythema (37%), and swelling (22%); the most

[a] Assessed in children 24 months through 35 months of age

[b] Assessed in children 6 months through 23 months of age

common solicited systemic adverse reactions were irritability (54%)[b], abnormal crying (41%)[b], malaise (38%)[a], drowsiness (38%)[b], appetite loss (32%)[b], myalgia (27%)[a], vomiting (15%)[b], and fever (14%). In children 3 years through 8 years of age, the most common (≥10%) injection-site reactions were pain (67%), erythema (34%), and swelling (25%); the most common solicited systemic adverse reactions were myalgia (39%), malaise (32%), and headache (23%). In adults 18 years and older, the most common (≥10%) injection-site reaction was pain (47%); the most common solicited systemic adverse reactions were myalgia (24%), headache (16%), and malaise (11%). In adults 65 years of age and older, the most common (≥10%) injection-site reaction was pain (33%); the most common solicited systemic adverse reactions were myalgia (18%), headache (13%), and malaise (11%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse event rates observed in the clinical trial(s) of a vaccine cannot be directly compared to rates in the clinical trial(s) of another vaccine and may not reflect the rates observed in practice.

Children 6 Months Through 8 Years of Age

Study 1 (NCT01240746, see http://clinicaltrials.gov) was a single-blind, randomized, active-controlled multi-center safety and immunogenicity study conducted in the US. In this study, children 6 months through 35 months of age received one or two 0.25 mL doses of either Fluzone Quadrivalent or one of two formulations of a comparator trivalent influenza vaccine (TIV-1 or TIV-2), and children 3 years through 8 years of age received one or two 0.5 mL doses of either Fluzone Quadrivalent, TIV-1, or TIV-2. Each of the trivalent formulations contained an influenza

type B virus that corresponded to one of the two type B viruses in Fluzone Quadrivalent (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). For participants who received two doses, the doses were administered approximately 4 weeks apart. The safety analysis set included 1841 children 6 months through 35 months of age and 2506 children 3 years through 8 years of age. Among participants 6 months through 8 years of age in the three vaccine groups combined, 49.3% were female (Fluzone Quadrivalent, 49.2%; TIV-1, 49.8%; TIV-2, 49.4%), 58.4% Caucasian (Fluzone Quadrivalent, 58.4%; TIV-1, 58.9%; TIV-2, 57.8%), 20.2% Black (Fluzone Quadrivalent, 20.5%; TIV-1, 19.9%; TIV-2, 19.1%), 14.1% Hispanic (Fluzone Quadrivalent, 14.3%; TIV-1, 13.2%; TIV-2, 14.7%), and 7.3% were of other racial/ethnic groups (Fluzone Quadrivalent, 6.8%; TIV-1, 8.0%; TIV-2, 8.5%). Table 2 and Table 3 summarize solicited injection-site and systemic adverse reactions reported within 7 days post-vaccination via diary cards. Participants were monitored for unsolicited adverse events for 28 days after each dose and serious adverse events (SAEs) during the 6 months following the last dose.

Table 2: Study 1[a]: Percentage of Solicited Injection-site and Systemic Adverse Reactions Within 7 Days After Vaccination in Children 6 Months Through 35 Months of Age (Safety Analysis Set)[b]

	Fluzone Quadrivalent[c, d] (N^g=1223)			**TIV-1[d, e] (B Victoria) (N^g=310)**			**TIV-2[d, f] (B Yamagata) (N^g=308)**		
	Any (%)	**Grade 2[h] (%)**	**Grade 3[i] (%)**	**Any (%)**	**Grade 2[h] (%)**	**Grade 3[i] (%)**	**Any (%)**	**Grade 2[h] (%)**	**Grade 3[i] (%)**
Injection-site adverse reactions									
Pain[j]	57.0	10.2	1.0	52.3	11.5	0.8	50.3	5.4	2.7
Tenderness[k]	54.1	11.3	1.9	48.4	8.2	1.9	49.7	10.3	0.0
Erythema	37.3	1.5	0.2	32.9	1.0	0.0	33.3	1.0	0.0
Swelling	21.6	0.8	0.2	19.7	1.0	0.0	17.3	0.0	0.0
Systemic									

	Fluzone Quadrivalent[c, d] (N[g]=1223)			TIV-1[d, e] (B Victoria) (N[g]=310)			TIV-2[d, f] (B Yamagata) (N[g]=308)		
	Any (%)	Grade 2[h] (%)	Grade 3[i] (%)	Any (%)	Grade 2[h] (%)	Grade 3[i] (%)	Any (%)	Grade 2[h] (%)	Grade 3[i] (%)
adverse reactions									
Fever (≥100.4°F)[l]	14.3	5.5	2.1	16.0	6.6	1.7	13.0	4.1	2.0
Malaise[j]	38.1	14.5	4.6	35.2	14.8	4.7	32.4	12.8	6.8
Myalgia[j]	26.7	6.6	1.9	26.6	9.4	1.6	25.0	6.8	2.7
Headache[j]	8.9	2.5	0.6	9.4	3.9	0.0	12.2	4.7	0.0
Irritability[k]	54.0	26.4	3.2	52.8	20.1	3.1	53.5	22.9	2.8
Crying abnormal[k]	41.2	12.3	3.3	36.5	8.2	1.9	29.9	10.4	2.1
Drowsiness[k]	37.7	8.4	1.3	32.1	3.8	0.6	31.9	5.6	0.7
Appetite loss[k]	32.3	9.1	1.8	33.3	5.7	1.9	25.0	8.3	0.7
Vomiting[k]	14.8	6.2	1.0	11.3	4.4	0.6	13.9	6.3	0.0

[a]NCT01240746

[b]The safety analysis set includes all persons who received at least one dose of study vaccine

[c]Fluzone Quadrivalent (0.25 mL) containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d] Participants received 1 or 2 doses according to ACIP recommendations

[e]2010-2011 Fluzone TIV (0.25 mL) containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[f]Investigational TIV (0.25 mL) containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[g]N is the number of participants in the safety analysis set

[h]Grade 2 - Injection-site pain: sufficiently discomforting to interfere with normal behavior or activities; Injection-site tenderness: cries and protests when injection-site is touched; Injection-site erythema, Injection-site swelling: ≥2.5 cm to <5 cm; Fever: >101.3°F to ≤103.1°F (6 months through 23 months); ≥101.2°F to ≤102.0°F (24 months through 35 months); Malaise, Myalgia, and Headache: some interference with activity; Irritability: requiring increased attention; Crying abnormal: 1 to 3 hours; Drowsiness: not interested in surroundings or did not wake up for a feed/meal; Appetite loss: missed 1 or 2 feeds/meals completely; Vomiting: 2 to 5 episodes per 24 hours

[i]Grade 3 - Injection-site pain: incapacitating, unable to perform usual activities; Injection-site tenderness: cries when injected limb is moved, or the movement of the injected limb is reduced; Injection-site erythema, Injection-site swelling: ≥5 cm; Fever: >103.1°F (6 months through 23 months); ≥102.1°F (24 months through 35 months); Malaise, Myalgia, and Headache: Significant; prevents daily activity; Irritability: inconsolable; Crying abnormal: >3 hours; Drowsiness: sleeping most of the time or difficult to wake up; Appetite loss: refuses ≥3 feeds/meals or refuses most feeds/meals; Vomiting: ≥6 episodes per 24 hours or requiring parenteral hydration

[j]Assessed in children 24 months through 35 months of age

[k]Assessed in children 6 months through 23 months of age

[l]Fever measured by any route

Table 3: Study 1[a]: Percentage of Solicited Injection-site and Systemic Adverse Reactions Within 7 Days After Vaccination in Children 3 Years Through 8 Years of Age (Safety Analysis Set)[b]

	Fluzone Quadrivalent[c] (N[f]=1669)			TIV-1[d] (B Victoria) (N[f]=424)			TIV-2[e] (B Yamagata) (N[f]=413)		
	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)
Injection-site adverse reactions									
Pain	66.6	15.8	2.1	64.6	9.5	2.0	63.8	11.6	2.8
Erythema	34.1	2.9	1.8	36.8	3.4	1.2	35.2	2.5	1.8
Swelling	24.8	2.8	1.4	25.4	1.5	1.2	25.9	2.5	1.8
Systemic adverse reactions									
Fever (≥100.4°F)[i]	7.0	2.1	2.1	7.1	2.2	1.2	7.6	2.8	0.8
Headache	23.1	6.8	2.2	21.2	5.1	2.7	24.4	7.5	2.0
Malaise	31.9	11.2	5.5	32.8	11.4	5.6	33.4	10.8	5.0
Myalgia	38.6	12.2	3.3	34.1	9.0	2.7	38.4	11.1	2.8

[a]NCT01240746

[b]The safety analysis set includes all persons who received at least one dose of study vaccine

[c]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d]2010-2011 Fluzone TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[e]Investigational TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[f]N is the number of participants in the safety analysis set

[g]Grade 2 - Injection-site pain: sufficiently discomforting to interfere with normal behavior or activities; Injection-site erythema, Injection-site swelling: ≥2.5 cm to <5 cm; Fever: ≥101.2°F to ≤102.0°F; Headache, Malaise, and Myalgia: some interference with activity

[h]Grade 3 - Injection-site pain: incapacitating, unable to perform usual activities; Injection-site erythema, Injection-site swelling: ≥5 cm; Fever: ≥102.1°F; Headache, Malaise, and Myalgia: Significant; prevents daily activity

[i]Fever measured by any route

Among children 6 months through 8 years of age, unsolicited non-serious adverse events were reported in 1360 (47.0%) recipients in the Fluzone Quadrivalent group, 352 (48.0%) recipients in

the TIV-1 group, and 346 (48.0%) recipients in the TIV-2 group. The most commonly reported unsolicited non-serious adverse events were cough, vomiting, and pyrexia. During the 28 days following vaccination, a total of 16 (0.6%) recipients in the Fluzone Quadrivalent group, 4 (0.5%) recipients in the TIV-1 group, and 4 (0.6%) recipients in the TIV-2 group, experienced at least one SAE. Throughout the study period, a total of 41 (1.4%) recipients in the Fluzone Quadrivalent group, 7 (1.0%) recipients in the TIV-1 group, and 14 (1.9%) recipients in the TIV-2 group, experienced at least one SAE. Three SAEs were considered to be possibly related to vaccination: croup in a Fluzone Quadrivalent recipient and 2 episodes of febrile seizure, 1 each in a TIV-1 recipient and a TIV-2 recipient.

0.5-mL Dose of Fluzone Quadrivalent in Children 6 Months through 35 Months of Age

Study 2 (NCT02915302 see http://clinicaltrials.gov) was a randomized, observer-blinded, 2-arm, multi-center safety and immunogenicity study conducted in the US. In this study, 1950 children 6 months through 35 months of age were randomly assigned to receive Fluzone Quadrivalent administered in either a volume of 0.25 mL (Group 1) or 0.5 mL (Group 2). For participants recommended to receive two doses of influenza vaccine as per Advisory Committee on Immunization Practices guidance, the same dose was administered 4 weeks after the first. The safety analysis set included 1941 participants who received at least 1 dose of study vaccine. Of these participants, 49.7% were female, 74.3% were Caucasian, 19.2% were Black, 6.5% were of other racial groups, and 22.0% were Hispanic/Latino.

Table 4 summarizes solicited injection-site and systemic adverse reactions reported within 7 days post-vaccination via diary cards for the 0.25 mL and 0.5 mL volumes of Fluzone Quadrivalent in children 6 months through 35 months of age.

Table 4: Study 2[a]: Percentage of Solicited Injection-site and Systemic Adverse Reactions Within 7 Days After Vaccination in Children 6 Months Through 35 Months of Age (Safety Analysis Set)[b]

	Fluzone Quadrivalent 0.25 mL[c] (N[d]=949)		**Fluzone Quadrivalent 0.5 mL[c] (N[d]=992)**	
	Any (%)	**Grade 3[e] (%)**	**Any (%)**	**Grade 3[e] (%)**
Injection-site adverse reactions				
Tenderness	47.3	1.7	50.4	1.2
Redness	23.1	0.0	24.3	0.2
Swelling	12.9	0.1	14.7	0.0
Systemic adverse reactions				
Irritability	47.4	3.6	48.6	4.0
Abnormal Crying	33.3	3.1	34.1	2.6
Drowsiness	31.9	2.1	31.3	1.6
Loss of Appetite	27.3	1.4	28.3	2.2
Fever (≥100.4°F)[f]	11.3	0.6	12.2	1.2
Vomiting	10.0	0.4	10.2	0.5

[a]NCT02915302

[b]The safety analysis set includes all persons who received at least one dose of study vaccine

[c]Participants received 1 or 2 doses according to ACIP recommendations

[d]N is the number of participants in the safety analysis set

[e]Grade 3 - Injection-site tenderness: Cries when injected limb is moved, or the movement of the injected limb is reduced; Injection-site redness, Injection-site swelling: ≥50 mm; Irritability: inconsolable; Abnormal Crying: >3 hours; Drowsiness: sleeping most of the time or difficult to wake up; Loss of Appetite: refuses ≥3 feeds/meals or refuses most feeds/meals; Fever: >103.1°F; Vomiting: ≥6 episodes per 24 hours or requiring parenteral hydration

[f]Fever measured by any route

The difference in fever rate (Group 2 minus Group 1) was 0.84% (95% CI: -2.13%; 3.80%), meeting the prespecified non-inferiority criterion (upper limit of the 2-sided 95% CI of the difference in fever rates <5%). Participants were monitored for unsolicited adverse events and SAEs during the 28 days following vaccination. Unsolicited non-serious adverse events were reported in 417 (44%) participants in Group 1 and 394 (40%) participants in Group 2. The most commonly reported unsolicited non-serious adverse events in both groups were cough and rhinorrhea. Ten SAEs were reported during the 28-day follow-up period: 5 (0.5%) in Group 1 and 5 (0.5%) in Group 2.

Adults

In Study 3 (NCT00988143, see http://clinicaltrials.gov), a multi-centered randomized, open-label trial conducted in the US, adults 18 years of age and older received one dose of either Fluzone Quadrivalent or one of two formulations of comparator trivalent influenza vaccine (TIV-1 or TIV-2). Each of the trivalent formulations contained an influenza type B virus that corresponded to one of the two type B viruses in Fluzone Quadrivalent (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). The safety analysis set included 570 recipients, half aged 18-60 years and half aged 61 years or older. Among participants in the three vaccine groups combined, 67.2% were female (Fluzone Quadrivalent, 68.4%; TIV-1, 67.9%; TIV-2, 65.3%), 88.4% Caucasian (Fluzone Quadrivalent, 91.1%; TIV-1, 86.8%; TIV-2, 87.4%), 9.6% Black (Fluzone Quadrivalent, 6.8%; TIV-1, 12.1%; TIV-2, 10.0%), 0.4% Hispanic (Fluzone Quadrivalent, 0.0%; TIV-1, 0.5%; TIV-2, 0.5%), and 1.7% were of other racial/ethnic groups (Fluzone Quadrivalent, 2.1%; TIV-1, 0.5%; TIV-2, 2.2%). Table 5 summarizes solicited injection-site and systemic

adverse reactions reported within 3 days post-vaccination via diary cards. Participants were monitored for unsolicited adverse events and SAEs during the 21 days following vaccination.

Table 5: Study 3[a]: Percentage of Solicited Injection-site and Systemic Adverse Reactions Within 3 Days After Vaccination in Adults 18 Years of Age and Older (Safety Analysis Set)[b]

	Fluzone Quadrivalent[c] (N[f]=190)			TIV-1[d] (B Victoria) (N[f]=190)			TIV-2[e] (B Yamagata) (N[f]=190)		
	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)
Injection-site adverse reactions									
Pain	47.4	6.8	0.5	52.1	7.9	0.5	43.2	6.3	0.0
Erythema	1.1	0.0	0.0	1.6	0.5	0.0	1.6	0.5	0.0
Swelling	0.5	0.0	0.0	3.2	0.5	0.0	1.1	0.0	0.0
Induration	0.5	0.0	0.0	1.6	0.5	0.0	0.5	0.0	0.0
Ecchymosis	0.5	0.0	0.0	0.5	0.0	0.0	0.5	0.0	0.0
Systemic adverse reactions									
Myalgia	23.7	5.8	0.0	25.3	5.8	0.0	16.8	5.8	0.0
Headache	15.8	3.2	0.5	18.4	6.3	0.5	18.0	4.2	0.0
Malaise	10.5	1.6	1.1	14.7	3.2	1.1	12.1	4.7	0.5
Shivering	2.6	0.5	0.0	5.3	1.1	0.0	3.2	0.5	0.0
Fever (≥100.4°F)[i]	0.0	0.0	0.0	0.5	0.5	0.0	0.5	0.5	0.0

[a]NCT00988143

[b]The safety analysis set includes all persons who received study vaccine

[c]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d]2009-2010 Fluzone TIV containing A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[e]2008-2009 Fluzone TIV containing A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Florida/04/2006 (Yamagata lineage), licensed

[f]N is the number of participants in the safety analysis set

[g]Grade 2 - Injection-site pain: Some interference with activity; Injection-site erythema, Injection-site swelling, Injection-site induration, and Injection-site ecchymosis: ≥5.1 to ≤10 cm; Fever: ≥101.2°F to ≤102.0°F; Myalgia, Headache, Malaise, and Shivering: some interference with activity

[h]Grade 3 - Injection-site pain: Significant; prevents daily activity; Injection-site erythema, Injection-site swelling, Injection-site induration, and Injection-site ecchymosis: >10 cm; Fever: ≥102.1°F; Myalgia, Headache, Malaise, and Shivering: Significant; prevents daily activity

[i]Fever measured by any route

Unsolicited non-serious adverse events were reported in 33 (17.4%) recipients in the Fluzone Quadrivalent group, 45 (23.7%) recipients in the TIV-1 group, and 45 (23.7%) recipients in the TIV-2 group. The most commonly reported unsolicited non-serious adverse events were headache, cough, and oropharyngeal pain. In the follow-up period, there were two SAEs, 1 (0.5%) in the Fluzone Quadrivalent group and 1 (0.5%) in the TIV-2 group.

Geriatric Adults

In Study 4 (NCT01218646, see http://clinicaltrials.gov), a multi-center, randomized, double-blind trial conducted in the US, adults 65 years of age and older received one dose of either Fluzone Quadrivalent, or one of two formulations of comparator trivalent influenza vaccine (TIV-1 or TIV-2). Each of the trivalent formulations contained an influenza type B virus that corresponded to one of the two type B viruses in Fluzone Quadrivalent (a type B virus of the Victoria lineage or a type B virus of the Yamagata lineage). The safety analysis set included 675 recipients. Among participants in the three vaccine groups combined, 55.7% were female (Fluzone Quadrivalent, 57.3%; TIV-1, 56.0%; TIV-2, 53.8%), 89.5% Caucasian (Fluzone Quadrivalent, 87.6%; TIV-1, 89.8%; TIV-2, 91.1%), 2.2% Black (Fluzone Quadrivalent, 4.0%; TIV-1, 1.8%; TIV-2, 0.9%), 7.4% Hispanic (Fluzone Quadrivalent, 8.4%; TIV-1, 7.6%; TIV-2, 6.2%) and 0.9% were of other racial/ethnic groups (Fluzone Quadrivalent, 0.0%; TIV-1, 0.9%; TIV-2, 1.8%).

Table 6 summarizes solicited injection-site and systemic adverse reactions reported within 7 days post-vaccination via diary cards. Participants were monitored for unsolicited adverse events and SAEs during the 21 days following vaccination.

Table 6: Study 4[a]: Percentage of Solicited Injection-site and Systemic Adverse Reactions Within 7 Days After Vaccination in Adults 65 Years of Age and Older (Safety Analysis Set)[b]

	Fluzone Quadrivalent[c] (N[f]=225)			TIV-1[d] (B Victoria) (N[f]=225)			TIV-2[e] (B Yamagata) (N[f]=225)		
	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)	Any (%)	Grade 2[g] (%)	Grade 3[h] (%)
Injection-site adverse reactions									
Pain	32.6	1.3	0.9	28.6	2.7	0.0	23.1	0.9	0.0
Erythema	2.7	0.9	0.0	1.3	0.0	0.0	1.3	0.4	0.0
Swelling	1.8	0.4	0.0	1.3	0.0	0.0	0.0	0.0	0.0
Systemic adverse reactions									
Myalgia	18.3	4.0	0.4	18.3	4.0	0.0	14.2	2.7	0.4
Headache	13.4	1.3	0.4	11.6	1.3	0.0	11.6	1.8	0.4
Malaise	10.7	4.5	0.4	6.3	0.4	0.0	11.6	2.7	0.9
Fever (≥100.4°F)[i]	1.3	0.0	0.4	0.0	0.0	0.0	0.9	0.4	0.4

[a]NCT01218646

[b]The safety analysis set includes all persons who received study vaccine

[c]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d]2010-2011 Fluzone TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[e]Investigational TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[f]N is the number of participants in the safety analysis set

[g]Grade 2 - Injection-site pain: some interference with activity; Injection-site erythema and Injection-site swelling: ≥5.1 to ≤10 cm; Fever: ≥101.2°F to ≤102.0°F; Myalgia, Headache, and Malaise: some interference with activity

[h]Grade 3 - Injection-site pain: Significant; prevents daily activity; Injection-site erythema and Injection-site swelling: >10 cm; Fever: ≥102.1°F; Myalgia, Headache, and Malaise: Significant; prevents daily activity

[i]Fever measured by any route

Unsolicited non-serious adverse events were reported in 28 (12.4%) recipients in the Fluzone Quadrivalent group, 22 (9.8%) recipients in the TIV-1 group, and 22 (9.8%) recipients in the TIV-2 group. The most commonly reported adverse events were oropharyngeal pain, rhinorrhea, injection-site induration, and headache. Three SAEs were reported during the follow-up period, 2 (0.9%) in the TIV-1 group and 1 (0.4%) in the TIV-2 group.

6.2 Post-Marketing Experience

The following events have been spontaneously reported during the post-approval use of Fluzone (trivalent) or Fluzone Quadrivalent. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure. Adverse events were included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Fluzone (trivalent) or Fluzone Quadrivalent.

- *Blood and Lymphatic System Disorders*: Thrombocytopenia, lymphadenopathy
- *Immune System Disorders*: Anaphylaxis, other allergic/hypersensitivity reactions (including urticaria, angioedema)
- *Eye Disorders*: Ocular hyperemia
- *Nervous System Disorders*: Guillain-Barré syndrome (GBS), convulsions, febrile convulsions, myelitis (including encephalomyelitis and transverse myelitis), facial palsy (Bell's palsy), optic neuritis/neuropathy, brachial neuritis, syncope (shortly after vaccination), dizziness, paresthesia

- *Vascular Disorders*: Vasculitis, vasodilatation/flushing
- *Respiratory, Thoracic and Mediastinal Disorders*: Dyspnea, cough, wheezing, throat tightness, oropharyngeal pain, rhinorrhea
- *Skin and Subcutaneous Tissue Disorders*: Rash, pruritus, and Stevens-Johnson syndrome
- *General Disorders and Administration Site Conditions*: Asthenia/fatigue, pain in extremities, chest pain
- *Gastrointestinal Disorders*: Vomiting

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

Sanofi Pasteur Inc. is maintaining a prospective pregnancy exposure registry to collect data on pregnancy outcomes following vaccination with Fluzone Quadrivalent during pregnancy. Healthcare providers are encouraged to enroll women who receive Fluzone Quadrivalent during pregnancy in Sanofi Pasteur Inc.'s vaccination pregnancy registry by calling 1-800-822-2463.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Available data with Fluzone Quadrivalent use in pregnant women are insufficient to inform vaccine-associated risk of adverse developmental outcomes.

A developmental and reproductive toxicity study was performed in female rabbits given a 0.5 mL/dose of Fluzone Quadrivalent prior to mating and during gestation (a single human dose is 0.5 mL). This study revealed no adverse effects to the fetus or pre-weaning development due to Fluzone Quadrivalent *[see Animal Data (8.1)]*.

Data

Animal Data: In a developmental and reproductive toxicity study female rabbits were administered a 0.5 mL/dose of Fluzone Quadrivalent by intramuscular injection 24 and 10 days before insemination, and on Days 6, 12, and 27 of gestation (a single human dose is 0.5 mL). There were no adverse effects on pre-weaning development or vaccine-related fetal malformations noted in this study.

Clinical Considerations

Disease-associated Maternal and/or Embryo/Fetal Risk

Pregnant women are at increased risk of complications associated with influenza infection compared to non-pregnant women. Pregnant women who contract influenza may be at increased risk for adverse pregnancy outcomes, including preterm labor and delivery.

8.2 Lactation

Risk Summary

It is not known whether Fluzone Quadrivalent is excreted in human milk. Data are not available to assess the effects of Fluzone Quadrivalent on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Fluzone Quadrivalent and any potential adverse effects on the breastfed child from Fluzone Quadrivalent or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to the disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of Fluzone Quadrivalent in children below the age of 6 months have not been established.

8.5 Geriatric Use

Safety and immunogenicity of Fluzone Quadrivalent were evaluated in adults 65 years of age and older. [See *Clinical Studies* (14.6).] Antibody responses to Fluzone Quadrivalent are lower in persons ≥65 years of age than in younger adults.

11 DESCRIPTION

Fluzone Quadrivalent (Influenza Vaccine) for intramuscular injection is an inactivated influenza vaccine, prepared from influenza viruses propagated in embryonated chicken eggs. The virus-containing allantoic fluid is harvested and inactivated with formaldehyde. Influenza virus is concentrated and purified in a linear sucrose density gradient solution using a continuous flow centrifuge. The virus is then chemically disrupted using a non-ionic surfactant, octylphenol ethoxylate (Triton® X-100), producing a “split virus”. The split virus is further purified and then

suspended in sodium phosphate-buffered isotonic sodium chloride solution. The Fluzone Quadrivalent process uses an additional concentration factor after the ultrafiltration step in order to obtain a higher hemagglutinin (HA) antigen concentration. Antigens from the four strains included in the vaccine are produced separately and then combined to make the quadrivalent formulation.

Fluzone Quadrivalent suspension for injection is clear and slightly opalescent in color.

Antibiotics are not used in the manufacture of Fluzone Quadrivalent.

The Fluzone Quadrivalent prefilled syringe and vial presentations are not made with natural rubber latex.

Fluzone Quadrivalent is standardized according to United States Public Health Service requirements and is formulated to contain HA of each of the following four influenza strains recommended for the 2022-2023 influenza season: A/Victoria/2570/2019 IVR-215 (H1N1), A/Darwin/9/2021 SAN-010 (H3N2), B/Phuket/3073/2013 (B Yamagata lineage), and B/Michigan/01/2021 (a B/Austria/1359417/2021-like virus, B Victoria lineage).

The amounts of HA and other ingredients per dose of vaccine are listed in Table 7. The single-dose, pre-filled syringe (0.5 mL) and the single-dose vial (0.5 mL) are manufactured and formulated without thimerosal or any other preservative. The 5 mL multi-dose vial presentation contains thimerosal, a mercury derivative, added as a preservative. Each 0.5 mL dose from the multi-dose vial contains 25 mcg mercury. Each 0.25 mL dose from the multi-dose vial contains

12.5 mcg mercury.

Table 7: Fluzone Quadrivalent Ingredients

Ingredient	Quantity (per dose)	
	Fluzone Quadrivalent 0.25 mL Dose	**Fluzone Quadrivalent 0.5 mL Dose**
Active Substance: Split influenza virus, inactivated strains[a]:	30 mcg HA total	60 mcg HA total
A (H1N1)	7.5 mcg HA	15 mcg HA
A (H3N2)	7.5 mcg HA	15 mcg HA
B/(Victoria lineage)	7.5 mcg HA	15 mcg HA
B/(Yamagata lineage)	7.5 mcg HA	15 mcg HA
Other:		
Sodium phosphate-buffered isotonic sodium chloride solution	QS[b] to appropriate volume	QS[b] to appropriate volume
Formaldehyde	≤50 mcg	≤100 mcg
Octylphenol ethoxylate	≤125 mcg	≤250 mcg
Preservative		
Single-dose presentations	-	-
Multi-dose presentation (thimerosal)	12.5 mcg mercury	25 mcg mercury

[a]per United States Public Health Service (USPHS) requirement

[b]Quantity Sufficient

"-" Indicates information is not applicable

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Influenza illness and its complications follow infection with influenza viruses. Global surveillance of influenza identifies yearly antigenic variants. Since 1977, antigenic variants of influenza A (H1N1 and H3N2) viruses and influenza B viruses have been in global circulation. Since 2001, two distinct lineages of influenza B (Victoria and Yamagata lineages) have co-circulated worldwide. Protection from influenza virus infection has not been correlated with a specific level of hemagglutination inhibition (HI) antibody titer post-vaccination. However, in some human

studies, antibody titers ≥1:40 have been associated with protection from influenza illness in up to 50% of subjects. (See ref. 2) (See ref. 3)

Antibodies against one influenza virus type or subtype confer limited or no protection against another. Furthermore, antibodies to one antigenic variant of influenza virus might not protect against a new antigenic variant of the same type or subtype. Frequent development of antigenic variants through antigenic drift is the virologic basis for seasonal epidemics and the reason for the usual change of one or more new strains in each year's influenza vaccine. Therefore, influenza vaccines are standardized to contain the hemagglutinins of influenza virus strains representing the influenza viruses likely to be circulating in the US during the influenza season.

Annual vaccination with the influenza vaccine is recommended because immunity during the year after vaccination declines and because circulating strains of influenza virus change from year to year.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Fluzone Quadrivalent has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Vaccination of female rabbits with Fluzone Quadrivalent revealed no evidence of impaired female fertility *[see Animal Data (8.1)]*.

14 CLINICAL STUDIES

The effectiveness of Fluzone Quadrivalent was demonstrated based on clinical endpoint efficacy data for Fluzone (trivalent influenza vaccine) and on an evaluation of serum HI antibody

responses to Fluzone Quadrivalent. Fluzone Quadrivalent, an inactivated influenza vaccine that contains the hemagglutinins of two influenza A subtype viruses and two influenza type B viruses, is manufactured according to the same process as Fluzone.

14.1 Efficacy of Fluzone (Trivalent Influenza Vaccine) in Children 6 through 24 Months of Age

A randomized, double-blind, placebo-controlled study was conducted at a single US center during the 1999-2000 (Year 1) and 2000-2001 (Year 2) influenza seasons. The intent-to-treat analysis set included a total of 786 children 6 through 24 months of age. Participants received two 0.25 mL doses of either Fluzone (N = 525) or a placebo (N = 261). Among all randomized participants in both years, the mean age was 13.8 months; 52.5% were male, 50.8% were Caucasian, 42.0% were Black, and 7.2% were of other racial groups. Cases of influenza were identified through active and passive surveillance for influenza-like illness or acute otitis media and confirmed by culture. Influenza-like illness was defined as fever with signs or symptoms of an upper respiratory infection. Vaccine efficacy against all influenza viral types and subtypes was a secondary endpoint and is presented in Table 8.

Table 8: Estimated Efficacy of Fluzone (Trivalent Influenza Vaccine) Against Culture-Confirmed Influenza in Children Aged 6 through 24 Months during the 1999-2000 and 2000-2001 Influenza Seasons – Intent-to-Treat Analysis Set[a]

	Fluzone[b]				Placebo[c]				Fluzone vs. Placebo	
Year	n[d]	N[e]	Rate (n/N)[f]	(95% CI)	n[d]	N[e]	Rate (n/N)[f]	(95% CI)	Relative Risk (95% CI)	Percent Relative Reduction[g] (95% CI)
Year 1[h] (1999-2000)	15	273	5.5	(3.1; 8.9)	22	138	15.9	(10.3; 23.1)	0.34 (0.18; 0.64)	66 (36; 82)
Year 2[i] (2000-2001)	9	252	3.6	(1.6; 6.7)	4	123	3.3	(0.9; 8.1)	1.10 (0.34; 3.50)	-10 (-250; 66)

[a]The intent-to-treat analysis set includes all enrolled participants who were randomly assigned to receive Fluzone or placebo and vaccinated
[b]Fluzone (0.25 mL): 1999-2000 formulation containing A/Beijing/262/95 (H1N1), A/Sydney/15/97 (H3N2), and B/Yamanashi/166/98 (Yamagata lineage) and 2000-2001 formulation containing A/New Caledonia/20/99 (H1N1), A/Panama/2007/99 (H3N2), and B/Yamanashi/166/98 (Yamagata lineage)
[c]Placebo: 0.4% NaCl
[d]n is the number of participants with culture-confirmed influenza for the given year of study as listed in the first column
[e]N is the number of participants randomly assigned to receive Fluzone or placebo for the given year of study as listed in the column headers (intent-to-treat analysis set)
[f]Rate (%) = (n/N) * 100
[g]Relative reduction in vaccine efficacy was defined as (1-relative risk) x 100
[h]Includes all culture confirmed influenza cases throughout the study duration for Year 1 (12 months of follow-up)
[i]Includes all culture-confirmed influenza cases throughout the study duration for Year 2 (6 months of follow-up)

14.2 Efficacy of Fluzone (Trivalent Influenza Vaccine) in Adults

A randomized, double-blind, placebo-controlled study was conducted in a single US center during the 2007-2008 influenza season. Participants received one dose of either Fluzone vaccine (N = 813), an active comparator (N = 814), or placebo (N = 325). The intent-to-treat analysis set included 1138 healthy adults who received Fluzone or placebo. Participants were 18 through 49 years of age (mean age was 23.3 years); 63.3% were female, 83.1% were Caucasian, and 16.9% were of other racial/ethnic groups. Cases of influenza were identified through active and passive surveillance and confirmed by cell culture and/or real-time polymerase chain reaction (PCR).

Influenza-like illness was defined as an illness with at least 1 respiratory symptom (cough or nasal congestion) and at least 1 constitutional symptom (fever or feverishness, chills, or body aches).

Vaccine efficacy of Fluzone against all influenza viral types and subtypes is presented in Table 9.

Table 9: Estimated Efficacy of Fluzone (Trivalent Influenza Vaccine) Against Influenza in Adults Aged 18 through 49 Years during the 2007-2008 Influenza Season – Intent-to-Treat Analysis Set[a,b]

Laboratory-Confirmed Symptomatic Influenza	Fluzone[c] (N=813)[e]			Placebo[d] (N=325)[e]			Fluzone vs. Placebo	
	n[f]	Rate (%)[g]	(95% CI)	n[f]	Rate (%)[g]	(95% CI)	Relative Risk (95% CI)	Percent Relative Reduction[h] (95% CI)
Positive culture	21	2.6	(1.6; 3.9)	31	9.5	(6.6; 13.3)	0.27 (0.16; 0.46)	73 (54; 84)
Positive PCR	28	3.4	(2.3; 4.9)	35	10.8	(7.6; 14.7)	0.32 (0.20; 0.52)	68 (48; 80)
Positive culture, positive PCR, or both	28	3.4	(2.3; 4.9)	35	10.8	(7.6; 14.7)	0.32 (0.20; 0.52)	68 (48; 80)

[a]NCT00538512
[b]The intent-to-treat analysis set includes all enrolled participants who were randomly assigned to receive Fluzone or placebo and vaccinated
[c]Fluzone: 2007-2008 formulation containing A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005 (H3N2), and B/Malaysia/2506/2004 (Victoria lineage)
[d]Placebo: 0.9% NaCl
[e]N is the number of participants randomly assigned to receive Fluzone or placebo
[f]n is the number of participants satisfying the criteria listed in the first column
[g]Rate (%) = (n/N) * 100
[h]Relative reduction in vaccine efficacy was defined as (1 - relative risk) x 100

14.3 Immunogenicity of Fluzone Quadrivalent in Children 6 Months through 8 Years of Age

In Study 1 (NCT01240746) [see *Adverse Reactions* (6.1)], 1419 children 6 months through 35 months of age and 2101 children 3 years through 8 years of age were included in the per-protocol immunogenicity analysis. Participants 6 months through 35 months of age received one or two 0.25 mL doses and participants 3 years through 8 years of age received one or two 0.5 mL doses of Fluzone Quadrivalent, TIV-1, or TIV-2. For participants who received two doses, the doses were administered approximately 4 weeks apart. The distribution of demographic characteristics was similar to that of the safety analysis set [see *Adverse Reactions* (6.1)].

HI antibody geometric mean titers (GMTs) and seroconversion rates 28 days following vaccination with Fluzone Quadrivalent were non-inferior to those following each TIV for all four strains, based on pre-specified criteria (see Table 10 and Table 11).

Table 10: Study 1[a]: Non-inferiority of Fluzone Quadrivalent Relative to TIV for Each Strain by HI Antibody GMTs at 28 Days Post-Vaccination, Persons 6 Months Through 8 Years of Age[b] (Per-protocol Analysis Set)[c]

Antigen Strain	**Fluzone Quadrivalent[d] N[e]=2339**	**Pooled TIV[f] N[e]=1181**		**GMT Ratio (95% CI)[g]**
	GMT	**GMT**		
A (H1N1)	1124	1096		1.03 (0.93; 1.14)
A (H3N2)	822	828		0.99 (0.91; 1.08)
	Fluzone Quadrivalent[d] N[e]=2339	**TIV-1[h] (B Victoria) N[e]=582**	**TIV-2[i] (B Yamagata) N[e]=599**	**GMT Ratio (95% CI)[g]**
	GMT	**GMT**	**GMT**	
B/Brisbane/60/2008 (B Victoria)	86.1	64.3	(19.5)[j]	1.34 (1.20; 1.50)
B/Florida/04/2006 (B Yamagata)	61.5	(16.3)[k]	58.3	1.06 (0.94; 1.18)

[a]NCT01240746

[b]Participants 6-35 months old received 1 or 2 doses (0.25 mL) and participants 3-8 years old received 1 or 2 doses (0.5 mL) as per ACIP recommendation

[c]Per-protocol analysis set included all persons who had no study protocol deviations

[d]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[e]N is the number of participants in the per-protocol analysis set

[f]Pooled TIV group includes participants vaccinated with either TIV-1 or TIV-2

[g]Non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI of the ratio of GMTs (Fluzone Quadrivalent divided by pooled TIV for the A strains, or the TIV containing the corresponding B strain) was >0.66

[h]2010-2011 Fluzone TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[i]Investigational TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[j]TIV-2 did not contain B/Brisbane/60/2008

[k]TIV-1 did not contain B/Florida/60/2006

Table 11: Study 1[a]: Non-inferiority of Fluzone Quadrivalent Relative to TIV for Each Strain by Seroconversion Rates at 28 Days Post-Vaccination, Persons 6 Months Through 8 Years of Age[b](Per-protocol Analysis Set)[c]

Antigen Strain	Fluzone Quadrivalent[d] N[e]=2339	Pooled TIV[f] N[e]=1181		Difference of Seroconversion Rates (95% CI)[h]
	Seroconversion[g] (%)			
A (H1N1)	92.4	91.4		0.9 (-0.9; 3.0)
A (H3N2)	88.0	84.2		3.8 (1.4; 6.3)
	Fluzone Quadrivalent[d] N[e]=2339	**TIV-1[i] (B Victoria) N[e]=582**	**TIV-2[j] (B Yamagata) N[e]=599**	**Difference of Seroconversion Rates (95% CI)[h]**
	Seroconversion[g] (%)			
B/Brisbane/60/2008 (B Victoria)	71.8	61.1	(20.0)[k]	10.7 (6.4; 15.1)
B/Florida/04/2006 (B Yamagata)	66.1	(17.9)[l]	64.0	2.0 (-2.2; 6.4)

[a]NCT01240746

[b]Participants 6-35 months old received 1 or 2 doses (0.25 mL) and participants 3-8 years old received 1 or 2 doses (0.5 mL) as per ACIP recommendations

[c]Per-protocol analysis set included all persons who had no study protocol deviations

[d]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[e]N is the number of participants in the per-protocol analysis set

[f]Pooled TIV group includes participants vaccinated with either TIV-1 or TIV-2

[g]Seroconversion: Paired samples with pre-vaccination HI titer <1:10 and post-vaccination titer ≥1:40 or a minimum 4-fold increase for participants with pre-vaccination titer ≥1:10

[h]Non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI of the difference in seroconversion rates (Fluzone Quadrivalent minus pooled TIV for the A strains, or the TIV containing the corresponding B strain) was >-10%

[i]2010-2011 Fluzone TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[j]Investigational TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[k]TIV-2 did not contain B/Brisbane/60/2008

[l]TIV-1 did not contain B/Florida/04/2006

Non-inferiority immunogenicity criteria based on HI antibody GMTs and seroconversion rates were also met when age subgroups (6 months to <36 months and 3 years to <9 years) were examined. In addition, HI antibody GMTs and seroconversion rates following Fluzone Quadrivalent were higher than those following TIV for the B strain not contained in each respective TIV based on pre-specified criteria (the lower limit of the 2-sided 95% CI of the ratio of the GMTs [Fluzone Quadrivalent divided by TIV] >1.5 for each B strain in Fluzone Quadrivalent compared with the corresponding B strain not contained in each TIV and the lower limit of the two 2-sided 95% CI of the difference of the seroconversion rates [Fluzone Quadrivalent minus TIV] >10% for each B strain in Fluzone Quadrivalent compared with the corresponding B strain not contained in each TIV).

14.4 Immunogenicity of the 0.5 mL Dose of Fluzone Quadrivalent in Children 6 Months through 35 Months of Age

In Study 2 (NCT02915302) [see *Adverse Reactions* (6.1)], 1027 children, 6 months through 35 months of age, were included in the per-protocol immunogenicity analysis. The distribution of demographic characteristics was similar to that of the safety analysis set [see *Adverse Reactions* (6.1)].

In this study, children 6 months through 35 months of age received one or two doses of either 0.25 mL or 0.5 mL of Fluzone Quadrivalent. Non-inferiority of the 0.5 mL dose(s) relative to the 0.25 mL dose(s) of Fluzone Quadrivalent was demonstrated for all four strains based on pre-specified criteria (lower limit of the 2-sided 95% CI of the ratio of GMTs between groups > 0.667; lower limit of the 2-sided 95% CI of the difference in seroconversion rates >-10%). GMT ratios ($GMT_{0.5\text{-mL dose}}$ divided by $GMT_{0.25\text{-mL dose}}$) for the A/H1N1, A/H3N2, B Victoria lineage, and B Yamagata lineage strains were 1.42 (95% CI: 1.16; 1.74), 1.48 (95% CI: 1.21; 1.82), 1.33 (95% CI: 1.09; 1.62), and 1.41 (95% CI: 1.17; 1.70), respectively. Seroconversion rate (SCR) differences ($SCR_{0.5\text{-mL dose}}$ minus $SCR_{0.25\text{-mL dose}}$) for the A/H1N1, A/H3N2, B Victoria lineage, and B Yamagata lineage strains were 4.6% (95% CI: -0.4%; 9.6%), 5.1% (95% CI: 0.4%; 9.8%), 1.3% (95% CI: -2.9%; 5.6%), and 2.6% (95% CI: -1.4%; 6.5%).

14.5 Immunogenicity of Fluzone Quadrivalent in Adults ≥18 Years of Age

In Study 3 (NCT00988143) [see *Adverse Reactions* (6.1)], 565 adults 18 years of age and older who had received one dose of Fluzone Quadrivalent, TIV-1, or TIV-2 were included in the per-protocol immunogenicity analysis. The distribution of demographic characteristics was similar to that of the safety analysis set [see *Adverse Reactions* (6.1)].

HI antibody GMTs 21 days following vaccination with Fluzone Quadrivalent were non-inferior to those following each TIV for all four strains, based on pre-specified criteria (see Table 12).

Table 12: Study 3[a]: Non-inferiority of Fluzone Quadrivalent Relative to TIV for Each Strain by HI Antibody GMTs at 21 Days Post-Vaccination, Adults 18 Years of Age and Older (Per-protocol Analysis Set)[b]

Antigen Strain	Fluzone Quadrivalent[c] N[d]=190	Pooled TIV[e] N[d]=375		GMT Ratio (95% CI)[f]
	GMT	GMT		
A (H1N1)	161	151		1.06 (0.87; 1.31)
A (H3N2)	304	339		0.90 (0.70; 1.15)
	Fluzone Quadrivalent[c] N[d]=190	**TIV-1[g] (B Victoria) N[d]=187**	**TIV-2[h] (B Yamagata) N[d]=188**	**GMT Ratio (95% CI)[f]**
	GMT	**GMT**	**GMT**	
B/Brisbane/60/2008 (B Victoria)	101	114	(44.0)[i]	0.89 (0.70; 1.12)
B/Florida/04/2006 (B Yamagata)	155	(78.1)[j]	135	1.15 (0.93; 1.42)

[a]NCT00988143

[b]Per-protocol analysis set included all persons who had no study protocol deviations

[c]Fluzone Quadrivalent containing A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d]N is the number of participants in the per-protocol analysis set

[e]Pooled TIV group includes participants vaccinated with either TIV-1 or TIV-2

[f]Non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI of the ratio of GMTs (Fluzone Quadrivalent divided by pooled TIV for the A strains, or the TIV containing the corresponding B strain) was >2/3

[g]2009-2010 Fluzone TIV containing A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[h]2008-2009 Fluzone TIV containing A/Brisbane/59/2007 (H1N1), A/Uruguay/716/2007 (H3N2), and B/Florida/04/2006 (Yamagata lineage), licensed

[i]TIV-2 did not contain B/Brisbane/60/2008

[j]TIV-1 did not contain B/Florida/04/2006

14.6 Immunogenicity of Fluzone Quadrivalent in Geriatric Adults ≥65 Years of Age

In Study 4 (NCT01218646) [see *Adverse Reactions* (6.1)], 660 adults 65 years of age and older were included in the per-protocol immunogenicity analysis. The distribution of demographic characteristics was similar to that of the safety analysis set [see *Adverse Reactions* (6.1)].

HI antibody GMTs 21 days following vaccination with Fluzone Quadrivalent were non-inferior to those following TIV for all four strains, based on pre-specified criteria (see Table 13).

Seroconversion rates 21 days following Fluzone Quadrivalent were non-inferior to those following TIV for H3N2, B/Brisbane, and B/Florida, but not for H1N1 (see Table 14). The HI antibody GMT following Fluzone Quadrivalent was higher than that following TIV-1 for B/Florida but not higher than that following TIV-2 for B/Brisbane, based on pre-specified criteria (the lower limit of the 2-sided 95% CI of the ratio of the GMTs [Fluzone Quadrivalent divided by TIV] >1.5 for each B strain in Fluzone Quadrivalent compared with the corresponding B strain not contained in each TIV). Seroconversion rates following Fluzone Quadrivalent were higher than those following TIV for the B strain not contained in each respective TIV, based on pre-specified criteria (the lower limit of the two 2-sided 95% CI of the difference of the seroconversion rates [Fluzone Quadrivalent minus TIV] >10% for each B strain in Fluzone Quadrivalent compared with the corresponding B strain not contained in each TIV).

Table 13: Study 4[a]: Non-inferiority of Fluzone Quadrivalent Relative to TIV for Each Strain by HI Antibody GMTs at 21 Days Post-Vaccination, Adults 65 Years of Age and Older (Per-protocol Analysis Set)[b]

Antigen Strain	**Fluzone Quadrivalent[c] N[d]=220**	**Pooled TIV[e] N[d]=440**		**GMT Ratio (95% CI)[f]**
	GMT	**GMT**		
A (H1N1)	231	270		0.85 (0.67; 1.09)
A (H3N2)	501	324		1.55 (1.25; 1.92)
	Fluzone Quadrivalent[c] N[d]=220	**TIV-1[g] (B Victoria) N[d]=219**	**TIV-2[h] (B Yamagata) N[d]=221**	**GMT Ratio (95% CI)[f]**
	GMT	**GMT**	**GMT**	
B/Brisbane/60/2008 (B Victoria)	73.8	57.9	(42.2)[i]	1.27 (1.05; 1.55)
B/Florida/04/2006 (B Yamagata)	61.1	(28.5)[j]	54.8	1.11 (0.90; 1.37)

[a]NCT01218646

[b]Per-protocol analysis set included all persons who had no study protocol deviations

[c]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d]N is the number of participants in the per-protocol analysis set

[e]Pooled TIV group includes participants vaccinated with either TIV-1 or TIV-2

[f]Non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI of the ratio of GMTs (Fluzone Quadrivalent divided by pooled TIV for the A strains, or the TIV containing the corresponding B strain) was >0.66

[g]2010-2011 Fluzone TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[h]Investigational TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[i]TIV-2 did not contain B/Brisbane/60/2008

[j]TIV-1 did not contain B/Florida/04/2006

Table 14: Study 4[a]: Non-inferiority of Fluzone Quadrivalent Relative to TIV for Each Strain by Seroconversion Rates at 21 Days Post-Vaccination, Adults 65 Years of Age and Older (Per-protocol Analysis Set)[b]

Antigen Strain	Fluzone Quadrivalent[c] N[d]=220	Pooled TIV[e] N[d]=440		Difference of Seroconversion Rates (95% CI)[f]
	Seroconversion[g] (%)			
A (H1N1)	65.91	69.77		-3.86 (-11.50; 3.56)
A (H3N2)	69.09	59.32		9.77 (1.96; 17.20)
	Fluzone Quadrivalent[c] N[d]=220	TIV-1[h] (B Victoria) N[d]=219	TIV-2[i] (B Yamagata) N[d]=221	Difference of Seroconversion Rates (95% CI)[f]
	Seroconversion[g] (%)			
B/Brisbane/60/2008 (B Victoria)	28.64	18.72	(8.60)[j]	9.91 (1.96; 17.70)
B/Florida/04/2006 (B Yamagata)	33.18	(9.13)[k]	31.22	1.96 (-6.73; 10.60)

[a]NCT01218646

[b]Per-protocol analysis set included all persons who had no study protocol deviations

[c]Fluzone Quadrivalent containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (Victoria lineage), and B/Florida/04/2006 (Yamagata lineage)

[d]N is the number of participants in the per-protocol analysis set

[e]Pooled TIV group includes participants vaccinated with either TIV-1 or TIV-2

[f]Non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI of the difference in seroconversion rates (Fluzone Quadrivalent minus pooled TIV for the A strains, or the TIV containing the corresponding B strain) was >-10%

[g]Seroconversion: Paired samples with pre-vaccination HI titer <1:10 and post-vaccination titer ≥1:40 or a minimum 4-fold increase for participants with pre-vaccination titer ≥1:10

[h]2010-2011 Fluzone TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Brisbane/60/2008 (Victoria lineage), licensed

[i]Investigational TIV containing A/California/07/2009 (H1N1), A/Victoria/210/2009 (H3N2), and B/Florida/04/2006 (Yamagata lineage), non-licensed

[j]TIV-2 did not contain B/Brisbane/60/2008

[k]TIV-1 did not contain B/Florida/04/2006

15 REFERENCES

1 Lasky T, Terracciano GJ, Magder L, et al. The Guillain-Barré syndrome and the 1992-1993 and 1993-1994 influenza vaccines. N Engl J Med 1998;339:1797-802.

2 Hannoun C, Megas F, Piercy J. Immunogenicity and protective efficacy of influenza vaccination. Virus Res 2004;103:133-138.

3 Hobson D, Curry RL, Beare AS, Ward-Gardner A. The role of serum haemagglutination-inhibiting antibody in protection against challenge infection with influenza A2 and B viruses. J Hyg Camb 1972;70:767-777.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Single-dose, prefilled syringe (clear plunger rod), without needle, 0.5 mL (NDC 49281-422-88) (not made with natural rubber latex). Supplied as package of 10 (NDC 49281-422-50).

Single-dose vial, 0.5 mL (NDC 49281-422-58) (not made with natural rubber latex). Supplied as package of 10 (NDC 49281-422-10).

Multi-dose vial, 5 mL (NDC 49281-637-78) (not made with natural rubber latex). Supplied as package of 1 (NDC 49281-637-15). A maximum of ten doses can be withdrawn from the multi-dose vial.

16.2 Storage and Handling

Store all Fluzone Quadrivalent presentations refrigerated at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Discard if vaccine has been frozen.

Do not use after the expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

See FDA-approved patient labeling (Patient Information). Inform the vaccine recipient or guardian:

- Fluzone Quadrivalent contains killed viruses and cannot cause influenza.
- Fluzone Quadrivalent stimulates the immune system to protect against influenza, but does not prevent other respiratory infections.
- Annual influenza vaccination is recommended.
- Report adverse reactions to their healthcare provider and/or to the Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967.
- Sanofi Pasteur Inc. is maintaining a prospective pregnancy exposure registry to collect data on pregnancy outcomes and newborn health status following vaccination with Fluzone Quadrivalent during pregnancy. Women who receive Fluzone Quadrivalent during pregnancy are encouraged to contact Sanofi Pasteur Inc. directly or have their healthcare provider contact Sanofi Pasteur Inc. at 1-800-822-2463.

Vaccine Information Statements must be provided to vaccine recipients or their guardians, as required by the National Childhood Vaccine Injury Act of 1986 prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Fluzone is a registered trademark of Sanofi Pasteur Inc.

Manufactured by:

Sanofi Pasteur Inc.

Swiftwater, PA 18370 USA

Patient Information Sheet
Fluzone® Quadrivalent
Influenza Vaccine

Please read this information sheet before getting Fluzone Quadrivalent. This summary is not intended to take the place of talking with your healthcare provider. If you have questions or would like more information, please talk with your healthcare provider.

What is Fluzone Quadrivalent?

Fluzone Quadrivalent is a vaccine that helps protect against influenza illness (flu).

Fluzone Quadrivalent is for people who are 6 months of age and older.

Vaccination with Fluzone Quadrivalent may not protect all people who receive the vaccine.

Who should not get Fluzone Quadrivalent?

You should not get Fluzone Quadrivalent if you:

- ever had a severe allergic reaction to eggs or egg products.
- ever had a severe allergic reaction after getting any flu vaccine.
- are younger than 6 months of age.

Tell your healthcare provider if you or your child have or have had:

- Guillain-Barré syndrome (severe muscle weakness) after getting a flu vaccine.
- problems with your immune system as the immune response may be diminished.

How is the Fluzone Quadrivalent given?

Fluzone Quadrivalent is a shot given into the muscle of the arm.

For infants, Fluzone Quadrivalent is a shot given into the muscle of the thigh.

What are the possible side effects of Fluzone Quadrivalent?

The most common side effects of Fluzone Quadrivalent are:

- pain, redness, and swelling where you got the shot
- muscle aches
- tiredness
- headache
- fever

These are not all of the possible side effects of Fluzone Quadrivalent. You can ask your healthcare provider for a list of other side effects that is available to healthcare professionals.

Call your healthcare provider for advice about any side effects that concern you. You may report side effects to the Vaccine Adverse Event Reporting System (VAERS) at 1-800-822-7967 or *http://vaers.hhs.gov.* Sanofi Pasteur Inc. is collecting information on pregnancy outcomes and the health of newborns following vaccination with Fluzone Quadrivalent during pregnancy. Women who receive Fluzone Quadrivalent during pregnancy are encouraged to contact Sanofi Pasteur Inc. directly or have their healthcare provider contact Sanofi Pasteur Inc. at 1-800-822-2463.

What are the ingredients in Fluzone Quadrivalent?

Fluzone Quadrivalent contains 4 killed flu virus strains.

Inactive ingredients include formaldehyde and octylphenol ethoxylate. The preservative thimerosal is only in the multi-dose vial of Fluzone Quadrivalent.

Manufactured by:

Sanofi Pasteur Inc.

Swiftwater, PA 18370 USA

Measles / Mumps / Rubella (MMR)

M-M-R® II
(MEASLES, MUMPS, and RUBELLA VIRUS VACCINE LIVE)

DESCRIPTION

M-M-R® II (Measles, Mumps, and Rubella Virus Vaccine Live) is a live virus vaccine for vaccination against measles (rubeola), mumps, and rubella (German measles).

M-M-R II is a sterile lyophilized preparation of (1) ATTENUVAX® (Measles Virus Vaccine Live), a more attenuated line of measles virus, derived from Enders' attenuated Edmonston strain and propagated in chick embryo cell culture; (2) MUMPSVAX® (Mumps Virus Vaccine Live), the Jeryl Lynn™ (B level) strain of mumps virus propagated in chick embryo cell culture; and (3) MERUVAX® II (Rubella Virus Vaccine Live), the Wistar RA 27/3 strain of live attenuated rubella virus propagated in WI-38 human diploid lung fibroblasts.{1,2}

The growth medium for measles and mumps is Medium 199 (a buffered salt solution containing vitamins and amino acids and supplemented with fetal bovine serum) containing SPGA (sucrose, phosphate, glutamate, and recombinant human albumin) as stabilizer and neomycin.

The growth medium for rubella is Minimum Essential Medium (MEM) [a buffered salt solution containing vitamins and amino acids and supplemented with fetal bovine serum] containing recombinant human albumin and neomycin. Sorbitol and hydrolyzed gelatin stabilizer are added to the individual virus harvests.

The cells, virus pools, and fetal bovine serum are all screened for the absence of adventitious agents.

The reconstituted vaccine is for subcutaneous administration. Each 0.5 mL dose contains not less than 1,000 $TCID_{50}$ (tissue culture infectious doses) of measles virus; 12,500 $TCID_{50}$ of mumps virus; and 1,000 $TCID_{50}$ of rubella virus. Each dose of the vaccine is calculated to contain sorbitol (14.5 mg), sodium phosphate, sucrose (1.9 mg), sodium chloride, hydrolyzed gelatin (14.5 mg), recombinant human albumin (≤0.3 mg), fetal bovine serum (<1 ppm), other buffer and media ingredients and approximately 25 mcg of neomycin. The product contains no preservative.

Before reconstitution, the lyophilized vaccine is a light yellow compact crystalline plug. M-M-R II, when reconstituted as directed, is clear yellow.

CLINICAL PHARMACOLOGY

Measles, mumps, and rubella are three common childhood diseases, caused by measles virus, mumps virus (paramyxoviruses), and rubella virus (togavirus), respectively, that may be associated with serious complications and/or death. For example, pneumonia and encephalitis are caused by measles. Mumps is associated with aseptic meningitis, deafness and orchitis; and rubella during pregnancy may cause congenital rubella syndrome in the infants of infected mothers.

The impact of measles, mumps, and rubella vaccination on the natural history of each disease in the United States can be quantified by comparing the maximum number of measles, mumps, and rubella cases reported in a given year prior to vaccine use to the number of cases of each disease reported in 1995. For measles, 894,134 cases reported in 1941 compared to 288 cases reported in 1995 resulted in a 99.97% decrease in reported cases; for mumps, 152,209 cases reported in 1968 compared to 840 cases reported in 1995 resulted in a 99.45% decrease in reported cases; and for rubella, 57,686 cases reported in 1969 compared to 200 cases reported in 1995 resulted in a 99.65% decrease.{3}

Clinical studies of 284 triple seronegative children, 11 months to 7 years of age, demonstrated that M-M-R II is highly immunogenic and generally well tolerated. In these studies, a single injection of the vaccine induced measles hemagglutination-inhibition (HI) antibodies in 95%, mumps neutralizing antibodies in 96%, and rubella HI antibodies in 99% of susceptible persons. However, a small percentage (1-5%) of vaccinees may fail to seroconvert after the primary dose (see also INDICATIONS AND USAGE, *Recommended Vaccination Schedule*).

A study{4} of 6-month-old and 15-month-old infants born to vaccine-immunized mothers demonstrated that, following vaccination with ATTENUVAX, 74% of the 6-month-old infants developed detectable neutralizing antibody (NT) titers while 100% of the 15-month-old infants developed NT. This rate of seroconversion is higher than that previously reported for 6-month-old infants born to naturally immune mothers tested by HI assay. When the 6-month-old infants of immunized mothers were revaccinated at 15

months, they developed antibody titers equivalent to the 15-month-old vaccinees. The lower seroconversion rate in 6-month-olds has two possible explanations: 1) Due to the limit of the detection level of the assays (NT and enzyme immunoassay [EIA]), the presence of trace amounts of undetectable maternal antibody might interfere with the seroconversion of infants; or 2) The immune system of 6-month-olds is not always capable of mounting a response to measles vaccine as measured by the two antibody assays.

There is some evidence to suggest that infants who are born to mothers who had wild-type measles and who are vaccinated at less than one year of age may not develop sustained antibody levels when later revaccinated. The advantage of early protection must be weighed against the chance for failure to respond adequately on reimmunization.{5,6}

Efficacy of measles, mumps, and rubella vaccines was established in a series of double-blind controlled field trials which demonstrated a high degree of protective efficacy afforded by the individual vaccine components.{7-12} These studies also established that seroconversion in response to vaccination against measles, mumps, and rubella paralleled protection from these diseases.{13-15}

Following vaccination, antibodies associated with protection can be measured by neutralization assays, HI, or ELISA (enzyme linked immunosorbent assay) tests. Neutralizing and ELISA antibodies to measles, mumps, and rubella viruses are still detectable in most individuals 11 to 13 years after primary vaccination.{16-18} See INDICATIONS AND USAGE, *Non-Pregnant Adolescent and Adult Females*, for Rubella Susceptibility Testing.

The RA 27/3 rubella strain in M-M-R II elicits higher immediate post-vaccination HI, complement-fixing and neutralizing antibody levels than other strains of rubella vaccine{19-25} and has been shown to induce a broader profile of circulating antibodies including anti-theta and anti-iota precipitating antibodies.{26,27} The RA 27/3 rubella strain immunologically simulates natural infection more closely than other rubella vaccine viruses.{27-29} The increased levels and broader profile of antibodies produced by RA 27/3 strain rubella virus vaccine appear to correlate with greater resistance to subclinical reinfection with the wild virus,{27,29-31} and provide greater confidence for lasting immunity.

INDICATIONS AND USAGE

Recommended Vaccination Schedule

M-M-R II is indicated for simultaneous vaccination against measles, mumps, and rubella in individuals 12 months of age or older.

Individuals first vaccinated at 12 months of age or older should be revaccinated prior to elementary school entry. Revaccination is intended to seroconvert those who do not respond to the first dose. The Advisory Committee on Immunization Practices (ACIP) recommends administration of the first dose of M-M-R II at 12 to 15 months of age and administration of the second dose of M-M-R II at 4 to 6 years of age.{32} In addition, some public health jurisdictions mandate the age for revaccination. Consult the complete text of applicable guidelines regarding routine revaccination including that of high-risk adult populations.

Measles Outbreak Schedule

Infants Between 6 to 12 Months of Age

Local health authorities may recommend measles vaccination of infants between 6 to 12 months of age in outbreak situations. This population may fail to respond to the components of the vaccine. Safety and effectiveness of mumps and rubella vaccine in infants less than 12 months of age have not been established. The younger the infant, the lower the likelihood of seroconversion (see CLINICAL PHARMACOLOGY). Such infants should receive a second dose of M-M-R II between 12 to 15 months of age followed by revaccination at elementary school entry.{32}

Unnecessary doses of a vaccine are best avoided by ensuring that written documentation of vaccination is preserved and a copy given to each vaccinee's parent or guardian.

Other Vaccination Considerations

Non-Pregnant Adolescent and Adult Females

Immunization of susceptible non-pregnant adolescent and adult females of childbearing age with live attenuated rubella virus vaccine is indicated if certain precautions are observed (see below and PRECAUTIONS). Vaccinating susceptible postpubertal females confers individual protection against subsequently acquiring rubella infection during pregnancy, which in turn prevents infection of the fetus and consequent congenital rubella injury.{33}

Women of childbearing age should be advised not to become pregnant for 3 months after vaccination and should be informed of the reasons for this precaution.

The ACIP has stated "If it is practical and if reliable laboratory services are available, women of childbearing age who are potential candidates for vaccination can have serologic tests to determine susceptibility to rubella. However, with the exception of premarital and prenatal screening, routinely performing serologic tests for all women of childbearing age to determine susceptibility (so that vaccine is given only to proven susceptible women) can be effective but is expensive. Also, 2 visits to the health-care provider would be necessary — one for screening and one for vaccination. Accordingly, rubella vaccination of a woman who is not known to be pregnant and has no history of vaccination is justifiable without serologic testing — and may be preferable, particularly when costs of serology are high and follow-up of identified susceptible women for vaccination is not assured."{33}

Postpubertal females should be informed of the frequent occurrence of generally self-limited arthralgia and/or arthritis beginning 2 to 4 weeks after vaccination (see ADVERSE REACTIONS).

Postpartum Women

It has been found convenient in many instances to vaccinate rubella-susceptible women in the immediate postpartum period (see PRECAUTIONS, *Nursing Mothers*).

Other Populations

Previously unvaccinated children older than 12 months who are in contact with susceptible pregnant women should receive live attenuated rubella vaccine (such as that contained in monovalent rubella vaccine or in M-M-R II) to reduce the risk of exposure of the pregnant woman.

Individuals planning travel outside the United States, if not immune, can acquire measles, mumps, or rubella and import these diseases into the United States. Therefore, prior to international travel, individuals known to be susceptible to one or more of these diseases can either receive the indicated monovalent vaccine (measles, mumps, or rubella), or a combination vaccine as appropriate. However, M-M-R II is preferred for persons likely to be susceptible to mumps and rubella; and if monovalent measles vaccine is not readily available, travelers should receive M-M-R II regardless of their immune status to mumps or rubella.{34-36}

Vaccination is recommended for susceptible individuals in high-risk groups such as college students, health-care workers, and military personnel.{33,34,37}

According to ACIP recommendations, most persons born in 1956 or earlier are likely to have been infected with measles naturally and generally need not be considered susceptible. All children, adolescents, and adults born after 1956 are considered susceptible and should be vaccinated, if there are no contraindications. This includes persons who may be immune to measles but who lack adequate documentation of immunity such as: (1) physician-diagnosed measles, (2) laboratory evidence of measles immunity, or (3) adequate immunization with live measles vaccine on or after the first birthday.{34}

The ACIP recommends that "Persons vaccinated with inactivated vaccine followed within 3 months by live vaccine should be revaccinated with two doses of live vaccine. Revaccination is particularly important when the risk of exposure to wild-type measles virus is increased, as may occur during international travel."{34}

Post-Exposure Vaccination

Vaccination of individuals exposed to wild-type measles may provide some protection if the vaccine can be administered within 72 hours of exposure. If, however, vaccine is given a few days before exposure, substantial protection may be afforded.{34,38,39} There is no conclusive evidence that vaccination of individuals recently exposed to wild-type mumps or wild-type rubella will provide protection.{33,37}

Use With Other Vaccines

See DOSAGE AND ADMINISTRATION, *Use With Other Vaccines*.

CONTRAINDICATIONS

Hypersensitivity to any component of the vaccine, including gelatin.{40}

Do not give M-M-R II to pregnant females; the possible effects of the vaccine on fetal development are unknown at this time. If vaccination of postpubertal females is undertaken, pregnancy should be avoided for three months following vaccination (see INDICATIONS AND USAGE, *Non-Pregnant Adolescent and Adult Females* and PRECAUTIONS, *Pregnancy*).

Anaphylactic or anaphylactoid reactions to neomycin (each dose of reconstituted vaccine contains approximately 25 mcg of neomycin).

Febrile respiratory illness or other active febrile infection. However, the ACIP has recommended that all vaccines can be administered to persons with minor illnesses such as diarrhea, mild upper respiratory infection with or without low-grade fever, or other low-grade febrile illness.{41}

Patients receiving immunosuppressive therapy. This contraindication does not apply to patients who are receiving corticosteroids as replacement therapy, e.g., for Addison's disease.

Individuals with blood dyscrasias, leukemia, lymphomas of any type, or other malignant neoplasms affecting the bone marrow or lymphatic systems.

Primary and acquired immunodeficiency states, including patients who are immunosuppressed in association with AIDS or other clinical manifestations of infection with human immunodeficiency viruses;{41-43} cellular immune deficiencies; and hypogammaglobulinemic and dysgammaglobulinemic states. Measles inclusion body encephalitis{44} (MIBE), pneumonitis{45} and death as a direct consequence of disseminated measles vaccine virus infection have been reported in immunocompromised individuals inadvertently vaccinated with measles-containing vaccine.

Individuals with a family history of congenital or hereditary immunodeficiency, until the immune competence of the potential vaccine recipient is demonstrated.

WARNINGS

Due caution should be employed in administration of M-M-R II to persons with a history of cerebral injury, individual or family histories of convulsions, or any other condition in which stress due to fever should be avoided. The physician should be alert to the temperature elevation which may occur following vaccination (see ADVERSE REACTIONS).

Hypersensitivity to Eggs

Live measles vaccine and live mumps vaccine are produced in chick embryo cell culture. Persons with a history of anaphylactic, anaphylactoid, or other immediate reactions (e.g., hives, swelling of the mouth and throat, difficulty breathing, hypotension, or shock) subsequent to egg ingestion may be at an enhanced risk of immediate-type hypersensitivity reactions after receiving vaccines containing traces of chick embryo antigen. The potential risk to benefit ratio should be carefully evaluated before considering vaccination in such cases. Such individuals may be vaccinated with extreme caution, having adequate treatment on hand should a reaction occur (see PRECAUTIONS).{46}

However, the AAP has stated, "Most children with a history of anaphylactic reactions to eggs have no untoward reactions to measles or MMR vaccine. Persons are not at increased risk if they have egg allergies that are not anaphylactic, and they should be vaccinated in the usual manner. In addition, skin testing of egg-allergic children with vaccine has not been predictive of which children will have an immediate hypersensitivity reaction...Persons with allergies to chickens or chicken feathers are not at increased risk of reaction to the vaccine."{47}

Hypersensitivity to Neomycin

The AAP states, "Persons who have experienced anaphylactic reactions to topically or systemically administered neomycin should not receive measles vaccine. Most often, however, neomycin allergy manifests as a contact dermatitis, which is a delayed-type (cell-mediated) immune response rather than anaphylaxis. In such persons, an adverse reaction to neomycin in the vaccine would be an erythematous, pruritic nodule or papule, 48 to 96 hours after vaccination. A history of contact dermatitis to neomycin is not a contraindication to receiving measles vaccine."{47}

Thrombocytopenia

Individuals with current thrombocytopenia may develop more severe thrombocytopenia following vaccination. In addition, individuals who experienced thrombocytopenia with the first dose of M-M-R II (or its component vaccines) may develop thrombocytopenia with repeat doses. Serologic status may be evaluated to determine whether or not additional doses of vaccine are needed. The potential risk to benefit ratio should be carefully evaluated before considering vaccination in such cases (see ADVERSE REACTIONS).

PRECAUTIONS

General

Adequate treatment provisions, including epinephrine injection (1:1000), should be available for immediate use should an anaphylactic or anaphylactoid reaction occur.

Special care should be taken to ensure that the injection does not enter a blood vessel.

Children and young adults who are known to be infected with human immunodeficiency viruses and are not immunosuppressed may be vaccinated. However, vaccinees who are infected with HIV should be monitored closely for vaccine-preventable diseases because immunization may be less effective than for uninfected persons (see CONTRAINDICATIONS).{42,43}

Vaccination should be deferred for 3 months or longer following blood or plasma transfusions, or administration of immune globulin (human).{47}

Excretion of small amounts of the live attenuated rubella virus from the nose or throat has occurred in the majority of susceptible individuals 7 to 28 days after vaccination. There is no confirmed evidence to indicate that such virus is transmitted to susceptible persons who are in contact with the vaccinated individuals. Consequently, transmission through close personal contact, while accepted as a theoretical possibility, is not regarded as a significant risk.{33} However, transmission of the rubella vaccine virus to infants via breast milk has been documented (see *Nursing Mothers*).

There are no reports of transmission of live attenuated measles or mumps viruses from vaccinees to susceptible contacts.

It has been reported that live attenuated measles, mumps and rubella virus vaccines given individually may result in a temporary depression of tuberculin skin sensitivity. Therefore, if a tuberculin test is to be done, it should be administered either before or simultaneously with M-M-R II.

Children under treatment for tuberculosis have not experienced exacerbation of the disease when immunized with live measles virus vaccine;{48} no studies have been reported to date of the effect of measles virus vaccines on untreated tuberculous children. However, individuals with active untreated tuberculosis should not be vaccinated.

As for any vaccine, vaccination with M-M-R II may not result in protection in 100% of vaccinees.

The health-care provider should determine the current health status and previous vaccination history of the vaccinee.

The health-care provider should question the patient, parent, or guardian about reactions to a previous dose of M-M-R II or other measles-, mumps-, or rubella-containing vaccines.

Information for Patients

The health-care provider should provide the vaccine information required to be given with each vaccination to the patient, parent, or guardian.

The health-care provider should inform the patient, parent, or guardian of the benefits and risks associated with vaccination. For risks associated with vaccination see WARNINGS, PRECAUTIONS, and ADVERSE REACTIONS.

Patients, parents, or guardians should be instructed to report any serious adverse reactions to their health-care provider who in turn should report such events to the U.S. Department of Health and Human Services through the Vaccine Adverse Event Reporting System (VAERS), 1-800-822-7967.{49}

Pregnancy should be avoided for 3 months following vaccination, and patients should be informed of the reasons for this precaution (see INDICATIONS AND USAGE, *Non-Pregnant Adolescent and Adult Females*, CONTRAINDICATIONS, and PRECAUTIONS, *Pregnancy*).

Laboratory Tests

See INDICATIONS AND USAGE, *Non-Pregnant Adolescent and Adult Females*, for Rubella Susceptibility Testing, and CLINICAL PHARMACOLOGY.

Drug Interactions

See DOSAGE AND ADMINISTRATION, *Use With Other Vaccines*.

Immunosuppressive Therapy

The immune status of patients about to undergo immunosuppressive therapy should be evaluated so that the physician can consider whether vaccination prior to the initiation of treatment is indicated (see CONTRAINDICATIONS and PRECAUTIONS).

The ACIP has stated that "patients with leukemia in remission who have not received chemotherapy for at least 3 months may receive live virus vaccines. Short-term (<2 weeks), low- to moderate-dose systemic corticosteroid therapy, topical steroid therapy (e.g. nasal, skin), long-term alternate-day treatment with low to moderate doses of short-acting systemic steroid, and intra-articular, bursal, or tendon injection of corticosteroids are not immunosuppressive in their usual doses and do not contraindicate the administration of [measles, mumps, or rubella vaccine]."{33,34,37}

Immune Globulin

Administration of immune globulins concurrently with M-M-R II may interfere with the expected immune response.{33,34,47}

See also PRECAUTIONS, *General*.

Carcinogenesis, Mutagenesis, Impairment of Fertility

M-M-R II has not been evaluated for carcinogenic or mutagenic potential, or potential to impair fertility.

Pregnancy

Animal reproduction studies have not been conducted with M-M-R II. It is also not known whether M-M-R II can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Therefore, the vaccine should not be administered to pregnant females; furthermore, pregnancy should be avoided for 3 months following vaccination (see INDICATIONS AND USAGE, *Non-Pregnant Adolescent and Adult Females* and CONTRAINDICATIONS).

In counseling women who are inadvertently vaccinated when pregnant or who become pregnant within 3 months of vaccination, the physician should be aware of the following: (1) In a 10-year survey involving over 700 pregnant women who received rubella vaccine within 3 months before or after conception (of whom 189 received the Wistar RA 27/3 strain), none of the newborns had abnormalities compatible with congenital rubella syndrome;{50} (2) Mumps infection during the first trimester of pregnancy may increase the rate of spontaneous abortion. Although mumps vaccine virus has been shown to infect the placenta and fetus, there is no evidence that it causes congenital malformations in humans;{37} and (3) Reports have indicated that contracting wild-type measles during pregnancy enhances fetal risk. Increased rates of spontaneous abortion, stillbirth, congenital defects and prematurity have been observed subsequent to infection with wild-type measles during pregnancy.{51,52} There are no adequate studies of the attenuated (vaccine) strain of measles virus in pregnancy. However, it would be prudent to assume that the vaccine strain of virus is also capable of inducing adverse fetal effects.

Nursing Mothers

It is not known whether measles or mumps vaccine virus is secreted in human milk. Recent studies have shown that lactating postpartum women immunized with live attenuated rubella vaccine may secrete the virus in breast milk and transmit it to breast-fed infants.{53} In the infants with serological evidence of rubella infection, none exhibited severe disease; however, one exhibited mild clinical illness typical of acquired rubella.{54,55} Caution should be exercised when M-M-R II is administered to a nursing woman.

Pediatric Use

Safety and effectiveness of measles vaccine in infants below the age of 6 months have not been established (see also CLINICAL PHARMACOLOGY). Safety and effectiveness of mumps and rubella vaccine in infants less than 12 months of age have not been established.

Geriatric Use

Clinical studies of M-M-R II did not include sufficient numbers of seronegative subjects aged 65 and over to determine whether they respond differently from younger subjects. Other reported clinical experience has not identified differences in responses between the elderly and younger subjects.

ADVERSE REACTIONS

The following adverse reactions are listed in decreasing order of severity, without regard to causality, within each body system category and have been reported during clinical trials, with use of the marketed vaccine, or with use of monovalent or bivalent vaccine containing measles, mumps, or rubella:

Body as a Whole

Panniculitis; atypical measles; fever; syncope; headache; dizziness; malaise; irritability.

Cardiovascular System

Vasculitis.

Digestive System

Pancreatitis; diarrhea; vomiting; parotitis; nausea.

Endocrine System

Diabetes mellitus.

Hemic and Lymphatic System

Thrombocytopenia (see WARNINGS, *Thrombocytopenia*); purpura; regional lymphadenopathy; leukocytosis.

Immune System

Anaphylaxis and anaphylactoid reactions have been reported as well as related phenomena such as angioneurotic edema (including peripheral or facial edema) and bronchial spasm in individuals with or without an allergic history.

Musculoskeletal System

Arthritis; arthralgia; myalgia.

Arthralgia and/or arthritis (usually transient and rarely chronic), and polyneuritis are features of infection with wild-type rubella and vary in frequency and severity with age and sex, being greatest in adult females and least in prepubertal children. This type of involvement as well as myalgia and paresthesia, have also been reported following administration of MERUVAX II.

Chronic arthritis has been associated with wild-type rubella infection and has been related to persistent virus and/or viral antigen isolated from body tissues. Only rarely have vaccine recipients developed chronic joint symptoms.

Following vaccination in children, reactions in joints are uncommon and generally of brief duration. In women, incidence rates for arthritis and arthralgia are generally higher than those seen in children (children: 0-3%; women: 12-26%),{17,56,57} and the reactions tend to be more marked and of longer duration. Symptoms may persist for a matter of months or on rare occasions for years. In adolescent girls, the reactions appear to be intermediate in incidence between those seen in children and in adult women. Even in women older than 35 years, these reactions are generally well tolerated and rarely interfere with normal activities.

Nervous System

Encephalitis; encephalopathy; measles inclusion body encephalitis (MIBE) (see CONTRAINDICATIONS); subacute sclerosing panencephalitis (SSPE); Guillain-Barré Syndrome (GBS); acute disseminated encephalomyelitis (ADEM); transverse myelitis; febrile convulsions; afebrile convulsions or seizures; ataxia; polyneuritis; polyneuropathy; ocular palsies; paresthesia.

Encephalitis and encephalopathy have been reported approximately once for every 3 million doses of M-M-R II or measles-, mumps-, and rubella-containing vaccine administered since licensure of these vaccines.

The risk of serious neurological disorders following live measles virus vaccine administration remains less than the risk of encephalitis and encephalopathy following infection with wild-type measles (1 per 1000 reported cases).{58,59}

In severely immunocompromised individuals who have been inadvertently vaccinated with measles-containing vaccine; measles inclusion body encephalitis, pneumonitis, and fatal outcome as a direct consequence of disseminated measles vaccine virus infection have been reported (see CONTRAINDICATIONS). In this population, disseminated mumps and rubella vaccine virus infection have also been reported.

There have been reports of subacute sclerosing panencephalitis (SSPE) in children who did not have a history of infection with wild-type measles but did receive measles vaccine. Some of these cases may have resulted from unrecognized measles in the first year of life or possibly from the measles vaccination. Based on estimated nationwide measles vaccine distribution, the association of SSPE cases to measles vaccination is about one case per million vaccine doses distributed. This is far less than the association with infection with wild-type measles, 6-22 cases of SSPE per million cases of measles. The results of a retrospective case-controlled study conducted by the Centers for Disease Control and Prevention suggest that the overall effect of measles vaccine has been to protect against SSPE by preventing measles with its inherent higher risk of SSPE.{60}

Cases of aseptic meningitis have been reported to VAERS following measles, mumps, and rubella vaccination. Although a causal relationship between the Urabe strain of mumps vaccine and aseptic meningitis has been shown, there is no evidence to link Jeryl Lynn™ mumps vaccine to aseptic meningitis.

Respiratory System

Pneumonia; pneumonitis (see CONTRAINDICATIONS); sore throat; cough; rhinitis.

Skin

Stevens-Johnson syndrome; erythema multiforme; urticaria; rash; measles-like rash; pruritis.

Local reactions including burning/stinging at injection site; wheal and flare; redness (erythema); swelling; induration; tenderness; vesiculation at injection site; Henoch-Schönlein purpura; acute hemorrhagic edema of infancy.

Special Senses — Ear

Nerve deafness; otitis media.

Special Senses — Eye

Retinitis; optic neuritis; papillitis; retrobulbar neuritis; conjunctivitis.

Urogenital System

Epididymitis; orchitis.

Other

Death from various, and in some cases unknown, causes has been reported rarely following vaccination with measles, mumps, and rubella vaccines; however, a causal relationship has not been established in healthy individuals (see CONTRAINDICATIONS). No deaths or permanent sequelae were reported in a published post-marketing surveillance study in Finland involving 1.5 million children and adults who were vaccinated with M-M-R II during 1982 to 1993.{61}

Under the National Childhood Vaccine Injury Act of 1986, health-care providers and manufacturers are required to record and report certain suspected adverse events occurring within specific time periods after vaccination. However, the U.S. Department of Health and Human Services (DHHS) has established a Vaccine Adverse Event Reporting System (VAERS) which will accept all reports of suspected events.{49} A VAERS report form as well as information regarding reporting requirements can be obtained by calling VAERS 1-800-822-7967.

DOSAGE AND ADMINISTRATION

FOR SUBCUTANEOUS ADMINISTRATION

Do not inject intravascularly.

The dose for any age is 0.5 mL administered subcutaneously, preferably into the outer aspect of the upper arm.

The recommended age for primary vaccination is 12 to 15 months.

Revaccination with M-M-R II is recommended prior to elementary school entry. See also INDICATIONS AND USAGE, *Recommended Vaccination Schedule*.

Children first vaccinated when younger than 12 months of age should receive another dose between 12 to 15 months of age followed by revaccination prior to elementary school entry.{32} See also INDICATIONS AND USAGE, *Measles Outbreak Schedule*.

Immune Globulin (IG) is not to be given concurrently with M-M-R II (see PRECAUTIONS, *General* and PRECAUTIONS, *Drug Interactions*).

CAUTION: A sterile syringe free of preservatives, antiseptics, and detergents should be used for each injection and/or reconstitution of the vaccine because these substances may inactivate the live virus vaccine. A 25 gauge, 5/8" needle is recommended.

To reconstitute, use only the diluent supplied, since it is free of preservatives or other antiviral substances which might inactivate the vaccine.

Single Dose Vial — First withdraw the entire volume of diluent into the syringe to be used for reconstitution. Inject all the diluent in the syringe into the vial of lyophilized vaccine, and agitate to mix thoroughly. If the lyophilized vaccine cannot be dissolved, discard. Withdraw the entire contents into a syringe and inject the total volume of restored vaccine subcutaneously.

It is important to use a separate sterile syringe and needle for each individual patient to prevent transmission of hepatitis B and other infectious agents from one person to another.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. M-M-R II, when reconstituted, is clear yellow.

Use With Other Vaccines

M-M-R II should be given one month before or after administration of other live viral vaccines.

M-M-R II has been administered concurrently with VARIVAX® [Varicella Virus Vaccine Live (Oka/Merck)], and PedvaxHIB® [*Haemophilus b* Conjugate Vaccine (Meningococcal Protein Conjugate)] using separate injection sites and syringes. No impairment of immune response to individually tested vaccine antigens was demonstrated. The type, frequency, and severity of adverse experiences observed with M-M-R II were similar to those seen when each vaccine was given alone.

Routine administration of DTP (diphtheria, tetanus, pertussis) and/or OPV (oral poliovirus vaccine) concurrently with measles, mumps and rubella vaccines is not recommended because there are limited data relating to the simultaneous administration of these antigens.

However, other schedules have been used. The ACIP has stated "Although data are limited concerning the simultaneous administration of the entire recommended vaccine series (i.e., DTaP [or DTwP], IPV [or OPV], Hib with or without Hepatitis B vaccine, and varicella vaccine), data from numerous studies have

indicated no interference between routinely recommended childhood vaccines (either live, attenuated, or killed). These findings support the simultaneous use of all vaccines as recommended."{62}

HOW SUPPLIED

No. 4681 — M-M-R II is supplied as follows: (1) a box of 10 single-dose vials of lyophilized vaccine (package A), **NDC** 0006-4681-00; and (2) a box of 10 vials of diluent (package B). To conserve refrigerator space, the diluent may be stored separately at room temperature.

Storage

To maintain potency, M-M-R II must be stored between -58°F and +46°F (-50°C to +8°C). Use of dry ice may subject M-M-R II to temperatures colder than -58°F (-50°C).

Protect the vaccine from light at all times, since such exposure may inactivate the viruses.

Before reconstitution, store the lyophilized vaccine at 36°F to 46°F (2°C to 8°C). The diluent may be stored in the refrigerator with the lyophilized vaccine or separately at room temperature. **Do not freeze the diluent.**

It is recommended that the vaccine be used as soon as possible after reconstitution. Store reconstituted vaccine in the vaccine vial in a dark place at 36°F to 46°F (2°C to 8°C) and discard if not used within 8 hours.

For information regarding stability under conditions other than those recommended, call 1-800-MERCK-90.

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For patent information: www.merck.com/product/patent/home.html

Revised: 05/2017

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HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use PRIORIX safely and effectively. See full prescribing information for PRIORIX.

PRIORIX (Measles, Mumps, and Rubella Vaccine, Live), suspension for subcutaneous injection

Initial U.S. Approval: 2022

INDICATIONS AND USAGE

PRIORIX is a vaccine indicated for active immunization for the prevention of measles, mumps, and rubella in individuals 12 months of age and older. (1)

DOSAGE AND ADMINISTRATION

For subcutaneous injection only.

Each dose is approximately 0.5 mL.

- The first dose is administered at 12 through 15 months of age. (2.1)
- The second dose is administered at 4 through 6 years of age. (2.1)

DOSAGE FORMS AND STRENGTHS

PRIORIX is a suspension for injection supplied as a single-dose vial of lyophilized antigen component to be reconstituted with the accompanying prefilled syringe of sterile water diluent component. A single dose after reconstitution is approximately 0.5 mL. (3)

CONTRAINDICATIONS

- Severe allergic reaction (e.g., anaphylaxis) to any component of PRIORIX, or after a previous dose of any measles, mumps, and rubella virus-containing vaccine. (4.1)
- Severe immunodeficiency. (4.2)
- Pregnancy. (4.3, 8.1)

WARNINGS AND PRECAUTIONS

- There is a risk of febrile seizure following administration of PRIORIX. (5.2)
- Thrombocytopenia and thrombocytopenic purpura have been reported following vaccination with PRIORIX. (5.3)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including PRIORIX. Procedures should be in place to avoid injury from fainting. (5.4)
- The tip caps of the prefilled syringes contain natural rubber latex, which may cause allergic reactions. (5.5)

ADVERSE REACTIONS

Most common solicited adverse reactions in clinical trials participants:

- 12 through 15 months of age: local reactions were pain (26%) and redness (25%); systemic reactions were irritability (63%), loss of appetite (45%), drowsiness (45%), and fever (35%). (6.1)
- 4 through 6 years of age: local reactions were pain (41%), redness (22%), and swelling (11%); systemic reactions were loss of appetite (21%), drowsiness (27%), and fever (24%). (6.1)
- 7 years of age and older: local reactions were pain (12%) and redness (12%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

DRUG INTERACTIONS

- Administration of immune globulins and other blood products concurrently with PRIORIX may interfere with the expected immune response to the vaccine. (7.1)
- PRIORIX may result in a temporary suppression of tuberculin reactivity. (7.2)

USE IN SPECIFIC POPULATIONS

- Do not use during pregnancy. (8.1)
- Avoid pregnancy for 1 month following vaccination with PRIORIX. (8.1)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 6/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

PRIORIX is a vaccine indicated for active immunization for the prevention of measles, mumps, and rubella in individuals 12 months of age and older.

2 DOSAGE AND ADMINISTRATION

For subcutaneous injection only.

2.1 Dose and Schedule

After reconstitution, a single dose of PRIORIX is approximately 0.5 mL.

Administer according to the following schedule:

- First dose – 12 through 15 months of age
- Second dose – 4 through 6 years of age

If PRIORIX is not administered according to this schedule and 2 doses of measles-, mumps- and rubella-virus vaccine are recommended for an individual, there should be a minimum of 4 weeks between the first and second dose.

PRIORIX may be administered as a second dose to individuals who have received a first dose of another measles, mumps and rubella virus-containing vaccine.

2.2 Preparation

Reconstitute the Lyophilized Antigen Component, Live only with the accompanying Sterile Water Diluent Component to form PRIORIX. The reconstituted vaccine should be a clear peach- to fuchsia pink-colored suspension. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, do not administer the vaccine.

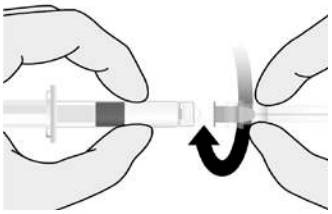

Figure 1. Hold the prefilled syringe by the barrel and unscrew the syringe cap by twisting it counterclockwise. Align the needle to the axis of the syringe and attach by gently connecting the needle hub into the Luer Lock Adaptor (LLA) and rotate a quarter turn clockwise until you feel it lock.

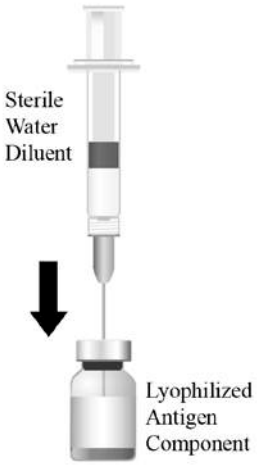

Figure 2. Cleanse the vial stopper. Transfer the entire contents of the prefilled syringe into the lyophilized antigen component vial.

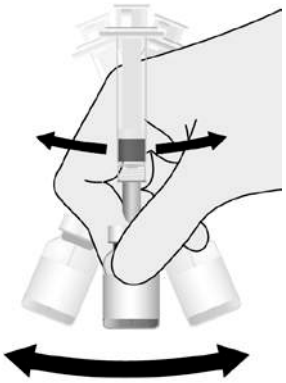

Figure 3. Shake the vial well until the powder is completely dissolved. Do not invert the vial while shaking.

Figure 4. After reconstitution, **withdraw the entire contents** of the reconstituted vaccine into the same syringe and after changing the needle, administer **subcutaneously**.

2.3 Administration

Administer PRIORIX immediately after reconstitution. If not used immediately, store refrigerated between 36° and 46° F (2° and 8°C) and administer within 8 hours. Discard reconstituted vaccine if not used within 8 hours.

3 DOSAGE FORMS AND STRENGTHS

PRIORIX is a suspension for injection supplied as a single-dose vial of lyophilized antigen component to be reconstituted with the accompanying prefilled syringe of sterile water diluent. A single dose after reconstitution is approximately 0.5 mL.

4 CONTRAINDICATIONS

4.1 Severe Allergic Reactions

Do not administer PRIORIX to individuals with a history of severe allergic reactions (e.g., anaphylaxis) to any component of the vaccine or after a previous dose of any measles, mumps, and rubella virus-containing vaccine *[see Description (11)]*.

4.2 Immunosuppression

Due to the risk of disseminated vaccine virus infection, do not administer PRIORIX to individuals with severe humoral or cellular (primary or acquired) immunodeficiency.

4.3 Pregnancy

Do not administer PRIORIX to individuals who are pregnant. Pregnancy should be avoided for 1 month after vaccination *[see Use in Specific Populations (8.1)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Allergic Vaccine Reactions

Appropriate medical treatment used to manage immediate allergic reactions must be available in the event an acute anaphylactic reaction occurs following administration of PRIORIX.

5.2 Febrile Seizures

There is a risk of febrile seizure following immunization with PRIORIX *[see Adverse Reactions (6.1)]*.

5.3 Thrombocytopenia

Thrombocytopenia and thrombocytopenic purpura have been reported following vaccination with PRIORIX *[see Adverse Reactions (6.2)]*.

5.4 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including PRIORIX. Procedures should be in place to avoid injury from fainting.

5.5 Latex

The tip caps of the prefilled syringes of diluent contain natural rubber latex, which may cause allergic reactions.

5.6 Risk of Vaccine Virus Transmission

Live attenuated rubella vaccine virus has been detected in the nose and throat of individuals 7 to 28 days after vaccination with a rubella virus containing vaccine. No documented confirmed cases of transmitted rubella vaccine virus have been reported.[1]

5.7 Limitation of Vaccine Effectiveness

Vaccination with PRIORIX may not protect all susceptible individuals.

6 ADVERSE REACTIONS

The most commonly reported (≥10%) solicited adverse reactions in the following age groups in clinical trials were:

- Age 12 through 15 months – local: pain (26%) and redness (25%); systemic: irritability (63%), loss of appetite (45%), drowsiness (45%), and fever (35%)
- Age 4 through 6 years – local: pain (41%), redness (22%), and swelling (11%); systemic: loss of appetite (21%), drowsiness (27%), and fever (24%)
- Age 7 years and older – local: pain (12%) and redness (12%)

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of PRIORIX was evaluated in 6 clinical studies, in which a total of 12,151 participants (6,391 in the United States) received at least 1 dose of PRIORIX: 8,780 children (4,148 in the United States) 12 through 15 months of age; 2,917 children (1,950 in the United States) 4 through 6 years of age; and 454 adults and children (293 in the United States) 7 years of age and older. Across the 6 studies, participants who received PRIORIX are as follows: 51.6% were male; 64.6% were White, 18.4% were Asian, 6.1% were Black, and 10.9% were of other racial groups (including American Indian/Native American, Native Hawaiian/Pacific Islander, Arabic/North African and Other); and 14.3% were of Hispanic/Latino ethnicity. The racial/ethnic distribution of participants who received PRIORIX and M-M-R II was similar.

Children 12 through 15 Months of Age Who Received PRIORIX as a First Dose

In a randomized, observer-blind, controlled clinical study (Study 1, NCT01702428) conducted in 5 countries (United States [including Puerto Rico], Estonia, Finland, Mexico and Spain), 5,003 participants 12 through 15 months of age received a first dose of PRIORIX (n = 3,714) or M-M-R II (n = 1,289) given concomitantly with HAVRIX (Hepatitis A Vaccine) and VARIVAX (Varicella Virus Vaccine Live, Merck & Co., Inc.); children enrolled in the United States also received PREVNAR 13 (Pneumococcal 13-valent Conjugate Vaccine, Pfizer Inc.) concomitantly. In the overall population, 51.3% were male; 75.6% were White, 4.8% were Black, 3.5% were Asian, 16.1% were of other racial groups (including American Indian/Native American, Native Hawaiian/Pacific Islander, Arabic/North African and Other); and 18.6% were of Hispanic/Latino ethnicity. The median age of participants was 12 months (range: 11 to 16 months). Local solicited adverse reactions were recorded by parents or guardians using standardized diary cards for 4 days. Systemic solicited adverse reactions of drowsiness, loss of

appetite, and irritability were collected for 15 days, and fever, rash, parotid/salivary gland swelling, febrile convulsions, and signs of meningeal irritation (i.e., neck stiffness with or without photophobia or headache) were collected for 43 days (Table 1). Unsolicited adverse events that occurred within 43 days following vaccination were recorded using diary cards supplemented by medical review. Data on solicited adverse reactions and unsolicited adverse events were transcribed into the study database during an on-site visit on Day 42 and via telephone contact on Day 180.

Table 1. Incidence of Solicited Local and Systemic Adverse Reactions after the First Dose of PRIORIX Compared with M-M-R II in Children 12 through 15 Months of Age (Study 1, NCT01702428, Total Vaccinated Cohort)[a]

Adverse Reaction	PRIORIX n (%)	M-M-R II n (%)
Local (within 4 Days[b])	**N = 3,555**	**N = 1,242**
Pain	919 (25.9%)	349 (28.1%)
Redness	870 (24.5%)	313 (25.2%)
Swelling	318 (8.9%)	133 (10.7%)
Systemic (within 15 Days[b])	**N = 3,566**	**N = 1,243**
Drowsiness	1601 (44.9%)	586 (47.1%)
Irritability	2258 (63.3%)	819 (65.9%)
Loss of appetite	1608 (45.1%)	548 (44.1%)
Systemic (within 43 Days[b])	**N = 3,566**	**N = 1,243**
Measles/rubella-like rash	235 (6.6%)	77 (6.2%)
Fever (defined as temperature ≥38°C/100.4°F)	1244 (34.9%)	412 (33.1%)
Parotid/ salivary gland swelling	0	0
Febrile convulsions	7 (0.2%)	3 (0.2%)
Signs of meningeal irritation[c]	3 (0.1%)	0

Total vaccinated cohort for safety included all vaccinated participants for whom safety data were available.

N = Number of participants.

n = Number of participants presenting with solicited adverse reaction described.

[a] HAVRIX and VARIVAX were administered concomitantly with PRIORIX or M-M-R II; participants in the U.S. also received PREVNAR 13 concomitantly with PRIORIX (n = 1,847) or M-M-R II (n = 654).

[b] 4 Days, 15 Days, and 43 Days included the day of vaccination and the subsequent 3, 14, and 42 days, respectively.

[c] Neck stiffness with or without photophobia or headache.

Children 12 through 15 Months of Age Who Received a Second Dose of PRIORIX 6 Weeks after the First Dose

In a randomized, observer-blind, controlled clinical study (Study 2, NCT01681992) conducted in six countries (United States [including Puerto Rico], Czech Republic, Finland, Malaysia, Spain and Thailand), 4,516 participants 12 through 15 months of age received a first dose of PRIORIX (n = 2,990) or M-M-R II (n = 1,526) followed by a second dose of the same vaccine 6 weeks later. The first dose was given concomitantly with HAVRIX and VARIVAX; children enrolled in the United States (including Puerto Rico) also received PREVNAR 13 concomitantly. In the overall population, 51.7% were male; 68.4% were White, 24.4% were Asian, 3.2% were Black, and 4.0% were of other racial groups (including American Indian/Native American, Native Hawaiian/Pacific Islander, Arabic/North African and Other); and 5.6% were of Hispanic/Latino ethnicity. The median age of participants was 12 months (range: 11 to 16 months). Local solicited adverse reactions were recorded by parents or guardians using standardized diary cards for 4 days, and systemic adverse reactions of fever, rash, parotid/salivary gland swelling, febrile convulsions, and signs of meningeal irritation (i.e., neck stiffness with or without photophobia or headache) were collected for 43 days. Unsolicited adverse events that occurred within 43 days following vaccination were recorded using diary cards supplemented by medical review. Data on solicited adverse reactions and unsolicited adverse events were transcribed into the study database during on-site visits on Day 42, Day 84, and Day 222. The safety profile of PRIORIX following the second dose was similar to the safety profile following the first dose of PRIORIX.

Children 4 through 6 Years of Age Who Received PRIORIX as a Second Dose of Measles, Mumps, and Rubella Vaccine

In a randomized, observer-blind, controlled clinical study (Study 3, NCT01621802) conducted in 3 countries (United States, South Korea, and Taiwan), 4,007 participants 4 through 6 years of age received PRIORIX (n = 2,917) or M-M-R II (n = 1,090) as a second dose following administration of an initial dose of a combined measles, mumps, and rubella virus-containing vaccine in the second year of life. PRIORIX and M-M-R II were given concomitantly with KINRIX (DTaP-IPV) [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed and Inactivated Poliovirus Vaccine] and VARIVAX in a subset of subjects (n = 802 receiving PRIORIX, n = 298 receiving M-M-R II) enrolled in the United States. In the overall population, 52.5% were male; 42.4% were White, 37.2% were Asian, 8.2% were Black, and 12.3% were of other racial groups (including American Indian/Native American, Native Hawaiian/Pacific Islander, Arabic/North African and Other) and 17.2% were of Hispanic/Latino ethnicity. The median age of participants was 4 years (range: 3 to 6 years). In a subset of participants who received concomitantly administered vaccines, data on local solicited adverse reactions were recorded by parents or guardians using standardized diary cards for 4 days. Systemic solicited adverse reactions of drowsiness and loss of appetite were collected for 4 days, and fever, rash, parotid/salivary gland swelling, febrile convulsions, and signs of meningeal irritation (i.e., neck stiffness with or without photophobia or headache) were collected for 43 days (Table 2).

Unsolicited adverse events that occurred within 43 days following vaccination were recorded using diary cards supplemented by medical review. Data on solicited adverse reactions and unsolicited adverse events were transcribed into the study database during an on-site visit on Day 42 and via telephone contact on Day 180.

Table 2. Incidence of Solicited Local and Systemic Adverse Reactions after the Second Dose of PRIORIX Compared with M-M-R II Concomitantly Administered with KINRIX and VARIVAX in Children 4 through 6 Years of Age (Study 3, NCT01621802, Total Vaccinated Cohort)

Adverse Reaction	PRIORIX n (%)	M-M-R II n (%)
Local (within 4 Days[a])	**N = 727**	**N = 267**
Pain	295 (40.6%)	109 (40.8%)
Redness	157 (21.6%)	69 (25.8%)
Swelling	82 (11.3%)	28 (10.5%)
Systemic (within 4 Days[a])	**N = 731**	**N = 268**
Drowsiness	199 (27.2%)	72 (26.9%)
Loss of appetite	154 (21.1%)	59 (22.0%)
Systemic (within 43 Days[a])	**N = 731**	**N = 268**
Measles/rubella-like rash	14 (1.9%)	5 (1.9%)
Fever (defined as temperature ≥38°C/100.4°F)	177 (24.2%)	67 (25.0%)
Parotid/ salivary gland swelling	0	0
Febrile convulsions	0	0
Signs of meningeal irritation[b]	0	2 (0.7%)

Total vaccinated cohort for safety included all vaccinated participants for whom safety data were available.

N = Number of participants.

n = Number of participants presenting with solicited adverse reaction described.

[a] 4 Days and 43 Days included the day of vaccination and the subsequent 3 and 42 days, respectively.

[b] Neck stiffness with or without photophobia or headache.

Individuals 7 Years of Age and Older Who Received PRIORIX as a Second Dose of Measles, Mumps, and Rubella Vaccine.

In a randomized, observer-blind, controlled clinical study (Study 4, NCT02058563) conducted in 3 countries (United States, Slovakia, and Estonia), 860 participants 7 years of age and older received PRIORIX (n = 426) or M-M-R II (n = 434) as a second dose following previous administration of a combined measles, mumps, and rubella virus-containing vaccine. Participants 7 through 17 years were enrolled if they had received one dose of a combined measles, mumps, and rubella virus-containing vaccine on or after their first birthday and participants 18 years of

age or older were enrolled if they previously received at least one dose of a combined measles, mumps, and rubella virus-containing vaccine. In the overall population, 46.2% were male; 73.8% were White, 0.2% were Asian, 24.0% were Black, and 1.9% were of other racial groups (including American Indian/Native American, Native Hawaiian/Pacific Islander, Arabic/North African and Other) and 13.3% were of Hispanic/Latino ethnicity. The median age of participants was 26 years (range: 7 to 59 years). Data on solicited local and systemic adverse reactions were recorded by the participants or their parents or guardians using standardized diary cards for 4 days and 43 days, respectively (Table 3). Unsolicited adverse events that occurred within 43 days following vaccination were recorded using diary cards supplemented by medical review. Data on solicited adverse reactions and unsolicited adverse events were transcribed into the study database during an on-site visit on Day 42 and via telephone contact on Day 180.

Table 3. Incidence of Solicited Local and Systemic Adverse Reactions after PRIORIX as a Second Dose Compared with M-M-R II in Individuals 7 Years of Age and Older (Study 4, NCT02058563, Total Vaccinated Cohort)[a]

	PRIORIX n (%)	**M-M-R II n (%)**
Local (within 4 Days[b])	**N = 405**	**N = 422**
Pain	49 (12.1%)	47 (11.1%)
Redness	48 (11.9%)	50 (11.8%)
Swelling	23 (5.7%)	29 (6.9%)
Systemic (within 43 Days[b])	**N = 405**	**N = 422**
Fever (defined as temperature ≥38°C/100.4°F)	11 (2.7%)	23 (5.5%)
Measles/rubella-like rash	0	2 (0.5%)
Joint pain (arthralgia/arthritis)	8 (2.0%)	4 (0.9%)
Parotid/ salivary gland swelling	1 (0.2%)	0
Signs of meningeal irritation[c]	1 (0.2%)	1 (0.2%)

Total vaccinated cohort for safety included all vaccinated participants for whom safety data were available.

N = Number of participants.

n = Number of participants presenting with solicited adverse reaction described.

[a] Participants received a first dose of either M-M-R II, PRIORIX, or a non-U.S. combined measles, mumps, rubella and varicella virus vaccine.

[b] 4 Days and 43 Days included the day of vaccination and the subsequent 3 and 42 days, respectively.

[c] Neck stiffness with or without photophobia or headache.

6.2 Postmarketing Experience

In addition to adverse reactions reported from clinical trials, the following adverse reactions have been identified during postmarketing use of PRIORIX. Because these reactions are reported

voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccination with PRIORIX.

Blood and Lymphatic System Disorders

Thrombocytopenia, thrombocytopenic purpura.

Vascular Disorders

Vasculitis (including Henoch-Schönlein purpura and Kawasaki syndrome).

Immune system Disorders

Anaphylactic reactions.

Infections and Infestations

Meningitis, measles-like illness, mumps-like illness (including orchitis, epididymitis, and parotitis).

Musculoskeletal and Connective Tissue Disorders

Arthralgia, arthritis.

Nervous System Disorders

Encephalitis, cerebellitis, cerebellitis-like symptoms (including transient gait disturbance and transient ataxia), Guillain-Barré syndrome, transverse myelitis, peripheral neuritis, afebrile seizures, syncope.

Skin and Subcutaneous Tissue Disorders

Erythema multiforme.

7 DRUG INTERACTIONS

7.1 Immune Globulins and Blood Products

Immune globulins and other blood products administered concomitantly with PRIORIX contain antibodies that may interfere with vaccine virus replication and decrease the expected immune response. The Advisory Committee on Immunization Practices (ACIP) has specific recommendations for intervals between administration of antibody containing products and live virus vaccines.

7.2 Tuberculin Skin Testing

PRIORIX may result in a temporary suppression of tuberculin reactivity. Therefore, if a tuberculin test is to be done, it should be administered either any time before, simultaneously with, or at least 4 weeks after PRIORIX to avoid false-negative results.

7.3 Use With Other Live Viral Vaccines

PRIORIX can be administered concomitantly with other live viral vaccines. If not given concomitantly, PRIORIX should be given 1 month before or 1 month after administration of other live viral vaccines to avoid potential for immune interference.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

PRIORIX contains live attenuated measles, mumps, and rubella viruses. The vaccine is contraindicated for use in pregnant women because infection during pregnancy with the wild-type viruses is associated with maternal and fetal adverse outcomes. Pregnancy should be avoided for 1 month after vaccination *[see Contraindications (4.3), Patient Counseling Information (17)]*.

Reports have indicated that contracting wild-type measles during pregnancy enhances fetal risk, including increased rates of spontaneous abortion, stillbirth, premature delivery and congenital defects.[2,3] Wild-type mumps virus infection during the first trimester of pregnancy may increase the rate of spontaneous abortion. Pregnant women infected with wild-type rubella virus are at increased risk for miscarriage or stillbirth, and their infants are at risk for congenital rubella syndrome.[1]

Available data on inadvertent administration of PRIORIX to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

There are no animal studies with PRIORIX to inform use during pregnancy.

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

8.2 Lactation

Risk Summary

It is not known whether the vaccine components of PRIORIX are excreted in human milk. Data are not available to assess the effects of PRIORIX on the breastfed infant or on milk production/excretion. Studies have shown that lactating postpartum women vaccinated with live attenuated rubella vaccine may secrete the virus in breast milk and transmit it to breast-fed infants.[4,5] In the breast-fed infants with serological evidence of rubella virus vaccine strain antibodies, none exhibited severe disease; however, one exhibited mild clinical illness typical of acquired rubella.[6,7]

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for PRIORIX and any potential adverse effects on the breastfed child from

PRIORIX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of PRIORIX in infants younger than 12 months have not been established.

8.5 Geriatric Use

Clinical studies of PRIORIX did not include participants 65 years of age and older to determine whether they respond differently from younger participants.

11 DESCRIPTION

PRIORIX (Measles, Mumps, and Rubella Vaccine, Live) is a suspension for subcutaneous injection. PRIORIX is supplied as a sterile, lyophilized antigen component which is reconstituted at the time of use with the accompanying sterile water diluent. The lyophilized antigen component is a whitish to slightly pink powder.

PRIORIX contains the Schwarz strain of live attenuated measles virus, the RIT 4385 strain of live attenuated mumps virus (derived from the Jeryl Lynn strain), both propagated in chick-embryo fibroblasts from embryonated eggs of specific pathogen-free flocks and the Wistar RA 27/3 strain of live attenuated rubella virus propagated in MRC-5 human diploid cells. The 3 virus strains are cultured in media containing amino acids, a small amount of neomycin sulfate and bovine serum albumin and are stabilized after multiple washing steps in media free from antibiotics and albumin. The attenuated measles, mumps and rubella viruses are then mixed with a stabilizer prior to lyophilization.

After reconstitution, each approximately 0.5-mL dose contains not less than 3.4 $\log_{10}$ Cell Culture Infective Dose 50% ($CCID_{50}$) of measles virus, 4.2 $\log_{10}$ $CCID_{50}$ of mumps virus, and 3.3 $\log_{10}$ $CCID_{50}$ of rubella virus. Each dose also contains 32 mg of anhydrous lactose, 9 mg of sorbitol, 9 mg of amino acids, and 8 mg of mannitol. Each dose may also contain residual amounts of neomycin sulphate (≤25 mcg), ovalbumin (≤60 ng), and bovine serum albumin (≤50 ng), from the manufacturing process. After reconstitution, PRIORIX is a clear peach- to fuchsia pink-colored suspension.

PRIORIX contains no preservative.

The tip caps of the prefilled syringes of sterile water diluent contain natural rubber latex. The plungers of the syringes and the stoppers of the lyophilized antigen component vials are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Humoral immune responses against measles, mumps, and rubella viruses induced by PRIORIX were measured by enzyme-linked immunosorbent assays (ELISAs). IgG antibodies measured by the ELISAs used in clinical studies of PRIORIX have been shown to correlate with the presence of neutralizing antibodies that have been associated with protection *[see Clinical Studies (14)]*.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

PRIORIX has not been evaluated for carcinogenic or mutagenic potential or for impairment of fertility.

14 CLINICAL STUDIES

The effectiveness of PRIORIX is based on a comparison of antibody responses relative to M-M-R II. Antibody responses to measles, mumps, and rubella viruses were measured by ELISAs. Analyses evaluated antibody geometric mean concentrations (GMC) and seroresponse rates (SRR). Seroresponse thresholds are 200 mIU/mL, 10 ELU/mL, and 10 IU/mL for anti-measles virus, anti-mumps virus, and anti-rubella virus antibodies, respectively.

14.1 Antibody Responses to Measles, Mumps and Rubella Viruses

Children 12 through 15 Months of Age Who Received PRIORIX as a First Dose

In Study 1 (NCT01702428), 5,003 participants 12 through 15 months of age received a first dose of PRIORIX (n = 3,714) or M-M-R II (n = 1,289) *[see Adverse Reactions (6.1)]*. Antibody responses to measles, mumps, and rubella viruses were measured by ELISAs using sera obtained 42 days following the first dose of either PRIORIX or M-M-R II. Non-inferiority of the immune response after the first dose of PRIORIX compared with M-M-R II was demonstrated in terms of SRR and GMC to measles, mumps, and rubella viruses. The immune responses measured in the U.S. study participants were similar to those in the overall study population. A summary of immune responses is shown in Table 4.

Table 4. Immune Responses after the First Dose of PRIORIX Compared with M-M-R II (Study 1, NCT01702428, According-to-Protocol Population)

Parameter	Virus Antigen	PRIORIX N = 3,187-3,248	M-M-R II N = 1,107-1,137	Difference (PRIORIX minus M-M-R II) (95% CI)
SRR[a] (%)	Measles	98	98	0.18 (-0.68, 1.25)
	Mumps	98	98	0.81 (-0.10, 1.96)
	Rubella	97	99	-1.15 (-2.00, -0.15)
		PRIORIX N = 3,187-3,248	**M-M-R II N = 1,107-1,137**	**Ratio (PRIORIX/M-M-R II) (95% CI)**
GMC[b]	Measles (mIU/mL)	3,165	3,215	0.98 (0.93, 1.05)
	Mumps (ELU/mL)	76	73	1.05 (0.99, 1.11)
	Rubella (IU/mL)	53	60	0.87 (0.83, 0.92)

According-to-Protocol cohort included all vaccinated participants who met protocol-defined criteria for immunogenicity analysis.
PRIORIX or M-M-R II was administered concomitantly with HAVRIX and VARIVAX; U.S. participants also received PREVNAR 13.
N = Number of participants.
SRR = Seroresponse rate (percentage of initially seronegative participants with concentration above seroresponse threshold for each assay).
GMC = Geometric mean antibody concentration adjusted for country.
CI = Confidence Interval
[a] Non-inferiority criterion met for all antigens (lower limit of 2-sided 95% CI for the difference [group receiving PRIORIX minus group receiving M-M-R II] was ≥-5%).
[b] Non-inferiority criterion met for all antigens (lower limit of 2-sided 95% CI for the ratio [group receiving PRIORIX over group receiving M-M-R II] was ≥0.67).

Children 12 through 15 Months of Age Who Received a Second Dose of PRIORIX 6 Weeks after the First Dose

In Study 2 (NCT01681992), 4,516 participants 12 through 15 months of age received a first dose of PRIORIX (n = 2,990) or M-M-R II (n = 1,526) followed by a second dose of the same vaccine 6 weeks later *[see Adverse Reactions (6.1)]*. Antibody responses to measles, mumps, and rubella

viruses were measured in a subset of participants (n = 199 – 259 PRIORIX; n = 212 – 257 M-M-R II) in sera obtained 42 days following the second dose of either PRIORIX or M-M-R II. In a descriptive analysis, the immune response after a second dose was similar between the group receiving PRIORIX and the group receiving M-M-R II in terms of antibody SRR and GMC for all antigens.

Children 4 through 6 Years of Age Who Received PRIORIX as a Second Dose of Measles, Mumps, and Rubella Virus Vaccine.

In Study 3 (NCT01621802), 4,007 participants 4 through 6 years of age received PRIORIX (n = 2,917) or M-M-R II (n = 1,090) as a second dose following administration of an initial dose of a combined measles, mumps, and rubella virus-containing vaccine in the second year of life *[see Adverse Reactions (6.1)]*. Prior to vaccination, the percentages of participants with antibody levels above the seroresponse thresholds were 98.0% for measles, 95.7% for mumps, and 98.7% for rubella. Antibody responses to measles, mumps, and rubella viruses were measured by ELISAs using sera obtained 42 days following of either PRIORIX or M-M-R II as a second dose. The non-inferiority of PRIORIX to M-M-R II when administered with KINRIX and VARIVAX was demonstrated in terms of SRR and GMC to measles, mumps, and rubella viruses at Day 42 (Table 5).

Table 5. Immune Responses to PRIORIX Compared with M-M-R II as a Second Dose in Children 4 through 6 Years of Age (Study 3, NCT01621802, According-to-Protocol Population)

Parameter	Virus Antigen	PRIORIX N = 690-698	M-M-R II N = 245-250	Difference (PRIORIX minus M-M-R II) (97.5% CI)
SRR[a] (%)	Measles	100	100	0.00 (-0.72, 1.98)
	Mumps	100	100	0.00 (-0.72, 1.97)
	Rubella	100	100	-0.14 (-0.98, 1.84)
		PRIORIX N = 690-691	**M-M-R-II N = 245-248**	**Ratio (PRIORIX/ M-M-R II) (97.5% CI)**
GMC[b]	Measles (mIU/mL)	4,285	4,333	0.99 (0.92, 1.06)
	Mumps (ELU/mL)	171	188	0.91 (0.83, 1.00)
	Rubella (IU/mL)	97	94	1.03 (0.97, 1.09)

According-to-Protocol cohort included all vaccinated participants who met protocol-defined criteria for immunogenicity analysis.
N = Number of participants.
SRR = Seroresponse rate (percentage of participants with concentration above seroresponse threshold for each assay).
GMC = Geometric mean antibody concentration adjusted for pre-vaccination concentration.
CI = Confidence Interval.
[a] Non-inferiority criterion met for all antigens (lower limit of 2-sided 97.5% CI for the difference [group receiving PRIORIX minus group receiving M-M-R II] was ≥-5%).
[b] Non-inferiority criterion met for all antigens (lower limit of 2-sided 97.5% CI for the ratio [group receiving PRIORIX over group receiving M-M-R II] was ≥0.67).

Individuals 7 Years of Age and Older Who Received PRIORIX as a Second Dose of Measles, Mumps, and Rubella Vaccine

In Study 4 (NCT02058563), 860 participants 7 years of age and older received PRIORIX (n = 426) or M-M-R II (n = 434) as a second dose following previous administration of a combined measles, mumps, and rubella virus-containing vaccine *[see Adverse Reactions (6.1)]*. Prior to vaccination, the percentages of participants with antibody levels above the seroresponse

thresholds were 93.1% for measles, 88.0% for mumps, and 81.9% for rubella. Antibody responses to measles, mumps, and rubella viruses were measured in sera obtained 42 days following the second dose of either PRIORIX or M-M-R II. The non-inferiority of the immune response after the second dose of PRIORIX compared with M-M-R II was demonstrated in terms of SRR and antibody GMC to measles, mumps, and rubella antigens. A summary of immune responses is shown in Table 6.

Table 6. Immune Responses to PRIORIX as a Second Dose Compared with M-M-R II (Study 4, NCT02058563, According-to-Protocol Population)

Parameter	Virus Antigen	PRIORIX N = 405	M-M-R II N = 414	Difference (PRIORIX minus M-M-R II) (95% CI)
SRR[a] (%)	Measles	99	99	-0.51 (-2.22, 1.02)
	Mumps	98	100	-1.25 (-3.10, 0.23)
	Rubella	100	100	-0.25 (-1.57, 0.90)
		PRIORIX N = 404	M-M-R II N = 413	Ratio (PRIORIX / M-M-R II) (95% CI)
GMC[b]	Measles (mIU/mL)	1,754	1,783	0.98 (0.89, 1.09)
	Mumps (ELU/mL)	114	110	1.04 (0.94, 1.15)
	Rubella (IU/mL)	76	74	1.03 (0.94, 1.12)

According-to-Protocol cohort included all vaccinated participants who met protocol-defined criteria for immunogenicity analysis.
N = Number of participants.
SRR = Seroresponse rate (percentage of participants with concentration above seroresponse threshold for each assay).
GMC = Geometric mean antibody concentration adjusted for gender, age, country, and pre-vaccination concentration.
CI = Confidence Intervals.
[a] Non-inferiority criterion met for all antigens (lower limit of 2-sided 95% CI for the difference [group receiving PRIORIX minus group receiving M-M-R II] was ≥-5%).
[b] Non-inferiority criterion met for all antigens (lower limit of 2-sided 95% CI for the ratio [group receiving PRIORIX over group receiving M-M-R II] was ≥0.67).

14.2 Concomitant Administration

Concomitant Administration with HAVRIX, VARIVAX, and PREVNAR 13

The concomitant use of PRIORIX or M-M-R II with HAVRIX and VARIVAX was evaluated in Study 1 (NCT01702428) in children 12 through 15 months of age. All participants received PRIORIX or M-M-R II administered concomitantly with HAVRIX and VARIVAX. Children enrolled in the U.S. also received PREVNAR 13 concomitantly.

In subsets of participants in Study 1, immune responses to the antigens contained in HAVRIX, VARIVAX, and PREVNAR 13 were measured in sera obtained 42 days after concomitant administration of PRIORIX or M-M-R II. There was no evidence that PRIORIX interfered with the antibody responses to these vaccines relative to the antibody responses when M-M-R II was concomitantly administered.

Concomitant Administration with KINRIX and VARIVAX

The concomitant use of PRIORIX or M-M-R II with KINRIX and VARIVAX was evaluated in Study 3 (NCT01621802) in children 4 through 6 years of age. A subset of participants received PRIORIX or M-M-R II administered concomitantly with KINRIX and VARIVAX.

Immune responses to the antigens contained in KINRIX and VARIVAX were measured in sera obtained 42 days after concomitant administration of PRIORIX or M-M-R II. There was no evidence that PRIORIX interfered with the antibody responses to these vaccines relative to the antibody responses when M-M-R II was concomitantly administered.

15 REFERENCES

1. Centers for Disease Control and Prevention. Measles, Mumps, and Rubella- Vaccine Use and Strategies for Elimination of Measles, Rubella, and Congenital Rubella Syndrome and Control of Mumps: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR*. 2013; 62(4);1-34.
2. Eberhart-Phillips, J.E.; et al: Measles in pregnancy: a descriptive study of 58 cases. Obstetrics and Gynecology, 82(5): 797-801, November 1993.
3. Jespersen, C.S.; et al: Measles as a cause of fetal defects: A retrospective study of ten measles epidemics in Greenland. Acta Paediatr Scand. 66: 367-372, May 1977.
4. Losonsky, G.A.; Fishaut, J.M.; Strussenber, J.; Ogra, P.L.: Effect of immunization against rubella on lactation products. II. Maternal-neonatal interactions, J. Infect. Dis. 145: 661-666, 1982.
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6. Landes, R.D.; Bass, J.W.; Millunchick, E.W.; Oetgen, W.J.: Neonatal rubella following postpartum maternal immunization, J. Pediatr. 97: 465-467, 1980.

7. Lerman, S.J.: Neonatal rubella following postpartum maternal immunization, J. Pediatr. 98: 668, 1981. (Letter)

16 HOW SUPPLIED/STORAGE AND HANDLING

PRIORIX is supplied in a box (NDC 58160-824-15) containing:

- 10 single-dose vials of lyophilized antigen component: NDC 58160-831-03
- 10 single-dose prefilled ungraduated syringes of sterile water diluent (packaged without needles): NDC 58160-833-02

After reconstitution, each vial contains one dose (approximately 0.5 mL) of PRIORIX.

16.1 Storage before Reconstitution

Vials of lyophilized antigen component: Store refrigerated between 36° and 46°F (2° and 8°C). Protect vials from light.

Prefilled ungraduated syringes of sterile water diluent: Store refrigerated between 36° and 46°F (2° and 8°C) or at controlled room temperature up to 77°F (25°C).

Do not freeze lyophilized antigen component or sterile water diluent.

16.2 Storage after Reconstitution

Administer PRIORIX immediately after reconstitution. If not used immediately, store refrigerated between 36° and 46°F (2° and 8°C) and administer within 8 hours. Discard reconstituted vaccine if not used within 8 hours.

Do not freeze. Discard if the reconstituted vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

- Inform vaccine recipients, parents, or guardians of the potential benefits and risks of vaccination with PRIORIX.
- Question individuals of reproductive potential regarding the possibility of pregnancy prior to administration of PRIORIX. Instruct these individuals to avoid pregnancy for 1 month following vaccination *[see Contraindications (4.3), Use in Specific Populations (8.1)]*.
- Inform vaccine recipients, parents, or guardians about the potential for adverse reactions that have been observed following administration of PRIORIX.
- Provide the Vaccine Information Statements, which are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

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Rixensart, Belgium, U.S. License 1617

Distributed by **GlaxoSmithKline**, Research Triangle Park, NC 27709

PRX:1PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ProQuad safely and effectively. See full prescribing information for ProQuad.

ProQuad®
Measles, Mumps, Rubella and Varicella Virus Vaccine Live
Suspension for subcutaneous injection
Initial U.S. Approval: 2005

----------INDICATIONS AND USAGE----------
ProQuad is a vaccine indicated for active immunization for the prevention of measles, mumps, rubella, and varicella in children 12 months through 12 years of age. (1)

---------- DOSAGE AND ADMINISTRATION ----------
Administer a 0.5-mL dose of ProQuad subcutaneously. (2.1)
- The first dose is administered at 12 to 15 months of age. (2.1)
- The second dose is administered at 4 to 6 years of age. (2.1)

---------- DOSAGE FORMS AND STRENGTHS ----------
Suspension for injection (0.5-mL dose) supplied as a lyophilized vaccine to be reconstituted using accompanying sterile diluent. (2.2, 3)

----------CONTRAINDICATIONS----------
- Hypersensitivity to any component of the vaccine. (4.1)
- Immunosuppression. (4.2)
- Moderate or severe febrile illness. (4.3)
- Active untreated tuberculosis. (4.4)
- Pregnancy. (4.5, 8.1)

---------- WARNINGS AND PRECAUTIONS----------
- Administration of ProQuad (dose 1) to children 12 to 23 months old who have not been previously vaccinated against measles, mumps, rubella, or varicella, nor had a history of the wild-type infections, is associated with higher rates of fever and febrile seizures at 5 to 12 days after vaccination when compared to children vaccinated with M-M-R® II and VARIVAX® administered separately. Exercise caution when administering ProQuad to persons with an individual or family history of febrile seizures. (5.1, 6.1, 6.3)
- Use caution when administering ProQuad to children with anaphylaxis or immediate hypersensitivity following egg ingestion. (5.2)
- Use caution when administering ProQuad to children with thrombocytopenia. (5.3)
- Evaluate individuals for immune competence prior to administration of ProQuad if there is a family history of congenital or hereditary immunodeficiency. (5.4)
- Avoid close contact with high-risk individuals susceptible to varicella because of possible transmission of varicella vaccine virus. (5.6)
- Immune Globulins (IG) and other blood products should not be given concurrently with ProQuad. (5.7, 7.1)
- Avoid using salicylates for 6 weeks after vaccination with ProQuad. (5.8, 7.2, 17)

---------- ADVERSE REACTIONS ----------
- The most frequent vaccine-related adverse events reported in ≥5% of subjects vaccinated with ProQuad were:
  - injection-site reactions (pain/tenderness/soreness, erythema, and swelling)
  - fever
  - irritability. (6.1)
- Systemic vaccine-related adverse events that were reported at a significantly greater rate in recipients of ProQuad than in recipients of the component vaccines administered concomitantly were:
  - fever
  - measles-like rash. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------DRUG INTERACTIONS----------
- Administration of immune globulins and other blood products concurrently with ProQuad vaccine may interfere with the expected immune response. (7.1)
- ProQuad vaccination may result in a temporary depression of purified protein derivative (PPD) tuberculin skin sensitivity. (7.4)
- ProQuad may be administered concomitantly with *Haemophilus influenzae* type b conjugate vaccine and/or hepatitis B vaccine at separate injection sites. (7.5)
- ProQuad may be administered concomitantly with pneumococcal 7-valent conjugate vaccine and/or hepatitis A vaccine (inactivated) at separate injection sites. (7.5)

---------- USE IN SPECIFIC POPULATIONS ----------
Pregnancy: Do not administer ProQuad to females who are pregnant. Pregnancy should be avoided for 3 months following vaccination with ProQuad. (4.5, 8.1, 17)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 04/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ProQuad® is a vaccine indicated for active immunization for the prevention of measles, mumps, rubella, and varicella in children 12 months through 12 years of age.

2 DOSAGE AND ADMINISTRATION

FOR SUBCUTANEOUS ADMINISTRATION ONLY

2.1 Recommended Dose and Schedule

Each 0.5-mL dose of ProQuad is administered subcutaneously.

The first dose is administered at 12 to 15 months of age but may be given anytime through 12 years of age.

The second dose is administered at 4 to 6 years of age. At least 1 month should elapse between a dose of a measles-containing vaccine and a dose of ProQuad. At least 3 months should elapse between a dose of varicella-containing vaccine and ProQuad.

2.2 Preparation for Administration

Use a sterile syringe free of preservatives, antiseptics, and detergents for each injection and/or reconstitution of the vaccine because these substances may inactivate the live virus vaccine. To reconstitute, use only the diluent supplied with the vaccine since it is free of preservatives or other antiviral substances which might inactivate the vaccine.

To reconstitute the vaccine, withdraw the entire volume of the supplied diluent from its vial and inject into lyophilized vaccine vial. Agitate to dissolve completely. Discard if the lyophilized vaccine cannot be dissolved.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Visually inspect the vaccine before and after reconstitution prior to administration. Do not use the product if particulates are present or if it appears discolored. Before reconstitution, the lyophilized vaccine is a white to pale yellow compact crystalline plug. ProQuad, when reconstituted, is a clear pale yellow to light pink liquid.

Withdraw the entire amount of the reconstituted vaccine from the vial into the same syringe, inject the entire volume, and discard vial.

To minimize loss of potency, the vaccine should be administered immediately after reconstitution. If not used immediately, the reconstituted vaccine may be stored at room temperature, protected from light, for up to 30 minutes. Discard reconstituted vaccine if it is not used within 30 minutes.

2.3 Method of Administration

Inject the vaccine subcutaneously into the outer aspect of the deltoid region of the upper arm or into the higher anterolateral area of the thigh.

3 DOSAGE FORMS AND STRENGTHS

ProQuad is a suspension for injection supplied as a single dose vial of lyophilized vaccine to be reconstituted using the accompanying sterile diluent *[see Dosage and Administration (2.2) and How Supplied/Storage and Handling (16)]*. A single dose after reconstitution is 0.5 mL.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Do not administer ProQuad to individuals with a history of hypersensitivity to any component of the vaccine (including gelatin) {1} or to a previous dose of M-M-R II® (Measles, Mumps, Rubella, Live), ProQuad or VARIVAX® (Varicella Virus Vaccine Live) vaccine, or any other measles, mumps, and rubella or varicella-containing vaccine. Do not administer ProQuad to individuals with a history of anaphylaxis to neomycin *[see Description (11)]*.

4.2 Immunosuppression

Do not administer ProQuad vaccine to individuals who are immunodeficient or immunosuppressed due to disease or medical therapy. Measles inclusion body encephalitis {2} (MIBE), pneumonitis {3} and death as a direct consequence of disseminated measles vaccine virus infection have been reported in

immunocompromised individuals inadvertently vaccinated with measles-containing vaccine. In this population, disseminated mumps and rubella vaccine virus infection have also been reported. Disseminated varicella disease and extensive vaccine-associated rash have been reported in individuals who are immunosuppressed or immunodeficient who were inadvertently vaccinated with a varicella-containing vaccine {4}.

4.3 Moderate or Severe Febrile Illness

Do not administer ProQuad to individuals with an active febrile illness with fever >101.3°F (>38.5°C).

4.4 Active Untreated Tuberculosis

Do not administer ProQuad vaccine to individuals with active untreated tuberculosis (TB).

4.5 Pregnancy

Do not administer ProQuad to individuals who are pregnant or planning on becoming pregnant in the next 3 months *[see Use in Specific Populations (8.1) and Patient Counseling Information (17)].*

5 WARNINGS AND PRECAUTIONS

5.1 Fever and Febrile Seizures

Administration of ProQuad (dose 1) to children 12 to 23 months old who have not been previously vaccinated against measles, mumps, rubella, or varicella, nor had a history of the wild-type infections, is associated with higher rates of fever and febrile seizures at 5 to 12 days after vaccination when compared to children vaccinated with a first dose of M-M-R II and VARIVAX administered concomitantly *[see Adverse Reactions (6.3)]*. Exercise caution when administering ProQuad to persons with an individual or family history of febrile seizures.

5.2 Hypersensitivity to Eggs

Individuals with a history of anaphylactic, anaphylactoid, or other immediate reactions (*e.g.*, hives, swelling of the mouth and throat, difficulty breathing, hypotension, or shock) subsequent to egg ingestion may be at an enhanced risk of immediate-type hypersensitivity reactions after receiving ProQuad vaccine. The potential risks and known benefits should be evaluated before considering vaccination in these individuals *[see Contraindications (4.1)]* {5}.

5.3 Thrombocytopenia

Transient thrombocytopenia has been reported within 4-6 weeks following vaccination with measles, mumps and rubella vaccine. Carefully evaluate the potential risk and benefit of vaccination in children with thrombocytopenia or in those who experienced thrombocytopenia after vaccination with a previous dose of a measles, mumps, and rubella-containing vaccine *[see Adverse Reactions (6.2)]* {6-8}.

5.4 Family History of Immunodeficiency

Vaccination should be deferred in individuals with a family history of congenital or hereditary immunodeficiency until the individual's immune status has been evaluated and the individual has been found to be immunocompetent.

5.5 Use in HIV-Infected Individuals

The Advisory Committee on Immunization Practices (ACIP) has recommendations on the use of varicella vaccine in HIV-infected individuals.

5.6 Risk of Vaccine Virus Transmission

Post-licensing experience suggests that transmission of varicella vaccine virus (Oka/Merck) resulting in varicella infection including disseminated disease may occur between vaccine recipients (who develop or do not develop a varicella-like rash) and contacts susceptible to varicella including healthy as well as high-risk individuals.

High-risk individuals susceptible to varicella include:

- Immunocompromised individuals;
- Pregnant women without documented positive history of varicella (chickenpox) or laboratory evidence of prior infection;
- Newborn infants of mothers without documented positive history of varicella or laboratory evidence of prior infection and all newborn infants born at <28 weeks gestation regardless of maternal varicella immunity.

Vaccine recipients should attempt to avoid, to the extent possible, close association with high-risk individuals susceptible to varicella for up to 6 weeks following vaccination. In circumstances where contact with high-risk individuals susceptible to varicella is unavoidable, the potential risk of transmission

of the varicella vaccine virus should be weighed against the risk of acquiring and transmitting wild-type varicella virus.

Excretion of small amounts of the live, attenuated rubella virus from the nose or throat has occurred in the majority of susceptible individuals 7 to 28 days after vaccination. There is no confirmed evidence to indicate that such virus is transmitted to susceptible persons who are in contact with the vaccinated individuals. Consequently, transmission through close personal contact, while accepted as a theoretical possibility, is not regarded as a significant risk. However, transmission of the rubella vaccine virus to infants via breast milk has been documented *[see Use in Specific Populations (8.2)]*.

There are no reports of transmission of the more attenuated Enders' Edmonston strain of measles virus or the Jeryl Lynn™ strain of mumps virus from vaccine recipients to susceptible contacts.

5.7 Immune Globulins and Transfusions

Immune Globulins (IG) and other blood products should not be given concurrently with ProQuad *[see Drug Interactions (7.1)]*. These products may contain antibodies that interfere with vaccine virus replication and decrease the expected immune response.

The ACIP has specific recommendations for intervals between administration of antibody containing products and live virus vaccines.

5.8 Salicylate Therapy

Avoid the use of salicylates (aspirin) or salicylate-containing products in children and adolescents 12 months through 12 years of age, for six weeks following vaccination with ProQuad due to the association of Reye syndrome with salicylate therapy and wild-type varicella infection *[see Drug Interactions (7.2) and Patient Counseling Information (17)]*.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. Vaccine-related adverse reactions reported during clinical trials were assessed by the study investigators to be possibly, probably, or definitely vaccine-related and are summarized below.

Children 12 Through 23 Months of Age Who Received a Single Dose of ProQuad

ProQuad was administered to 4497 children 12 through 23 months of age involved in 4 randomized clinical trials without concomitant administration with other vaccines. The safety of ProQuad was compared with the safety of M-M-R II and VARIVAX given concomitantly (N=2038) at separate injection sites. The safety profile for ProQuad was similar to the component vaccines. Children in these studies were monitored for up to 42 days postvaccination using vaccination report card-aided surveillance. Safety follow-up was obtained for 98% of children in each group. Few subjects (<0.1%) who received ProQuad discontinued the study due to an adverse reaction. The race distribution of the study subjects across these studies following a first dose of ProQuad was as follows: 65.2% White; 13.1% African-American; 11.1% Hispanic; 5.8% Asian/Pacific; 4.5% other; and 0.2% American Indian. The racial distribution of the control group was similar to that of the group who received ProQuad. The gender distribution across the studies following a first dose of ProQuad was 52.5% male and 47.5% female. The gender distribution of the control group was similar to that of the group who received ProQuad. Vaccine-related injection-site and systemic adverse reactions observed among recipients of ProQuad or M-M-R II and VARIVAX at a rate of at least 1% are shown in Table 1. Systemic vaccine-related adverse reactions that were reported at a significantly greater rate in individuals who received a first dose of ProQuad than in individuals who received first doses of M-M-R II and VARIVAX concomitantly at separate injection sites were fever (≥102°F [≥38.9°C] oral equivalent or abnormal) (21.5% versus 14.9%, respectively, risk difference 6.6%, 95% CI: 4.6, 8.5), and measles-like rash (3.0% versus 2.1%, respectively, risk difference 1.0%, 95% CI: 0.1, 1.8). Both fever and measles-like rash usually occurred within 5 to 12 days following the vaccination, were of short duration, and resolved with no long-term sequelae. Pain/tenderness/soreness at the injection site was reported at a statistically lower rate in individuals who received ProQuad than in individuals who received M-M-R II and VARIVAX concomitantly at separate injection sites (22.0% versus 26.8%, respectively, risk difference -4.8%, 95% CI: -7.1, -2.5). The only vaccine-related injection-site adverse reaction that was more frequent among recipients of ProQuad than recipients of M-M-R II and

VARIVAX was rash at the injection site (2.4% versus 1.6%, respectively, risk difference 0.9%, 95% CI: 0.1, 1.5).

Table 1: Vaccine-Related Injection-Site and Systemic Adverse Reactions Reported in ≥1% of Children Who Received ProQuad Dose 1 or M-M-R II and VARIVAX at 12 to 23 Months of Age (0 to 42 Days Postvaccination)

Adverse Reactions	ProQuad (N=4497) (n=4424) %	M-M-R II and VARIVAX (N=2038) (n=1997) %
*Injection Site**		
Pain/tenderness/soreness†	22.0	26.7
Erythema†	14.4	15.8
Swelling†	8.4	9.8
Ecchymosis	1.5	2.3
Rash	2.3	1.5
Systemic		
Fever†,‡	21.5	14.9
Irritability	6.7	6.7
Measles-like rash†	3.0	2.1
Varicella-like rash†	2.1	2.2
Rash (not otherwise specified)	1.6	1.4
Upper respiratory infection	1.3	1.1
Viral exanthema	1.2	1.1
Diarrhea	1.2	1.3

* Injection-site adverse reactions for M-M-R II and VARIVAX are based on occurrence with either of the vaccines administered.
† Designates a solicited adverse reaction. Injection-site adverse reactions were solicited only from Days 0 to 4 postvaccination.
‡ Temperature reported as elevated (≥102°F, oral equivalent) or abnormal.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

Rubella-like rashes were observed in <1% of subjects following a first dose of ProQuad.

In these clinical trials, two cases of herpes zoster were reported among 2108 healthy subjects 12 through 23 months of age who were vaccinated with their first dose of ProQuad and followed for 1 year. Both cases were unremarkable and no sequelae were reported.

Children 15 to 31 Months of Age Who Received a Second Dose of ProQuad

In 5 clinical trials, 2780 healthy children were vaccinated with ProQuad (dose 1) at 12 to 23 months of age and then administered a second dose approximately 3 to 9 months later. The race distribution of the study subjects across these studies following a second dose of ProQuad was as follows: 64.4% White; 14.1% African-American; 12.0% Hispanic; 5.9% other; 3.5% Asian/Pacific; and 0.1% American Indian. The gender distribution across the studies following a second dose of ProQuad was 51.5% male and 48.5% female. Children in these open-label studies were monitored for at least 28 days postvaccination using vaccination report card-aided surveillance. Safety follow-up was obtained for approximately 97% of children overall. Vaccine-related injection-site and systemic adverse reactions observed after Dose 1 and 2 of ProQuad at a rate of at least 1% are shown in Table 2. In these trials, the overall rates of systemic adverse reactions after ProQuad (dose 2) were comparable to, or lower than, those seen with the first dose. In the subset of children who received both ProQuad dose 1 and dose 2 in these trials (N=2408) with follow-up for fever, fever ≥102.2°F (≥38.9°C) was observed significantly less frequently days 1 to 28 after the second dose (10.8%) than after the first dose (19.1%) (risk difference 8.3%, 95% CI: 6.4, 10.3). Fevers ≥102.2°F (≥38.9°C) days 5 to 12 after vaccinations were also reported significantly less frequently after dose 2 (3.9%) than after dose 1 (13.6%) (risk difference 9.7%, 95% CI: 8.1, 11.3). In the subset of children who received both doses and for whom injection-site reactions were reported (N=2679), injection-site erythema was noted significantly more frequently after ProQuad (dose 2) as compared to ProQuad (dose 1) (12.6% and 10.8%, respectively, risk difference -1.8, 95% CI: -3.3, -0.3); however, pain and tenderness at the injection site was significantly lower after dose 2 (16.1%) as compared with after dose 1 (21.9%) (risk difference, 5.8%, 95% CI: 4.1, 7.6). Two children had febrile seizures after ProQuad (dose 2); both febrile seizures were thought to be related to a concurrent viral illness *[see Adverse Reactions (6.3) and Clinical Studies (14)]*. These studies were not designed or statistically powered to

detect a difference in rates of febrile seizure between recipients of ProQuad as compared to M-M-R II and VARIVAX. The risk of febrile seizure has not been evaluated in a clinical study comparing the incidence rate after ProQuad (dose 2) with the incidence rate after concomitant M-M-R II (dose 2) and VARIVAX (dose 2) *[see Adverse Reactions (6.1), Children 4 to 6 Years of Age Who Received ProQuad After Primary Vaccination with M-M-R II and VARIVAX].*

Table 2: Vaccine-Related Injection-Site and Systemic Adverse Reactions Reported in ≥1% of Children Who Received ProQuad Dose 1 at 12 to 23 Months of Age and Dose 2 at 15 to 31 Months of Age (1 to 28 Days Postvaccination)

Adverse Reactions	ProQuad Dose 1 (N=3112) (n=3019) %	ProQuad Dose 2 (N=2780) (n=2695) %
Injection-Site		
Pain/tenderness/soreness*	21.4	15.9
Erythema*	10.7	12.4
Swelling*	8.0	8.5
Injection-site bruising	1.1	0.0
Systemic		
Fever*,†	20.4	8.3
Irritability	6.0	2.4
Measles-like/Rubella-like rash	4.3	0.9
Varicella-like/Vesicular rash	1.5	0.1
Diarrhea	1.3	0.6
Upper respiratory infection	1.3	1.4
Rash (not otherwise specified)	1.2	0.6
Rhinorrhea	1.1	1.0

* Designates a solicited adverse reaction. Injection-site adverse reactions were solicited only from Days 1 to 5 postvaccination.
† Temperature reported as elevated or abnormal.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

Children 4 to 6 Years of Age Who Received ProQuad After Primary Vaccination with M-M-R II and VARIVAX

In a double-blind clinical trial, 799 healthy 4- to 6-year-old children who received M-M-R II and VARIVAX at least 1 month prior to study entry were randomized to receive ProQuad and placebo (N=399), M-M-R II and placebo concomitantly (N=205) at separate injection sites, or M-M-R II and VARIVAX (N=195) concomitantly at separate injection sites *[see Clinical Studies (14)]*. Children in these studies were monitored for up to 42 days postvaccination using vaccination report card-aided surveillance. Safety follow-up was obtained for >98% of children in each group. The race distribution of the study subjects following a dose of ProQuad was as follows: 78.4% White; 12.3% African-American; 3.8% Hispanic; 3.5% other; and 2.0% Asian/Pacific. The gender distribution following a dose of ProQuad was 52.1% male and 47.9% female. Injection-site and systemic adverse reactions observed after Dose 1 and 2 of ProQuad at a rate of at least 1% are shown in Table 3 *[see Clinical Studies (14)].*

Table 3: Vaccine-Related Injection-Site and Systemic Adverse Reactions Reported in ≥1% of Children Previously Vaccinated with M-M-R II and VARIVAX Who Received ProQuad + Placebo, M-M-R II + Placebo, or M-M-R II + VARIVAX at 4 to 6 Years of Age (1 to 43 Days Postvaccination)

Adverse Reactions	ProQuad + Placebo (N=399) (n=397) %	M-M-R II + Placebo (N=205) (n=205) %	M-M-R II + VARIVAX (N=195) (n=193) %
Systemic			
Fever*,†	2.5	2.0	4.1
Cough	1.3	0.5	0.5
Irritability	1.0	0.5	1.0
Headache	0.8	1.5	1.6
Rhinorrhea	0.5	1.0	0.5
Nasopharyngitis	0.3	1.0	1.0
Vomiting	0.3	1.0	0.5

Upper respiratory infection	0.0		0.0		1.0	
	ProQuad %	Placebo %	M-M-R II %	Placebo %	M-M-R II %	VARIVAX %
Injection-Site						
Pain*	41.1	34.5	36.6	34.1	35.2	36.8
Erythema*	24.4	13.4	15.6	14.1	14.5	15.5
Swelling*	15.6	8.1	10.2	8.8	7.8	10.9
Bruising	3.5	3.8	2.4	3.4	1.6	2.1
Rash	1.5	1.3	0.0	0.0	0.5	0.0
Pruritus	1.0	0.3	0.0	0.0	0.0	1.0
Nodule	0.0	0.0	0.0	0.0	0.0	1.0

* Designates a solicited adverse reaction. Injection-site adverse reactions were solicited only from Days 1 to 5 postvaccination.
† Temperature reported as elevated (≥102°F, oral equivalent) or abnormal.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

Safety in Trials That Evaluated Concomitant Use with Other Vaccines

ProQuad Administered with Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) and Haemophilus influenzae type b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine

In an open-label clinical trial, 1434 children were randomized to receive ProQuad given with diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP) and *Haemophilus influenzae* type b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant) vaccine concomitantly (N=949) or non-concomitantly with ProQuad given first and the other vaccines 6 weeks later (N=485). No clinically significant differences in adverse events were reported between treatment groups *[see Clinical Studies (14)]*. The race distribution of the study subjects who received ProQuad was as follows: 70.7% White; 10.9% Asian/Pacific; 10.7% African-American; 4.5% Hispanic; 3.0% other; and 0.2% American Indian. The gender distribution of the study subjects who received ProQuad was 53.6% male and 46.4% female.

ProQuad Administered with Pneumococcal 7-valent Conjugate Vaccine and/or Hepatitis A Vaccine, Inactivated

In an open-label clinical trial, 1027 healthy children 12 to 23 months of age were randomized to receive ProQuad (dose 1) and pneumococcal 7-valent conjugate vaccine (dose 4) concomitantly (N=510) or non-concomitantly at different clinic visits (N=517). The race distribution of the study subjects was as follows: 65.2% White; 15.1% African-American; 10.0% Hispanic; 6.6% other; and 3.0% Asian/Pacific. The gender distribution of the study subjects was 54.5% male and 45.5% female. Injection-site and systemic adverse reactions observed among recipients of ProQuad administered concomitantly or non-concomitantly with pneumococcal 7-valent conjugate vaccine at a rate of at least 1% are shown in Table 4. No clinically significant differences in adverse reactions were reported between the concomitant and non-concomitant treatment groups *[see Clinical Studies (14)]*.

Table 4: Vaccine-Related Injection-Site and Systemic Adverse Reactions Reported in ≥1% of Children Who Received ProQuad (dose 1) Concomitantly or Non-Concomitantly with PCV7* (dose 4) at the First Visit (1 to 28 Days Postvaccination)

Adverse Reactions	ProQuad + PCV7 (N=510) (n=498) %	PCV7 (N=258) (n=250) %	ProQuad (N=259) (n=255) %
Injection-Site - ProQuad			
Pain†	24.9	N/A	24.7
Erythema†	12.4	N/A	11.0
Swelling†	10.8	N/A	7.5
Bruising	2.0	N/A	1.6
Injection-Site - PCV7			
Pain†	30.5	29.6	N/A
Erythema†	21.1	24.4	N/A
Swelling†	17.9	20.0	N/A
Bruising	1.6	1.2	N/A
Systemic			
Fever†,‡	15.5	10.0	15.3
Measles-like rash	4.4	0.8	5.1

Irritability	3.8	3.6	3.5
Upper respiratory infection	1.6	0.8	1.2
Varicella-like/vesicular rash	1.6	0.0	1.2
Diarrhea	0.8	1.2	1.2
Vomiting	0.6	0.8	1.2
Rash	0.4	0.0	1.2
Somnolence	0.0	0.0	1.2

* PCV7 = Pneumococcal 7-valent conjugate vaccine, dose 4.
† Designates a solicited adverse reaction. Injection-site adverse reactions were solicited only from Days 1 to 5 postvaccination.
‡ Temperature reported as elevated (≥102°F, oral equivalent) or abnormal.
N/A = Not applicable.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

In an open-label clinical trial, 699 healthy children 12 to 23 months of age were randomized to receive 2 doses of VAQTA® (hepatitis A vaccine, inactivated) (N=352) or 2 doses of VAQTA concomitantly with 2 doses of ProQuad (N=347) at least 6 months apart. An additional 1101 subjects received 2 doses of VAQTA alone at least 6 months apart (non-randomized), resulting in 1453 subjects receiving 2 doses of VAQTA alone (1101 non-randomized and 352 randomized) and 347 subjects receiving 2 doses of VAQTA concomitantly with ProQuad (all randomized). The race distribution of the study subjects following a dose of ProQuad was as follows: 47.3% White; 42.7% Hispanic; 5.5% other; 2.9% African-American; and 1.7% Asian/Pacific. The gender distribution of the study subjects following a dose of ProQuad was 49.3% male and 50.7% female. Vaccine-related injection-site adverse reactions (days 1 to 5 postvaccination) and systemic adverse events (days 1 to 14 post VAQTA and days 1 to 28 post ProQuad vaccination) observed among recipients of VAQTA and ProQuad administered concomitantly with VAQTA at a rate of at least 1% are shown in Tables 5 and 6, respectively. In addition, among the randomized cohort, in the 14 days after each vaccination, the rates of fever (including all vaccine- and non-vaccine-related reports) were significantly higher in subjects who received ProQuad with VAQTA concomitantly after dose 1 (22.0%) as compared to subjects given dose 1 of VAQTA without ProQuad (10.8%). However, rates of fever were not significantly higher in subjects who received ProQuad with VAQTA concomitantly after dose 2 (12.5%) as compared to subjects given dose 2 of VAQTA without ProQuad (9.4%). In post-hoc analyses, these rates were significantly different for dose 1 (relative risk (RR) 2.03 [95% CI: 1.42, 2.94]), but not dose 2 (RR 1.32 [95% CI: 0.82, 2.13]). Rates of injection-site adverse reactions and other systemic adverse events were lower following a second dose than following the first dose of both vaccines given concomitantly.

Table 5: Vaccine-Related Injection-Site Adverse Reactions Reported in ≥1% of Children Who Received VAQTA or ProQuad Concomitantly with VAQTA 1 to 5 Days After Vaccination with VAQTA or VAQTA and ProQuad

	Dose 1		Dose 2	
Adverse Reactions	**VAQTA (N=1453) (n=1412) %**	**ProQuad + VAQTA (N=347) (n=328) %**	**VAQTA (N=1301) (n=1254) %**	**ProQuad + VAQTA (N=292) (n=264) %**
Injection-Site - VAQTA				
Pain/tenderness*	29.2	27.1	30.1	25.0
Erythema*	13.5	12.5	14.3	11.7
Swelling*	7.1	9.1	9.0	8.0
Injection-site bruising	1.9	2.4	1.0	0.8
Injection-Site - ProQuad				
Pain/tenderness*	N/A	30.5	N/A	26.2
Erythema*	N/A	13.4	N/A	12.9
Swelling*	N/A	6.7	N/A	6.5
Injection-site bruising	N/A	1.5	N/A	0.4

* Designates a solicited adverse reaction. Injection-site adverse reactions were solicited only from Days 1 to 5 postvaccination.
N/A = Not applicable.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

Table 6: Vaccine-Related Systemic Adverse Reactions

Reported in ≥1% of Children Who Received VAQTA* or ProQuad Concomitantly with VAQTA 1 to 14 Days After VAQTA or Vaccination with ProQuad and VAQTA and 1 to 28 Days After Vaccination with ProQuad and VAQTA

Adverse Reactions	Dose 1			Dose 2		
	Days 1 to 14		Days 1 to 28	Days 1 to 14		Days 1 to 28
	VAQTA† (N=1453) (n=1412) %	ProQuad + VAQTA† (N=347) (n=328) %	ProQuad + VAQTA (N=347) (n=328) %	VAQTA (N=1301) (n=1254) %	ProQuad + VAQTA† (N=292) (n=264) %	ProQuad + VAQTA† (N=291) (n=263) %
Fever‡,§	5.7	14.9	15.2	4.1	8.0	8.4
Irritability	5.8	7.0	7.3	3.5	5.3	5.3
Measles-like rash	0.0	3.4	3.4	0.0	1.1	1.1
Rhinorrhea	0.6	2.7	3.0	0.6	1.1	2.7
Diarrhea	1.5	1.8	2.4	1.7	0.4	0.8
Cough	0.6	2.1	2.1	0.2	0.8	1.5
Vomiting	1.1	0.3	0.9	0.6	0.8	1.1

* Systemic adverse events for subjects given VAQTA alone were collected for 14 days postvaccination.
† Safety follow-up for systemic adverse reactions was 14 days for VAQTA and 28 days for ProQuad + VAQTA.
‡ Designates a solicited adverse reaction.
§ Temperature reported as elevated or abnormal.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

In an open-label clinical trial, 653 children 12 to 23 months of age were randomized to receive a first dose of ProQuad with VAQTA and pneumococcal 7-valent conjugate vaccine concomitantly (N=330) or a first dose of ProQuad and pneumococcal 7-valent conjugate vaccine concomitantly and then vaccinated with VAQTA 6 weeks later (N=323). Approximately 6 months later, subjects received either the second doses of ProQuad and VAQTA concomitantly or the second doses of ProQuad and VAQTA separately. The race distribution of the study subjects was as follows: 60.3% White; 21.6% African-American; 9.5% Hispanic; 7.2% other; 1.1% Asian/Pacific; and 0.3% American Indian. The gender distribution of the study subjects was 50.7% male and 49.3% female. Vaccine-related injection-site and systemic adverse reactions observed among recipients of concomitant ProQuad, VAQTA, and pneumococcal 7-valent conjugate vaccine and ProQuad and pneumococcal 7-valent conjugate vaccine at a rate of at least 1% are shown in Tables 7 and 8. In the 28 days after vaccination with the first dose of ProQuad, the rates of fever (including all vaccine- and non-vaccine-related reports) were comparable in subjects who received the 3 vaccines together (38.6%) as compared with subjects given ProQuad and pneumococcal 7-valent conjugate vaccine (42.7%). The rates of fever in the 28 days following the second dose of ProQuad were also comparable in subjects who received ProQuad and VAQTA together (17.4%) as compared with subjects given ProQuad separately from VAQTA (17.0%). In a post-hoc analysis, these differences were not statistically significant after ProQuad (dose 1) (RR 0.90 [95% CI: 0.75, 1.09]) nor after dose 2 (RR 1.02 [95% CI: 0.70, 1.51]). No clinically significant differences in adverse reactions were reported among treatment groups *[see Clinical Studies (14)]*.

Table 7: Vaccine-Related Injection-Site Adverse Reactions Reported in ≥1% of Children Who Received ProQuad + VAQTA + PCV7* Concomitantly or VAQTA Alone Followed by ProQuad + PCV7 Concomitantly (1 to 5 Days After a Dose of ProQuad)

Adverse Reactions	Dose 1		Dose 2	
	VAQTA + ProQuad + PCV7 (N=330) (n=311) %	VAQTA Alone Followed by ProQuad + PCV7 (N=323) (n=302) %	VAQTA + ProQuad (N=273) (n=265) %	VAQTA Alone Followed by ProQuad (N=240) (n=230) %
Injection-Site - ProQuad				
Pain/tenderness†	21.2	24.2	18.1	17.0
Erythema†	13.5	11.9	10.6	13.0
Swelling†	7.4	10.9	8.3	11.7

Bruising	1.9	1.3	0.8	0.4
Injection-Site - VAQTA				
Pain/tenderness†	20.6	15.3	17.5	20.3
Erythema†	9.6	11.7	9.1	12.7
Swelling†	6.8	9.5	6.1	7.6
Bruising	1.3	1.1	1.1	1.6
Rash	1.0	0.0	0.4	0.4
Injection-Site - PCV7				
Pain/tenderness†	25.4	27.6	N/A	N/A
Erythema†	16.4	16.6	N/A	N/A
Swelling†	13.2	14.3	N/A	N/A
Bruising	0.6	1.7	N/A	N/A

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
† Designates a solicited adverse reaction. Injection-site adverse reactions were solicited only from Days 1 to 5 postvaccination at each vaccine injection site.
N/A = Not applicable.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

Table 8: Vaccine-Related Systemic Adverse Reactions Reported in ≥1% of Children Who Received ProQuad + VAQTA + PCV7* Concomitantly, or VAQTA Alone Followed by ProQuad + PCV7 Concomitantly (1 to 28 Days After a Dose of ProQuad)

Adverse Reactions	Dose 1		Dose 2	
	VAQTA + ProQuad + PCV7 (N=330) (n=311) %	VAQTA Alone Followed by ProQuad + PCV7 (N=323) (n=302) %	VAQTA + ProQuad (N=273) (n=265) %	VAQTA Alone Followed by ProQuad (N=240) (n=230) %
Fever†,‡	26.4	27.2	9.1	9.6
Irritability	4.8	6.3	1.9	1.3
Measles-like rash†	2.3	4.0	0.0	0.0
Varicella-like rash†	1.0	1.7	0.0	0.0
Rash (not otherwise specified)	1.3	1.3	0.0	0.9
Diarrhea	1.3	1.3	0.4	1.3
Upper respiratory infection	1.0	1.3	1.1	0.9
Viral infection	1.0	0.7	0.0	0.0
Rhinorrhea	0.0	0.7	1.1	0.0

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
† Designates a solicited adverse reaction.
‡ Temperature reported as elevated or abnormal.
N = number of subjects vaccinated.
n = number of subjects with safety follow-up.

6.2 Post-Marketing Experience

The following adverse events have been identified during post-approval use of either the components of ProQuad or ProQuad. Because the events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Infections and infestations

Subacute sclerosing panencephalitis, encephalitis, aseptic meningitis, meningitis, measles, atypical measles, pneumonia, respiratory infection, infection, varicella (vaccine strain), influenza, herpes zoster, orchitis, epididymitis, cellulitis, skin infection, retinitis, bronchitis, parotitis, sinusitis, impetigo, herpes simplex, candidiasis, rhinitis.

The vaccine virus (Oka/Merck strain) contained in ProQuad may establish latency of varicella zoster virus in immunocompetent individuals, with the potential for later development of herpes zoster.

Cases of encephalitis or meningitis caused by vaccine strain varicella virus have been reported in immunocompetent individuals previously vaccinated with VARIVAX (same varicella vaccine strain as in ProQuad) months to years after vaccination. Reported cases were commonly associated with preceding or concurrent herpes zoster rash.

Blood and the lymphatic system disorders

Aplastic anemia, thrombocytopenia, regional lymphadenopathy, lymphadenitis.

Immune system disorders

Anaphylaxis and related phenomena such as angioneurotic edema, facial edema, and peripheral edema, anaphylactoid reaction.

Psychiatric disorders

Agitation, apathy, nervousness.

Nervous system disorders

Measles inclusion body encephalitis, acute disseminated encephalomyelitis, transverse myelitis, cerebrovascular accident, encephalopathy, Guillain-Barré syndrome, optic neuritis, Bell's palsy, polyneuropathy, ataxia, hypersomnia, afebrile convulsions or seizures, febrile seizure, headache, syncope, dizziness, tremor, paresthesia.

Eye disorders

Necrotizing retinitis (in immunocompromised individuals), retrobulbar neuritis, ocular palsies, edema of the eyelid, irritation eye.

Ear and labyrinth disorders

Nerve deafness, ear pain.

Vascular disorders

Extravasation blood.

Respiratory, thoracic and mediastinal disorders

Pneumonitis, pulmonary congestion, wheezing, bronchial spasm, epistaxis, sore throat.

Gastrointestinal disorders

Hematochezia, abdominal pain, mouth ulcer.

Skin and subcutaneous tissue disorders

Stevens-Johnson syndrome, Henoch-Schönlein purpura, erythema multiforme, acute hemorrhagic edema of infancy, purpura, skin induration, panniculitis, pruritus.

Musculoskeletal, connective tissue and bone disorders

Arthritis, arthralgia, pain of the hip, leg, or neck; myalgia; musculoskeletal pain.

General disorders and administration site conditions

Injection-site complaints including wheal and flare, warm to touch, stiffness, warm sensation, inflammation, injection-site hemorrhage, injection-site injury.

6.3 Post-Marketing Observational Safety Surveillance Study

Safety was evaluated in an observational study that included 69,237 children vaccinated with ProQuad 12 months to 12 years old. A historical comparison group included 69,237 age-, gender-, and date-of-vaccination- (day and month) matched subjects who were given M-M-R II and VARIVAX concomitantly. The primary objective was to assess the incidence of febrile seizures occurring within various time intervals after vaccination in 12- to 60-month-old children who had neither been vaccinated against measles, mumps, rubella, or varicella, nor had a history of the wild-type infections (N=31,298 vaccinated with ProQuad, including 31,043 who were 12 to 23 months old). The incidence of febrile seizures was also assessed in a historical control group of children who had received their first vaccination with M-M-R II and VARIVAX concomitantly (N=31,298, including 31,019 who were 12 to 23 months old). The secondary objective was to assess the general safety of ProQuad in the 30-day period after vaccination in children 12 months to 12 years old.

In pre-licensure clinical studies, an increase in fever was observed 5 to 12 days after vaccination with ProQuad (dose 1) compared to M-M-R II and VARIVAX (dose 1) given concomitantly. In the post-marketing observational surveillance study, results from the primary safety analysis revealed an approximate two-fold increase in the risk of febrile seizures in the same 5 to 12 day timeframe after vaccination with ProQuad (dose 1). The incidence of febrile seizures 5 to 12 days after ProQuad (dose 1) (0.70 per 1000 children) was higher than that in children receiving M-M-R II and VARIVAX concomitantly (0.32 per 1000 children) [RR 2.20, 95% confidence interval (CI): 1.04, 4.65]. The incidence of febrile seizures 0 to 30 days after ProQuad (dose 1) (1.41 per 1000 children) was similar to that observed in children receiving M-M-R II and VARIVAX concomitantly [RR 1.10 (95% CI: 0.72, 1.69)]. See Table 9. General safety analyses revealed that the risks of fever (RR=1.89; 95% CI: 1.67, 2.15) and skin eruption (RR=1.68; 95% CI: 1.07, 2.64) were significantly higher after ProQuad (dose 1) compared with those who received concomitant first doses of M-M-R II and VARIVAX, respectively. All medical events that resulted

in hospitalization or emergency room visits were compared between the group given ProQuad and the historical comparison group, and no other safety concerns were identified in this study.

Table 9: Confirmed Febrile Seizures Days 5 to 12 and 0 to 30 After Vaccination with ProQuad (dose 1) Compared to Concomitant Vaccination with M-M-R II and VARIVAX (dose 1) in Children 12 to 60 Months of Age

Time Period	ProQuad cohort (N=31,298)		MMR+V cohort (N=31,298)		Relative risk (95% CI)
	n	Incidence per 1000	n	Incidence per 1000	
5 to 12 Days	22	0.70	10	0.32	2.20 (1.04, 4.65)
0 to 30 Days	44	1.41	40	1.28	1.10 (0.72, 1.69)

In this observational post-marketing study, no case of febrile seizure was observed during the 5 to 12 day postvaccination time period among 26,455 children who received ProQuad as a second dose of M-M-R II and VARIVAX. In addition, detailed general safety data were available from more than 25,000 children who received ProQuad as a second dose of M-M-R II and VARIVAX, most of them (95%) between 4 and 6 years of age, and an analysis of these data by an independent, external safety monitoring committee did not identify any specific safety concern.

7 DRUG INTERACTIONS

7.1 Immune Globulins and Transfusions

Administration of immune globulins and other blood products concurrently with ProQuad vaccine may interfere with the expected immune response *[see Warnings and Precautions (5.7)]* {9-11}. The ACIP has specific recommendations for intervals between administration of antibody containing products and live virus vaccines.

7.2 Salicylates

Reye syndrome has been reported following the use of salicylates during wild-type varicella infection. Vaccine recipients should avoid use of salicylates for 6 weeks after vaccination with ProQuad *[see Warnings and Precautions (5.8) and Patient Counseling Information (17)].*

7.3 Corticosteroids and Immunosuppressive Drugs

ProQuad vaccine should not be administered to individuals receiving immunosuppressive therapy, including high dose corticosteroids. Vaccination with ProQuad vaccine can result in disseminated disease and extensive vaccine-associated rash in individuals on immunosuppressive drugs *[see Contraindications (4.2)].*

7.4 Drug/Laboratory Test Interactions

Live, attenuated measles, mumps, and rubella virus vaccines given individually may result in a temporary depression of tuberculin skin sensitivity. Therefore, if a tuberculin test is to be done, it should be administered either any time before, simultaneously with, or at least 4 to 6 weeks after ProQuad.

7.5 Use with Other Vaccines

At least 1 month should elapse between a dose of a measles-containing vaccine and a dose of ProQuad, and at least 3 months should elapse between administration of 2 doses of ProQuad or varicella-containing vaccines.

ProQuad may be administered concomitantly with *Haemophilus influenzae* type b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant). Additionally, ProQuad may be administered concomitantly with pneumococcal 7-valent conjugate vaccine, and/or hepatitis A (inactivated) vaccines *[see Clinical Studies (14)].*

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

ProQuad vaccine contains live attenuated measles, mumps, rubella and varicella viruses. The vaccine is contraindicated for use in pregnant women because infection during pregnancy with the wild-type viruses is associated with maternal and fetal adverse outcomes.

For women who are inadvertently vaccinated when pregnant or who become pregnant within 3 months of administration of ProQuad, the healthcare provider should be aware of the following: (1) Reports have

indicated that contracting wild-type measles during pregnancy enhances fetal risk. Increased rates of spontaneous abortion, stillbirth, congenital defects, and prematurity have been observed subsequent to infection with wild-type measles during pregnancy. There are no adequate studies of the attenuated (vaccine) strain of measles virus in pregnancy; (2) Mumps infection during the first trimester of pregnancy may increase the rate of spontaneous abortion. Although mumps vaccine virus has been shown to infect the placenta and fetus, there is no evidence that it causes congenital malformations in humans {12}; (3) In a 10-year survey involving over 700 pregnant women who received rubella vaccine within 3 months before or after conception (of whom 189 received the Wistar RA 27/3 strain), none of the newborns had abnormalities compatible with congenital rubella syndrome {13}; and (4) Wild-type varicella, if acquired during pregnancy, can sometimes cause congenital varicella syndrome.

Available data on inadvertent administration of ProQuad to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

There are no relevant animal data.

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4%, and 15% to 20%, respectively {14,15}.

Data

Human Data

In a 10-year CDC survey involving over 700 pregnant women who received rubella vaccine within 3 months before or after conception (of whom 189 received the Wistar RA 27/3 strain), none of the newborns had abnormalities compatible with congenital rubella syndrome {13}.

8.2 Lactation

Risk Summary

It is not known whether varicella, measles, or mumps vaccine virus is excreted in human milk. Studies have shown that lactating postpartum women vaccinated with live rubella vaccine may secrete the virus in breast milk and transmit it to breastfed infants *[see Warnings and Precautions (5.6)]* {16,17}.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for ProQuad, and any potential adverse effects on the breastfed child from ProQuad or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Do not administer ProQuad to infants younger than 12 months of age or to children 13 years and older. Safety and effectiveness of ProQuad in infants younger than 12 months of age and in children 13 years and older have not been established *[see Clinical Studies (14)].*

8.5 Geriatric Use

ProQuad is not indicated for use in the geriatric population (≥age 65).

11 DESCRIPTION

ProQuad (Measles, Mumps, Rubella and Varicella Virus Vaccine Live) is a combined, attenuated, live virus vaccine containing measles, mumps, rubella, and varicella viruses. ProQuad is a sterile lyophilized preparation of (1) the components of M-M-R II (Measles, Mumps, and Rubella Virus Vaccine Live): Measles Virus Vaccine Live, a more attenuated line of measles virus, derived from Enders' attenuated Edmonston strain and propagated in chick embryo cell culture; Mumps Virus Vaccine Live, the Jeryl Lynn™ (B level) strain of mumps virus propagated in chick embryo cell culture; Rubella Virus Vaccine Live, the Wistar RA 27/3 strain of live attenuated rubella virus propagated in WI-38 human diploid lung fibroblasts; and (2) Varicella Virus Vaccine Live (Oka/Merck), the Oka/Merck strain of varicella-zoster virus propagated in MRC-5 cells. The cells, virus pools, bovine serum, and recombinant human albumin used in manufacturing are all tested to provide assurance that the final product is free of potential adventitious agents.

ProQuad, when reconstituted as directed, is a sterile suspension for subcutaneous administration. Each 0.5-mL dose contains not less than 3.00 $\log_{10}$ $TCID_{50}$ of measles virus; 4.30 $\log_{10}$ $TCID_{50}$ of mumps virus; 3.00 $\log_{10}$ $TCID_{50}$ of rubella virus; and not less than 3.99 $\log_{10}$ PFU of Oka/Merck varicella virus.

After reconstitution, each 0.5-mL dose of the vaccine also contains 21 mg of sucrose, 11 mg of hydrolyzed gelatin, 2.4 mg of sodium chloride, 1.8 mg of sorbitol, 0.40 mg of monosodium L-glutamate,

0.34 mg of sodium phosphate dibasic, 0.31 mg of recombinant human albumin, 0.17 mg of sodium bicarbonate, 72 mcg of potassium phosphate monobasic, 60 mcg of potassium chloride; 36 mcg of potassium phosphate dibasic; and residual components from the manufacturing process: MRC-5 cells including DNA and protein; <16 mcg of neomycin, ≤0.5 mcg of bovine calf serum, and other buffer and media ingredients. The product contains no preservative.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

ProQuad has been shown to induce measles-, mumps-, rubella-, and varicella-specific immunity, which is thought to be the mechanism by which it protects against these four childhood diseases.

The efficacy of ProQuad was established through the use of immunological correlates for protection against measles, mumps, rubella, and varicella. Results from efficacy studies or field effectiveness studies that were previously conducted for the component vaccines were used to define levels of serum antibodies that correlated with protection against measles, mumps, and rubella. Also, in previous studies with varicella vaccine, antibody responses against varicella virus ≥5 gpELISA units/mL in a glycoprotein enzyme-linked immunosorbent assay (gpELISA) (not commercially available) similarly correlated with long-term protection. In these efficacy studies, the clinical endpoint for measles and mumps was a clinical diagnosis of either disease confirmed by a 4-fold or greater rise in serum antibody titers between either postvaccination or acute and convalescent titers; for rubella, a 4-fold or greater rise in antibody titers with or without clinical symptoms of rubella; and for varicella, varicella-like rash that occurred >42 days postvaccination and for which varicella was not excluded by either viral cultures of the lesion or serological tests. Specific laboratory evidence of varicella either by serology or culture was not required to confirm the diagnosis of varicella. Clinical studies with a single dose of ProQuad have shown that vaccination elicited rates of antibody responses against measles, mumps, and rubella that were similar to those observed after vaccination with a single dose of M-M-R II *[see Clinical Studies (14)]* and seroresponse rates for varicella virus were similar to those observed after vaccination with a single dose of VARIVAX *[see Clinical Studies (14)]*. The duration of protection from measles, mumps, rubella, and varicella infections after vaccination with ProQuad is unknown.

12.6 Persistence of Antibody Responses after Vaccination

The persistence of antibody at 1 year after vaccination was evaluated in a subset of 2107 children enrolled in the clinical trials. Antibody was detected in 98.9% (1722/1741) for measles, 96.7% (1676/1733) for mumps, 99.6% (1796/1804) for rubella, and 97.5% (1512/1550) for varicella (≥5 gpELISA units/mL) of vaccinees following a single dose of ProQuad.

Experience with M-M-R II demonstrates that neutralizing and ELISA antibodies to measles, mumps, and rubella viruses are still detectable in 95-100%, 74-91%, and 90-100% of individuals respectively, 11 to 13 years after primary vaccination series {18-24}. Varicella antibodies were present for up to ten years postvaccination in most of the individuals tested who received 1 dose of VARIVAX.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

ProQuad has not been evaluated for its carcinogenic, mutagenic, or teratogenic potential, or its potential to impair fertility.

14 CLINICAL STUDIES

Formal studies to evaluate the clinical efficacy of ProQuad have not been performed.

Efficacy of the measles, mumps, rubella, and varicella components of ProQuad was previously established in a series of clinical studies with the monovalent vaccines. A high degree of protection from infection was demonstrated in these studies {25-32}.

Immunogenicity in Children 12 Months to 6 Years of Age

Prior to licensure, immunogenicity was studied in 5845 healthy children 12 months to 6 years of age with a negative clinical history of measles, mumps, rubella, and varicella who participated in 5 randomized clinical trials. The immunogenicity of ProQuad was similar to that of its individual component vaccines (M-M-R II and VARIVAX), which are currently used in routine vaccination.

The presence of detectable antibody was assessed by an appropriately sensitive enzyme-linked immunosorbent assay (ELISA) for measles, mumps (wild-type and vaccine-type strains), and rubella, and by gpELISA for varicella. For evaluation of vaccine response rates, a positive result in the measles ELISA corresponded to measles antibody concentrations of ≥255 mIU/mL when compared to the WHO II (66/202) Reference Immunoglobulin for Measles.

Children were positive for mumps antibody if the antibody level was ≥10 ELISA units/mL. A positive result in the rubella ELISA corresponded to concentrations of ≥10 IU rubella antibody/mL when compared to the WHO International Reference Serum for Rubella; children with varicella antibody levels ≥5 gpELISA units/mL were considered to be seropositive since a response rate based on ≥5 gpELISA units/mL has been shown to be highly correlated with long-term protection.

Immunogenicity in Children 12 to 23 Months of Age After a Single Dose

In 4 randomized clinical trials, 5446 healthy children 12 to 23 months of age were administered ProQuad, and 2038 children were vaccinated with M-M-R II and VARIVAX given concomitantly at separate injection sites. Subjects enrolled in each of these trials had a negative clinical history, no known recent exposure, and no vaccination history for varicella, measles, mumps, and rubella. Children were excluded from study participation if they had an immune impairment or had a history of allergy to components of the vaccine(s). Except for in 1 trial *[see ProQuad Administered with Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) and Haemophilus influenzae type b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine below]*, no concomitant vaccines were permitted during study participation. The race distribution of the study subjects across these studies following a first dose of ProQuad was as follows: 66.3% White; 12.7% African-American; 9.9% Hispanic; 6.7% Asian/Pacific; 4.2% other; and 0.2% American Indian. The gender distribution of the study subjects across these studies following a first dose of ProQuad was 52.6% male and 47.4% female. A summary of combined immunogenicity results 6 weeks following administration of a single dose of ProQuad or M-M-R II and VARIVAX is shown in Table 10. These results were similar to the immune response rates induced by concomitant administration of single doses of M-M-R II and VARIVAX at separate injection sites (lower bound of the 95% CI for the risk difference in measles, mumps, and rubella seroconversion rates were >-5.0 percentage points and the lower bound of the 95% CI for the risk difference in varicella seroprotection rates was either >-15 percentage points [one study] or >-10.0 percentage points [three studies]).

Table 10: Summary of Combined Immunogenicity Results 6 Weeks Following the Administration of a Single Dose of ProQuad (Varicella Virus Potency ≥3.97 $\log_{10}$ PFU) or M-M-R II and VARIVAX (Per-Protocol Population)

Group	Antigen	n	Observed Response Rate (95% CI)	Observed GMT (95% CI)
ProQuad (N=5446*)	Varicella	4381	91.2% (90.3%, 92.0%)	15.5 (15.0, 15.9)
	Measles	4733	97.4% (96.9%, 97.9%)	3124.9 (3038.9, 3213.3)
	Mumps (OD cutoff)†	973	98.8% (97.9%, 99.4%)	105.3 (98.0, 113.1)
	Mumps (wild-type ELISA)†	3735	95.8% (95.1%, 96.4%)	93.1 (90.2, 96.0)
	Rubella	4773	98.5% (98.1%, 98.8%)	91.8 (89.6, 94.1)
M-M-R II + VARIVAX (N=2038*)	Varicella	1417	94.1% (92.8%, 95.3%)	16.6 (15.9, 17.4)
	Measles	1516	98.2% (97.4%, 98.8%)	2239.6 (2138.3, 2345.6)
	Mumps (OD cutoff)†	501	99.4% (98.3%, 99.9%)	87.5 (79.7, 96.0)
	Mumps (wild-type ELISA)†	1017	98.0% (97.0%, 98.8%)	90.8 (86.2, 95.7)
	Rubella	1528	98.5% (97.7%, 99.0%)	102.2 (97.8, 106.7)

* Includes ProQuad + Placebo followed by ProQuad (Visit 1) (Protocol 009), ProQuad Middle and High Doses (Visit 1) (Protocol 011), ProQuad (Lot 1, Lot 2, Lot 3) (Protocol 012), both the Concomitant and Non-concomitant groups (Protocol 013).
† The mumps antibody response was assessed by a vaccine-strain ELISA in Protocols 009 and 011 and by a wild-type ELISA in Protocols 012 and 013. In the former assay, the serostatus was based on the OD cutoff of the assay. In the latter assay, 10 mumps ELISA units was used as the serostatus cutoff.
n = Number of per-protocol subjects with evaluable serology.
CI = Confidence interval.
GMT = Geometric mean titer.
ELISA = Enzyme-linked immunosorbent assay.
PFU = Plaque-forming units.
OD = Optical density.

Immunogenicity in Children 15 to 31 Months of Age After a Second Dose of ProQuad

In 2 of the 4 randomized clinical trials described above, a subgroup (N=1035) of the 5446 children administered a single dose of ProQuad were administered a second dose of ProQuad approximately 3 to 9 months after the first dose. Children were excluded from receiving a second dose of ProQuad if they were recently exposed to or developed varicella, measles, mumps, and/or rubella prior to receipt of the second dose. No concomitant vaccines were administered to these children. The race distribution across these studies following a second dose of ProQuad was as follows: 67.3% White; 14.3% African-American; 8.3% Hispanic; 5.4% Asian/Pacific; 4.4% other; 0.2% American Indian; and 0.10% mixed. The gender distribution of the study subjects across these studies following a second dose of ProQuad was 50.4% male and 49.6% female. A summary of immune responses following a second dose of ProQuad is presented in Table 11. Results from this study showed that 2 doses of ProQuad administered at least 3 months apart elicited a positive antibody response to all four antigens in greater than 98% of subjects. The geometric mean titers (GMTs) following the second dose of ProQuad increased approximately 2-fold each for measles, mumps, and rubella, and approximately 41-fold for varicella.

Table 11: Summary of Immune Response to a First and Second Dose of ProQuad in Subjects <3 Years of Age Who Received ProQuad with a Varicella Virus Dose ≥3.97 Log_{10} PFU*

		Dose 1 N=1097			Dose 2 N=1097		
Antigen	**Serostatus Cutoff/ Response Criteria**	**n**	**Observed Response Rate (95% CI)**	**Observed GMT (95% CI)**	**n**	**Observed Response Rate (95% CI)**	**Observed GMT (95% CI)**
Measles	≥120 mIU/mL†	915	98.1% (97.0%, 98.9%)	2956.8 (2786.3, 3137.7)	915	99.5% (98.7%, 99.8%)	5958.0 (5518.9, 6432.1)
	≥255 mIU/mL	943	97.8% (96.6%, 98.6%)	2966.0 (2793.4, 3149.2)	943	99.4% (98.6%, 99.8%)	5919.3 (5486.2, 6386.6)
Mumps	≥OD Cutoff (ELISA antibody units)	920	98.7% (97.7%, 99.3%)	106.7 (99.1, 114.8)	920	99.9% (99.4%, 100%)	253.1 (237.9, 269.2)
Rubella	≥10 IU/mL	937	97.7% (96.5%, 98.5%)	91.1 (85.9, 96.6)	937	98.3% (97.2%, 99.0%)	158.8 (149.1, 169.2)
Varicella	<1.25 to ≥5 gpELISA units	864	86.6% (84.1%, 88.8%)	11.6 (10.9, 12.3)	864	99.4% (98.7%, 99.8%)	477.5 (437.8, 520.7)
	≥OD Cutoff (gpELISA units)	695	87.2% (84.5%, 89.6%)	11.6 (10.9, 12.4)	695	99.4% (98.5%, 99.8%)	478.7 (434.8, 527.1)

* Includes the following treatment groups: ProQuad + Placebo followed by ProQuad (Visit 1) (Protocol 009) and ProQuad (Middle and High Dose) (Protocol 011).
† Samples from Protocols 009 and 011 were assayed in the legacy format Measles ELISA, which reported antibody titers in Measles ELISA units. To convert titers from ELISA units to mIU/mL, titers for these 2 protocols were divided by 0.1025.

The lowest measurable titer postvaccination is 207.5 mIU/mL. The response rate for measles in the legacy format is the percent of subjects with a negative baseline measles antibody titer, as defined by the optical density (OD) cutoff, with a postvaccination measles antibody titer ≥207.5 mIU/mL.
Samples from Protocols 009 and 011 were assayed in the legacy format Rubella ELISA, which reported antibody titers in Rubella ELISA units. To convert titers from ELISA units to IU/mL, titers for these 2 protocols were divided by 1.28.
ProQuad (Middle Dose) = ProQuad containing a varicella virus dose of 3.97 $\log_{10}$ PFU.
ProQuad (High Dose) = ProQuad containing a varicella virus dose of 4.25 $\log_{10}$ PFU.
ELISA = Enzyme-linked immunosorbent assay.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay.
N = Number vaccinated at baseline.
n = Number of subjects who were per-protocol Postdose 1 and Postdose 2 and satisfied the given prevaccination serostatus cutoff.
CI = Confidence interval.
GMT = Geometric mean titer.
PFU = Plaque-forming units.

Immunogenicity in Children 4 to 6 Years of Age Who Received a First Dose of ProQuad After Primary Vaccination With M-M-R II and VARIVAX

In a clinical trial, 799 healthy 4- to 6-year-old children who had received M-M-R II and VARIVAX at least 1 month prior to study entry were randomized to receive ProQuad and placebo (N=399), M-M-R II and placebo concomitantly at separate injection sites (N=205), or M-M-R II and VARIVAX concomitantly at separate injection sites (N=195). Children were eligible if they were previously administered primary doses of M-M-R II and VARIVAX, either concomitantly or non-concomitantly, at 12 months of age or older. Children were excluded if they were recently exposed to measles, mumps, rubella, and/or varicella, had an immune impairment, or had a history of allergy to components of the vaccine(s). No concomitant vaccines were permitted during study participation *[see Adverse Reactions (6.1) for ethnicity and gender information].*

A summary of antibody responses to measles, mumps, rubella, and varicella at 6 weeks postvaccination in subjects who had previously received M-M-R II and VARIVAX is shown in Table 12. Results from this study showed that a first dose of ProQuad after primary vaccination with M-M-R II and VARIVAX elicited a positive antibody response to all four antigens in greater than 98% of subjects. Postvaccination GMTs for recipients of ProQuad were similar to those following a second dose of M-M-R II and VARIVAX administered concomitantly at separate injection sites (the lower bound of the 95% CI around the fold difference in measles, mumps, rubella, and varicella GMTs excluded 0.5). Additionally, GMTs for measles, mumps, and rubella were similar to those following a second dose of M-M-R II given concomitantly with placebo (the lower bound of the 95% CI around the fold difference for the comparison of measles, mumps, and rubella GMTs excluded 0.5).

Table 12: Summary of Antibody Responses to Measles, Mumps, Rubella, and Varicella at 6 Weeks Postvaccination in Subjects 4 to 6 Years of Age Who Had Previously Received M-M-R II and VARIVAX (Per-Protocol Population)

Group Number (Description)	n	GMT (95% CI)	Seropositivity Rate (95% CI)	% ≥4-Fold Rise in Titer (95% CI)	Geometric Mean Fold Rise (95% CI)
		Measles*			
Group 1 (N=399) (ProQuad + placebo)	367	1985.9 (1817.6, 2169.9)	100% (99.0%, 100%)	4.9% (2.9%, 7.6%)	1.21 (1.13, 1.30)
Group 2 (N=205) (M-M-R II + placebo)	185	2046.9 (1815.2, 2308.2)	100% (98.0%, 100%)	4.3% (1.9%, 8.3%)	1.28 (1.17, 1.40)
Group 3 (N=195) (M-M-R II + VARIVAX)	171	2084.3 (1852.3, 2345.5)	99.4% (96.8%, 100%)	4.7% (2.0%, 9.0%)	1.31 (1.17, 1.46)
		Mumps†			
Group 1 (N=399) (ProQuad + placebo)	367	206.0 (188.2, 225.4)	99.5% (98.0%, 99.9%)	27.2% (22.8%, 32.1%)	2.43 (2.19, 2.69)
Group 2 (N=205) (M-M-R II + placebo)	185	308.5 (269.6, 352.9)	100% (98.0%, 100%)	41.1% (33.9%, 48.5%)	3.69 (3.14, 4.32)
Group 3 (N=195) (M-M-R II + VARIVAX)	171	295.9 (262.5, 333.5)	100% (97.9%, 100%)	41.5% (34.0%, 49.3%)	3.36 (2.84, 3.97)
		Rubella‡			

Group 1 (N=399) (ProQuad + placebo)	367	217.3 (200.1, 236.0)	100% (99.0%, 100%)	32.7% (27.9%, 37.8%)	3.00 (2.72, 3.31)
Group 2 (N=205) (M-M-R II + placebo)	185	174.0 (157.3, 192.6)	100% (98.0%, 100%)	31.9% (25.2%, 39.1%)	2.81 (2.41, 3.27)
Group 3 (N=195) (M-M-R II + VARIVAX)	171	154.1 (138.9, 170.9)	99.4% (96.8%, 100%)	26.9% (20.4%, 34.2%)	2.47 (2.17, 2.81)
		Varicella[§]			
Group 1 (N=399) (ProQuad + placebo)	367	322.2 (278.9, 372.2)	98.9% (97.2%, 99.7%)	80.7% (76.2%, 84.6%)	12.43 (10.63, 14.53)
Group 2 (N=205) (M-M-R II + placebo)	185	N/A	N/A	N/A	N/A
Group 3 (N=195) (M-M-R II + VARIVAX)	171	209.3 (171.2, 255.9)	99.4% (96.8%, 100%)	71.9% (64.6%, 78.5%)	8.50 (6.69, 10.81)

* Measles GMTs are reported in mIU/mL; seropositivity corresponds to ≥120 mIU/mL.
† Mumps GMTs are reported in mumps Ab units/mL; seropositivity corresponds to ≥10 Ab units/mL.
‡ Rubella titers obtained by the legacy format were converted to their corresponding titers in the modified format. Rubella serostatus was determined after the conversion to IU/mL: seropositivity corresponds to ≥10 IU/mL.
§ Varicella GMTs are reported in gpELISA units/mL; seropositivity rate is reported by % of subjects with postvaccination antibody titers ≥5 gpELISA units/mL. Percentages are calculated as the number of subjects who met the criterion divided by the number of subjects contributing to the per-protocol analysis.
gpELISA = Glycoprotein enzyme-linked immunosorbent assay; ELISA = Enzyme-linked immunosorbent assay; CI = Confidence interval; GMT = Geometric mean titer; N/A = Not applicable; N = Number of subjects vaccinated; n = number of subjects in the per-protocol analysis.

Immunogenicity Following Concomitant Use with Other Vaccines

ProQuad with Pneumococcal 7-valent Conjugate Vaccine and/or VAQTA

In a clinical trial, 1027 healthy children 12 to 15 months of age were randomized to receive ProQuad and pneumococcal 7-valent conjugate vaccine concomitantly (N=510) at separate injection sites or ProQuad and pneumococcal 7-valent conjugate vaccine non-concomitantly (N=517) at separate clinic visits *[see Adverse Reactions (6.1) for ethnicity and gender information].* The statistical analysis of non-inferiority in antibody response rates to measles, mumps, rubella, and varicella at 6 weeks postvaccination for subjects are shown in Table 13. In the per-protocol population, seroconversion rates were not inferior in children given ProQuad and pneumococcal 7-valent conjugate vaccine concomitantly when compared to seroconversion rates seen in children given these vaccines non-concomitantly for measles, mumps, and rubella. In children with baseline varicella antibody titers <1.25 gpELISA units/mL, the varicella seroprotection rates were not inferior when rates after concomitant and non-concomitant vaccination were compared 6 weeks postvaccination. Statistical analysis of non-inferiority in GMTs to *S. pneumoniae* serotypes at 6 weeks postvaccination are shown in Table 14. Geometric mean antibody titers (GMTs) for *S. pneumoniae* types 4, 6B, 9V, 14, 18C, 19F, and 23F were not inferior when antibody titers in the concomitant and non-concomitant groups were compared 6 weeks postvaccination.

Table 13: Statistical Analysis of Non-Inferiority in Antibody Response Rates to Measles, Mumps, Rubella, and Varicella at 6 Weeks Postvaccination for Subjects Initially Seronegative to Measles, Mumps, or Rubella, or With Varicella Antibody Titer <1.25 gpELISA units at Baseline in the ProQuad + PCV7* Treatment Group and the ProQuad Followed by PCV7 Control Group (Per-Protocol Analysis)

Assay Parameter	ProQuad + PCV7 (N=510) n	ProQuad + PCV7 (N=510) Estimated Response†	ProQuad followed by PCV7 (N=259) n	ProQuad followed by PCV7 (N=259) Estimated Response†	Difference (percentage points)†,‡ (95% CI)
Measles % ≥255 mIU/mL	406	97.3%	204	99.5%	-2.2 (-4.6, 0.2)
Mumps % ≥10 Ab units/mL	403	96.6%	208	98.6%	-1.9 (-4.5, 1.0)
Rubella % ≥10 IU/mL	377	98.7%	195	97.9%	0.9 (-1.3, 4.1)
Varicella % ≥5 gpELISA units/mL	379	92.5%	192	87.9%	4.5 (-0.4, 10.4)

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
Seronegative defined as baseline measles antibody titer <255 mIU/mL for measles, baseline mumps antibody titer <10 ELISA Ab units/mL for mumps, and baseline rubella antibody titer <10 IU/mL for rubella.
† Estimated responses and their differences were based on statistical analysis models adjusting for study center.
‡ ProQuad + PCV7 - ProQuad followed by PCV7.
The conclusion of non-inferiority is based on the lower bound of the 2-sided 95% CI on the risk difference being greater than -10 percentage points (*i.e.*, excluding a decrease equal to or more than the prespecified criterion of 10.0 percentage points). This indicates that the difference is statistically significantly less than the prespecified clinically relevant decrease of 10.0 percentage points at the 1-sided alpha = 0.025 level.
N = Number of subjects vaccinated in each treatment group.
n = Number of subjects with measles antibody titer <255 mIU/mL, mumps antibody titer <10 ELISA Ab units/mL, rubella antibody titer <10 IU/mL, or varicella antibody titer <1.25 gpELISA units/mL at baseline and with postvaccination serology contributing to the per-protocol analysis.
Ab = antibody; ELISA = Enzyme-linked immunosorbent assay; gpELISA = Glycoprotein enzyme-linked immunosorbent assay; CI = Confidence interval.

Table 14: Statistical Analysis of Non-Inferiority in GMTs to *S. pneumoniae* Serotypes at 6 Weeks Postvaccination in the ProQuad + PCV7* Treatment Group and the PCV7 Followed by ProQuad Control Group (Per-Protocol Analysis)

		Group 1 ProQuad + PCV7 (N=510)		Group 2 PCV7 followed by ProQuad (N=258)		
Serotype	Parameter	n	Estimated Response†	n	Estimated Response†	Fold-Difference*,‡ (95% CI)
4	GMT	410	1.5	193	1.3	1.2 (1.0, 1.4)
6B	GMT	410	8.9	192	8.4	1.1 (0.9, 1.2)
9V	GMT	409	2.9	193	2.5	1.2 (1.0, 1.3)
14	GMT	408	6.5	193	5.7	1.1 (1.0, 1.3)
18C	GMT	408	2.3	193	2.0	1.2 (1.0, 1.3)
19F	GMT	408	3.5	192	3.1	1.1 (1.0, 1.3)
23F	GMT	413	4.1	197	3.7	1.1 (1.0, 1.3)

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
† Estimated responses and their fold-difference were based on statistical analysis models adjusting for study center and prevaccination titer.
‡ ProQuad + PCV7 / PCV7 followed by ProQuad.
The conclusion of non-inferiority is based on the lower bound of the 2-sided 95% CI on the fold-difference being greater than 0.5, (*i.e.*, excluding a decrease of 2-fold or more). This indicates that the fold-difference is statistically significantly less than the pre-specified clinically relevant 2-fold difference at the 1-sided alpha = 0.025 level.
N = Number of subjects vaccinated in each treatment group; n = Number of subjects contributing to the per-protocol analysis for the given serotype; GMT = geometric mean titer; CI = Confidence interval.

In a clinical trial, 653 healthy children 12 to 15 months of age were randomized to receive VAQTA, ProQuad, and pneumococcal 7-valent conjugate vaccine concomitantly (N=330) or ProQuad and pneumococcal 7-valent conjugate vaccine concomitantly followed by VAQTA 6 weeks later (N=323) *[see Adverse Reactions (6.1) for ethnicity and gender information].* Statistical analysis of non-inferiority of the response rate for varicella antibody at 6 weeks postvaccination among subjects who received VAQTA concomitantly or non-concomitantly with ProQuad and pneumococcal 7-valent conjugate vaccine is shown in Table 15. For the varicella component of ProQuad, in subjects with baseline antibody titers <1.25 gpELISA units/mL, the proportion with a titer ≥5 gpELISA units/mL 6 weeks after their first dose of ProQuad was non-inferior when ProQuad was administered with VAQTA and pneumococcal 7-valent conjugate vaccine as compared to the proportion with a titer ≥5 gpELISA units/mL when ProQuad was administered with pneumococcal 7-valent conjugate vaccine alone. Statistical analysis of non-inferiority of the seropositivity rate for hepatitis A antibody at 4 weeks postdose 2 of VAQTA among subjects who received VAQTA concomitantly or non-concomitantly with ProQuad and pneumococcal 7-valent conjugate vaccine is shown in Table 16. The seropositivity rate to hepatitis A 4 weeks after a second dose of VAQTA given concomitantly with ProQuad and pneumococcal 7-valent conjugate vaccine (defined as the percent of subjects with a titer ≥10 mIU/mL) was non-inferior to the seropositivity rate observed when VAQTA was administered separately from ProQuad and pneumococcal 7-valent conjugate vaccine. Statistical analysis of non-inferiority in GMT to *S. pneumoniae* serotypes at 6 weeks postvaccination among subjects who received VAQTA concomitantly or non-concomitantly with ProQuad and pneumococcal 7-valent conjugate vaccine is shown in Table 17. Additionally, the GMTs for *S.*

pneumoniae types 4, 6B, 9V, 14, 18C, 19F, and 23F 6 weeks after vaccination with pneumococcal 7-valent conjugate vaccine administered concomitantly with ProQuad and VAQTA were non-inferior as compared to GMTs observed in the group given pneumococcal 7-valent conjugate vaccine with ProQuad alone. An earlier clinical study involving 617 healthy children provided data that indicated that the seroresponse rates 6 weeks post vaccination for measles, mumps, and rubella in those given M-M-R II and VAQTA concomitantly (N=309) were non-inferior as compared to historical controls.

Table 15: Statistical Analysis of Non-Inferiority of the Response Rate for Varicella Antibody at 6 Weeks Postvaccination Among Subjects Who Received VAQTA Concomitantly or Non-Concomitantly With ProQuad and PCV7* (Per-Protocol Analysis Set)

Parameter	Group 1: Concomitant VAQTA with ProQuad + PCV7 (N=330)		Group 2: Non-concomitant VAQTA separate from ProQuad + PCV7 (N=323)		Difference† (percentage points): Group 1 – Group 2 (95% CI)
	n	Estimated Response†	n	Estimated Response†	
% ≥5 gpELISA units/mL‡	225§	93.2%	232§	98.3%	-5.1 (-9.3, -1.4)

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
N = Number of subjects enrolled/randomized; n = Number of subjects contributing to the per-protocol analysis for varicella; CI = Confidence interval.
† Estimated responses and their differences were based on a statistical analysis model adjusting for combined study center.
‡ 6 weeks following Dose 1.
§ Initial Serostatus <1.25 gpELISA units/ mL.
The conclusion of similarity (non-inferiority) was based on the lower bound of the 2-sided 95% CI on the risk difference excluding a decrease of 10 percentage points or more (lower bound >-10.0). This indicated that the risk difference was statistically significantly greater than the pre-specified clinically relevant difference of -10 percentage points at the 1-sided alpha = 0.025 level.

Table 16: Statistical Analysis of Non-Inferiority of the Seropositivity Rate (SPR) for Hepatitis A Antibody at 4 Weeks Postdose 2 of VAQTA Among Subjects Who Received VAQTA Concomitantly or Non-Concomitantly With ProQuad and PCV7* (Per-Protocol Analysis Set)

Parameter	Group 1: Concomitant VAQTA with ProQuad + PCV7 (N=330)		Group 2: Non-concomitant VAQTA separate from ProQuad + PCV7 (N=323)		Difference† (percentage points): Group 1 - Group 2 (95% CI)
	n	Estimated Response†	n	Estimated Response†	
% ≥10 mIU/mL‡	182§	100.0%	159§	99.3%	0.7 (-1.4, 3.8)

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
CI = Confidence interval; N = Number of subjects enrolled/randomized; n = Number of subjects contributing to the per-protocol analysis for hepatitis A.
† Estimated responses and their differences were based on a statistical analysis model adjusting for combined study center.
‡ 4 weeks following receipt of 2 doses of VAQTA.
§ Regardless of initial serostatus.
The conclusion of non-inferiority was based on the lower bound of the 2-sided 95% CI on the risk difference being greater than -10 percentage points (*i.e.*, excluding a decrease of 10 percentage points or more) (lower bound >-10.0). This indicated that the risk difference was statistically significantly greater than the pre-specified clinically relevant difference of -10 percentage points at the 1-sided alpha = 0.025 level.

Table 17: Statistical Analysis of Non-Inferiority in Geometric Mean Titers (GMT) to *S. pneumoniae* Serotypes at 6 Weeks Postvaccination Among Subjects Who Received VAQTA Concomitantly or Non-Concomitantly With ProQuad and PCV7* (Per-Protocol Analysis Set)

Serotype	Group 1: Concomitant VAQTA with ProQuad + PCV7 (N=330)		Group 2: Non-concomitant VAQTA separate from ProQuad + PCV7 (N=323)		Fold-Difference† (95% CI)
	n	Estimated Response†	n	Estimated Response†	

4	246	1.9	247	1.7	1.1 (0.9, 1.3)
6B	246	9.9	246	9.9	1.0 (0.8, 1.2)
9V	247	3.7	247	4.2	0.9 (0.8, 1.0)
14	248	7.8	247	7.6	1.0 (0.9, 1.2)
18C	247	2.9	247	2.7	1.1 (0.9, 1.3)
19F	248	4.0	248	3.8	1.1 (0.9, 1.2)
23F	247	5.1	247	4.4	1.1 (1.0, 1.3)

* PCV7 = Pneumococcal 7-valent conjugate vaccine.
CI = Confidence interval; GMT = Geometric mean titer; N = Number of subjects enrolled/randomized; n = Number of subjects contributing to the per-protocol analysis for *S. pneumoniae* serotypes.
† Estimated responses and their fold-difference were based on statistical analysis models adjusting for combined study center and prevaccination titer.
The conclusion of non-inferiority was based on the lower bound of the 2-sided 95% CI on the fold-difference being greater than 0.5 (*i.e.*, excluding a decrease of 2-fold or more). This indicates that the fold-difference was statistically significantly less than the prespecified clinically relevant 2-fold difference at the 1-sided alpha = 0.025 level.

ProQuad Administered with Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) and Haemophilus influenzae type b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine

In a clinical trial, 1913 healthy children 12 to 15 months of age were randomized to receive ProQuad plus diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP) and *Haemophilus influenzae* type b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant) vaccine concomitantly at separate injection sites (N=949), ProQuad at the initial visit followed by DTaP and *Haemophilus* b conjugate and hepatitis B (recombinant) vaccine given concomitantly 6 weeks later (N=485), or M-M-R II and VARIVAX given concomitantly at separate injection sites (N=479) at the first visit *[see Adverse Reactions (6.1) for ethnicity and gender information].* Seroconversion rates and antibody titers for measles, mumps, rubella, varicella, anti-PRP, and hepatitis B were comparable between the 2 groups given ProQuad at approximately 6 weeks postvaccination indicating that ProQuad and *Haemophilus* b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant) vaccine may be administered concomitantly at separate injection sites (see Table 18 below). Response rates for measles, mumps, rubella, varicella, *Haemophilus influenzae* type b, and hepatitis B were not inferior in children given ProQuad plus *Haemophilus influenzae* type b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant) vaccines concomitantly when compared to ProQuad at the initial visit and *Haemophilus influenzae* type b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant) vaccines given concomitantly 6 weeks later. There are insufficient data to support concomitant vaccination with diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (data not shown).

Table 18: Summary of the Comparison of the Immunogenicity Endpoints for Measles, Mumps, Rubella, Varicella, *Haemophilus influenzae* type b, and Hepatitis B Responses Following Vaccination with ProQuad, *Haemophilus influenzae* type b Conjugate (Meningococcal Protein Conjugate), and Hepatitis B (Recombinant) Vaccine and DTaP Administered Concomitantly Versus Non-Concomitant Vaccination with ProQuad Followed by These Vaccines

		Concomitant Group	Non-Concomitant Group		
		N=949	N=485		
Vaccine Antigen	**Parameter**	**Response**	**Response**	**Risk Difference (95% CI)**	**Criterion for Non-inferiority**
Measles	% ≥120 mIU/mL	97.8%	98.7%	-0.9 (-2.3, 0.6)	LB >-5.0
Mumps	% ≥10 ELISA Ab units/mL	95.4%	95.1%	0.3 (-1.7, 2.6)	LB >-5.0
Rubella	% ≥10 IU/mL	98.6%	99.3%	-0.7 (-1.8, 0.5)	LB >-5.0
Varicella	% ≥5 gpELISA units/mL	89.6%	90.8%	-1.2 (-4.1, 2.0)	LB >-10.0
HiB-PRP	% ≥1.0 mcg/mL	94.6%	96.5%	-1.9 (-4.1, 0.8)	LB >-10.0
HepB	% ≥10 mIU/mL	95.9%	98.8%	-2.8	LB >10.0

				(-4.8, -0.8)	

HiB-PRP = *Haemophilus influenzae* type b, polyribosyl phosphate; HepB = hepatitis B; LB = lower bound, limit for non-inferiority comparison.

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16 HOW SUPPLIED/STORAGE AND HANDLING

No. 4171 — ProQuad is supplied as follows:

(1) a package of 10 single-dose vials of lyophilized vaccine, NDC 0006-4171-00 (package A)

(2) a separate package of 10 vials of sterile water diluent (package B).

Storage

To maintain potency, ProQuad must be stored frozen between -58°F and +5°F (-50°C to -15°C). Use of dry ice may subject ProQuad to temperatures colder than -58°F (-50°C).

Before reconstitution, store the lyophilized vaccine in a freezer at a temperature between −58°F and +5°F (−50°C and −15°C) for up to 18 months. Any freezer (*e.g.*, chest, frost-free) that reliably maintains an average temperature between −58°F and +5°F (−50°C and −15°C) and has a separate sealed freezer door is acceptable for storing ProQuad. Routine defrost cycling of a frost-free freezer is acceptable.

ProQuad may be stored at refrigerator temperature (36° to 46°F, 2° to 8°C) for up to 72 hours prior to reconstitution. Discard any ProQuad vaccine stored at 36° to 46°F which is not used within 72 hours of removal from 5°F (-15°C) storage.

Protect the vaccine from light at all times since such exposure may inactivate the vaccine viruses.

IF NOT USED IMMEDIATELY, THE RECONSTITUTED VACCINE MAY BE STORED AT ROOM TEMPERATURE, PROTECTED FROM LIGHT, FOR UP TO 30 MINUTES.

DISCARD RECONSTITUTED VACCINE IF IT IS NOT USED WITHIN 30 MINUTES.

DO NOT FREEZE RECONSTITUTED VACCINE.

Diluent should be stored separately at room temperature (68° to 77°F, 20° to 25°C), or in a refrigerator (36° to 46°F, 2° to 8°C).

For information regarding the product or questions regarding storage conditions, call 1-800-MERCK-90.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Instructions

Provide the required vaccine information to the patient, parent, or guardian.

Inform the patient, parent, or guardian of the benefits and risks associated with vaccination.

Inform the patient, parent, or guardian that the vaccine recipient should avoid use of salicylates for 6 weeks after vaccination with ProQuad *[see Warnings and Precautions (5.8) and Drug Interactions (7.2)]*.

Instruct postpubertal females to avoid pregnancy for 3 months following vaccination *[see Indications and Usage (1), Contraindications (4.5) and Use in Specific Populations (8.1)].*

Inform patients, parents, or guardians that vaccination with ProQuad may not offer 100% protection from measles, mumps, rubella, and varicella infection.

Instruct patients, parents, or guardians to report any adverse reactions to their health care provider. The U.S. Department of Health and Human Services has established a Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine, including but not limited to the reporting of events required by the National Childhood Vaccine Injury Act of 1986. For information or a copy of the vaccine reporting form, call the VAERS toll-free number at 1-800-822-7967, or report online at www.vaers.hhs.gov.

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For patent information: www.merck.com/product/patent/home.html

uspi-v221-i-fro-rha-2104r012

Meningococcal

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Menactra® safely and effectively. See full prescribing information for Menactra vaccine.

Menactra®, Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine
Solution for Intramuscular Injection

Initial U.S. Approval: 2005

----------**RECENT MAJOR CHANGES** ----------
Warnings and Precautions, Altered Immunocompetence (5.3) 4/2018

----------**INDICATIONS AND USAGE**----------
Menactra is indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y and W-135. Menactra is approved for use in individuals 9 months through 55 years of age. Menactra does not prevent *N meningitidis* serogroup B disease. (1)

----------**DOSAGE AND ADMINISTRATION**----------
A 0.5 mL dose for intramuscular injection. (2)

Primary Vaccination:
- Children 9 through 23 months of age: Two doses, three months apart.
- Individuals 2 through 55 years of age: A single dose.

Booster Vaccination:
- A single booster dose may be given to individuals 15 through 55 years of age at continued risk for meningococcal disease, if at least 4 years have elapsed since the prior dose.

----------**DOSAGE FORMS AND STRENGTHS**----------
Solution supplied in 0.5 mL single-dose vials (3)

----------**CONTRAINDICATIONS**----------
- Severe allergic reaction (eg, anaphylaxis) after a previous dose of a meningococcal capsular polysaccharide-, diphtheria toxoid- or CRM_{197}-containing vaccine, or to any component of Menactra. (4)

----------**WARNINGS AND PRECAUTIONS**----------
- Persons previously diagnosed with Guillain-Barré syndrome (GBS) may be at increased risk of GBS following receipt of Menactra. The decision to give Menactra should take into account the potential benefits and risks. (5.1)

----------**ADVERSE REACTIONS**----------
- Common (≥10%) solicited adverse events in infants and toddlers 9 and 12 months of age were injection site tenderness, erythema, and swelling; irritability, abnormal crying, drowsiness, appetite loss, vomiting, and fever. (6)
- Common (≥10%) solicited adverse events in individuals 2 through 55 years of age who received a single dose were injection site pain, redness, induration, and swelling; anorexia and diarrhea. Other common solicited adverse events were irritability and drowsiness (2-10 years of age), headache, fatigue, malaise, and arthralgia (11-55 years of age). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc. at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

----------**DRUG INTERACTIONS**----------
- When Menactra and DAPTACEL® (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed) are to be administered to children 4 through 6 years of age, preference should be given to simultaneous administration of the 2 vaccines or administration of Menactra prior to DAPTACEL. Administraton of Menactra one month after DAPTACEL has been shown to reduce meningococcal antibody responses to Menactra. (7.1)
- Pneumococcal antibody responses to some serotypes in Prevnar (PCV7) were decreased following co-administration of Menactra and PCV7. (7.1)

----------**USE IN SPECIFIC POPULATIONS**----------
- Safety and effectiveness of Menactra have not been established in children younger than 9 months of age, pregnant women, nursing mothers, and adults older than 55 years of age. (8.1, 8.2, 8.4, 8.5)
- A pregnancy registry is available. Contact Sanofi Pasteur Inc. at 1-800-822-2463. (8.1)

See **17 PATIENT_COUNSELING_INFORMATION**.
Revised: April 2018

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION:

1 INDICATIONS AND USAGE

Menactra®, Meningococcal (Groups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine, is indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y and W-135. Menactra is approved for use in individuals 9 months through 55 years of age. Menactra does not prevent *N meningitidis* serogroup B disease.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

Menactra is a clear to slightly turbid solution. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If any of these conditions exist, the vaccine should not be administered.

Withdraw the 0.5 mL dose of vaccine from the single-dose vial using a sterile needle and syringe.

2.2 Dose and Schedule

Menactra is administered as a 0.5 mL dose by intramuscular injection. Do not administer this product intravenously or subcutaneously.

Primary Vaccination:

- In children 9 through 23 months of age, Menactra is given as a 2-dose series three months apart.

- Individuals 2 through 55 years of age, Menactra is given as a single dose.

Booster Vaccination:

- A single booster dose may be given to individuals 15 through 55 years of age at continued risk for meningococcal disease, if at least 4 years have elapsed since the prior dose.

3 DOSAGE FORMS AND STRENGTHS

Menactra is a solution supplied in 0.5 mL single-dose vials. [See *Description (11)* for a complete listing of ingredients.]

4 CONTRAINDICATIONS

Severe allergic reaction (eg, anaphylaxis) after a previous dose of a meningococcal capsular polysaccharide-, diphtheria toxoid- or CRM_{197}-containing vaccine, or to any component of Menactra [see *Description (11)*].

5 WARNINGS AND PRECAUTIONS

5.1 Guillain-Barré Syndrome

Persons previously diagnosed with Guillain-Barré syndrome (GBS) may be at increased risk of GBS following receipt of Menactra. The decision to give Menactra should take into account the potential benefits and risks.

GBS has been reported in temporal relationship following administration of Menactra (1) (2). The risk of GBS following Menactra vaccination was evaluated in a post-marketing retrospective cohort study [see *Post-Marketing Experience (6.2)*].

5.2 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions to allow an assessment of benefits and risks. Epinephrine and other appropriate agents used for the control of immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur.

5.3 Altered Immunocompetence

- *Reduced Immune Response*

Some individuals with altered immunocompetence, including some individuals receiving immunosuppressant therapy, may have reduced immune responses to Menactra.

- *Complement Deficiency*

Persons with certain complement deficiencies and persons receiving treatment that inhibits terminal complement activation (for example, eculizumab) are at increased risk for invasive disease caused by *N meningitidis*, including invasive disease caused by serogroups A, C, Y and W-135, even if they develop antibodies following vaccination with Menactra. [See *Clinical Pharmacology (12).*]

5.4 Limitations of Vaccine Effectiveness

Menactra may not protect all recipients.

5.5 Syncope

Syncope (fainting) has been reported following vaccination with Menactra. Procedures should be in place to prevent falling injury and manage syncopal reactions.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

Children 9 Through 12 Months of Age

The safety of Menactra was evaluated in four clinical studies that enrolled 3721 participants who received Menactra at 9 and 12 months of age. At 12 months of age these children also received one or more other recommended vaccines [Measles, Mumps, Rubella and Varicella Virus Vaccine Live (MMRV) or Measles, Mumps, and Rubella Virus Vaccine (MMR) and Varicella Virus Vaccine Live (V) each manufactured by Merck & Co., Inc., Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein) manufactured by Wyeth Pharmaceuticals Inc. (PCV7), Hepatitis A Vaccine manufactured by Merck & Co., Inc. (HepA). A control group of 997 children

was enrolled at 12 months of age and received two or more childhood vaccines [MMRV (or MMR+V), PCV7, HepA] at 12 months of age [see *Concomitant Vaccine Administration (14.3)*]. Three percent of individuals received MMR and V, instead of MMRV, at 12 months of age.

The primary safety study was a controlled trial that enrolled 1256 children who received Menactra at 9 and 12 months of age. At 12 months of age these children received MMRV (or MMR+V), PCV7 and HepA. A control group of 522 children received MMRV, PCV7 and HepA. Of the 1778 children, 78% of participants (Menactra, N=1056; control group, N=322) were enrolled at United States (US) sites and 22% at a Chilean site. (Menactra, N=200; control group, N=200).

Individuals 2 Through 55 Years of Age

The safety of Menactra was evaluated in eight clinical studies that enrolled 10,057 participants aged 2-55 years who received Menactra and 5,266 participants who received Menomune® – A/C/Y/W-135, Meningococcal Polysaccharide Vaccine, Groups A, C, Y and W-135 Combined. There were no substantive differences in demographic characteristics between the vaccine groups. Among Menactra recipients 2-55 years of age 24.0%, 16.2%, 40.4% and 19.4% were in the 2-10, 11-14, 15-25 and 26-55-year age groups, respectively. Among Menomune – A/C/Y/W-135 recipients 2-55 years of age 42.3%, 9.3%, 30.0% and 18.5% were in the 2-10, 11-14, 15-25 and 26-55-year age groups, respectively. The three primary safety studies were randomized, active-controlled trials that enrolled participants 2-10 years of age (Menactra, N=1713; Menomune – A/C/Y/W-135, N=1519), 11-18 years of age (Menactra, N=2270; Menomune – A/C/Y/W-135, N=972) and 18-55 years of age (Menactra, N=1384; Menomune – A/C/Y/W-135, N=1170), respectively. Of the 3232 children 2-10 years of age, 68% of participants (Menactra, N=1164;

Menomune – A/C/Y/W-135, N=1031) were enrolled at US sites and 32% (Menactra, N=549; Menomune – A/C/Y/W-135, N=488) of participants at a Chilean site. The median ages in the Chilean and US subpopulations were 5 and 6 years, respectively. All adolescents and adults were enrolled at US sites. As the route of administration differed for the two vaccines (Menactra given intramuscularly, Menomune – A/C/Y/W-135 given subcutaneously), study personnel collecting the safety data differed from personnel administering the vaccine.

Booster Vaccination Study

In an open-label trial conducted in the US, 834 individuals were enrolled to receive a single dose of Menactra 4-6 years after a prior dose. The median age of participants was 17.1 years at the time of the booster dose.

Safety Evaluation

Participants were monitored after each vaccination for 20 or 30 minutes for immediate reactions, depending on the study. Solicited injection site and systemic reactions were recorded in a diary card for 7 consecutive days after each vaccination. Participants were monitored for 28 days (30 days for infants and toddlers) for unsolicited adverse events and for 6 months post-vaccination for visits to an emergency room, unexpected visits to an office physician, and serious adverse events. Unsolicited adverse event information was obtained either by telephone interview or at an interim clinic visit. Information regarding adverse events that occurred in the 6-month post-vaccination time period was obtained via a scripted telephone interview.

Serious Adverse Events in All Safety Studies

Serious adverse events (SAEs) were reported during a 6-month time period following vaccinations in individuals 9 months through 55 years of age. In children who received Menactra at 9 months and at 12 months of age, SAEs occurred at a rate of 2.0% - 2.5%. In participants who received one or more childhood vaccine(s) (without co-administration of Menactra) at 12 months of age, SAEs occurred at a rate of 1.6% - 3.6%, depending on the number and type of vaccines received. In children 2-10 years of age, SAEs occurred at a rate of 0.6% following Menactra and at a rate of 0.7% following Menomune – A/C/Y/W-135. In adolescents 11 through 18 years of age and adults 18 years through 55 years of age, SAEs occurred at a rate of 1.0% following Menactra and at a rate of 1.3% following Menomune – A/C/Y/W-135. In adolescents and adults, SAEs occurred at a rate of 1.3% following booster vaccination with Menactra.

Solicited Adverse Events in the Primary Safety Studies

The most frequently reported solicited injection site and systemic adverse reactions within 7 days following vaccination in children 9 months and 12 months of age (Table 1) were injection site tenderness and irritability.

The most frequently reported solicited injection site and systemic adverse reactions in US children aged 2-10 years of age (Table 2) were injection site pain and irritability. Diarrhea, drowsiness, and anorexia were also common.

The most commonly reported solicited injection site and systemic adverse reactions in adolescents, ages 11-18 years (Table 3), and adults, ages 18-55 years (Table 4), after a single dose

were injection site pain, headache and fatigue. Except for redness in adults, injection site reactions were more frequently reported after Menactra vaccination than after Menomune – A/C/Y/W-135 vaccination.

Table 1: Percentage of US Participants Reporting Solicited Adverse Reactions Within 7 Days Following Vaccine Administration at 9 Months and 12 Months of Age

	Menactra at 9 months of age N[d]=998 - 1002			Menactra + PCV7[a]+ MMRV[b] + HepA[c] at 12 months of age N[d]=898 - 908			PCV7[a] + MMRV[b] + HepA[c] at 12 months of age N[d]=302 - 307		
Reaction	**Any**	**Grade 2**	**Grade 3**	**Any**	**Grade 2**	**Grade 3**	**Any**	**Grade 2**	**Grade 3**
Local/Injection Site									
Tenderness[e]									
Menactra Site	37.4	4.3	0.6	48.5	7.5	1.3	-	-	-
PCV7 Site	-	-	-	45.6	9.4	1.6	45.7	8.3	0.3
MMRV Site	-	-	-	38.9	7.1	1.0	43.0	5.2	0.0
HepA Site	-	-	-	43.4	8.7	1.4	40.9	4.6	0.3
Erythema[f]									
Menactra Site	30.2	2.5	0.3	30.1	1.3	0.1	-	-	-
PCV7 Site	-	-	-	29.4	2.6	0.2	32.6	3.0	0.7
MMRV Site	-	-	-	22.5	0.9	0.3	33.2	5.9	0.0
HepA Site	-	-	-	25.1	1.1	0.0	26.6	0.7	0.0
Swelling[f]									
Menactra Site	16.8	0.9	0.2	16.2	0.9	0.1	-	-	-
PCV7 Site	-	-	-	19.5	1.3	0.4	16.6	1.3	0.7
MMRV Site	-	-	-	12.1	0.4	0.1	14.1	0.3	0.0
HepA Site	-	-	-	16.4	0.7	0.2	13.5	0.0	0.3
Systemic									
Irritability[g]	56.8	23.1	2.9	62.1	25.7	3.7	64.8	28.7	4.2
Abnormal crying[h]	33.3	8.3	2.0	40.0	11.5	2.4	39.4	10.1	0.7
Drowsiness[i]	30.2	3.5	0.7	39.8	5.3	1.1	39.1	5.2	0.7
Appetite loss[j]	30.2	7.1	1.2	35.7	7.6	2.6	31.9	6.5	0.7
Vomiting[k]	14.1	4.6	0.3	11.0	4.4	0.2	9.8	2.0	0.0
Fever[l]	12.2	4.5	1.1	24.5	11.9	2.2	21.8	7.3	2.6

[a] PCV7 (Prevnar®) = Pneumococcal 7-valent Conjugate Vaccine

[b] MMRV (ProQuad®) = Measles, Mumps, Rubella and Varicella Virus Vaccine Live

[c] HepA (VAQTA®) = Hepatitis A Vaccine, Inactivated

[d] N = The number of participants with available data.

[e] Grade 2: cries and protests when injection site is touched, Grade 3: cries when injected limb is moved, or the movement of the injected limb is reduced.

[f] Grade 2: ≥1.0 inches to <2.0 inches, Grade 3: ≥2.0 inches.

[g] Grade 2: requires increased attention, Grade 3: inconsolable.

[h] Grade 2: 1 to 3 hours, Grade 3: >3 hours.

[i] Grade 2: not interested in surroundings or did not wake up for a feed/meal, Grade 3: sleeping most of the time or difficult to wake up.

[j] Grade 2: missed 1 or 2 feeds/meals completely, Grade 3: refuses ≥3 feeds/meals or refuses most feeds/meals.

[k] Grade 2: 2 to 5 episodes per 24 hours, Grade 3: ≥6 episodes per 24 hours or requiring parenteral hydration.

[l] Grade 2: >38.5°C to ≤39.5°C, Grade 3: >39.5°C.

Table 2: Percentage of US Participants 2 Years Through 10 Years of Age Reporting Solicited Adverse Reactions Within 7 Days Following Vaccine Administration

	Menactra N[a]=1156 - 1157			Menomune – A/C/Y/W-135 N[a]=1027		
Reaction	**Any**	**Grade 2**	**Grade 3**	**Any**	**Grade 2**	**Grade 3**
Local/Injection Site						
Pain[b]	45.0	4.9	0.3	26.1	2.5	0.0
Redness[c]	21.8	4.6	3.9	7.9	0.5	0.0
Induration[c]	18.9	3.4	1.4	4.2	0.6	0.0
Swelling[c]	17.4	3.9	1.9	2.8	0.3	0.0
Systemic						
Irritability[d]	12.4	3.0	0.3	12.2	2.6	0.6
Diarrhea[e]	11.1	2.1	0.2	11.8	2.5	0.3
Drowsiness[f]	10.8	2.7	0.3	11.2	2.5	0.5
Anorexia[g]	8.2	1.7	0.4	8.7	1.3	0.8
Arthralgia[h]	6.8	0.5	0.2	5.3	0.7	0.0
Fever[i]	5.2	1.7	0.3	5.2	1.7	0.2
Rash[j]	3.4	-	-	3.0	-	-
Vomiting[k]	3.0	0.7	0.3	2.7	0.7	0.6
Seizure[j]	0.0	-	-	0.0	-	-

[a] N = The total number of participants reporting at least one solicited reaction. The median age of participants was 6 years in both vaccine groups.

[b] Grade 2: interferes with normal activities, Grade 3: disabling, unwilling to move arm.

[c] Grade 2: 1.0-2.0 inches, Grade 3: >2.0 inches.

[d] Grade 2: 1-3 hours duration, Grade 3: >3 hours duration.

[e] Grade 2: 3-4 episodes, Grade 3: ≥5 episodes.

[f] Grade 2: interferes with normal activities, Grade 3: disabling, unwilling to engage in play or interact with others.

[g] Grade 2: skipped 2 meals, Grade 3: skipped ≥3 meals.

[h] Grade 2: decreased range of motion due to pain or discomfort, Grade 3: unable to move major joints due to pain.

[i] Oral equivalent temperature; Grade 2: 38.4°C to 39.4ºC, Grade 3: ≥39.5ºC.

[j] These solicited adverse events were reported as present or absent only.

[k] Grade 2: 2 episodes, Grade 3: ≥3 episodes.

Note: During the study Grade 1, Grade 2, and Grade 3 were collected as Mild, Moderate, and Severe respectively.

Table 3: Percentage of Participants 11 Years Through 18 Years of Age Reporting Solicited Adverse Reactions Within 7 Days Following Vaccine Administration With a Single Dose

	Menactra Na=2264 - 2265			Menomune – A/C/Y/W-135 Na=970		
Reaction	**Any**	**Grade 2**	**Grade 3**	**Any**	**Grade 2**	**Grade 3**
Local/Injection Site						
Pain[b]	59.2[c]	12.8[c]	0.3	28.7	2.6	0.0
Induration[d]	15.7[c]	2.5[c]	0.3	5.2	0.5	0.0
Redness[d]	10.9[c]	1.6[c]	0.6[c]	5.7	0.4	0.0
Swelling[d]	10.8[c]	1.9[c]	0.5[c]	3.6	0.3	0.0
Systemic						
Headache[e]	35.6[c]	9.6[c]	1.1	29.3	6.5	0.4
Fatigue[e]	30.0[c]	7.5	1.1[c]	25.1	6.2	0.2
Malaise[e]	21.9[c]	5.8[c]	1.1	16.8	3.4	0.4
Arthralgia[e]	17.4[c]	3.6[c]	0.4	10.2	2.1	0.1
Diarrhea[f]	12.0	1.6	0.3	10.2	1.3	0.0
Anorexia[g]	10.7[c]	2.0	0.3	7.7	1.1	0.2
Chills[e]	7.0[c]	1.7[c]	0.2	3.5	0.4	0.1
Fever[h]	5.1[c]	0.6	0.0	3.0	0.3	0.1
Vomiting[i]	1.9	0.4	0.3	1.4	0.5	0.3
Rash[j]	1.6	-	-	1.4	-	-
Seizure[j]	0.0	-	-	0.0	-	-

[a] N = The number of participants with available data.

[b] Grade 2: interferes with or limits usual arm movement, Grade 3: disabling, unable to move arm.

[c] Denotes $p < 0.05$ level of significance. The *p*-values were calculated for each category and severity using Chi Square test.

[d] Grade 2: 1.0-2.0 inches, Grade 3: >2.0 inches.

[e] Grade 2: interferes with normal activities, Grade 3: requiring bed rest.

[f] Grade 2: 3-4 episodes, Grade 3: ≥5 episodes.

[g] Grade 2: skipped 2 meals, Grade 3: skipped ≥3 meals.

[h] Oral equivalent temperature; Grade 2: 38.5°C to 39.4ºC, Grade 3: ≥39.5ºC.

[i] Grade 2: 2 episodes, Grade 3: ≥3 episodes.

[j] These solicited adverse events were reported as present or absent only.

Note: During the study Grade 1, Grade 2, and Grade 3 were collected as Mild, Moderate, and Severe respectively.

Table 4: Percentage of Participants 18 Years Through 55 Years of Age Reporting Solicited Adverse Reactions Within 7 Days Following Vaccine Administration With a Single Dose

	Menactra Na=1371			Menomune – A/C/Y/W-135 Na=1159		
Reaction	**Any**	**Grade 2**	**Grade 3**	**Any**	**Grade 2**	**Grade 3**
Local/Injection Site						
Pain[b]	53.9[c]	11.3[c]	0.2	48.1	3.3	0.1
Induration[d]	17.1[c]	3.4[c]	0.7[c]	11.0	1.0	0.0
Redness[d]	14.4	2.9	1.1[c]	16.0	1.9	0.1
Swelling[d]	12.6[c]	2.3[c]	0.9[c]	7.6	0.7	0.0
Systemic						
Headache[e]	41.4	10.1	1.2	41.8	8.9	0.9
Fatigue[e]	34.7	8.3	0.9	32.3	6.6	0.4
Malaise[e]	23.6	6.6[c]	1.1	22.3	4.7	0.9
Arthralgia[e]	19.8[c]	4.7[c]	0.3	16.0	2.6	0.1
Diarrhea[f]	16.0	2.6	0.4	14.0	2.9	0.3
Anorexia[g]	11.8	2.3	0.4	9.9	1.6	0.4
Chills[e]	9.7[c]	2.1[c]	0.6[c]	5.6	1.0	0.0
Vomiting[h]	2.3	0.4	0.2	1.5	0.2	0.4
Fever[i]	1.5[c]	0.3	0.0	0.5	0.1	0.0
Rash[j]	1.4	-	-	0.8	-	-
Seizure[j]	0.0	-	-	0.0	-	-

[a] N = The number of participants with available data.

[b] Grade 2: interferes with or limits usual arm movement, Grade 3: disabling, unable to move arm.

[c] Denotes $p < 0.05$ level of significance. The *p*-values were calculated for each category and severity using Chi Square test.

[d] Grade 2: 1.0-2.0 inches, Grade 3: >2.0 inches.

[e] Grade 2: interferes with normal activities, Grade 3: requiring bed rest.

[f] Grade 2: 3-4 episodes, Grade 3: ≥5 episodes.

[g] Grade 2: skipped 2 meals, Grade 3: skipped ≥3 meals.

[h] Grade 2: 2 episodes, Grade 3: ≥3 episodes.

[i] Oral equivalent temperature; Grade 2: 39.0°C to 39.9ºC, Grade 3: ≥40.0ºC.

[j] These solicited adverse events were reported as present or absent only.

Note: During the study Grade 1, Grade 2, and Grade 3 were collected as Mild, Moderate, and Severe respectively.

Solicited Adverse Events in a Booster Vaccination Study

For a description of the study design and number of participants, [see *Clinical Trials Experience, Booster Vaccination Study (6.1)*]. The most common solicited injection site and systemic reactions within 7 days of vaccination were pain (60.2%) and myalgia (42.8%), respectively. Overall rates of solicited injection site reactions and solicited systemic reactions were similar to those observed in adolescents and adults after a single Menactra dose. The majority of solicited reactions were Grade 1 or 2 and resolved within 3 days.

Adverse Events in Concomitant Vaccine Studies

Solicited Injection Site and Systemic Reactions when Given with Routine Pediatric Vaccines

For a description of the study design and number of participants, [see *Clinical Trials Experience (6.1), Concomitant Vaccine Administration (14.3)*]. In the primary safety study, 1378 US children were enrolled to receive Menactra alone at 9 months of age and Menactra plus one or more other routinely administered vaccines (MMRV, PCV7 and HepA) at 12 months of age (N=961). Another group of children received two or more routinely administered vaccines (MMRV, PCV7 and HepA) (control group, n=321) at 12 months of age. The frequency of occurrence of solicited adverse events is presented in Table 1. Participants who received Menactra and the concomitant

vaccines at 12 months of age described above reported similar frequencies of tenderness, redness and swelling at the Menactra injection site and at the concomitant vaccine injection sites. Tenderness was the most frequent injection site reaction (48%, 39%, 46% and 43% at the Menactra, MMRV, PCV7 and HepA sites, respectively). Irritability was the most frequent systemic reaction, reported in 62% of recipients of Menactra plus concomitant vaccines, and 65% of the control group. [See *Concomitant Vaccine Administration (14.3).*]

In a randomized, parallel group, US multi-center clinical trial conducted in children 4 through 6 years of age, Menactra was administered as follows: 30 days after concomitant DAPTACEL®, Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed, (DTaP), manufactured by Sanofi Pasteur Limited + IPOL®, Poliovirus Vaccine Inactivated, (IPV), manufactured by Sanofi Pasteur SA [Group A]; concomitantly with DAPTACEL followed 30 days later by IPV [Group B]; concomitantly with IPV followed 30 days later by DAPTACEL [Group C]. Solicited injection site and systemic reactions were recorded in a diary card for 7 consecutive days after each vaccination. For all study groups, the most frequently reported solicited local reaction at the Menactra site was pain: 52.2%, 60.9% and 56.0% of participants in Groups A, B and C, respectively. For all study groups, the most frequently reported systemic reaction following the administration of Menactra alone or with the respective concomitant vaccines was myalgia: 24.2%, 37.3% and 26.7% of participants in Groups A, B and C, respectively. Fever >39.5ºC occurred at <1.0% in all groups. [See *Concomitant Vaccine Administration (14.3).*]

Solicited Injection Site and Systemic Reactions when Given with Tetanus and Diphtheria Toxoid Adsorbed Vaccine

In a clinical study, rates of local and systemic reactions after Menactra and Tetanus and Diphtheria Toxoid Adsorbed (Td) vaccine manufactured by Sanofi Pasteur Inc. were compared [see *Drug Interactions (7)*, and *Concomitant Vaccine Administration (14.3)* for study description]. Injection site pain was reported more frequently after Td vaccination than after Menactra vaccination (71% versus 53%). The overall rate of systemic adverse events was higher when Menactra and Td vaccines were given concomitantly than when Menactra was administered 28 days after Td vaccine (59% versus 36%). In both groups, the most common reactions were headache (Menactra + Td vaccine, 36%; Td vaccine + Placebo, 34%; Menactra alone, 22%) and fatigue (Menactra + Td vaccine, 32%; Td vaccine + Placebo, 29%; Menactra alone, 17%). Fever ≥40.0ºC occurred at ≤0.5% in all groups.

Solicited Injection Site and Systemic Reactions when Given with Typhoid Vi Polysaccharide Vaccine

In a clinical study, rates of local and systemic reactions after Menactra and Typhim Vi® [Typhoid Vi Polysaccharide Vaccine] (Typhoid), produced by Sanofi Pasteur SA were compared [see *Drug Interactions (7)* and *Concomitant Vaccine Administration (14.3)*] for a description of the concomitantly administered vaccine, study design and number of participants. More participants experienced pain after Typhoid vaccination than after Menactra vaccination (Typhoid + Placebo, 76% versus Menactra + Typhoid, 47%). The majority (70%-77%) of injection site solicited reactions for both groups at either injection site were reported as Grade 1 and resolved within 3 days post-vaccination. In both groups, the most common systemic reaction was headache (Menactra +

Typhoid, 41%; Typhoid + Placebo, 42%; Menactra alone, 33%) and fatigue (Menactra + Typhoid, 38%; Typhoid + Placebo, 35%; Menactra alone, 27%). Fever ≥40.0ºC and seizures were not reported in either group.

6.2 Post-Marketing Experience

In addition to reports in clinical trials, worldwide voluntary adverse events reports received since market introduction of Menactra are listed below. This list includes serious events and/or events which were included based on severity, frequency of reporting or a plausible causal connection to Menactra. Because these events were reported voluntarily from a population of uncertain size, it is not possible to reliably estimate their frequency or establish a causal relationship to vaccination.

- *Blood and Lymphatic System Disorders*

  Lymphadenopathy

- *Immune System Disorders*

  Hypersensitivity reactions such as anaphylaxis/anaphylactic reaction, wheezing, difficulty breathing, upper airway swelling, urticaria, erythema, pruritus, hypotension

- *Nervous System Disorders*

  Guillain-Barré syndrome, paraesthesia, vasovagal syncope, dizziness, convulsion, facial palsy, acute disseminated encephalomyelitis, transverse myelitis

- *Musculoskeletal and Connective Tissue Disorders*

Myalgia

- *General Disorders and Administrative Site Conditions*

Large injection site reactions, extensive swelling of the injected limb (may be associated with erythema, warmth, tenderness or pain at the injection site).

Post-marketing Safety Study

The risk of GBS following receipt of Menactra was evaluated in a US retrospective cohort study using healthcare claims data from 9,578,688 individuals 11 through 18 years of age, of whom 1,431,906 (15%) received Menactra. Of 72 medical chart-confirmed GBS cases, none had received Menactra within 42 days prior to symptom onset. An additional 129 potential cases of GBS could not be confirmed or excluded due to absent or insufficient medical chart information. In an analysis that took into account the missing data, estimates of the attributable risk of GBS ranged from 0 to 5 additional cases of GBS per 1,000,000 vaccinees within the 6-week period following vaccination.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

Menactra vaccine was concomitantly administered with Typhim Vi® [Typhoid Vi Polysaccharide Vaccine] (Typhoid) and Tetanus and Diphtheria Toxoids Adsorbed, For Adult Use (Td) vaccine, in individuals 18 through 55 and 11 through 17 years of age, respectively. In children 4 through 6 years of age, Menactra was co-administered with DAPTACEL, and in children younger than 2

years of age, Menactra was co-administered with one or more of the following vaccines: PCV7, MMR, V, MMRV, or HepA [see *Clinical Studies (14)* and *Adverse Reactions (6)*].

When Menactra and DAPTACEL are to be administered to children 4 through 6 years of age, preference should be given to simultaneous administration of the 2 vaccines or administration of Menactra prior to DAPTACEL. Administration of Menactra one month after DAPTACEL has been shown to reduce meningococcal antibody responses to Menactra. Data are not available to evaluate the immune response to Menactra administered to younger children following DAPTACEL or to Menactra administered to persons <11 years of age following other diphtheria toxoid-containing vaccines [see *Clinical Studies (14.3)*].

Pneumococcal antibody responses to some serotypes in PCV7 were decreased following co-administration of Menactra and PCV7 [see *Concomitant Vaccine Administration (14.3)*].

Do not mix Menactra with other vaccines in the same syringe. When Menactra is administered concomitantly with other injectable vaccines, the vaccines should be administered with different syringes and given at separate injection sites.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses) may reduce the immune response to vaccines.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to Menactra during pregnancy. To enroll in or obtain information about the registry, call Sanofi Pasteur at 1-800-822-2463.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no adequate and well-controlled studies of Menactra administration in pregnant women in the US. Available data suggest that rates of major birth defects and miscarriage in women who received Menactra 30 days prior to pregnancy or during pregnancy are consistent with estimated background rates.

A developmental toxicity study was performed in female mice given 0.1 mL (in divided doses) of Menactra prior to mating and during gestation (a single human dose is 0.5 mL). The study revealed no evidence of harm to the fetus due to Menactra [see *Animal Data (8.1)*].

Data

Human Data

A pregnancy registry spanning 11 years (2005-2016) included 222 reports of exposure to Menactra from 30 days before or at any time during pregnancy. Of these reports, 87 had known

pregnancy outcomes available and were enrolled in the pregnancy registry prior to the outcomes being known. Outcomes among these prospectively followed pregnancies included 2 major birth defects and 6 miscarriages.

Animal Data

A developmental toxicity study was performed in female mice. The animals were administered 0.1 mL of Menactra (in divided doses) at each of the following time points: 14 days prior to mating, and on Days 6 and 18 of gestation (a single human dose is 0.5 mL). There were no vaccine-related fetal malformations or variations, and no adverse effects on pre-weaning development observed in the study.

8.2 Lactation

Risk Summary

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Menactra and any potential adverse effects on the breastfed child from Menactra. Data are not available to assess the effects of Menactra on the breastfed infant or on milk production/excretion.

8.4 Pediatric Use

Menactra is not approved for use in infants under 9 months of age. Available data show that infants administered three doses of Menactra (at 2, 4, and 6 months of age) had diminished responses to each meningococcal vaccine serogroup compared to older children given two doses at 9 and 12 months of age.

8.5 Geriatric Use

Safety and effectiveness of Menactra in adults older than 55 years of age have not been established.

11 DESCRIPTION

Menactra is a sterile, intramuscularly administered vaccine that contains *N meningitidis* serogroup A, C, Y and W-135 capsular polysaccharide antigens individually conjugated to diphtheria toxoid protein. *N meningitidis* A, C, Y and W-135 strains are cultured on Mueller Hinton agar (3) and grown in Watson Scherp (4) media containing casamino acid. The polysaccharides are extracted from the *N meningitidis* cells and purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction and diafiltration. To prepare the polysaccharides for conjugation, they are depolymerized, derivatized, and purified by diafiltration. Diphtheria toxin is derived from *Corynebacterium diphtheriae* grown in modified culture medium containing hydrolyzed casein (5) and is detoxified using formaldehyde. The diphtheria toxoid protein is purified by ammonium sulfate fractionation and diafiltration. The derivatized polysaccharides are covalently linked to diphtheria toxoid and purified by serial diafiltration. The four meningococcal components, present as individual serogroup-specific glycoconjugates, compose the final formulated vaccine. No preservative or adjuvant is added during manufacture. Each 0.5 mL dose may contain residual amounts of formaldehyde of less than 2.66 mcg (0.000532%), by calculation. Potency of Menactra is determined by quantifying the amount of each polysaccharide antigen that is conjugated to diphtheria toxoid protein and the amount of unconjugated polysaccharide present.

Menactra is manufactured as a sterile, clear to slightly turbid liquid. Each 0.5 mL dose of vaccine is formulated in sodium phosphate buffered isotonic sodium chloride solution to contain 4 mcg each of meningococcal A, C, Y and W-135 polysaccharides conjugated to approximately 48 mcg of diphtheria toxoid protein carrier.

The vial stopper is not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The presence of bactericidal anti-capsular meningococcal antibodies has been associated with protection from invasive meningococcal disease (6) (7). Menactra induces the production of bactericidal antibodies specific to the capsular polysaccharides of serogroups A, C, Y and W-135.

13 NON-CLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Menactra has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility. A developmental animal toxicity study showed that Menactra had no effects on female fertility in mice [see *Pregnancy* (*8.1*)].

14 CLINICAL STUDIES

14.1 Efficacy

The serum bactericidal assay (SBA) used to test sera contained an exogenous complement source that was either human (SBA-H) or baby rabbit (SBA-BR). (8)

The response to vaccination following two doses of vaccine administered to children 9 and 12 months of age and following one dose of vaccine administered to children 2 through 10 years of age was evaluated by the proportion of participants having an SBA-H antibody titer of 1:8 or greater, for each serogroup. In individuals 11 through 55 years of age, the response to vaccination with a single dose of vaccine was evaluated by the proportion of participants with a 4-fold or greater increase in bactericidal antibody to each serogroup as measured by SBA-BR. For individuals 2 through 55 years of age, vaccine efficacy after a single dose was inferred from the demonstration of immunologic equivalence to a US-licensed meningococcal polysaccharide vaccine, Menomune – A/C/Y/W-135 vaccine as assessed by SBA.

14.2 Immunogenicity

Children 9 through 12 Months of Age

In a randomized, US, multi-center trial, children received Menactra at 9 months and 12 months of age. The first Menactra dose was administered alone, followed by a second Menactra dose given alone (N=404), or with MMRV (N=302), or with PCV7 (N=422). For all participants, sera were obtained approximately 30 days after last vaccination. There were no substantive differences in demographic characteristics between the vaccine groups. The median age range for administration of the first dose of Menactra was 278-279 days of age.

Table 5: Bactericidal Antibody Responses[a] 30 Days Following a Second Dose of Menactra Administered Alone or Concomitantly Administered with MMRV or PCV7 at 12 Months of Age

		Vaccinations administered at 12 months of age following a dose of Menactra at 9 months of age					
		Menactra		Menactra + MMRV		Menactra + PCV7	
		(N=272-277)[b]		(N=177-180)[b]		(N=264-267)[b]	
Serogroup			(95% CI)[c]		(95% CI)[c]		(95% CI)[c]
A	% ≥1:8[d]	95.6	(92.4; 97.7)	92.7	(87.8; 96.0)	90.5	(86.3; 93.8)
	GMT	54.9	(46.8; 64.5)	52.0	(41.8; 64.7)	41.0	(34.6; 48.5)
C	% ≥1:8[d]	100.0	(98.7; 100.0)	98.9	(96.0; 99.9)	97.8	(95.2; 99.2)
	GMT	141.8	(123.5; 162.9)	161.9	(136.3; 192.3)	109.5	(94.1; 127.5)
Y	%≥1:8[d]	96.4	(93.4; 98.2)	96.6	(92.8; 98.8)	95.1	(91.8; 97.4)
	GMT	52.4	(45.4; 60.6)	60.2	(50.4; 71.7)	39.9	(34.4; 46.2)
W-135	%≥1:8[d]	86.4	(81.8; 90.3)	88.2	(82.5; 92.5)	81.2	(76.0; 85.7)
	GMT	24.3	(20.8; 28.3)	27.9	(22.7; 34.3)	17.9	(15.2; 21.0)

[a] Serum bactericidal assay with an exogenous human complement (SBA-H) source.

[b] N=Number of participants with at least one valid serology result from a blood sample obtained between Days 30 to 44 post vaccination.

[c] 95% CIs for the proportions are calculated based on the Clopper-Pearson Exact method and normal approximation for that of the GMTs.

[d] The proportion of participants achieving an SBA-H titer of at least 1:8 thirty days after the second dose of Menactra.

Administration of Menactra to children at 12 months and 15 months of age was evaluated in a US study. Prior to the first dose, 33.3% [n=16/48] of participants had an SBA-H titer ≥1:8 to Serogroup A, and 0-2% [n=0-1 of 50-51] to Serogroups C, Y and W-135. After the second dose, percentages of participants with an SBA-H titer ≥1:8 were: 85.2%, Serogroup A [n=46/54]; 100.0%, Serogroup C [n=54/54]; 96.3%, Serogroup Y [n=52/54]; 96.2%, Serogroup W-135 [n=50/52].

Individuals 2 through 55 Years of Age

Immunogenicity was evaluated in three comparative, randomized, US, multi-center, active controlled clinical trials that enrolled children (2 through 10 years of age), adolescents (11 through 18 years of age), and adults (18 through 55 years of age). Participants received a single dose of Menactra (N=2526) or Menomune – A/C/Y/W-135 (N=2317). For all age groups studied, sera were obtained before and approximately 28 days after vaccination. [Blinding procedures for safety assessments are described in *Adverse Reactions (6)*.]

In each of the trials, there were no substantive differences in demographic characteristics between the vaccine groups, between immunogenicity subsets or the overall study population. In the study of children 2 through 10 years of age, the median age of participants was 3 years; 95% completed the study. In the adolescent trial, the median age for both groups was 14 years; 99% completed the study. In the adult trial, the median age for both groups was 24 years; 94% completed the study.

Immunogenicity in Children 2 through 10 Years of Age

Of 1408 enrolled children 2 through 10 years of age, immune responses evaluated in a subset of Menactra participants (2 through 3 years of age, n=52; 4-10 years of age, n=84) and Menomune – A/C/Y/W-135 participants (2 through 3 years of age, n=53; 4-10 years of age, n=84) were comparable for all four serogroups (Table 6).

Table 6: Comparison of Bactericidal Antibody Responses[a] to Menactra and Menomune – A/C/Y/W-135 28 Days after Vaccination for a Subset of Participants 2 through 3 Years of Age and 4 through 10 Years of Age

		Ages 2 through 3 Years				Ages 4 through 10 Years			
		Menactra		Menomune – A/C/Y/W-135		Menactra		Menomune – A/C/Y/W-135	
		N[b]=48-52		N[b]=50-53		N[b]=84		N[b]=84	
Serogroup			(95% CI)[c]		(95% CI)[c]		(95% CI)[c]		(95% CI)[c]
A	% ≥1:8[d]	73	(59,84)	64	(50,77)	81	(71,89)	55	(44,66)
	GMT	10	(8,13)	10	(7,12)	19	(14,26)	7	(6,9)
C	% ≥1:8[d]	63	(48,76)	38	(25,53)	79	(68,87)	48	(37,59)
	GMT	27	(14,52)	11	(5,21)	28	(19,41)	12	(7,18)
Y	% ≥1:8[d]	88	(75,95)	73	(59,84)	99	(94,100)	92	(84,97)
	GMT	51	(31,84)	18	(11,27)	99	(75,132)	46	(33,66)
W-135	% ≥1:8[d]	63	(47,76)	33	(20,47)	85	(75,92)	79	(68,87)
	GMT	15	(9,25)	5	(3,6)	24	(18,33)	20	(14,27)

[a] Serum bactericidal assay with an exogenous human complement (SBA-H) source.

[b] N=Number of subset participants with at least one valid serology result at Day 0 and Day 28.

[c] The 95% CI for the Geometric Mean Titer (GMT) was calculated based on an approximation to the normal distribution.

[d] The proportion of participants achieving an SBA-H titer of at least 1:8 was assessed using a 10% non-inferiority margin and a one-sided Type 1 error rate of 0.025.

In the subset of participants 2 through 3 years of age with undetectable pre-vaccination titers (ie, SBA-H titers <1:4 at Day 0), seroconversion rates (defined as the proportions of participants with

SBA-H titers ≥1:8 by Day 28) were similar between the Menactra and Menomune – A/C/Y/W-135 recipients. Menactra participants achieved seroconversion rates of: 57%, Serogroup A (n=12/21); 62%, Serogroup C (n=29/47); 84%, Serogroup Y (n=26/31); 53%, Serogroup W-135 (n=20/38). The seroconversion rates for Menomune – A/C/Y/W-135 recipients were: 55%, Serogroup A (n=16/29); 30%, Serogroup C (n=13/43); 57%, Serogroup Y (n=17/30); 26%, Serogroup W-135 (n=11/43).

In the subset of participants 4 through 10 years of age with undetectable pre-vaccination titers (ie, SBA-H titers <1:4 at Day 0), seroconversion rates (defined as the proportions of participants with SBA-H titers ≥1:8 by Day 28) were similar between the Menactra and Menomune – A/C/Y/W-135 recipients. Menactra participants achieved seroconversion rates of: 69%, Serogroup A (n=11/16); 81%, Serogroup C (n=50/62); 98%, Serogroup Y (n=45/46); 69%, Serogroup W-135 (n=27/39). The seroconversion rates for Menomune – A/C/Y/W-135 recipients were: 48%, Serogroup A (n=10/21); 38%, Serogroup C (n=19/50); 84%, Serogroup Y (n=38/45); 68%, Serogroup W-135 (n=26/38).

Immunogenicity in Adolescents 11 through 18 Years of Age

Results from the comparative clinical trial conducted in 881 adolescents aged 11 through 18 years showed that the immune responses to Menactra and Menomune – A/C/Y/W-135 were similar for all four serogroups (Table 7).

In participants with undetectable pre-vaccination titers (ie, SBA-BR titers <1:8 at Day 0), seroconversion rates (defined as the proportions of participants achieving a ≥4-fold rise in SBA-

BR titers by Day 28) were similar between the Menactra and Menomune – A/C/Y/W-135 recipients. Menactra participants achieved seroconversion rates of: 100%, Serogroup A (n=81/81); 99%, Serogroup C (n=153/155); 98%, Serogroup Y (n=60/61); 98%, Serogroup W-135 (n=161/164). The seroconversion rates for Menomune – A/C/Y/W-135 recipients were: 100%, Serogroup A (n=93/93); 99%, Serogroup C (n=151/152); 100%, Serogroup Y (n=47/47); 99%, Serogroup W-135 (n=138/139).

Immunogenicity in Adults 18 through 55 Years of Age

Results from the comparative clinical trial conducted in 2554 adults aged 18 through 55 years showed that the immune responses to Menactra and Menomune – A/C/Y/W-135 were similar for all four serogroups (Table 7).

Table 7: Comparison of Bactericidal Antibody Responses[a] to Menactra and Menomune – A/C/Y/W-135 28 Days after Vaccination for Participants 11 through 18 Years of Age and 18 through 55 Years of Age

		Ages 11 through 18 Years				Ages 18 through 55 Years			
		Menactra		Menomune – A/C/Y/W-135		Menactra		Menomune – A/C/Y/W-135	
		N[b]=423		N[b]=423		N[b]=1280		N[b]=1098	
Serogroup			(95% CI)[c]		(95% CI)[c]		(95% CI)[c]		(95% CI)[c]
A	% ≥4-fold rise[d]	92.7	(89.8, 95.0)	92.4	(89.5, 94.8)	80.5	(78.2, 82.6)	84.6	(82.3, 86.7)
	GMT	5483	(4920, 6111)	3246	(2910, 3620)	3897	(3647, 4164)	4114	(3832, 4417)
C	% ≥4-fold rise[d]	91.7	(88.7, 94.2)	88.7	(85.2, 91.5)	88.5	(86.6, 90.2)	89.7	(87.8, 91.4)
	GMT	1924	(1662, 2228)	1639	(1406, 1911)	3231	(2955, 3533)	3469	(3148, 3823)
Y	% ≥4-fold rise[d]	81.8	(77.8, 85.4)	80.1	(76.0, 83.8)	73.5	(71.0, 75.9)	79.4	(76.9, 81.8)
	GMT	1322	(1162, 1505)	1228	(1088, 1386)	1750	(1597, 1918)	2449	(2237, 2680)
W-135	% ≥4-fold rise[d]	96.7	(94.5, 98.2)	95.3	(92.8, 97.1)	89.4	(87.6, 91.0)	94.4	(92.8, 95.6)
	GMT	1407	(1232, 1607)	1545	(1384, 1725)	1271	(1172, 1378)	1871	(1723, 2032)

[a] Serum bactericidal assay with baby rabbit complement (SBA-BR).

[b] N=Number of subset participants with at least one valid serology result at Day 0 and Day 28.

[c] The 95% CI for the Geometric Mean Titer (GMT) was calculated based on an approximation to the normal distribution.

[d] Menactra was non-inferior to Menomune – A/C/Y/W-135. Non-inferiority was assessed by the proportion of participants with a 4-fold or greater rise in SBA-BR titer for *N meningitidis* serogroups A, C, Y and W-135 using a 10% non-inferiority margin and a one-sided Type I error rate of 0.05.

In participants with undetectable pre-vaccination titers (ie, SBA-BR titers <1:8 at Day 0), seroconversion rates (defined as the proportions of participants achieving a ≥4-fold rise in SBA-BR titers by Day 28) were similar between the Menactra and Menomune – A/C/Y/W-135 recipients. Menactra participants achieved seroconversion rates of: 100%, Serogroup A (n=156/156); 99%, Serogroup C (n=343/345); 91%, Serogroup Y (n=253/279); 97%, Serogroup W-135 (n=360/373). The seroconversion rates for Menomune – A/C/Y/W-135 recipients were: 99%, Serogroup A (n=143/144); 98%, Serogroup C (n=297/304); 97%, Serogroup Y (n=221/228); 99%, Serogroup W-135 (n=325/328).

Immunogenicity in Adolescents and Adults Following Booster Vaccination

For a description of the study design and number of participants, [see *Clinical Trials Experience, Booster Vaccination Study* (*6.1*).] Prior to revaccination, the percentage of participants (n=781) with an SBA-H titer ≥1:8 were 64.5%, 44.2%, 38.7%, and 68.5% for Serogroups A, C, Y and W-135, respectively. Among the subset of trial participants (n=112) for whom SBA-H responses at Day 6 were assessed, 86.6%, 91.1%, 94.6%, and 92.0% achieved a ≥4-fold rise in SBA-H titer for Serogroups A, C, Y and W-135, respectively. The proportions of participants (n=781) who achieved a ≥4-fold rise in SBA-H titer by Day 28 were 95.0%, 95.3%, 97.1%, and 96% for Serogroups A, C, Y and W-135, respectively. The proportions of participants who achieved an SBA-H titer ≥1:8 by Day 28 were >99% for each serogroup.

14.3 Concomitant Vaccine Administration

MMRV (or MMR + V) or PCV7

In a US, active-controlled trial, 1179 children received Menactra at 9 months and 12 months of age. At 12 months of age these children received Menactra concomitantly with MMRV (N=616), or MMR + V (N=48), or PCV7 (N=250). Another group of 12-month old children received MMRV + PCV7 (N=485). Sera were obtained approximately 30 days after the last vaccinations. Measles, mumps, rubella and varicella antibody responses among children who received Menactra and MMRV (or MMR and V) were comparable to corresponding antibody responses among children who received MMRV and PCV7.

When Menactra was given concomitantly with PCV7, the non-inferiority criteria for comparisons of pneumococcal IgG GMCs (upper limit of the two-sided 95% CI of the GMC ratio ≤2) were not met for 3 of 7 serotypes (4, 6B, 18C). In a subset of participants with available sera, pneumococcal opsonophagocytic assay GMT data were consistent with IgG GMC data.

Td Vaccine

In a double-blind, randomized, controlled trial, 1021 participants aged 11 through 17 years received Td vaccine and Menactra concomitantly (N=509), or Td vaccine followed one month later by Menactra (N=512). Sera were obtained approximately 28 days after each respective vaccination. The proportions of participants with a 4-fold or greater increase in SBA-BR titer to meningococcal Serogroups C, Y and W-135 were higher when Menactra was given concomitantly with Td vaccine (86%-96%) than when Menactra was given one month following Td vaccine (65%-91%). Anti-tetanus and anti-diphtheria antibody responses were similar in both study groups.

Typhim Vi

In a double-blind, randomized, controlled trial, 945 participants aged 18 through 55 years received Typhim Vi and Menactra concomitantly (N=469), or Typhim Vi followed one month later by Menactra (N=476). Sera were obtained approximately 28 days after each respective vaccination. The antibody responses to Menactra and to Typhim Vi components were similar in both study groups.

DAPTACEL and IPV

In a randomized, parallel group, US multi-center clinical trial conducted in children 4 through 6 years of age, Menactra was administered as follows: 30 days after concomitant DTaP (DAPTACEL®, Sanofi Pasteur Limited) + IPV (IPOL®, Sanofi Pasteur SA) [Group A]; concomitantly with DAPTACEL followed 30 days later by IPV [Group B]; concomitantly with IPV followed 30 days later by DAPTACEL [Group C]. Sera were obtained approximately 30 days after each respective vaccination. [See *Clinical Trials Experience (6.1)*.]

When Menactra was administered 30 days after DAPTACEL (and IPV) [Group A], significantly lower SBA-H GMTs to all 4 meningococcal serogroups were observed compared to Menactra (and IPV) administered 30 days prior to DAPTACEL [Group C]. When Menactra was administered concomitantly with DAPTACEL [Group B], SBA-H GMTs to meningococcal serogroups A, C, and W-135 were non-inferior to those observed after Menactra (and IPV) [Group C]. The non-inferiority criterion was marginally missed for meningococcal serogroup Y. Non-inferiority of SBA-H GMTs following concomitant administration of Menactra and

DAPTACEL compared to those after concomitant Menactra and IPV was concluded if the upper limit of the 2-sided 95% CI of ($GMT_{Group\ C}$ divided by $GMT_{Group\ B}$) computed separately for each of the serogroups was <2.

The respective SBA-H GMTs and proportion (%) of Group A, B, and C study participants achieving an SBA-H titer of ≥1:8 are displayed in Table 8.

Table 8: Bactericidal Antibody Responses[a] 30 Days Following Menactra Administered Alone or Concomitantly with DAPTACEL or IPV

		Vaccines administered at Visit 1 and 30 days later at Visit 2					
	Visit 1 Visit 2	Group A DAPTACEL + IPV Menactra		Group B Menactra + DAPTACEL IPV		Group C Menactra + IPV DAPTACEL	
		(N=250)[b]		(N=238)[b]		(N=121)[b]	
Serogroup			**(95% CI)[c]**		**(95% CI)[c]**		**(95% CI)[c]**
A	% ≥1:8[d]	49.6	(41.0; 58.3)	67.2	(58.4; 75.1)	64.4	(54.4; 73.6)
	GMT	6.7	(5.7; 8.0)	10.8	(8.7; 13.3)	10.4	(8.1; 13.3)
C	% ≥1:8[d]	20.3	(13.9; 28.0)	50.4	(41.5; 59.2)	50.5	(40.5; 60.5)
	GMT	3.3	(2.7; 3.9)	8.1	(6.3; 10.5)	7.8	(5.8; 10.7)
Y	%≥1:8[d]	44.2	(35.8; 52.9)	80.2	(72.3; 86.6)	88.5	(80.7; 93.9)
	GMT	6.5	(5.1; 8.2)	18.1	(14.2; 22.9)	26.2	(20.0; 34.4)
W-135	%≥1:8[d]	55.1	(46.4; 63.5)	87.8	(80.9; 92.9)	82.7	(74.0; 89.4)
	GMT	8.4	(6.7; 10.6)	22.8	(18.5; 28.1)	21.7	(16.6; 28.4)

[a] Serum bactericidal assay with an exogenous human complement (SBA-H) source.

[b] N=Total number of the subjects in the study population per group.

[c] 95% CIs for the proportions are calculated based on the Clopper-Pearson Exact method and normal approximation for that of the GMTs.

[d] The proportion of participants achieving an SBA-H titer of at least 1:8, 30 days after Menactra.

When Menactra was administered concomitantly with DAPTACEL, antibody responses to three of the pertussis antigens (pertussis toxin, filamentous hemagglutinin, and pertactin) (GMCs), tetanus toxin (% participants with antibody concentrations ≥1.0 IU/mL), and diphtheria toxin (% participants with antibody concentrations ≥1.0 IU/mL) were non-inferior to those observed after DAPTACEL and IPV. The pertussis anti-fimbriae GMCs were marginally lower when Menactra and DAPTACEL were administered concomitantly.

15 REFERENCES

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3 Mueller JH, et al. A protein-free medium for primary isolation of the gonococcus and meningococcus. Proc Soc Exp Biol Med 1941;48:330-333.

4 Watson RG, et al. The specific hapten of group C (group IIa) meningococcus. I. Preparation and immunological behavior. J Immunol 1958;81:331-336.

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7 Goldschneider I, et al. Human immunity to the meningococcus. I. The role of humoral antibodies. J Exp Med 1969;129:1307-1326.

8 Maslanka SE, et al. Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. Clin and Diag Lab Immunol 1997;4:156-167.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

- Single-dose vial, 0.5 mL (NDC 49281-589-58). Supplied as a package of 5 vials (NDC 49281-589-05).

16.2 Storage and Handling

Store at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Frozen/previously frozen product should not be used. Do not use after the expiration date.

17 PATIENT COUNSELING INFORMATION

Vaccine Information Statements are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization to the patient, parent, or guardian. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Inform the patients, parents or guardians about:

- Potential benefits and risks of immunization with Menactra.
- Potential for adverse reactions that have been temporally associated with administration of Menactra or other vaccines containing similar components.
- Reporting any adverse reactions to their healthcare provider.
- The Sanofi Pasteur Inc. Pregnancy Registry, as appropriate [see *Pregnancy* (*8.1*)].

Menactra® is a registered trademark of Sanofi, its affiliates and subsidiaries.

Manufactured by:

Sanofi Pasteur Inc.

Swiftwater PA 18370 USA

7186

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use MENQUADFI™ safely and effectively. See full prescribing information for MENQUADFI.

MenQuadfi™, Meningococcal (Groups A, C, Y, W) Conjugate Vaccine Solution for Intramuscular Injection

Initial U.S. Approval: 2020

INDICATIONS AND USAGE

MenQuadfi is a vaccine indicated for active immunization for the prevention of invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, W, and Y. MenQuadfi vaccine is approved for use in individuals 2 years of age and older. (1)
MenQuadfi does not prevent *N. meningitidis* serogroup B disease.

DOSAGE AND ADMINISTRATION

0.5 mL dose for intramuscular injection. (2)
Primary Vaccination
- Individuals 2 years of age and older: a single dose.

Booster Vaccination
- A single dose of MenQuadfi may be administered to individuals 15 years of age and older who are at continued risk for meningococcal disease if at least 4 years have elapsed since a prior dose of meningococcal (Groups A, C, W, Y) conjugate vaccine.

DOSAGE FORMS AND STRENGTHS

Solution for injection in 0.5 mL single-dose vial. (3)

CONTRAINDICATIONS

Severe allergic reaction to any component of the vaccine, or after a previous dose of MenQuadfi or any other tetanus toxoid-containing vaccine. (4)

ADVERSE REACTIONS

Most commonly reported adverse reactions (≥10%) following a primary dose were as follows:
- Children 2 through 9 years of age, pain (38.6%), erythema (22.6%), and swelling (13.8%) at the injection site; malaise (21.1%), myalgia (20.1%), and headache (12.5%). (6)
- Adolescents aged 10 through 17 years of age, injection site pain (34.8%–45.2%), myalgia (27.4%–35.3%), headache (26.5%–30.2%), and malaise (19.4%–26.0%). (6)
- Adults aged 18 through 55 years, injection site pain (41.9%), myalgia (35.6%), headache (29.0%), and malaise (22.9%). (6)
- Adults 56 years of age and older, pain at the injection site (25.5%), myalgia (21.9%), headache (19.0%), and malaise (14.5%). (6)

In adolescents and adults, rates of solicited adverse reactions following a booster dose were comparable to those observed following primary vaccination. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION

Revised: 04/2020

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

MenQuadfi™ is a vaccine indicated for active immunization for the prevention of invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, W, and Y. MenQuadfi is indicated for use in individuals 2 years of age and older.
MenQuadfi does not prevent *N. meningitidis* serogroup B disease.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

MenQuadfi is a clear solution.
Parenteral drug products should be inspected visually for particulate matter and/or discoloration prior to administration, whenever solution and container permit. If any of these conditions exist, the vaccine should not be administered. Discard the vial with any unused portion.

2.2 Dose and Schedule

Administer MenQuadfi as a single 0.5 mL injection intramuscularly.
Primary Vaccination
- Individuals 2 years of age and older receive a single dose.

Booster Vaccination
- A single dose of MenQuadfi may be administered to individuals 15 years of age and older who are at continued risk for meningococcal disease if at least 4 years have elapsed since a prior dose of meningococcal (Groups A, C, W, Y) conjugate vaccine.

3 DOSAGE FORMS AND STRENGTHS

MenQuadfi is a sterile solution for intramuscular injection supplied in 0.5 mL single-dose vials.

4 CONTRAINDICATIONS

Severe allergic reaction to any component of the vaccine, or after a previous dose of MenQuadfi or any other tetanus toxoid-containing vaccine [*see Description (11)*].

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate observation and medical treatment should always be readily available in case of an anaphylactic event following the administration of the vaccine.

5.2 Altered Immunocompetence

Reduced Immune Response
Some individuals with altered immunocompetence, including some individuals receiving immunosuppressant therapy, may have reduced immune responses to MenQuadfi.
Complement Deficiency
Persons with certain complement deficiencies and persons receiving treatment that inhibits terminal complement activation (for example, eculizumab) are at increased risk for invasive disease caused by *N. meningitidis*, including invasive disease caused by serogroups A, C, W, and Y, even if they develop antibodies following vaccination with MenQuadfi [*see Clinical Pharmacology (12.1)*].

5.3 Syncope

Syncope (fainting) can occur following, or even before, vaccination with MenQuadfi. Procedures should be in place to prevent falling and injury and to manage syncope.

5.4 Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) has been reported in temporal relationship following administration of another U.S.-licensed meningococcal quadrivalent polysaccharide conjugate vaccine. The decision by the healthcare professional to administer MenQuadfi to persons with a history of GBS should take into account the expected benefits and potential risks.

5.5 Tetanus Immunization

Immunization with MenQuadfi does not substitute for routine tetanus immunization.

5.6 Limitations of Vaccine Effectiveness

Vaccination with MenQuadfi may not protect all vaccine recipients.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trial(s) of a vaccine cannot be directly compared to rates in the clinical trial(s) of another vaccine and may not reflect the rates observed in practice. The safety of a single dose of MenQuadfi in individuals 2 years of age and older was evaluated in five randomized, active-controlled, multi-center clinical studies conducted in the US and Puerto Rico. In these studies, a total of 4,919 participants received either a

primary dose (N = 4517) or a booster dose (N = 402) of MenQuadfi and were included in the safety analyses.

Safety Monitoring

Participants were monitored for immediate reactions for 30 minutes following vaccination while at the study site. Solicited injection site and systemic reactions were recorded by participants or by parents/guardians in a diary card at home daily for 7 days following vaccination. All unsolicited adverse events that occurred within 30 days following vaccination were recorded by participants or by parents/guardians and collected by the study site at the next visit. Unsolicited adverse events that were medically attended (i.e., visits to an emergency room, or an unexpected visit to a health care provider), and all serious adverse events (SAEs) were collected for at least 6 months after vaccination.

Primary Vaccination Studies

Children 2 through 9 years of age

The safety of MenQuadfi in children 2 years through 9 years of age was evaluated in Study 1 (NCT03077438). The safety analysis set included 498 participants who received MenQuadfi and 494 participants who received Menveo (Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM_{197} Conjugate Vaccine). Of the participants 2 through 9 years of age who received MenQuadfi (N = 498), 50.2% were 2 through 5 years of age, 49.8% were 6 through 9 years of age, 49.0% were female, 80.5% were White, 13.3% were Black or African American, 0.4% were Asian, 5.2% were of other racial groups, and 22.9% were of Hispanic or Latino ethnicity. There were no substantive differences in demographic characteristics between the vaccine groups.

The rates and severity of the solicited adverse reactions that occurred within 7 days following MenQuadfi compared with Menveo (Study 1) are presented in Table 1.

SAEs occurred at a rate of 1.4% following MenQuadfi and at a rate of 0.6% following Menveo during the entire study period. Most SAEs occurred more than 30 days following vaccination and were commonly occurring events in the general population in this age group. No SAEs were determined to be vaccine related.

Table 1: Percentages of Solicited Injection-Site Reactions and Systemic Adverse Reactions within 7 Days after Vaccination with MenQuadfi or Menveo in Children 2 through 9 Years of Age (Study 1)*

	MenQuadfi (N†=484-487) %		Menveo (N†=479-486) %	
Adverse Reactions	**Any**	**Grade 3**	**Any**	**Grade 3**
Local Reactions				
Injection Site Pain‡	38.6	0.6	42.4	1.0
Injection Site Erythema§	22.6	3.1	31.5	9.9
Injection Site Swelling§	13.8	1.4	21.5	5.6
Systemic Reactions				
Myalgia¶	20.1	0.4	23.0	0.8
Malaise¶	21.1	1.8	20.4	1.0
Headache¶	12.5	0.0	11.5	0.4
Fever#	1.9	0.0	2.7	0.4

*Clinical trial identifier NCT03077438
†N is the number of vaccinated participants with available data for the events listed
‡Grade 3: Unable to perform usual activities
§Any: > 0 mm; Grade 3: ≥ 50 mm
¶Grade 3: Prevents daily activity
#Any: ≥ 100.4°F (38.0°C); Grade 3: ≥ 102.1°F (39.0°C)

Adolescents 10 through 17 years of age

The safety of MenQuadfi in adolescents 10 through 17 years of age was evaluated in two clinical trial studies Study 2 (NCT02199691) and Study 3 (NCT02842853). The safety analysis set in these two studies included 3,196 participants who received MenQuadfi alone (1,684 participants), MenQuadfi concomitantly with Adacel® (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed) (Tdap) and Gardasil® (Human Papillomavirus Quadrivalent (Types 6, 11, 16, and 18) Vaccine, Recombinant) (HPV) (392 participants), the concomitant vaccines without MenQuadfi (296 participants), or a U.S.-licensed comparator meningococcal vaccine (824 participants). The comparator meningococcal vaccine was either Menveo (501 participants) or Menactra (Meningococcal (Groups A, C, Y, and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine) (323 participants).

Of the participants 10 through 17 years of age who received MenQuadfi (N = 1,684), 49.6% were female. Among those with reported race and ethnicity, 79.3% were White, 14.2% were Black or African American, 1.1% were Asian, 5.4% were of other racial groups, and 21.5% were of Hispanic or Latino ethnicity. Mean age was 11.9 years at time of administration. There were no substantive differences in demographic characteristics between the vaccine groups.

The rates and severity of the solicited adverse reactions that occurred within 7 days following MenQuadfi compared with Menveo and Menactra are presented in Table 2. The most common injection site and systemic reactions occurring after MenQuadfi administration (in Study 2 and Study 3) were injection site pain (34.8% and 45.2%) and myalgia (27.4% and 35.3%), respectively.

In Study 2, SAEs occurred at a rate of 0.8% following MenQuadfi and 0.8% following Menveo. In Study 3, SAEs occurred at a rate of 0.3% following MenQuadfi and 0.9% following Menactra. No SAEs were determined to be vaccine related.

Table 2: Percentages of Solicited Injection-Site Reactions and Systemic Adverse Reactions within 7 Days after Vaccination with MenQuadfi or Menveo in Individuals 10 through 17 Years of Age Study 2* and MenQuadfi or Menactra in Individuals 10 through 17 Years of Age Study 3†

	Study 2				Study 3			
	MenQuadfi (N‡=494-496) %		Menveo (N‡=488-491) %		MenQuadfi (N‡=1129-1159) %		Menactra (N‡=310-314) %	
Adverse Reactions	**Any**	**Grade 3**	**Any**	**Grade 3**	**Any**	**Grade 3**	**Any**	**Grade 3**
Local Reactions								
Injection Site Pain§	45.2	1.4	42.5	1.0	34.8	1.8	41.4	2.2
Injection Site Erythema¶	5.0	0.4	7.5	1.2	4.5	0.3	4.5	0.3
Injection Site Swelling¶	5.4	0.2	6.5	0.4	4.1	<0.1	4.8	0.0
Systemic Reactions								
Myalgia§	35.3	1.6	35.2	1.8	27.4	1.9	31.2	1.9
Headache§	30.2	1.8	30.9	1.8	26.5	2.3	28.0	1.9
Malaise§	26.0	2.2	26.4	2.8	19.4	1.2	23.9	1.3
Fever#	1.4	0.4	1.2	0.6	0.7	0.2	0.6	0.0

*Clinical trial identifier NCT02199691
†Clinical trial identifier NCT02842853
‡N is the number of vaccinated participants with available data for the events listed
§Grade 3: Prevents daily activity
¶Any: > 25 mm; Grade 3: > 100 mm
#Any: ≥ 100.4°F (38.0°C); Grade 3: ≥ 102.1°F (39.0°C)

Among 296 participants who received Tdap and HPV concomitantly (without MenQuadfi) and 392 participants who received MenQuadfi concomitantly with Tdap and HPV, there were no notable differences in the rates of systemic solicited adverse reactions within 7 days following vaccination.

Dizziness within 30 minutes following vaccination was experienced by 1 (0.2%) participant who received MenQuadfi in Study 2 (NCT02199691) and 2 (0.2%) participants who received MenQuadfi in Study 3 (NCT02842853). Three participants in Study 2 experienced syncope within 30 minutes following vaccination: 1 (0.2%) participant who received Menveo, 1 (0.3%) participant who received MenQuadfi concomitantly with Tdap and HPV, and 1 (0.3%) participant who received Tdap and HPV concomitantly (without MenQuadfi). These events were non-serious and spontaneously resolved on the same day.

Adults 18 through 55 years of age

The safety of MenQuadfi in adults 18 through 55 years of age was evaluated in Study 3 (NCT02842853). The safety analysis set included 1,495 participants who received MenQuadfi and 312 participants who received Menactra. Of the participants 18 years through 55 years of age who received MenQuadfi (N = 1,495), 65.2% were female. Among those with reported race and ethnicity, 73.3% were White, 21.0% were Black or African American, 2.2% were Asian, 3.5% were of other racial groups, and 20.0% were of Hispanic or Latino ethnicity. Mean age was 39.4 years at time of administration.

The rates and severity of the solicited adverse reactions that occurred within 7 days following MenQuadfi compared with Menactra are presented in Table 3.

Dizziness within 30 minutes following vaccination was experienced by 5 (0.3%) participants who received MenQuadfi and 1 (0.3%) participant who received Menactra. These events were non-serious and spontaneously resolved on the same day.

SAEs occurred at a rate of 1.6% following MenQuadfi and at a rate of 0.6% following Menactra during the entire study period. No SAEs were determined to be vaccine related.

Table 3: Percentages of Solicited Injection-Site Reactions and Systemic Adverse Reactions within 7 Days after Vaccination with MenQuadfi or Menactra in Individuals 18 through 55 Years of Age (Study 3)*

	MenQuadfi (N†=1,441-1,460) %		Menactra (N†=297-301) %	
Adverse Reactions	**Any**	**Grade 3**	**Any**	**Grade 3**
Local Reactions				
Injection Site Pain‡	41.9	1.9	35.0	1.3
Injection Site Erythema§	5.1	0.3	3.7	0.3
Injection Site Swelling§	4.3	0.2	3.4	0.3
Systemic Reactions				
Myalgia‡	35.6	3.6	31.2	2.3

Table 3: Percentages of Solicited Injection-Site Reactions and Systemic Adverse Reactions within 7 Days after Vaccination with MenQuadfi or Menactra in Individuals 18 through 55 Years of Age (Study 3)* (continued)

	MenQuadfi (N†=1,441-1,460) %		Menactra (N†=297-301) %	
Adverse Reactions	**Any**	**Grade 3**	**Any**	**Grade 3**
Headache‡	29.0	2.9	27.6	2.7
Malaise‡	22.9	2.9	18.9	3.3
Fever¶	1.4	0.1	1.7	0.7

*Clinical trial identifier NCT02842853
†N is the number of vaccinated participants with available data for the events listed
‡Grade 3: Prevents daily activity
§Any: > 25 mm; Grade 3: > 100 mm
¶Any: ≥ 100.4°F (38.0°C); Grade 3: ≥ 102.1°F (39.0°C)

Adults 56 years of age and older

The safety of MenQuadfi in adults 56 years of age and older was evaluated in Study 4 (NCT02842866). The safety analysis set included 448 participants who received MenQuadfi intramuscularly and 453 participants who received a non-conjugate comparator meningococcal vaccine (Meningococcal Polysaccharide Vaccine, Groups A, C, Y, and W-135 Combined – Menomune, Sanofi Pasteur) subcutaneously. Of the participants 56 years of age and older who received MenQuadfi (N = 448), 44.4% were 56 through 64 years of age, 55.6% were 65 years of age and older, 57.6% were female, 86.6% were White, 11.6% were Black or African American, 1.1% were Asian, 0.4% were of other racial groups and 7.8% were of Hispanic or Latino ethnicity. Mean age was 67.0 years at time of administration.

The rates and severity of the solicited adverse reactions that occurred within 7 days following MenQuadfi compared with Menomune in Study 4 (NCT02842866) are presented in Table 4.

SAEs occurred at a rate of 3.3% following MenQuadfi and at a rate of 3.3% following Menomune during the entire study period. No SAEs were determined to be vaccine related.

Table 4: Percentages of Solicited Injection-Site Reactions and Systemic Adverse Reactions within 7 Days after Vaccination with MenQuadfi or Menomune in Individuals 56 Years of Age and Older Study 4*

	MenQuadfi (N†=436-443) %		Menomune‡ (N†=449-451) %	
Adverse Reactions	**Any**	**Grade 3**	**Any**	**Grade 3**
Local Reactions				
Injection Site Pain§	25.5	0.7	9.6	0.7
Injection Site Erythema¶	5.2	0.2	0.0	0.0
Injection Site Swelling¶	4.5	0.0	0.0	0.0
Systemic Reactions				
Myalgia§	21.9	1.6	15.3	1.3
Headache§	19.0	0.7	14.6	0.7
Malaise§	14.5	1.4	11.3	1.8
Fever#	2.1	0.2	0.4	0.0

*Clinical trial identifier NCT02842866
†N is the number of vaccinated participants with available data for the events listed
‡Menomune was given subcutaneously
§Grade 3: Prevents daily activity
¶Any: > 25 mm; Grade 3: > 100 mm
#Any: ≥ 100.4°F (38.0°C); Grade 3: ≥ 102.1°F (39.0°C)

Booster Vaccination Study

The safety of MenQuadfi in previously vaccinated adolescents and adults 15 years of age and older was evaluated in Study 5 (NCT02752906). All randomized participants had received a primary dose of either (Menveo or Menactra) 4 to 10 years previously. The safety analysis set included 402 participants who received a single booster dose of MenQuadfi (median age: 17.8 years) and 407 participants who received a single booster dose of Menactra (median age: 17.9 years). Of the participants who received MenQuadfi, 51.5% were female, 85.1% were White, 9.7% were Black, 2.7 % were Asian and 2.2 % were of other racial groups, and 15.7% were of Hispanic or Latino ethnicity.

The most commonly reported solicited adverse reactions (≥10%) within 7 days of MenQuadfi booster vaccination were injection site pain (44.7%) and headache (37.9%), myalgia (36.7%), and malaise (27.6%). The majority of solicited adverse reactions were Grade 1 or 2 and resolved within 3 days. Compared with recipients of a Menactra booster dose, recipients of a MenQuadfi booster dose had higher rates of injection site erythema (MenQuadfi 5.0%, Menactra 1.5%) and swelling (MenQuadfi 4.0%, Menactra 0.7%). Overall rates of solicited adverse reactions were comparable to those observed in unvaccinated adolescents and adults after a single MenQuadfi dose.

SAEs occurred at a rate of 1.2% following MenQuadfi and at a rate of 1.0% following Menactra during the entire study period. No SAEs were determined to be vaccine related.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

In a clinical trial in adolescents 10 through 17 years of age, MenQuadfi was administered concomitantly with Tdap and HPV [*see Adverse Reactions (6)* and *Clinical Studies (14.3)*]. Lower geometric mean antibody concentrations (GMCs) for antibodies to the pertussis antigens filamentous hemagglutinin (FHA), pertactin (PRN) and fimbriae (FIM) were observed when MenQuadfi was co-administered with Tdap and HPV, compared to concomitant administration of Tdap and HPV (without MenQuadfi) [*see Clinical Studies (14.3)*].

7.2 Immunosuppressive Treatments

Immunosuppressive therapies may reduce the immune response to MenQuadfi [*see Warnings and Precautions (5)*].

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to MenQuadfi during pregnancy. To enroll in or obtain information about the registry, call Sanofi Pasteur at 1-800-822-2463.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

There are no clinical studies of MenQuadfi in pregnant women. Available human data on MenQuadfi administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy. A developmental toxicity study in female rabbits administered a full human dose (0.5 mL) prior to mating and during gestation period revealed no evidence of harm to the fetus due to MenQuadfi (see *Animal Data*).

Data

Animal Data

In a developmental toxicity study, female rabbits received a human dose of MenQuadfi by intramuscular injection on five occasions: 30 days and 10 days prior to mating, gestation days 6, 12 and 27. No adverse effects on pre-weaning development up to post-natal day 35 were observed. There were no vaccine-related fetal malformations or variations observed.

8.2 Lactation

Risk Summary

It is not known whether MenQuadfi is excreted in human milk. Data are not available to assess the effects of MenQuadfi on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for MenQuadfi and any potential adverse effects on the breastfed child from MenQuadfi or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of MenQuadfi have not been established in individuals younger than 2 years of age in the US.

8.5 Geriatric Use

A total of 249 participants 65 years of age and older, including 71 participants 75 years of age or older, in Study 4 received one dose of MenQuadfi [*see Adverse Reactions (6.1)* and *Clinical Studies (14.1)*].

MenQuadfi recipients ≥ 65 years of age had lower GMTs and seroresponse rates for all serogroups compared to MenQuadfi recipients 56 through 64 years of age [*see Clinical Studies (14.1)*].

11 DESCRIPTION

MenQuadfi is a sterile liquid vaccine administered by intramuscular injection that contains *Neisseria meningitidis* serogroup A, C, W, and Y capsular polysaccharide antigens that are individually conjugated to tetanus toxoid protein. *N. meningitidis* A, C, W, and Y strains are cultured on Mueller Hinton agar medium and grown in Watson Scherp medium. The polysaccharides are extracted from the *N. meningitidis* cells and purified by centrifugation, detergent precipitation, alcohol precipitation, solvent extraction, and diafiltration. To prepare the polysaccharides for conjugation, Serogroup A is activated with carbonyldiimidazole (CDI), derivatized with adipic acid dihydrazide (ADH), and purified by diafiltration. Serogroups C, W, and Y are depolymerized, activated with periodate, and purified by diafiltration.

Clostridium tetani is fermented in media to generate tetanus toxin, which is purified by ammonium sulfate precipitation to yield purified tetanus toxin (PTT) and detoxified with formaldehyde to yield purified tetanus protein (PTP). The PTP is then concentrated and filtered to yield concentrated tetanus protein (CTP). The activated/derivatized polysaccharides are covalently linked to tetanus toxoid and purified by chromatography and serial diafiltration. The four meningococcal components, present as individual serogroup-specific glycoconjugates, compose the final formulated vaccine.

MenQuadfi is manufactured as a sterile, clear solution. Each 0.5 mL dose of vaccine contains 10 microgram each of meningococcal A, C, W, and Y polysaccharide antigens conjugated to approximately 55 micrograms tetanus toxoid protein carrier; 3.35 mg sodium chloride (0.67%), and 1.23 mg sodium acetate (30 mM). Potency of MenQuadfi is determined by quantifying the amount of each polysaccharide antigen that is conjugated to tetanus toxoid protein and the amount of unconjugated polysaccharide present.

No preservative or adjuvant is added during manufacture. Each 0.5 mL dose may contain residual amounts of formaldehyde of less than 3 mcg/mL, by calculation.

The vial in which the vaccine components are contained is composed of USP Type I borosilicate glass. The vial stopper is a chlorobutyl synthetic polyisoprene blend stopper (not made with natural rubber latex).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Invasive meningococcal disease (IMD) is caused by the bacterium *N. meningitidis*, a gram-negative diplococcus found exclusively in humans. The presence of bactericidal anti-capsular meningococcal antibodies in serum has been associated with protection from IMD. MenQuadfi induces the production of bactericidal antibodies specific to the capsular polysaccharides of *N. meningitidis* serogroups A, C, W, and Y.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

MenQuadfi has not been evaluated for carcinogenic or mutagenic potential or for impairment of male fertility. MenQuadfi administered to female rabbits had no effects on fertility [*see Use in Specific Population (8.1)*].

14 CLINICAL STUDIES

To infer effectiveness of MenQuadfi, the immunogenicity in persons 2 years of age and older was evaluated using a serogroup-specific serum bactericidal assay with exogenous human complement (hSBA). The hSBA responses following a single dose of MenQuadfi for primary vaccination were assessed in four studies, and the hSBA responses following a single dose of MenQuadfi for booster vaccination were assessed in one study. Serum was collected at baseline and 30 days post-vaccination to measure antibodies with hSBA. The hSBA geometric mean titers (GMTs) and proportion of participants who achieved hSBA seroresponse (defined below) were evaluated.

- Seroresponse rate for each serogroup: the proportion of participants with an hSBA
  - pre-vaccination titer < 1:8 who achieved a post-vaccination titer ≥ 1:16, or
  - pre-vaccination titer ≥ 1:8 who achieved a post-vaccination titer at least 4-fold greater than the pre-vaccination titer.

Non-inferiority of MenQuadfi seroresponse rates versus those for comparator vaccines was demonstrated for all 4 serogroups in individuals 2 years of age and older who received a primary vaccination and in individuals 15 years of age and older who received a booster vaccination at least 4 years following a previous dose of a meningococcal (Groups A, C, W, Y) conjugate vaccine.

14.1 Primary Vaccination

Immunogenicity in Children 2 through 9 Years of Age

Immunogenicity of MenQuadfi compared to Menveo in participants 2 through 9 years of age was evaluated in Study 1 (NCT03077438). The hSBA seroresponse rate and GMTs are presented in Table 5.

Immune non-inferiority, based on seroresponse rates, was demonstrated for MenQuadfi as compared to Menveo for all four serogroups.

Table 5: Comparison of Bactericidal Antibody Responses to MenQuadfi and Menveo 30 Days after Vaccination of Participants 2 through 9 Years of Age (Study 1)*

Endpoint†	MenQuadfi (95% CI)	Menveo (95% CI)	Percent difference MenQuadfi minus Menveo‡ (95% CI)
A	N=455-456	N=458	
% Participants achieving Seroresponse	55.4 (50.7; 60.0)	47.8 (43.2; 52.5)	7.6 (1.1, 14.0)
GMT	25 (22; 28)	23 (20; 26)	
C	N=458	N=458-459	
% Participants achieving Seroresponse	95.2 (92.8; 97.0)	47.8 (43.2; 52.5)	47.4 (42.2, 52.2)
GMT	238 (209; 270)	17.0 (14; 20)	
W	N=458	N=459	
% Participants achieving Seroresponse	78.8 (74.8; 82.5)	64.1 (59.5; 68.4)	14.8 (8.9, 20.5)
GMT	38 (34; 42)	26 (23; 30)	
Y	N=458	N=459	
% Participants achieving Seroresponse	91.5 (88.5; 93.9)	79.3 (75.3; 82.9)	12.2 (7.7, 16.7)
GMT	69 (61; 77)	44 (38; 50)	

N: number of participants in per-protocol analysis set with valid serology results.
95% CI of the single proportion calculated from the exact binomial method.
95% CI of the difference calculated from the Wilson Score method without continuity correction.
*Clinical trial identifier NCT03077438
†Seroresponse rate (primary end point) for each serogroup: the proportion of participants with an hSBA pre-vaccination titer < 1:8 who achieved a post-vaccination titer ≥ 1:16, or pre-vaccination titer ≥ 1:8 who achieved a post-vaccination titer at least 4-fold greater than the pre-vaccination titer.
‡Overall non-inferiority would be demonstrated if the lower limit of the 2-sided 95% CI is > -10% for all four serogroups.

Immunogenicity in Adolescents 10 through 17 Years of Age

Immunogenicity of MenQuadfi compared to Menveo in participants 10 through 17 years of age was evaluated in Study 2 (NCT02199691). Study 2 was conducted in healthy meningococcal vaccine naïve participants and evaluated seroresponse rates following administration with either MenQuadfi alone, Menveo alone, MenQuadfi co-administered with Tdap, and HPV, or Tdap and HPV alone. The hSBA seroresponse rate and GMTs for Study 2 are presented in Table 6.

Immune non-inferiority, based on seroresponse, was demonstrated for MenQuadfi as compared to Menveo for all four serogroups.

Study 2 (NCT02199691) was conducted in healthy meningococcal vaccine naïve male and female participants and evaluated seroresponses following administration with either MenQuadfi alone; Menveo alone; MenQuadfi co-administered with Tdap, and HPV; or Tdap and HPV alone. The hSBA seroresponse rate and GMTs for the MenQuadfi alone and Menveo alone groups are presented in Table 6.

Table 6: Comparison of Bactericidal Antibody Responses to MenQuadfi and Menveo 30 Days after Vaccination of Participants 10 through 17 Years of Age Study 2*

Endpoint†	MenQuadfi (95% CI)	Menveo (95% CI)	Percent difference MenQuadfi minus Menveo‡ (95% CI)
A	N=463	N=464	
% Participants achieving Seroresponse	75.6 (71.4; 79.4)	66.4 (61.9; 70.7)	9.2 (3.4; 15.0)
GMT	44 (39; 50)	35 (30; 41)	
C	N=462	N=463	
% Participants achieving Seroresponse	97.2 (95.2; 98.5)	72.6 (68.3; 76.6)	24.6 (20.3; 29.0)
GMT	387 (329; 456)	51 (41; 64)	
W	N=463	N=464	
% Participants achieving Seroresponse	86.2 (82.7; 89.2)	66.6 (62.1; 70.9)	19.6 (14.2; 24.8)
GMT	87 (78; 97)	36 (32; 41)	
Y	N=462-463	N=464	
% Participants achieving Seroresponse	97.0 (95.0; 98.3)	80.8 (76.9; 84.3)	16.2 (12.3; 20.2)
GMT	76 (66; 87)	28 (24; 32)	

N: number of participants in per-protocol analysis set with valid serology results.
95% CI of the single proportion calculated from the exact binomial method.
95% CI of the difference calculated from the Wilson Score method without continuity correction.
Overall non-inferiority would be demonstrated if the lower limit of the 2-sided 95% CI is > -10% for all four serogroups.
*Clinical trial identifier NCT02199691
†Seroresponse rate (primary end point) for each serogroup: the proportion of participants with an hSBA pre-vaccination titer < 1:8 who achieved a post-vaccination titer ≥ 1:16, or pre-vaccination titer ≥ 1:8 who achieved a post-vaccination titer at least 4-fold greater than the pre-vaccination titer.
‡post-vaccination hSBA titers ≥1:8 for participants with pre-vaccination hSBA titers < 1:8 or at least a 4-fold increase in hSBA titers from pre to post-vaccination for participants with pre-vaccination hSBA titers ≥1:8

Study 3 evaluated the immunogenicity of MenQuadfi (N=1097-1098) compared to Menactra (N=300) in healthy meningococcal-naïve participants 10 through 17 years of age. Seroresponse rates for MenQuadfi were noninferior to those of Menactra for all serogroups based on the same noninferiority criteria defined for Study 2.

Immunogenicity in Adults 18 through 55 Years of Age

Immunogenicity of MenQuadfi compared to Menactra in participants 18 through 55 years of age was evaluated in Study 3 (NCT02842853). The hSBA seroresponse rate and GMTs are presented in Table 7.

Immune non-inferiority, based on seroresponse rates, was demonstrated for MenQuadfi as compared to Menactra for all four serogroups.

Table 7: Comparison of Bactericidal Antibody Responses to MenQuadfi and Menactra 30 Days after Vaccination of Participants 18 through 55 Years of Age Study 3*

Endpoint†	MenQuadfi (95% CI)	Menactra (95% CI)	Percent difference MenQuadfi minus Menactra‡ (95% CI)
A	N=1,406-1,408	N=293	
% Participants achieving Seroresponse	73.5 (71.2; 75.8)	53.9 (48.0; 59.7)	19.6 (13.5; 25.8)
GMT	106 (97; 117)	52 (43; 64)	
C	N=1,406-1,408	N=293	
% Participants achieving Seroresponse	83.4 (81.4; 85.3)	42.3 (36.6; 48.2)	41.1 (35.0; 46.9)
GMT	234 (210; 261)	37 (29; 49)	
W	N=1,408-1,410	N=293	
% Participants achieving Seroresponse	77.0 (74.7; 79.2)	50.2 (44.3; 56.0)	26.8 (20.7; 32.9)
GMT	76 (69; 83)	33 (26; 42)	
Y	N=1,408-1,410	N=293	
% Participants achieving Seroresponse	88.1 (86.3; 89.8)	60.8 (54.9; 66.4)	27.4 (21.7; 33.3)
GMT	219 (200; 239)	55 (42; 70)	

N: number of participants in per-protocol analysis set with valid serology results.
95% CI of the single proportion calculated from the exact binomial method.
95% CI of the difference calculated from the Wilson Score method without continuity correction.
*Clinical trial identifier NCT02842853
†Seroresponse rate (primary end point) for each serogroup: the proportion of participants with an hSBA pre-vaccination titer < 1:8 who achieved a post-vaccination titer ≥ 1:16, or pre-vaccination titer ≥ 1:8 who achieved a post-vaccination titer at least 4-fold greater than the pre-vaccination titer.
‡The overall non-inferiority would be demonstrated if the lower limit of the 2-sided 95% CI is > -10% for all four serogroups.

Immunogenicity in Adults 56 Years of Age and Older

Immunogenicity of MenQuadfi compared to Menomune in participants 56 years and older was evaluated in Study 4 (NCT02842866).

Enrollment was stratified by age category: 56 through 64 years of age (44.3%), 65 through 74 years of age (39.7%), and 75 years of age and older (15.9%). The overall mean age of participants who received MenQuadfi was 66.9 years; range: 56 through 89.8 years of age. The mean age for participants in the 56 through 64 years age stratum who received MenQuadfi was 60.4 years, the mean age for participants ≥ 65 years age stratum who received MenQuadfi was 72.2 years.

The hSBA seroresponse rate and GMTs are presented in Table 8.

Immune non-inferiority, based on seroresponse rates, was demonstrated for MenQuadfi as compared to Menomune for all four serogroups.

Table 8: Comparison of Bactericidal Antibody Responses to MenQuadfi and Menomune in Naïve Older Adults and Elderly 30 Days after Vaccination Study 4*

Endpoint†	MenQuadfi (95% CI)	Menomune (95% CI)	Percent difference MenQuadfi minus Menomune‡ (95% CI)
A	N=433	N=431	
% Participants achieving Seroresponse	58.2 (53.4; 62.9)	42.5 (37.7; 47.3)	15.7 (9.08; 22.2)
GMT	55 (47; 65)	31 (27; 37)	
C	N=433	N=431	
% Participants achieving Seroresponse	77.1 (72.9; 81.0)	49.7 (44.8; 54.5)	27.5 (21.2; 33.5)

Table 8: Comparison of Bactericidal Antibody Responses to MenQuadfi and Menomune in Naïve Older Adults and Elderly 30 Days after Vaccination Study 4* (continued)

Endpoint†	MenQuadfi (95% CI)	Menomune (95% CI)	Percent difference MenQuadfi minus Menomune‡ (95% CI)
GMT	101 (84; 123)	25 (21; 30)	
W	N=433	N=431	
% Participants achieving Seroresponse	62.6 (57.8; 67.2)	44.8 (40.0; 49.6)	17.8 (11.2; 24.2)
GMT	28 (24; 33)	15 (13; 18)	
Y	N=433	N=431	
% Participants achieving Seroresponse	74.4 (70.0; 78.4)	43.4 (38.7; 48.2)	31.0 (24.6; 37.0)
GMT	69 (59; 81)	21 (17; 25)	

N: number of participants in per-protocol analysis set with valid serology results.
95% CI of the single proportion calculated from the exact binomial method.
95% CI of the difference calculated from the Wilson Score method without continuity correction.
*Clinical trial identifier NCT02842866
†Seroresponse rate (primary end point) for each serogroup: the proportion of participants with an hSBA pre-vaccination titer < 1:8 who achieved a post-vaccination titer ≥ 1:16, or pre-vaccination titer ≥ 1:8 who achieved a post-vaccination titer at least 4-fold greater than the pre-vaccination titer.
‡The overall non-inferiority would be demonstrated if the lower limit of the 2-sided 95% CI is > -10% for all four serogroups.

14.2 Booster

Immunogenicity of a booster dose of MenQuadfi compared to a booster dose of Menactra was evaluated in Study 5 (NCT02752906). The study-enrolled participants 15 years of age and older who had received a primary dose of Menveo or Menactra 4 to 10 years previously.

Immune non-inferiority, based on seroresponse rates, was demonstrated for MenQuadfi as compared to Menactra for all four serogroups.

For a description of study design and number of participants, see section 6.1 Booster Vaccination Study. The primary immunogenicity endpoint was hSBA seroresponse to each serogroup 30 days following booster vaccination with MenQuadfi or Menactra given to participants who received a prior dose of Menveo or Menactra 4 to 10 years ago. Seroresponse was defined as the proportion of participants with an hSBA pre-vaccination titer < 1:8 who achieved a post-vaccination titer ≥ 1:16, or pre-vaccination titer ≥ 1:8 who achieved a post-vaccination titer at least 4-fold greater than the pre-vaccination titer. The other endpoints included the proportions of participants with post-vaccination hSBA ≥1:8 and the hSBA GMTs for each serogroup. These endpoints were also evaluated at 6 days post vaccination in a subset.

Seroresponse rates at Day 30 following booster vaccination with MenQuadfi were 92.2% for serogroup A, 97.1% for serogroup C, 98.2% for serogroup W, and 97.4% for serogroup Y, as compared to 87.1% for serogroup A, 91.8% for serogroup C, 90.7% for serogroup W, and 95.6% for serogroup Y, following booster vaccination with Menactra. At Day 6, following booster vaccination with MenQuadfi, seroresponse rates were 72.7%, 83.6%, 94.5%, and 90.9% for serogroups A, C, W, and Y, respectively.

The hSBA GMTs were 173, 334, 499, and 302 for serogroups A, C, W, and Y at Day 6, and 497, 2618, 1747, and 2070, respectively, for the 4 serogroups at Day 30 following booster dose of MenQuadfi.

Overall, similar seroresponse rates were observed for those participants who received booster vaccination with Menactra.

14.3 Immunogenicity of Concomitantly Administered Vaccines

Concomitant administration of MenQuadfi with Tdap and HPV in adolescents 10 through 17 years was evaluated in Study 2 (NCT02199691). In this randomized study, 505 participants received MenQuadfi alone, 403 received MenQuadfi coadministered with Tdap and HPV, 300 received Tdap and HPV alone. A fourth group received Menveo alone (N=507).

No evidence of interference in hSBA seroresponse rates was observed when MenQuadfi was coadministered with Tdap and HPV. Antibody responses to HPV, and to the tetanus and diphtheria antigens were similar when Tdap and HPV were administered with and without MenQuadfi. Anti-pertussis GMC responses were non-inferior for the pertussis toxoid antigen, but did not meet non-inferiority for the FHA, PRN, and FIM antigens. The clinical relevance of the diminished responses to the pertussis antigens is unknown.

16 HOW SUPPLIED/STORAGE AND HANDLING

MenQuadfi is supplied in a single-dose vial (NDC 49281-590-58), in packages of 5 vials (NDC 49281-590-05).

The vial stopper is not made with natural rubber latex.

Store at 2°C to 8°C (35°F to 46°F). Do not freeze. Do not use vaccine that has been frozen. Do not use after expiration date.

17 PATIENT COUNSELING INFORMATION

Vaccine Information Statements are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization to the patient, parent, or guardian. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines). Inform the patients, parents or guardians about:

- Potential benefits and risks of immunization with MenQuadfi.
- Potential for adverse reactions that have been temporally associated with administration of MenQuadfi or other vaccines containing similar components.
- Reporting any adverse reactions to their healthcare provider.
- The Sanofi Pasteur Inc. Pregnancy Registry, as appropriate [*see Pregnancy (8.1)*].

Manufactured by:
Sanofi Pasteur Inc.
Swiftwater PA 18370 USA

R0-0420

MCV-FPLR-SL-APR20

Rx Only

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use MENVEO safely and effectively. See full prescribing information for MENVEO.

MENVEO [Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM_{197} Conjugate Vaccine] solution for injection, for intramuscular use

Initial U.S. Approval: 2010

----------RECENT MAJOR CHANGES----------
Dosage and Administration (2.1, 2.2, 2.3) 10/2022

---------- INDICATIONS AND USAGE----------
MENVEO is a vaccine indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y, and W-135 in individuals 2 months through 55 years of age.
MENVEO does not prevent *N. meningitidis* serogroup B infections. (1)

----------DOSAGE AND ADMINISTRATION----------
- For intramuscular injection only (0.5 mL). (2)

MENVEO is supplied as either:
- Two vials: A vial containing the MenCYW-135 liquid conjugate component (gray cap) and a vial containing the MenA lyophilized conjugate component (orange cap). The contents of the vials must be combined to form MENVEO prior to administration. This presentation is for use in individuals 2 months through 55 years of age. (2.1, 2.2),

OR
- One vial containing MENVEO (pink cap). This presentation does not require reconstitution before use. This presentation is for use in individuals 10 through 55 years of age. (2.1, 2.2)

Primary Vaccination
- In children initiating vaccination at 2 months of age, administer as a 4-dose series at 2, 4, 6, and 12 months of age. (2.4)
- In children initiating vaccination at 7 months through 23 months of age, administer as a 2-dose series with the second dose administered in the second year of life and at least 3 months after the first dose. (2.4)
- In individuals aged 2 through 55 years, administer as a single dose. (2.4)

Booster Vaccination
- A single booster dose of MENVEO may be administered to individuals aged 15 through 55 years who are at continued risk for meningococcal disease if at least 4 years have elapsed since a prior dose of a meningococcal (serogroups A, C, Y, W-135) conjugate vaccine. (2.4)

---------- DOSAGE FORMS AND STRENGTHS----------
Solution for injection. A single dose is 0.5 mL. (3)

---------- CONTRAINDICATIONS ----------
Severe allergic reaction (e.g., anaphylaxis) to a previous dose of MENVEO, to any component of this vaccine, or to any other diphtheria toxoid-containing vaccine. (4)

---------- WARNINGS AND PRECAUTIONS ----------
- Syncope (fainting) has occurred in association with administration of MENVEO. Procedures should be in place to avoid injury from fainting. (5.2)
- Apnea following intramuscular vaccination has been observed in some infants born prematurely. A decision about when to administer MENVEO to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.5)

---------- ADVERSE REACTIONS ----------
- Common solicited adverse reactions (≥10%) among children initiating vaccination at 2 months of age and receiving the 4-dose series were tenderness (24% to 41%) and erythema at injection site (11% to 15%), irritability (42% to 57%), sleepiness (29% to 50%), persistent crying (21% to 41%), change in eating habits (17% to 23%), vomiting (5% to 11%), and diarrhea (8% to 16%). (6.1)
- Common solicited adverse reactions (≥10%) among children initiating vaccination at 7 months through 23 months of age and receiving the 2-dose series were tenderness (10% to 16%) and erythema at injection site (12% to 15%), irritability (27% to 40%), sleepiness (17% to 29%), persistent crying (12-21%), change in eating habits (12% to 20%), and diarrhea (10% to 16%). (6.1)
- Common solicited adverse reactions (≥10%) among children aged 2 through 10 years who received MENVEO were injection site pain (31%), erythema (23%), irritability (18%), induration (16%), sleepiness (14%), malaise (12%), and headache (11%). (6.1)
- Common solicited adverse reactions (≥10%) among adolescents and adults who received a single dose of MENVEO were pain at the injection site (41%), headache (30%), myalgia (18%), malaise (16%), and nausea (10%). Similar rates of solicited adverse reactions were observed following a single booster dose. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 10/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

MENVEO is a vaccine indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y, and W-135 in individuals 2 months through 55 years of age.

MENVEO does not prevent *N. meningitidis* serogroup B infections.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 MENVEO Presentations

MENVEO is supplied in two presentations, a two-vial presentation and a one-vial presentation.

Two-Vial Presentation

The two-vial presentation includes a vial with a gray cap containing the MenCYW-135 liquid conjugate component and a vial with an orange cap containing the MenA lyophilized conjugate component. The contents of the vials must be combined to form MENVEO prior to administration. This presentation is for use in individuals 2 months through 55 years of age.

One-Vial Presentation

The one-vial presentation contains MENVEO in a single vial with a pink cap and does not require reconstitution before use. This presentation is for use in individuals 10 through 55 years of age.

2.2 Preparation

Reconstitution Instructions for MENVEO Two-Vial Presentation

Use the MenCYW-135 liquid conjugate component (Vial 1, gray cap) to reconstitute the MenA lyophilized conjugate component (Vial 2, orange cap) to form MENVEO. Invert Vial 2 and shake well until the lyophilized conjugate component is dissolved. After reconstitution, withdraw 0.5 mL from the vial containing the reconstituted vaccine. See Figures 1 through 4.

Administer MENVEO immediately **or** store between 36°F and 77°F (2°C and 25°C) for up to 8 hours. Shake well before using. Discard reconstituted vaccine if it has been frozen or not used within 8 hours.

Figure 1. Cleanse both vial stoppers. Using a sterile needle and sterile graduated syringe, withdraw the entire contents of Vial 1 containing the MenCYW-135 liquid conjugate component while slightly tilting the vial.

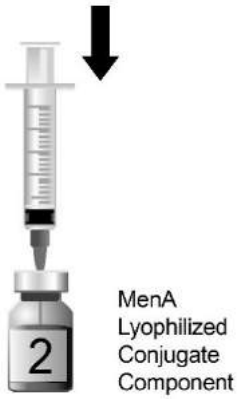


Figure 2. Slowly transfer entire contents of the syringe into Vial 2 containing the MenA lyophilized conjugate component.

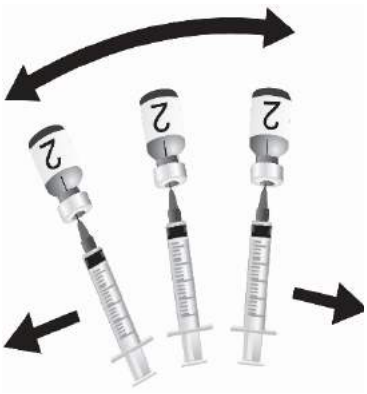


Figure 3. Invert the vial and shake well until lyophilized conjugate component is completely dissolved.

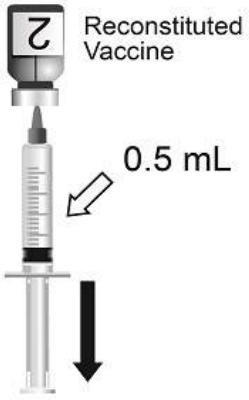


Figure 4. After reconstitution, withdraw 0.5 mL from the vial containing the reconstituted vaccine.

Instructions for MENVEO One-Vial Presentation

The MENVEO presentation that is supplied in a single vial with a pink cap does NOT require reconstitution. Withdraw 0.5 mL from the vial.

2.3 Administration

MENVEO is a clear, colorless solution, free from visible foreign particles. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If any of these conditions exist, MENVEO should not be administered.

Administer a single 0.5-mL dose by intramuscular injection.

2.4 Dosing Schedule

The dosing schedule is as follows:

Primary Vaccination

Table 1. Dosing Schedule for MENVEO Primary Vaccination

MENVEO Two-Vial Presentation	
Infants Aged 2 Months	4-dose series at 2, 4, 6, and 12 months of age
Children Aged 7 through 23 Months	2-dose series with the second dose administered in the second year of life and at least 3 months after the first dose
Children Aged 2 through 10 Years	A single dose For children aged 2 through 5 years at continued high risk of meningococcal disease, a second dose may be administered 2 months after the first dose.
Adolescents and Adults Aged 11 through 55 Years	A single dose
MENVEO One-Vial Presentation	
Adolescents and Adults Aged 10 through 55 Years	A single dose

Booster Vaccination

Adolescents and Adults Aged 15 through 55 Years: A single booster dose of MENVEO using either the two-vial presentation or the one-vial presentation may be administered to individuals who are at continued risk for meningococcal disease if at least 4 years have elapsed since a prior dose of a meningococcal (serogroups A, C, Y, W-135) conjugate vaccine.

3 DOSAGE FORMS AND STRENGTHS

MENVEO is a solution for intramuscular injection. A single dose is 0.5 mL.

4 CONTRAINDICATIONS

Do not administer MENVEO to individuals with a severe allergic reaction (e.g., anaphylaxis) to a previous dose of MENVEO, to any component of this vaccine, or to any other diphtheria toxoid-containing vaccine. *[See Description (11).]*

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate medical treatment must be available should an acute allergic reaction, including an anaphylactic reaction, occur following administration of MENVEO.

5.2 Syncope

Syncope (fainting) has occurred in association with administration of MENVEO. Procedures should be in place to avoid injury from fainting.

5.3 Altered Immunocompetence

Reduced Immune Response

Some individuals with altered immunocompetence, including some individuals receiving immunosuppressant therapy, may have reduced immune responses to MENVEO.

Complement Deficiency

Persons with certain complement deficiencies and persons receiving treatment that inhibits terminal complement activation (for example, eculizumab) are at increased risk for invasive disease caused by *N. meningitidis*, including invasive disease caused by serogroups A, C, Y, and W, even if they develop antibodies following vaccination with MENVEO. *[See Clinical Pharmacology (12.1).]*

5.4 Guillain-Barré Syndrome

Guillain-Barré syndrome (GBS) has been reported in temporal relationship following administration of another U.S.-licensed meningococcal quadrivalent polysaccharide conjugate vaccine. The decision by the healthcare professional to administer MENVEO to persons with a history of GBS should take into account the expected benefits and potential risks.

5.5 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. A decision about when to administer MENVEO to an infant born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

Overall, in clinical studies, 36,146 individuals 2 months through 55 years of age were administered at least one dose of MENVEO supplied in the two-vial presentation and 1,337 individuals 10 through 44 years of age were administered one dose of MENVEO supplied in the one-vial presentation. The safety data for the two-vial presentation are relevant to the safety of the one-vial presentation because each presentation contains the same meningococcal conjugated oligosaccharides. *[See Description (11).]*

Primary Vaccination Studies

Children Aged 2 through 23 Months: The safety of MENVEO in infants vaccinated at 2, 4, 6, and 12 months of age was evaluated in 3 randomized multicenter clinical studies (NCT00474526, NCT00806195, NCT01000311) conducted in the U.S., Australia, Canada, Taiwan, and several countries of Latin America in which 8,735 infants received at least 1 dose of MENVEO and routine infant vaccines (diphtheria toxoid; acellular pertussis; tetanus toxoid [DTaP]; inactivated polio types 1, 2, and 3 [IPV]; hepatitis B; *Haemophilus influenzae* type b (Hib) antigens; pentavalent rotavirus; and 7-valent pneumococcal conjugate [PCV7]). With Dose 4 of MENVEO, toddlers received concomitantly the following vaccines: 7-valent pneumococcal conjugate; measles, mumps, rubella, and varicella; and inactivated hepatitis A. A total of 2,864 infants in these studies received the routine infant/toddler vaccines only. The infants who received MENVEO were Caucasian (33%), Hispanic (44%), African American (8%), Asian (8%), and other racial/ethnic groups (7%); 51% were male, with a mean age of 65.1 days (Standard Deviation [SD]: 7.5 days) at the time of first vaccination.

Safety data for administration of 2 doses of MENVEO in children aged 6 through 23 months are available from 3 randomized studies (NCT00474526, NCT00310856, NCT00626327) conducted in the U.S., Latin America, and Canada, of which one U.S. study specifically addressed the safety of MENVEO administered concomitantly with measles, mumps, rubella, and varicella vaccine (MMRV). The 1,985 older infants and toddlers who received 2 doses of MENVEO were Caucasian (49%), Hispanic (32%), African American (11%), and other racial/ethnic groups (8%), 51% male, with a mean age of 10.1 months (SD: 2.0 months).

Children Aged 2 through 10 Years: The safety of MENVEO in children aged 2 through 10 years was evaluated in 4 clinical trials (NCT00310817, NCT00262028, NCT00329849, NCT00616421) conducted in North America (66%), Latin America (28%), and Europe (6%) in which 3,181 subjects received MENVEO and 2,116 subjects received comparator vaccines (either Meningococcal Polysaccharide Vaccine, Groups A, C, Y, and W-135 Combined - MENOMUNE, Sanofi Pasteur [n = 861], or Meningococcal (Groups A, C, Y, and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine - MENACTRA, Sanofi Pasteur [n = 1,255]). The subjects aged 2 through 10 years who received MENVEO were Caucasian (69%), Hispanic (13%), African American (7%), and other racial/ethnic groups (6%), 51% male, with a mean age of 5.2 years. The safety of a second dose of MENVEO administered 2 months following a first dose was studied in 351 children aged 2 through 5 years.

Adolescents and Adults: The safety of MENVEO in individuals aged 11 through 55 years was evaluated in 5 randomized controlled clinical trials (NCT01018732, NCT00329901, NCT00450437, NCT00474487, NCT00518180) in which 6,185 participants received MENVEO alone (5,286 participants), MENVEO concomitant with other vaccine(s) (899 participants), or a U.S.-licensed comparator vaccine (1,966 participants). In the concomitant trials (NCT00329901, NCT00518180) MENVEO was given with vaccines containing: tetanus toxoid, diphtheria toxoid, and pertussis (Tdap), or Tdap with human papillomavirus (HPV). The comparator

vaccine was either MENOMUNE (209 participants) or MENACTRA (1,757 participants). The trials were conducted in North America (46%), Latin America (41%), and Europe (13%). In 2 of the studies, subjects received concomitant vaccination with Tdap or with Tdap plus HPV. Overall, in these studies, subjects were Caucasian (50%), followed by Hispanic (40%), African American (7%), and other racial/ethnic groups (3%). Among recipients of MENVEO, 61%, 17%, and 22% were in the 11- through 18-year, 19- through 34-year, and 35- through 55-year age groups, respectively, with a mean age of 23.5 years (SD: 12.9 years). Among recipients of MENACTRA, 31%, 32%, and 37% were in the 11- through 18-year, 19- through 34-year, and 35- through 55-year age groups, respectively, with a mean age of 29.2 years (SD: 13.4 years). Among MENOMUNE recipients, 100% were in the 11- through 18-year age group, and the mean age was 14.2 years (SD: 1.8 years).

The safety of MENVEO one-vial presentation was evaluated in 2 randomized clinical trials (NCT03652610, NCT03433482). In these studies, 1,337 subjects aged 10 through 44 years were administered a single dose of MENVEO supplied in the one-vial presentation and contributed to study analyses and 1,332 subjects 10 through 40 years of age were administered MENVEO supplied in the two-vial presentation. The studies were conducted in Australia, Belgium, Canada, Germany, and Italy (NCT03652610), and in Brazil, Estonia, Finland, France, Mexico, Russian Federation, South Africa, Spain, and Turkey (NCT03433482). Overall, in these studies, subjects were White (80.8%), followed by Hispanic or Latino ethnicity (12.8%), other racial groups (11.4%), African American (4.3%), Asian (3.0%), American Indian or Alaskan Native (0.3%), and Native Hawaiian or other Pacific Islander (0.1%). Overall, 25.6% of individuals were aged 10 through 17 years, and 74.4% were aged 18 through 44 years.

Booster Vaccination Study

In a multicenter, open-label trial (NCT02986854) conducted in the U.S., 601 subjects aged 15 to 51 years received a single booster dose of MENVEO 4 to 6 years after prior vaccination with MENVEO (n = 301; median age: 16 years) or MENACTRA (n = 300; median age: 16 years). Across booster groups of MENVEO, 81% of subjects were White and 50% were female.

In most trials, solicited local and systemic adverse reactions were monitored daily for 7 days following each (one or more) vaccination and recorded on a diary card. Participants were monitored for unsolicited adverse events which included adverse events requiring a physician visit or Emergency Department visit (i.e., medically-attended) or which led to a subject's withdrawal from the study. Among children, adolescents, and adults aged 2 to 55 years, medically significant adverse events and serious adverse events (SAEs) were monitored for 6 months after vaccination. Across the studies of infants and toddlers aged 2 through 23 months, either all medically-attended or all medically-significant adverse events were collected in the period between the infant dose(s) and the toddler doses and during the 6-month period after the toddler dose.

Solicited Adverse Reactions in the Primary Vaccination Studies

The reported frequencies of solicited local and systemic adverse reactions from U.S. infants in the largest multinational safety study of MENVEO (NCT00806195) are presented in Table 2. Among the U.S. participants in the group receiving MENVEO with routine vaccines, 51% were female; 64% were Caucasian, 12% were African American, 15% were Hispanic, 2% were Asian, and 7% were of other racial/ethnic groups.

In infants initiating vaccination at 2 months of age and receiving the 4-dose series, common solicited adverse reactions (≥10%) were tenderness (24% to 41%) and erythema at injection site (11% to 15%), irritability (42% to 57%), sleepiness (29% to 50%), persistent crying (21% to 41%), change in eating habits (17% to 23%), vomiting (5% to 11%), and diarrhea (8% to 16%). The rates of solicited adverse reactions reported for subjects aged 2 months and older receiving MENVEO with routine vaccines at 2, 4, 6, and 12 months of age were comparable to rates among subjects who only received routine vaccines.

Table 2. Rates of Solicited Adverse Reactions Reported in U.S. Infants, Aged 2 Months and Older, during the 7 Days following Each Vaccination of MENVEO Administered with Routine Infant/Toddler Vaccines, or Routine Infant/Toddler Vaccines Alone at 2, 4, 6, and 12 Months of Age[a]

	Dose 1		**Dose 2**		**Dose 3**		**Dose 4**	
Adverse Reactions	**MENVEO with Routine[b]** %	**Routine Vaccines[b]** %	**MENVEO with Routine[b]** %	**Routine Vaccines[b]** %	**MENVEO with Routine[b]** %	**Routine Vaccines[b]** %	**MENVEO with Routine[b]** %	**Routine Vaccines[b]** %
Local Adverse Reactions[c]	**n = 1,250-1,252**	**n = 428**	**n = 1,205-1,207**	**n = 399**	**n = 1,056-1,058**	**n = 351-352**	**n = 1,054-1,055**	**n = 334-337**
Tenderness, any	41	45	31	36	24	32	29	39
Tenderness, severe[d]	3	5	2	2	1	3	1	1
Erythema, any	11	14	12	21	14	23	15	25
Erythema, >50 mm	<1	<1	0	0	0	0	0	0
Induration, any	8	16	9	17	8	19	8	21
Induration, >50 mm	0	<1	0	0	0	0	0	0

Systemic Adverse Reactions	n = 1,246-1,251	n = 427-428	n = 1,119-1,202	n = 396-398	n = 1,050-1,057	n = 349-350	n = 1,054-1,056	n = 333-337
Irritability, any	57	59	48	46	42	38	43	42
Irritability, severe[e]	2	2	1	3	1	1	2	1
Sleepiness, any	50	50	37	36	30	30	29	27
Sleepiness, severe[f]	2	1	1	1	<1	<1	1	0
Persistent crying, any	41	38	28	24	22	17	21	18
Persistent crying, ≥3 hours	2	2	2	2	1	1	1	1
Change in eating habits, any	23	24	18	17	17	13	19	16
Change in eating habits, severe[g]	1	1	1	1	1	<1	1	0
Vomiting, any	11	9	7	6	6	4	5	4
Vomiting, severe[h]	<1	0	<1	0	<1	0	<1	0
Diarrhea, any	16	11	11	8	8	6	13	9
Diarrhea, severe[i]	<1	<1	<1	<1	1	<1	1	1
Rash[j]	3	3	3	4	3	3	4	3
Fever ≥38.0°C[k]	3	2	4	6	7	6	9	7
Fever 38.0-38.9°C	3	2	4	5	7	6	6	5
Fever 39.0-39.9°C	0	0	1	1	<1	0	2	2
Fever ≥40.0°C	0	<1	0	<1	0	0	<1	0

Clinicaltrials.gov Identifier NCT00806195.

n = Number of subjects who completed the diary card for a given symptom at the specified vaccination.

[a] As-Treated Safety Subpopulation = U.S. children who received at least 1 dose of study vaccine and whose diary cards were completed per protocol and returned to the site.

[b] Routine infant/toddler vaccines include DTaP-IPV-Hib and PCV7 at Doses 1, 2, 3, and PCV7, MMRV, and Hepatitis A vaccines at Dose 4. HBV and rotavirus vaccines were allowed according to Advisory Committee on Immunization Practices (ACIP) recommendations.

[c] Local reactogenicity of MENVEO and PCV7 was assessed.

[d] Tenderness, severe = Cried when injected limb moved.

[e] Irritability, severe = Unable to console.

[f] Sleepiness, severe = Sleeps most of the time, hard to arouse.

[g] Change in eating habits, severe = Missed >2 feeds.

[h] Vomiting, severe = Little/no intake for more prolonged time.

[i] Diarrhea, severe = ≥6 liquid stools, no solid consistency.

[j] Rash was assessed only as present or not present, without a grading for severity.

[k] Axillary temperature.

The safety of a second dose of MENVEO administered at 12 months of age concomitantly with MMRV was investigated in a randomized, controlled, multicenter study (NCT00626327) conducted in the U.S. The rates of solicited adverse reactions reported were comparable between the concomitantly administered group (MENVEO with MMRV) and the group which received MMRV alone or MENVEO alone. The frequency and severity of solicited local and systemic reactions occurring within 7 days following vaccination at 12 months of age are shown in Table 3. In subjects who received both MENVEO and MMRV at 12 months of age local reactions at both injection sites were evaluated separately. Body temperature measurements were collected for 28 days following the 12-months-of-age visit, when MMRV was administered to the vaccinees. Common solicited adverse reactions (≥10%) among children initiating vaccination at 7 months through 23 months of age and receiving the 2-dose series were tenderness (10% to 16%) and erythema at injection site (12% to 15%), irritability (27% to 40%), sleepiness (17% to 29%), persistent crying (12% to 21%), change in eating habits (12% to 20%), and diarrhea (10% to 16%). An examination of the fever profile during this period showed that MENVEO administered with MMRV did not increase the frequency or intensity of fever above that observed for the MMRV-only group.

Table 3. Rates of Solicited Adverse Reactions Reported in U.S. Toddlers during the 7 Days following Vaccination with MENVEO Administered at 7-9 Months and 12 Months of Age, MENVEO Administered Alone at 7-9 Months and with MMRV at 12 Months of Age, and MMRV Administered Alone at 12 Months of Age[a]

	MENVEO		MENVEO + MMRV		MMRV
Adverse Reactions	**MENVEO 7-9 Months %**	**MENVEO 12 Months %**	**MENVEO 7-9 Months %**	**MENVEO with MMRV 12 Months %**	**MMRV 12 Months %**
Local Adverse Reactions– MENVEO	**n = 460-462**	**n = 381-384**	**n = 430-434**	**n = 386-387**	
Tenderness, any	11	10	11	16	N/A
Tenderness, severe[b]	<1	<1	<1	0	N/A
Erythema, any	15	13	13	12	N/A
Erythema, >50 mm	<1	<1	0	1	N/A
Induration, any	8	8	7	8	N/A
Induration, >50 mm	<1	<1	0	1	N/A
Local Adverse Reactions– MMRV				**n = 382-383**	**n = 518-520**
Tenderness, any	N/A	N/A	N/A	16	19
Tenderness, severe[b]	N/A	N/A	N/A	0	<1
Erythema, any	N/A	N/A	N/A	15	14
Erythema, >50 mm	N/A	N/A	N/A	1	<1
Induration, any	N/A	N/A	N/A	13	8
Induration, >50 mm	N/A	N/A	N/A	<1	0

Systemic Adverse Reactions	n = 461-463	n = 385-386	n = 430-434	n = 387-389	n = 522-524
Irritability, any	40	27	37	37	44
Irritability, severe[c]	2	2	2	1	3
Sleepiness, any	26	17	29	26	32
Sleepiness, severe[d]	2	1	1	1	2
Persistent crying, any	21	12	20	19	20
Persistent crying, ≥3 hours	2	1	1	1	2
Change in eating habits, any	17	12	17	20	20
Change in eating habits, severe[e]	<1	1	1	2	1
Vomiting, any	9	6	9	6	6
Vomiting, severe[f]	<1	<1	<1	<1	<1
Diarrhea, any	16	10	15	15	20
Diarrhea, severe[g]	2	1	<1	1	2
Rash[h]	3	5	6	6	8
Fever ≥38.0°C[i]	5	5	6	9	7
Fever 38.0-38.9°C	3	3	5	7	7
Fever 39.0-39.9°C	2	2	1	1	1
Fever ≥40.0°C	<1	1	<1	<1	0

Clinicaltrials.gov Identifier NCT00626327.

n = Number of subjects who completed the diary card for a given symptom at the specified vaccination.

[a] As-Treated Safety Subpopulation = U.S. children who received at least 1 dose of study vaccine and whose diary cards were completed per protocol and returned to the site.

[b] Tenderness, severe = Cried when injected limb moved.

[c] Irritability, severe = Unable to console.

[d] Sleepiness, severe = Sleeps most of the time, hard to arouse.

[e] Change in eating habits, severe = Missed >2 feeds.

[f] Vomiting, severe = Little/no intake for more prolonged time.

[g] Diarrhea, severe = ≥6 liquid stools, no solid consistency.

[h] Rash was assessed only as present or not present, without a grading for severity.

[i] Axillary temperature.

In clinical trials of children aged 2 through 10 years (NCT00310817, NCT00262028, NCT00329849, NCT00616421), the most frequently occurring adverse reactions (≥10%) among all subjects who received MENVEO were injection site pain (31%), erythema (23%), irritability (18%), induration (16%), sleepiness (14%), malaise (12%), and headache (11%). Among

subjects aged 11 through 55 years, the most frequently occurring adverse reactions (≥10%) among all subjects who received MENVEO were pain at the injection site (41%), headache (30%), myalgia (18%), malaise (16%), and nausea (10%).

The rates of solicited adverse reactions reported for subjects aged 2 through 5 years and 6 through 10 years who received a single dose of MENVEO or MENACTRA in a randomized, controlled, multicenter study (NCT00616421) conducted in the U.S. and Canada are shown in Table 4. Following a second dose of MENVEO administered to children aged 2 through 5 years, the most common solicited adverse reactions (≥10%) were pain at injection site (28%), erythema (22%), irritability (16%), induration (13%), and sleepiness (12%). The solicited adverse reactions from a separate randomized, controlled, multicenter study conducted in the U.S. in adolescents and adults (NCT00450437) are provided in Tables 5 and 6, respectively. In neither study were concomitant vaccines administered with the study vaccines.

Table 4. Rates of Solicited Adverse Reactions within 7 Days following a Single Vaccination in Children Aged 2 through 5 Years and 6 through 10 Years

	Participants Aged 2 through 5 Years					
	MENVEO n = 693 %			**MENACTRA n = 684 %**		
Adverse Reactions	**Any**	**Moderate**	**Severe**	**Any**	**Moderate**	**Severe**
Local Adverse Reactions						
Injection site pain[a]	33	6	1	35	8	0.4
Erythema[b]	27	5	1	25	3	0.3
Induration[b]	18	2	0.4	18	2	0.3
Systemic Adverse Reactions[e]						
Irritability[a]	21	6	1	22	7	1
Sleepiness[a]	16	3	1	18	5	1
Change in eating[a]	9	2	1	10	2	0.3
Diarrhea[a]	7	1	0.1	8	1	0
Headache[a]	5	1	0	6	1	0.3
Rash[c]	4	-	-	5	-	-
Arthralgia[a]	3	1	0.1	4	1	0
Vomiting[a]	3	1	0.1	3	1	0
Fever[d]	2	0.4	0	2	0.3	0

	Participants Aged 6 through 10 Years					
	MENVEO n = 582 %			MENACTRA n = 571 %		
Adverse Reactions	**Any**	**Moderate**	**Severe**	**Any**	**Moderate**	**Severe**
Local Adverse Reactions						
Injection site pain[a]	39	8	1	45	10	2
Erythema[b]	28	5	1	22	2	0.2
Induration[b]	17	2	0.3	13	2	0
Systemic Adverse Reactions[e]						
Headache[a]	18	3	1	13	2	1
Malaise[a]	14	3	1	11	3	1
Myalgia[a]	10	2	1	10	2	1
Nausea[a]	8	2	1	6	2	0.4
Arthralgia[a]	6	1	0	4	1	0.4
Chills[a]	5	1	0	5	1	0.4
Rash[c]	5	-	-	3	-	-
Fever[d]	2	1	0	2	0	0.4

Clinicaltrials.gov Identifier NCT00616421.

[a] Moderate: Some limitation in normal daily activity, Severe: Unable to perform normal daily activity.

[b] Moderate: ≥50-100 mm, Severe: >100 mm.

[c] Rash was assessed only as present or not present, without a grading for severity.

[d] Fever grading: Any: ≥38°C, Moderate: 39-39.9°C, Severe: ≥40°C. Parents reported the use of antipyretic medication to treat or prevent symptoms in 11% and 13% of subjects aged 2 through 5 years, 9% and 10% of subjects aged 6 through 10 years for MENVEO and MENACTRA, respectively.

[e] Different systemic reactions were solicited in different age groups.

Table 5. Rates of Solicited Adverse Reactions within 7 Days following Vaccination in Individuals Aged 11 through 18 Years

Adverse Reactions	MENVEO n = 1,631 %			MENACTRA n = 539 %		
	Any	Moderate	Severe	Any	Moderate	Severe
Local Adverse Reactions						
Injection site pain[a]	44	9	1	53	11	1
Erythema[b]	15	2	0.4	16	1	0
Induration[b]	12	2	0.2	11	1	0
Systemic Adverse Reactions						
Headache[a]	29	8	2	28	7	1
Myalgia[a]	19	4	1	18	5	0.4
Nausea[a]	12	3	1	9	2	1
Malaise[a]	11	3	1	12	5	1
Chills[a]	8	2	1	7	1	0.2
Arthralgia[a]	8	2	0.4	6	1	0
Rash[c]	3	-	-	3	-	-
Fever[d]	1	0.4	0	1	0	0

Clinicaltrials.gov Identifier NCT00450437.

[a] Moderate: Some limitation in normal daily activity, Severe: Unable to perform normal daily activity.

[b] Moderate: ≥50-100 mm, Severe: >100 mm.

[c] Rash was assessed only as present or not present, without a grading for severity.

[d] Fever grading: Any: ≥38°C, Moderate: 39-39.9°C, Severe: ≥40°C.

Table 6. Rates of Solicited Adverse Reactions within 7 Days following Vaccination in Individuals Aged 19 through 55 Years

Adverse Reactions	MENVEO n = 1,018 %			MENACTRA n = 336 %		
	Any	Moderate	Severe	Any	Moderate	Severe
Local Adverse Reactions						
Injection site pain[a]	38	7	0.3	41	6	0
Erythema[b]	16	2	1	12	1	0
Induration[b]	13	1	0.4	9	0.3	0
Systemic Adverse Reactions						
Headache[a]	25	7	2	25	7	1
Myalgia[a]	14	4	0.5	15	3	1
Malaise[a]	10	3	1	10	2	1
Nausea[a]	7	2	0.4	5	1	0.3
Arthralgia[a]	6	2	0.4	6	1	1
Chills[a]	4	1	0.1	4	1	0
Rash[c]	2	-	-	1	-	-
Fever[d]	1	0.3	0	1	0.3	0

Clinicaltrials.gov Identifier NCT00450437.

[a] Moderate: Some limitation in normal daily activity, Severe: Unable to perform normal daily activity.

[b] Moderate: ≥50-100 mm, Severe: >100 mm.

[c] Rash was assessed only as present or not present, without a grading for severity.

[d] Fever grading: Any: ≥38°C, Moderate: 39-39.9°C, Severe: ≥40°C.

In studies NCT03652610 and NCT03433482, there were no notable differences in frequency and severity of solicited adverse reactions within 7 days following vaccination in individuals who received the one-vial presentation compared to individuals who received the two-vial presentation.

Solicited Adverse Reactions in the Booster Vaccination Study (Adolescents and Adults)

A multicenter, open-label clinical trial (NCT02986854) was conducted in the U.S. in subjects aged 15 through 55 years *[see Clinical Studies (14.1)]*. The methodology for evaluating solicited adverse reactions, unsolicited adverse events, and SAEs after a booster dose of MENVEO was similar to the primary vaccination studies. The most common solicited local and systemic adverse reactions within 7 days of vaccination were pain at injection site (36%) and fatigue (38%), respectively.

Solicited Adverse Reactions following Concomitant Vaccine Administration

The safety of 4-dose series of MENVEO administered concomitantly with U.S.-licensed routine infant and toddler vaccines was evaluated in one pivotal trial (NCT00806195). The safety of a 2-dose series of MENVEO initiated at 7-9 months of age, with the second dose administered concomitantly with U.S.-licensed MMRV vaccine at 12 months of age, was evaluated in one pivotal trial (NCT00626327). Rates of solicited adverse reactions which occurred 7 days following vaccination are shown in Tables 2 and 3, respectively. There was no significant increase in the rates of solicited systemic or local reactions observed in recipients of routine childhood vaccines when concomitantly vaccinated with MENVEO. *[See Drug Interactions (7.1).]*

The safety of MENVEO administered concomitantly with Tdap and HPV was evaluated in a single-center study (NCT00518180) conducted in Costa Rica. Solicited local and systemic adverse reactions were reported as noted above. In this study, subjects aged 11 through 18 years received MENVEO concomitantly with Tdap and HPV (n = 540), or MENVEO followed 1 month later by Tdap and then 1 month later by HPV (n = 541), or Tdap followed 1 month later by MENVEO and then 1 month later by HPV (n = 539). Some solicited systemic adverse reactions were more frequently reported in the group that received MENVEO, Tdap, and HPV concomitantly, (headache 40%, malaise 25%, myalgia 27%, and arthralgia 17%) compared with the group that first received MENVEO alone (headache 36%, malaise 20%, myalgia 19%, and arthralgia 11%). Among subjects administered MENVEO alone (1 month prior to Tdap), 36% reported headache, 20% malaise, and 16% myalgia. Among subjects administered MENVEO 1 month after Tdap, 27% reported headache, 18% malaise, and 16% myalgia.

Serious Adverse Events in All Safety Studies

SAEs in subjects receiving a 4-dose series of MENVEO at 2, 4, 6, and 12 months were evaluated in 3 randomized, multicenter clinical studies (NCT00474526, NCT00806195, NCT01000311). In the 2 controlled studies (NCT00806195, NCT01000311), the proportions of infants randomized to receive the 4-dose series of MENVEO concomitantly with routine vaccinations and infants who received routine vaccinations alone that reported SAEs during different study periods were, respectively: a) 2.7% and 2.2% during the infant series, b) 2.5% and 2.5% between the infant series and the toddler dose, c) 0.3% and 0.3% in the 1 month following the toddler dose, and d) 1.6% and 2.2% during the 6-month follow-up period after the last dose. In the third study (NCT00474526), which was controlled up to the toddler dose, the proportions of infants randomized to dosing regimens that included receiving 4 doses of MENVEO concomitantly with routine vaccinations at 2, 4, 6, and 12 months and infants who received routine vaccinations alone that reported SAEs during different study periods were, respectively: a) 3.5% and 3.6% during the infant series, and b) 2.8% and 3.3% between the infant series and the toddler dose, and c) 0.5% and 0.7% in the 1 month following the toddler dose. In the same study, 1.9% of infants randomized to receive the 4-dose series of MENVEO concomitantly with routine vaccinations reported SAEs during the 6-month follow-up period after the toddler dose. The

most common SAEs reported in these 3 studies were wheezing, pneumonia, gastroenteritis, and convulsions, and most occurred at highest frequency after the infant series.

In a study of older infants (NCT00626327) randomized to receive the 2-dose series of MENVEO concomitantly with MMRV at 12 months of age, the rates of SAEs during the study, including the 6-month follow-up period after the last dose, were 3.6% and 3.8% for the groups receiving MENVEO with MMRV and MENVEO only, respectively. Infants receiving MMRV alone, who had a shorter period of study participation as they were enrolled at 12 months of age, had a lower rate of SAEs (1.5%). Among 1,597 study subjects included in the safety population, the most commonly reported SAEs in all study arms combined were dehydration (0.4%) and gastroenteritis (0.3%). Across the submitted studies of individuals aged 2 through 23 months within 28 days of vaccination, 2 deaths were reported in the groups receiving MENVEO (one case of sudden death and one case of sepsis), while no deaths were reported in the control group. None of the deaths was assessed as related to vaccination. Among subjects with symptom onset within 42 days of vaccination (Days 12, 25, 29), 3/12,049 (0.02%, 95% CI: [0.01%, 0.07%]) recipients of MENVEO and 0/2,877 (0%, 95% CI: [0%, 0.13%]) control recipients were diagnosed with Kawasaki Disease. One case of acute disseminated encephalomyelitis with symptom onset 29 days post Dose 4 was observed in a participant given MENVEO coadministered with routine U.S. childhood vaccines at 12 months of age (including measles, mumps, and rubella vaccine [MMR] and varicella vaccine).

The information regarding SAEs in subjects aged 2 through 10 years was derived from 3 randomized, controlled clinical trials (NCT00262028, NCT00329849, NCT00616421). Safety follow-up ranged from 6 through 12 months and included 2,883 subjects administered MENVEO. SAEs reported during the safety follow-up periods occurred in 21/2,883 (0.7%) subjects receiving MENVEO, in 7/1,255 (0.6%) MENACTRA subjects, and 2/861 (0.2%) MENOMUNE subjects. In the subjects receiving either 1 or 2 doses of MENVEO, there were 6 subjects with pneumonia, 3 subjects with appendicitis, and 2 subjects with dehydration; all other events were reported to occur in one subject. Among 1,255 subjects administered a single dose of MENACTRA and 861 subjects administered MENOMUNE, there were no events reported to occur in more than 1 subject. The SAEs occurring within the first 30 days after receipt of each vaccine were as follows: MENVEO (6/2,883 [0.2%]) — appendicitis, pneumonia, staphylococcal infection, dehydration, febrile convulsion, and tonic convulsion; MENACTRA (1/1255 [0.1%]) — inguinal hernia; MENOMUNE (2/861 [0.2%]) — abdominal pain, lobar pneumonia. In a supportive study (NCT00310817), 298 subjects received 1 or 2 doses of MENVEO and 22 (7%) had SAEs over a 13-month follow-up period including 13 subjects with varicella and 2 subjects with laryngitis. All other events were reported to occur in 1 subject. During the 30 days post vaccination in this study, 1 limb injury and 1 case of varicella were reported.

The information regarding SAEs in subjects aged 11 through 55 years was derived from 5 randomized, controlled clinical trials (NCT01018732, NCT00329901, NCT00450437,

NCT00474487, NCT00518180). SAEs reported within 6 months of vaccination occurred in 40/6,185 (0.6%) subjects receiving MENVEO, 13/1,757 (0.7%) MENACTRA subjects, and 5/209 (2.4%) MENOMUNE subjects. During the 6 months following immunization, SAEs reported by more than 1 subject were as follows: MENVEO - appendicitis (3 subjects), road traffic accident (3 subjects), and suicide attempt (5 subjects); MENACTRA - intervertebral disc protrusion (2 subjects); MENOMUNE - none. SAEs that occurred within 30 days of vaccination were reported by 7 of 6,185 (0.1%) subjects in the group receiving MENVEO, 4 of 1,757 (0.2%) subjects in the MENACTRA group, and by none of 209 subjects in the MENOMUNE group. The events that occurred during the first 30 days post immunization with MENVEO were: vitello-intestinal duct remnant, Cushing's syndrome, viral hepatitis, pelvic inflammatory disease, intentional multiple-drug overdose, simple partial seizure, and suicidal depression. The events that occurred during the first 30 days post immunization with MENACTRA were: herpes zoster, fall, intervertebral disc protrusion, and angioedema.

In the 2 clinical studies (NCT03652610, NCT03433482) which evaluated the safety of the one-vial presentation of MENVEO, SAEs were reported by 14 subjects (1.0%) who received the one-vial presentation of MENVEO and 14 subjects (1.1%) who received the two-vial presentation of MENVEO within the 6-month follow-up period after vaccination. No deaths were reported. None of the SAEs were related to the study vaccines.

6.2 Postmarketing Experience

In addition to reports in clinical trials, the following adverse reactions have been identified during postapproval use of MENVEO. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Blood and Lymphatic System Disorders

Local lymphadenopathy.

Ear and Labyrinth Disorders

Hearing impaired, ear pain, vertigo, vestibular disorder.

Eye Disorders

Eyelid ptosis.

General Disorders and Administration Site Conditions

Injection site pruritus; pain; erythema; inflammation; and swelling, including extensive swelling of the vaccinated limb; fatigue; malaise; pyrexia.

Immune System Disorders

Hypersensitivity reactions, including anaphylaxis.

Infections and Infestations

Vaccination site cellulitis.

Injury, Poisoning, and Procedural Complications

Fall, head injury.

Investigation

Alanine aminotransferase increased, body temperature increased.

Musculoskeletal and Connective Tissue Disorders

Arthralgia, bone pain.

Nervous System Disorders

Dizziness, syncope, tonic convulsion, headache, facial paresis, balance disorder.

Respiratory, Thoracic, and Mediastinal Disorders

Oropharyngeal pain.

Skin and Subcutaneous Tissue Disorders

Skin exfoliation.

Postmarketing Observational Safety Study

In a postmarketing observational safety study conducted in a U.S. health maintenance organization, data from electronic health records of 48,899 persons aged 11 through 21 years were used to evaluate pre-specified events of interest following vaccination with MENVEO. Using a self-controlled case series method, Bell's palsy showed a statistically significant increased risk in the period 1 to 84 days post vaccination compared with the control period, with an overall adjusted relative incidence of 2.9 (95% CI: 1.1-7.5). Among the 8 reported cases of Bell's palsy, 6 cases occurred in persons who received MENVEO concomitantly with one or more of the following vaccines: Tdap, HPV, and Influenza vaccine. All reported Bell's palsy cases resolved.

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

Do not mix MENVEO or any of its components with any other vaccine or diluent in the same syringe or vial.

In 2 clinical trials of infants initiating vaccination at 2 months of age (NCT00474526, NCT01000311), MENVEO was given concomitantly at 2, 4, and 6 months with routine infant vaccines: diphtheria toxoid; acellular pertussis; tetanus toxoid; inactivated polio types 1, 2, and 3; hepatitis B; *Haemophilus influenzae* type b antigens; pentavalent rotavirus; and 7-valent pneumococcal conjugate vaccine. For Dose 4 given at 12 months of age, MENVEO was given

concomitantly with the following vaccines: 7-valent pneumococcal conjugate, MMRV, or measles, mumps, and rubella vaccine and varicella vaccine (MMR+V), and inactivated hepatitis A. In a clinical trial of older infants (aged 7 months and older) and toddlers (NCT00626327), MENVEO was administered concomitantly with MMRV or MMR+V vaccine(s) at 12 months of age. No immune interference was observed for the concomitantly administered vaccines, including most pneumococcal vaccine serotypes (post Dose 3); no immune interference was observed post Dose 4 for any pneumococcal vaccine serotypes (NCT00474526, NCT01000311). *[See Clinical Studies (14.1).]*

For children aged 2 through 10 years, no data are available to evaluate safety and immunogenicity of other childhood vaccines when administered concomitantly with MENVEO.

In a clinical trial in adolescents (NCT00518180), MENVEO was given concomitantly with the following: Tdap and HPV; no interference was observed in meningococcal immune responses when compared with MENVEO given alone. Lower geometric mean antibody concentrations (GMCs) for antibodies to the pertussis antigens filamentous hemagglutinin (FHA) and pertactin were observed when MENVEO was administered concomitantly with Tdap and HPV as compared with Tdap alone. *[See Clinical Studies (14.1).]*

7.2 Immunosuppressive Treatments

Immunosuppressive therapies, such as irradiation, antimetabolite medications, alkylating agents, cytotoxic drugs, and corticosteroids (when used in greater than physiologic doses) may reduce the immune response to MENVEO. *[See Warnings and Precautions (5.3).]* The immunogenicity of MENVEO has not been evaluated in persons receiving such therapies.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no adequate and well-controlled studies of MENVEO in pregnant women in the U.S. There was a pregnancy exposure registry conducted from 2014-2017 that included 82 subjects. Available data do not suggest an increased risk of major birth defects and miscarriage in women who received MENVEO within 28 days prior to conception or during pregnancy *(see Data)*.

A developmental toxicity study was performed in female rabbits administered 0.5 mL (at each occasion) of MENVEO prior to mating and during gestation. A single human dose is 0.5 mL. This study revealed no adverse effects on fetal or pre-weaning development *(see Data)*.

Data

Human Data: A pregnancy exposure registry (2014 to 2017) included 82 pregnancies with known outcomes with exposure within 28 days prior to conception or during pregnancy. Miscarriage was reported for 12.2% of pregnancies with exposure to MENVEO within 28 days prior to conception or during pregnancy (10/82). Major birth defects were reported for 3.6% of live born infants whose mothers were exposed within 28 days prior to conception or during pregnancy (2/55). The rates of miscarriage and major birth defects were consistent with estimated background rates.

Animal Data: In a developmental toxicity study, female rabbits were administered MENVEO by intramuscular injection on Days 29, 15, and 1 prior to mating and on Gestation Days 7 and 20. The total dose was 0.5 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to Postnatal Day 29 were observed. There were no vaccine-related fetal malformations or variations observed.

8.2 Lactation

Risk Summary

It is not known whether the vaccine components of MENVEO are excreted in human milk. Data are not available to assess the effects of MENVEO in the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for MENVEO and any potential adverse effects on the breastfed child from MENVEO or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of MENVEO in children aged younger than 2 months have not been established.

Safety and effectiveness of the one-vial presentation of MENVEO in children aged younger than 10 years have not been established. *[See Dosage and Administration (2).]*

For children 2 months through 9 years of age, only the two-vial presentation is approved for use. *[See Dosage and Administration (2).]*

8.5 Geriatric Use

Safety and effectiveness of MENVEO in adults aged 65 years and older have not been established.

11 DESCRIPTION

MENVEO [Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM_{197} Conjugate Vaccine] is a sterile liquid vaccine administered by intramuscular injection that contains *N. meningitidis* serogroup A, C, Y, and W-135 oligosaccharides conjugated individually to *Corynebacterium diphtheriae* CRM_{197} protein. The polysaccharides are produced by bacterial fermentation of *N. meningitidis* (serogroups A, C, Y, or W-135). *N. meningitidis* strains A, C, Y, and W-135 are each cultured and grown on Franz Complete medium and treated with formaldehyde. MenA, MenW-135, and MenY polysaccharides are purified by several extraction and precipitation steps. MenC polysaccharide is purified by a combination of chromatography and precipitation steps.

The protein carrier (CRM_{197}) is produced by bacterial fermentation and is purified by a series of chromatography and ultrafiltration steps. *C. diphtheriae* is cultured and grown on CY medium containing yeast extracts and amino acids.

The oligosaccharides are prepared for conjugation from purified polysaccharides by hydrolysis, sizing, and reductive amination. After activation, each oligosaccharide is covalently linked to the CRM_{197} protein. The resulting glycoconjugates are purified to yield the 4 drug substances, which compose the final vaccine. The vaccine contains no preservative or adjuvant. Each dose of vaccine contains 10 mcg MenA oligosaccharide; 5 mcg of each of MenC, MenY, and MenW-135 oligosaccharides; and 25.4 to 65.5 mcg CRM_{197} protein. Residual formaldehyde per dose is estimated to be not more than 0.30 mcg.

The vials in which the vaccine components are contained are composed of Type I glass, USP.

The container closures (synthetic rubber stoppers) are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Neisseria meningitidis is a gram-negative diplococcus that causes life-threatening invasive disease such as meningitis and sepsis. Globally, 5 serogroups, A, B, C, Y, and W-135 cause almost all invasive meningococcal infections. The presence of serum bactericidal antibodies protects against invasive meningococcal disease.[1] Vaccination with MENVEO leads to the production of bactericidal antibodies directed against the capsular polysaccharides of serogroups A, C, Y, and W-135.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

MENVEO has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Vaccination of female rabbits with MENVEO had no effect on fertility. *[See Use in Specific Populations (8.1).]*

14 CLINICAL STUDIES

For all age groups, effectiveness has been inferred from the measurement of serogroup-specific anticapsular antibodies with bactericidal activity using pooled human serum that lacked bactericidal activity as the source of exogenous complement (hSBA).

14.1 Primary Vaccination Studies

In the absence of a licensed comparator vaccine for use in infants, the pre-specified endpoint for effectiveness of MENVEO in U.S. infants receiving a 4-dose series at 2, 4, 6, and 12 months of age was the proportion of subjects achieving an hSBA ≥1:8, with the lower limit of the 2-sided 95% CI for the point estimate being ≥80% of vaccinees for serogroup A, and ≥85% of vaccinees for serogroups C, W-135, and Y 1 month following the final dose.

The effectiveness of MENVEO in subjects aged 2 through 55 years was assessed by comparing the hSBA responses to immunization with MENVEO to those following immunization with the licensed meningococcal quadrivalent conjugate vaccine MENACTRA.

The primary effectiveness endpoint was hSBA seroresponse to each serogroup 28 days after vaccination. Seroresponse was defined as: a) post-vaccination hSBA ≥1:8 for subjects with a baseline hSBA <1:4; or, b) at least 4-fold higher than baseline titers for subjects with a pre-vaccination hSBA ≥1:4. Secondary endpoints included the proportion of subjects with post-vaccination hSBA ≥1:8 and the hSBA Geometric Mean Titer (GMT) for each serogroup. In a separate group of children aged 2 through 5 years randomized to receive 2 doses of MENVEO administered 2 months apart, seroresponse rate, proportion with post-vaccination hSBA ≥1:8, and GMT were determined for each serogroup.

Immunogenicity in Infants/Toddlers Aged 2 Months through 12 Months

The effectiveness of MENVEO in infants was assessed in a randomized, controlled, multicenter study (NCT01000311). Among the subjects receiving MENVEO who were included in the per-protocol analysis, the mean age at enrollment was 65 days, 51% were male, 67% were Caucasian, 6% were African American, 15% were Hispanic, 2% were Asian, and 9% were noted as other racial/ethnic groups. The pre-defined criteria for immunogenicity were met for all 4 serogroups A, C, W-135, and Y at 1 month following completion of a 4-dose series at 2, 4, 6, and 12 months of age (Table 7).

The percentage of subjects with hSBA ≥1:8 at 7 months was 94% to 98% for serogroups C, W-135, and Y and 76% for serogroup A.

Table 7. Bactericidal Antibody Responses following Administration of MENVEO with Routine Infant/Toddler Vaccines at 2, 4, 6, and 12 Months of Age

Serogroup		Post 3rd Dose	Post 4th Dose
A		**n = 202**	**n = 168**
	% ≥1:8 95% CI	76 (69, 81)	89 (83[a], 93)
	GMT 95% CI	21 (17, 26)	54 (44, 67)
C		**n = 199**	**n = 156**
	% ≥1:8 95% CI	94 (90, 97)	95 (90[a], 98)
	GMT 95% CI	74 (62, 87)	135 (107, 171)
W-135		**n = 194**	**n = 153**
	% ≥1:8 95% CI	98 (95, 99)	97 (93[a], 99)
	GMT 95% CI	79 (67, 92)	215 (167, 227)
Y		**n = 188**	**n = 153**
	% ≥1:8 95% CI	94 (89, 97)	96 (92[a], 99)
	GMT 95% CI	51 (43, 61)	185 (148, 233)

Clinicaltrials.gov Identifier NCT01000311.

% ≥1:8 = Proportions of subjects with hSBA ≥1:8 against a given serogroup; CI = Confidence interval; GMT = Geometric mean antibody titer; n = Number of infants eligible for inclusion in the Per-Protocol Immunogenicity population for whom serological results were available for the post-Dose 3 and post-Dose 4 evaluations.

Serum Bactericidal Assay with exogenous human complement source (hSBA).

[a] Pre-specified criteria for adequacy of immune response were met (lower limit of the 95% CI >80% for serogroup A and >85% for serogroups C, W, and Y).

The effectiveness of 2 doses of MENVEO given at 7-9 months and 12 months of age was assessed in a randomized, multicenter, controlled clinical trial (NCT00626327) conducted in the U.S. This study also investigated the concomitant administration of MENVEO and MMRV. The per-protocol population for assessing the response to 2 doses of MENVEO consisted of 386 subjects. Among subjects who completed the per-protocol analysis, their mean age at enrollment was 8.5 months (SD: 0.8 months), 50% were male; 61% were Caucasian, 15% were Hispanic, 10% were African American, 4% were Asian, and 10% were noted as other racial/ethnic groups.

Among the per-protocol population, after MENVEO administered at 7-9 and at 12 months, the proportions of subjects with hSBA ≥1:8 for serogroups A, C, W-135, and Y were respectively: 88% (84-91), 100% (98-100), 98% (96-100), 96% (93-99).

Immunogenicity in Children Aged 2 Years through 10 Years

Effectiveness in subjects aged 2 through 10 years was evaluated in a randomized, multicenter, active-controlled clinical study (NCT00616421) comparing hSBA responses following 1 dose of MENVEO or MENACTRA. The study was conducted in the U.S. and Canada and was stratified by age (2 through 5 years and 6 through 10 years). The per-protocol population evaluated after a single dose of vaccine consisted of 1,170 subjects who received MENVEO and 1,161 who received MENACTRA (Table 8) and included serological results for 89% to 95% of subjects, depending on serogroup and age group. Demographics for the 616 and 619 subjects aged 2 through 5 years for MENVEO and MENACTRA were as follows: mean age 3.6 years (SD: 1.1) vs. 3.6 years (SD: 1.1), 51% vs. 52% male, 62% vs. 62% Caucasian, 14% vs. 13% Hispanic, 12% vs. 13% African American, 6% vs. 4% Asian, and 7% vs. 8% other racial/ethnic groups, respectively. Demographics were for 554 and 542 per-protocol subjects aged 6 through 10 years for MENVEO and MENACTRA were as follows: mean age 7.9 years (SD: 1.4) vs. 8.1 years (SD: 1.4), 52% vs. 56% male, 66% vs. 66% Caucasian, 14% vs. 14% African American, 7% vs. 7% Hispanic, 5% vs. 6% Asian, and 8% vs. 8% other racial/ethnic groups, respectively. In a separate group of children aged 2 through 5 years randomized to receive 2 doses of MENVEO administered 2 months apart, the per-protocol population evaluated after 2 doses of MENVEO consisted of 297 subjects and included serologic results for 96% to 99% of subjects, depending on serogroup.

In study participants aged 2 through 5 years and 6 through 10 years, non-inferiority of MENVEO to MENACTRA for the proportion of subjects with a seroresponse was demonstrated for serogroups C, W-135, and Y, but not for serogroup A (Table 8).

Table 8. Comparison of Bactericidal Antibody Responses[a] to MENVEO and MENACTRA 28 Days after Vaccination of Subjects Aged 2 through 5 Years and 6 through 10 Years

	2-5 Years			6-10 Years		
Endpoint by Serogroup	**MENVEO (95% CI)**	**MENACTRA (95% CI)**	**Percent Difference (MENVEO – MENACTRA) or GMT Ratio (MENVEO/ MENACTRA) (95% CI)**	**MENVEO (95% CI)**	**MENACTRA (95% CI)**	**Percent Difference (MENVEO – MENACTRA) or GMT Ratio (MENVEO/ MENACTRA) (95% CI)**
A	**n = 606**	**n = 611**		**n = 551**	**n = 541**	
% Seroresponse[b]	72 (68, 75)	77 (73, 80)	-5 (-10, -0)[c]	77 (73, 80)	83 (79, 86)	-6 (-11, -1)[c]
% ≥1:8	72 (68, 75)	78 (74, 81)	-6 (-11, -1)	77 (74, 81)	83 (80, 86)	-6 (-11, -1)
GMT	26 (22, 30)	25 (21, 29)	1.04 (0.86, 1.27)	35 (29, 42)	35 (29, 41)	1.01 (0.83, 1.24)
C	**n = 607**	**n = 615**		**n = 554**	**n = 539**	
% Seroresponse[b]	60 (56, 64)	56 (52, 60)	4 (-2, 9)[d]	63 (59, 67)	57 (53, 62)	6 (0, 11)[d]
% ≥1:8	68 (64, 72)	64 (60, 68)	4 (-1, 10)	77 (73, 80)	74 (70, 77)	3 (-2, 8)
GMT	18 (15, 20)	13 (11, 15)	1.33 (1.11, 1.6)	36 (29, 45)	27 (21, 33)	1.36 (1.06, 1.73)
W-135	**n = 594**	**n = 605**		**n = 542**	**n = 533**	
% Seroresponse[b]	72 (68, 75)	58 (54, 62)	14 (9, 19)[d]	57 (53, 61)	44 (40, 49)	13 (7, 18)[d]
% ≥1:8	90 (87, 92)	75 (71, 78)	15 (11, 19)	91 (88, 93)	84 (81, 87)	7 (3, 11)
GMT	43 (38, 50)	21 (19, 25)	2.02 (1.71, 2.39)	61 (52, 72)	35 (30, 42)	1.72 (1.44, 2.06)
Y	**n = 593**	**n = 600**		**n = 545**	**n = 539**	
% Seroresponse[b]	66 (62, 70)	45 (41, 49)	21 (16, 27)[d]	58 (54, 62)	39 (35, 44)	19 (13, 24)[d]
% ≥1:8	76 (72, 79)	57 (53, 61)	19 (14, 24)	79 (76, 83)	63 (59, 67)	16 (11, 21)
GMT	24 (20, 28)	10 (8.68, 12)	2.36 (1.95, 2.85)	34 (28, 41)	14 (12, 17)	2.41 (1.95, 2.97)

Clinicaltrials.gov Identifier NCT00616421.
CI = Confidence interval; GMT = Geometric mean antibody titer.
[a] Serum Bactericidal Assay with exogenous human complement source (hSBA).
[b] Seroresponse was defined as: Subjects with a pre-vaccination hSBA <1:4, a post-vaccination titer of >1:8 and among subjects with a pre-vaccination hSBA ≥1:4, a post-vaccination titer at least 4-fold higher than baseline.
[c] Non-inferiority criterion not met (the lower limit of the 2-sided 95% CI ≤-10% for vaccine group differences).
[d] Non-inferiority criterion met (the lower limit of the 2-sided 95% CI >-10% for vaccine group differences [MENVEO minus MENACTRA]).

In the 297 per-protocol subjects aged 2 through 5 years observed at 1 month after the second dose of MENVEO, the proportions of subjects with seroresponse (95% CI) were: 91% (87-94), 98% (95-99), 89% (85-92), and 95% (91-97) for serogroups A, C, W-135, and Y, respectively. The proportion of subjects with hSBA ≥1:8 (95% CI) were 91% (88-94), 99% (97-100), 99% (98-100), and 98% (95-99) for serogroups A, C, W-135, and Y, respectively. The hSBA GMTs (95% CI) for this group were 64 (51-81), 144 (118-177), 132 (111-157), and 102 (82-126) for serogroups A, C, W-135, and Y, respectively.

Immunogenicity in Adolescents Aged 11 Years through 18 Years

Effectiveness in subjects aged 11 through 55 years was evaluated in a randomized, multicenter, active-controlled clinical study (NCT00450437) comparing the hSBA responses following 1 dose of MENVEO or MENACTRA. The study was conducted in the U.S. and stratified by age (11 through 18 years and 19 through 55 years). This study enrolled 3,539 participants, who were randomized to receive a dose of MENVEO (n = 2,663) or MENACTRA (n = 876). Among subjects who completed the per-protocol evaluation for immunogenicity (n = 3,393, MENVEO = 2,549, MENACTRA = 844), demographics for subjects receiving MENVEO and MENACTRA, respectively, were as follows: mean age 23.9 (SD: 13.6) vs. 23.7 (SD: 13.7), 42% vs. 42% male, 79% vs. 78% Caucasian, 8% vs. 9% African American, 7% vs. 7% Hispanic, 3% vs. 3% Asian, 2% vs. 3% other racial/ethnic groups. Immunogenicity for each serogroup was assessed in a subset of study participants (Tables 9 and 10).

In study participants aged 11 through 18 years, non-inferiority of MENVEO to MENACTRA was demonstrated for all 4 serogroups for the proportion of subjects with a seroresponse (Table 9).

Table 9. Comparison of Bactericidal Antibody Responses[a] to MENVEO and MENACTRA 28 Days after Vaccination of Subjects Aged 11 through 18 Years

Endpoint by Serogroup	Bactericidal Antibody Response[a]		Comparison of MENVEO and MENACTRA	
	MENVEO (95% CI)	MENACTRA (95% CI)	MENVEO/ MENACTRA (95% CI)	MENVEO minus MENACTRA (95% CI)
A	**n = 1,075**	**n = 359**		
% Seroresponse[b]	75 (72, 77)	66 (61, 71)		8 (3, 14)[c]
% ≥1:8	75 (73, 78)	67 (62, 72)	-	8 (3, 14)
GMT	29 (24, 35)	18 (14, 23)	1.63 (1.31, 2.02)	-
C	**n = 1,396**	**n = 460**		
% Seroresponse[b]	76 (73, 78)	73 (69, 77)		2 (-2, 7)[c]
% ≥1:8	85 (83, 87)	85 (81, 88)	-	0 (-4, 4)
GMT	50 (39, 65)	41 (30, 55)	1.22 (0.97, 1.55)	-
W-135	**n = 1,024**	**n = 288**		
% Seroresponse[b]	75 (72, 77)	63 (57, 68)		12 (6, 18)[c]
% ≥1:8	96 (95, 97)	88 (84, 92)	-	8 (4, 12)
GMT	87 (74, 102)	44 (35, 54)	2.00 (1.66, 2.42)	-
Y	**n = 1,036**	**n = 294**		
% Seroresponse[b]	68 (65, 71)	41 (35, 47)		27 (20, 33)[c]
% ≥1:8	88 (85, 90)	69 (63, 74)	-	19 (14, 25)
GMT	51 (42, 61)	18 (14, 23)	2.82 (2.26, 3.52)	-

Clinicaltrials.gov Identifier NCT00450437.

CI = Confidence interval; GMT = Geometric mean antibody titer.

[a] Serum Bactericidal Assay with exogenous human complement source (hSBA).

[b] Seroresponse was defined as: a) post-vaccination hSBA ≥1:8 for subjects with a pre-vaccination hSBA <1:4; or, b) at least 4-fold higher than baseline titers for subjects with a pre-vaccination hSBA ≥1:4.

[c] Non-inferiority criterion for the primary endpoint met (the lower limit of the 2-sided 95% CI >-10% for vaccine group differences [MENVEO minus MENACTRA]).

Immunogenicity in Adults Aged 19 Years through 55 Years

The study in subjects aged 11 through 55 years was a randomized, multicenter, active-controlled clinical trial (NCT00450437) conducted in the U.S. and stratified by age (11 through 18 years and 19 through 55 years) as described above.

In study participants aged 19 through 55 years, non-inferiority of MENVEO to MENACTRA was demonstrated for all 4 serogroups for the proportion of subjects with a seroresponse (Table 10).

Table 10. Comparison of Bactericidal Antibody Responses to MENVEO and MENACTRA 28 Days after Vaccination of Subjects Aged 19 through 55 Years

	Bactericidal Antibody Response[a]		**Comparison of MENVEO and MENACTRA**	
Endpoint by Serogroup	**MENVEO (95% CI)**	**MENACTRA (95% CI)**	**MENVEO/ MENACTRA (95% CI)**	**MENVEO minus MENACTRA (95% CI)**
A	**n = 963**	**n = 321**		
% Seroresponse[b]	67 (64, 70)	68 (63, 73)		-1 (-7, 5)[c]
% ≥1:8	69 (66, 72)	71 (65, 76)	-	-2 (-7, 4)
GMT	31 (27, 36)	30 (24, 37)	1.06 (0.82, 1.37)	-
C	**n = 902**	**n = 300**		
% Seroresponse[b]	68 (64, 71)	60 (54, 65)		8 (2, 14)[c]
% ≥1:8	80 (77, 83)	74 (69, 79)	-	6 (1, 12)
GMT	50 (43, 59)	34 (26, 43)	1.50 (1.14, 1.97)	-

W-135	**n = 484**	**n = 292**		
% Seroresponse[b]	50 (46, 55)	41 (35, 47)		9 (2, 17)[c]
% ≥1:8	94 (91, 96)	90 (86, 93)	-	4 (0, 9)
GMT	111 (93, 132)	69 (55, 85)	1.61 (1.24, 2.1)	-
Y	**n = 503**	**n = 306**		
% Seroresponse[b]	56 (51, 60)	40 (34, 46)		16 (9, 23)[c]
% ≥1:8	79 (76, 83)	70 (65, 75)	-	9 (3, 15)
GMT	44 (37, 52)	21 (17, 26)	2.10 (1.60, 2.75)	-

Clinicaltrials.gov Identifier NCT00450437.

CI = Confidence interval; GMT = Geometric mean antibody titer.

[a] Serum Bactericidal Assay with exogenous human complement source (hSBA).

[b] Seroresponse was defined as: a) post-vaccination hSBA >1:8 for subjects with a pre-vaccination hSBA <1:4; or, b) at least 4-fold higher than baseline titers for subjects with a pre-vaccination hSBA ≥1:4.

[c] Non-inferiority criterion for the primary endpoint met (the lower limit of the 2-sided 95% CI >-10% for vaccine group differences [MENVEO minus MENACTRA]).

Immunogenicity of MENVEO One-Vial Presentation in Individuals Aged 10 Years through 40 Years

Immunogenicity of MENVEO one-vial presentation was evaluated in an observer-blind randomized, multicenter, controlled clinical trial in individuals aged 10 to 40 years (NCT03433482). The study compared the immune response of the MENVEO one-vial presentation to the MENVEO two-vial presentation.

The primary analysis demonstrated non-inferiority of MenA serogroup hSBA GMTs at 28 days post-vaccination for the MENVEO one-vial presentation group compared to the MENVEO two-vial presentation group (lower limit of the two-sided 95% CI for the ratio of hSBA GMTs against serogroup A between the MENVEO one-vial presentation group and the MENVEO two-vial presentation group was greater than 0.5). Secondary analyses showed comparable immune responses against *N. meningitidis* serogroups C, W-135 and Y as measured by hSBA GMTs. Additional secondary analyses demonstrated comparable percentages of subjects with hSBA titers ≥8, and percentages of subjects with a ≥4-fold rise in titers compared to baseline for serogroups A, C, W-135 and Y.

14.2 Booster Vaccination Study

Immunogenicity in Adolescents and Adults Aged 15 Years through 55 Years

For a description of study design and number of participants, see section 6.1 Booster Vaccination Study. The co-primary immunogenicity endpoints were hSBA seroresponse to each serogroup 29 days a) following a booster vaccination with MENVEO given to subjects who received a prior dose of MENVEO, and b) following a booster vaccination with MENVEO given to subjects who received a prior dose of MENACTRA. Seroresponse was defined as: a) post-vaccination hSBA ≥1:16 for subjects with a baseline hSBA <1:4 or b) at least 4-fold higher than baseline titers for subjects with a pre-vaccination hSBA ≥1:4. Secondary endpoints included the proportions of subjects with post-vaccination hSBA ≥1:8, the hSBA GMTs for each serogroup, and antibody titers against each serogroup 4 to 6 years after a prior dose (as measured by percentages of subjects with hSBA titers ≥1:8 and hSBA GMTs prior to booster vaccination).

Seroresponse rates at Day 29 following a booster vaccination with MENVEO were 97% for serogroup A, 95% for serogroup C, 96% for serogroup W-135, and 97% for serogroup Y, in subjects who had received a prior dose of MENVEO (n = 290). At Day 6, following a booster vaccination, seroresponse rates were 39%, 51%, 50%, and 49% for serogroups A, C, W-135, and Y, respectively, in subjects who had received a prior dose of MENVEO.

The hSBA GMTs were 13, 92, 112, and 63 for serogroups A, C, W-135, and Y at Day 6, and 210, 1160, 1395, and 1067, respectively, for the 4 serogroups at Day 29 following a booster dose of MENVEO.

Overall, similar seroresponse rates and GMTs were observed for those subjects who received a booster vaccination with MENVEO following a prior dose of MENACTRA (n = 282).

Prior to booster vaccination, the percentage of subjects with hSBA titers >1:8 for serogroups A, C, W-135, and Y were 12%, 62%, 76%, and 54% for those who received a prior dose of MENVEO 4 to 6 years earlier, and 15%, 54%, 77%, and 47% for those who received a prior dose of MENACTRA 4 to 6 years earlier. The hSBA GMTs for serogroups A, C, W-135, and Y prior to booster vaccination were 3, 16, 23, and 9 following a prior vaccination with MENVEO and 3, 11, 23, and 8 following a prior vaccination with MENACTRA.

14.3 Immunogenicity of Concomitantly Administered Vaccines

In U.S. infants (NCT00474526, NCT01000311) who received MENVEO concomitantly with DTaP-IPV-Hib and PCV7 at 2, 4, and 6 months of age and HBV administered according to ACIP recommendations, there was no evidence for reduced antibody response to pertussis antigens (GMC to pertussis toxin, filamentous hemagglutinin, fimbriae, and pertactin), diphtheria toxoid (antibody levels ≥0.1 IU/mL), tetanus toxoid (antibody levels ≥0.1 IU/mL), poliovirus types 1, 2, and 3 (neutralizing antibody levels ≥1:8 to each virus), *Haemophilus influenzae* type b (anti-PRP antibody ≥0.15 mcg/mL), hepatitis B (anti-hepatitis B surface antigen ≥10 mIU/mL), or most serotypes of PCV7 (antibody levels ≥0.35 mcg/mL) relative to the response in infants

administered DTaP-IPV-Hib, PCV7, and HBV. The immune responses to DTaP-IPV-Hib, PCV7, and HBV were evaluated 1 month following Dose 3. No interference was observed for pertussis based on GMC ratios, or for the other concomitantly administered vaccines, with the exception of pneumococcal serotype 6B and 23F, for which interference was suggested post Dose 3. No interference was observed post Dose 4 for these serotypes.

There was no evidence for interference in the immune response to MMR and varicella vaccines (among initially seronegative children) in terms of percentages of children with anti-measles antibodies ≥255 mIU/mL, anti-mumps ≥10 ELISA antibody units, anti-rubella ≥10 IU/mL, and anti-varicella ≥5 gp ELISA units/mL, administered at 12 months of age (NCT00626327) concomitantly with MENVEO relative to these vaccines administered alone. The immune responses to MMR and varicella vaccines were evaluated 6 weeks post vaccination.

For children aged 2 through 10 years, no data are available for evaluating safety and immunogenicity of other childhood vaccines when administered concomitantly with MENVEO.

For individuals aged 11 through 18 years, the effect of concomitant administration of MENVEO with Tdap and HPV was evaluated in a study (NCT00518180) conducted in Costa Rica (see also section 6.1 for the safety results from this trial). Subjects were randomized to receive one of the following regimens at the start of the trial: MENVEO plus Tdap plus HPV (n = 540); MENVEO alone (n = 541); Tdap alone (n = 539). Subjects were healthy adolescents aged 11 through 18 years (mean age between groups was 13.8 to 13.9 years). For antigens of MENVEO, the proportion (95% CI) of subjects achieving an hSBA seroresponse among those who received MENVEO plus Tdap plus HPV vs. MENVEO alone, respectively, were: serogroup A 80% (76, 84) vs. 82% (78, 85); serogroup C 83% (80, 87) vs. 84% (80, 87); serogroup W-135 77% (73, 80) vs. 81% (77, 84); serogroup Y 83% (79, 86) vs. 82% (79, 86). Among subjects who received Tdap plus MENVEO plus HPV, compared with Tdap alone, the proportions (95% CI) of subjects who achieved an anti-tetanus or anti-diphtheria toxoids levels ≥1.0 IU/mL in the 2 groups, respectively, were 100% (99, 100) vs. 98% (96, 99) and 100% (99, 100) vs. 100% (99, 100). For pertussis antigens, among subjects who received Tdap plus MENVEO plus HPV, compared with Tdap alone, the responses respectively for anti-pertussis toxin GMCs (95% CI) were 51 (47, 55) vs. 63 (58, 69) ELISA Units (EU)/mL, for anti-filamentous hemagglutinin were 342 (310, 376) vs. 511 (464, 563) EU/mL, and for anti-pertactin were 819 (727, 923) vs. 1,197 (1,061, 1,350) EU/mL. Because there are no established serological correlates of protection for pertussis, the clinical implications of the lower pertussis antigen responses are unknown.

15 REFERENCES

1. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med*. (1969);129:1307-1326.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 MENVEO Two-Vial Presentation

MENVEO two-vial presentation is supplied in cartons containing:

- 5 Vials containing MenCYW-135 Liquid Conjugate Component (Vial 1; gray cap)
- 5 Vials containing MenA Lyophilized Conjugate Component (Vial 2; orange cap)

One vial of MenCYW-135 liquid conjugate component (Vial 1) and one vial of MenA lyophilized conjugate component (Vial 2) must be combined before use to form a single dose (0.5 mL) of MENVEO (packaged without syringes or needles). Each carton contains 5 doses of MENVEO.

The container closures (synthetic rubber stoppers) are not made with natural rubber latex.

Table 11. MENVEO: Two-Vial Presentation

Presentation	Carton NDC Number	MenCYW-135 Liquid Conjugate Component (Vial 1; gray cap) NDC Number	MenA Lyophilized Conjugate Component (Vial 2; orange cap) NDC Number
Carton of 10 vials (5 doses)	NDC 58160-955-09	5 Vials NDC 58160-959-01	5 Vials NDC 58160-958-01

Storage before Reconstitution

Do not freeze. Frozen/previously frozen product should be discarded.

Store refrigerated, away from the freezer compartment, at 36°F to 46°F (2°C to 8°C).

Protect from light. Vaccine must be maintained at 36°F to 46°F (2°C to 8°C) during transport.

Do not use after the expiration date.

Storage after Reconstitution

The reconstituted vaccine should be used immediately but may be held at 36°F to 77°F (2°C to 25°C) for up to 8 hours. Do not freeze. Discard reconstituted vaccine if it has been frozen or not used within 8 hours.

16.2 MENVEO One-Vial Presentation

MENVEO one-vial presentation is supplied in cartons containing:

- 10 Vials containing MENVEO (pink cap)

Each carton contains 10 single dose vials of MENVEO. Each dose is 0.5 mL.

The container closures (synthetic rubber stoppers) are not made with natural rubber latex.

Table 12. MENVEO: One-Vial Presentation

Presentation	Carton NDC Number	MENVEO (pink cap) NDC Number
Carton of 10 vials (10 doses)	NDC 58160-827-30	10 Vials NDC 58160-827-03

Do not freeze. Frozen/previously frozen product should be discarded.

Store refrigerated, away from the freezer compartment, at 36°F to 46°F (2°C to 8°C).

Protect from light. Vaccine must be maintained at 36°F to 46°F (2°C to 8°C) during transport.

Do not use after the expiration date.

17 PATIENT COUNSELING INFORMATION

Give the recipient, parent, or guardian the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Provide the following information to the vaccine recipient, parent, or guardian:

- Potential benefits and risks of immunization with MENVEO.
- The importance of completing the immunization series.
- Potential for adverse reactions that have been temporally associated with administration of MENVEO or other vaccines containing similar components.
- Reporting any adverse reactions to their healthcare provider.

Manufactured by:
GSK Vaccines, Srl
Bellaria-Rosia 53018, Sovicille (SI), Italy
US License No. 1617

Distributed by:
GlaxoSmithKline
Durham, NC 27701

MNV:7PI

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use BEXSERO safely and effectively. See full prescribing information for BEXSERO.

BEXSERO (Meningococcal Group B Vaccine) suspension, for intramuscular injection

Initial U.S. Approval: 2015

---------- INDICATIONS AND USAGE----------

BEXSERO is a vaccine indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroup B. BEXSERO is approved for use in individuals aged 10 through 25 years. (1)

Approval of BEXSERO is based on demonstration of immune response, as measured by serum bactericidal activity against three serogroup B strains representative of prevalent strains in the United States. The effectiveness of BEXSERO against diverse serogroup B strains has not been confirmed. (1)

----------DOSAGE AND ADMINISTRATION----------

For intramuscular use only. (2)

Administer 2 doses (0.5-mL each) of BEXSERO at least 1 month apart. (2.1)

---------- DOSAGE FORMS AND STRENGTHS----------

Suspension for intramuscular injection in 0.5-mL single-dose prefilled syringes. (3)

---------- CONTRAINDICATIONS ----------

Hypersensitivity, including severe allergic reaction, to any component of the vaccine, or after a previous dose of BEXSERO. (4)

---------- WARNINGS AND PRECAUTIONS ----------

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.3)

---------- ADVERSE REACTIONS ----------

The most common solicited adverse reactions observed in clinical trials were pain at the injection site (≥83%), myalgia (≥48%), erythema (≥45%), fatigue (≥35%), headache (≥33%), induration (≥28%), nausea (≥18%), and arthralgia (≥13%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 01/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

BEXSERO is a vaccine indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroup B. BEXSERO is approved for use in individuals aged 10 through 25 years.

Approval of BEXSERO is based on demonstration of immune response, as measured by serum bactericidal activity against three serogroup B strains representative of prevalent strains in the United States. The effectiveness of BEXSERO against diverse serogroup B strains has not been confirmed.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only.

2.1 Dose and Schedule

Administer 2 doses (0.5-mL each) of BEXSERO at least 1 month apart.

2.2 Administration

Shake the syringe immediately before use to form a homogeneous suspension. Do not use the vaccine if it cannot be resuspended. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if particulate matter or discoloration is found.

Administer BEXSERO as a 0.5-mL intramuscular injection into the deltoid muscle of the upper arm.

2.3 Use of BEXSERO with Other Meningococcal Group B Vaccines

Sufficient data are not available on the safety and effectiveness of using BEXSERO and other meningococcal group B vaccines interchangeably to complete the vaccination series.

3 DOSAGE FORMS AND STRENGTHS

BEXSERO is a suspension for intramuscular injection in 0.5-mL single-dose prefilled syringes.

4 CONTRAINDICATIONS

Hypersensitivity, including severe allergic reaction, to any component of the vaccine, or after a previous dose of BEXSERO *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Preventing and Managing Allergic Reactions

Appropriate observation and medical treatment should always be readily available in case of an anaphylactic reaction following the administration of the vaccine.

5.2 Syncope

Syncope (fainting) can occur in association with administration of BEXSERO. Ensure procedures are in place to avoid injury from falling associated with syncope.

5.3 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.4 Limitation of Vaccine Effectiveness

BEXSERO may not protect all vaccine recipients. BEXSERO may not provide protection against all meningococcal serogroup B strains *[see Clinical Pharmacology (12.1)]*.

5.5 Altered Immunocompetence

Some individuals with altered immunocompetence may have reduced immune responses to BEXSERO.

Complement Deficiency

Persons with certain complement deficiencies and persons receiving treatment that inhibits terminal complement activation (for example, eculizumab) are at increased risk for invasive disease caused by *N. meningitidis* serogroup B even if they develop antibodies following vaccination with BEXSERO. *[See Clinical Pharmacology (12.1).]*

6 ADVERSE REACTIONS

The most common solicited adverse reactions observed in clinical trials were pain at the injection site (≥83%), myalgia (≥48%), erythema (≥45%), fatigue (≥35%), headache (≥33%), induration (≥28%), nausea (≥18%), and arthralgia (≥13%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

In 4 clinical trials, 3,058 individuals aged 10 through 25 years received at least one dose of BEXSERO, 1,436 participants received only BEXSERO, 2,089 received only placebo or a control vaccine, and 1,622 participants received a mixed regimen (placebo or control vaccine and BEXSERO).

In a randomized controlled study[1] conducted in U.S. and Poland, 120 participants aged 10 through 25 years received at least 1 dose of BEXSERO, including 112 participants who received 2 doses of BEXSERO 2 months apart; 97 participants received saline placebo followed by MENVEO [Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM_{197} Conjugate Vaccine]. Across groups, median age was 13 years, males comprised 49%, and 60% were white, 34% were Hispanic, 4% were black, <1% were Asian, and 2% were other.

In a second randomized controlled study[2] conducted in Chile, all subjects (N = 1,622) aged 11 through 17 years received at least 1 dose of BEXSERO. This study included a subset of 810 subjects who received 2 doses of BEXSERO 1 or 2 months apart. A control group of 128 subjects received at least 1 dose of placebo containing aluminum hydroxide. A subgroup of 128 subjects received 2 doses of BEXSERO 6 months apart. In this study, median age was 14 years, males comprised 44%, and 99% were Hispanic.

In a third randomized controlled study[3] conducted in the United Kingdom (U.K.), 974 university students aged 18 through 24 years received at least 1 dose of BEXSERO, including 932 subjects who received 2 doses of BEXSERO 1 month apart. Comparator groups received 1 dose of MENVEO followed by 1 dose of placebo containing aluminum hydroxide (n = 956) or 2 doses

of IXIARO (Japanese Encephalitis Vaccine, Inactivated, Adsorbed) (n = 947). Across groups, median age was 20 years, males comprised 46%, and 88% were white, 5% were Asian, 2% were black, <1% were Hispanic, and 4% were other.

In an uncontrolled study[4] conducted in Canada and Australia, 342 participants aged 11 through 17 years received at least 1 dose of BEXSERO, including 338 participants who received 2 doses of BEXSERO 1 month apart. The median age was 13 years, males comprised 55%, and 80% were white, 10% were Asian, 4% were Native American/Alaskan, and 4% were other.

Local and systemic reactogenicity data were solicited from all participants in the studies conducted in Chile, U.S./Poland, Canada/Australia, and in a subset of participants in the U.K. study. Reports of unsolicited adverse events occurring within the first 7 days after each vaccination were collected in all studies. In the U.S./Poland study, reports of unsolicited adverse events were collected up to 1 month after the second vaccination.

Reports of all serious adverse events, medically attended adverse events, and adverse events leading to premature withdrawal were collected throughout the study period for the studies conducted in Chile (12 months), U.K. (12 months), U.S./Poland (8 months), and Canada/Australia (2 months).

Solicited Adverse Reactions

The reported rates of local and systemic reactions among participants aged 10 through 25 years following each dose of BEXSERO administered 2 months apart or control in the U.S./Polish study[1] are presented in Table 1.

Table 1. Percentage of U.S. and Polish Participants Aged 10 through 25 Years Reporting Solicited Local and Systemic Adverse Reactions within 7 Days after BEXSERO or Control, by Dose

		Dose 1		Dose 2[b]	
		BEXSERO	Placebo (Saline)	BEXSERO	MENVEO
Solicited Reaction[a]		n = 110-114	n = 94-96	n = 107-109	n = 90-92
Local Adverse Reactions					
Pain	Any	90	27	83	43
	Mild	27	20	18	26
	Moderate	44	5	37	9
	Severe	20	2	29	8

Erythema	Any	50	13	45	26
	1-25 mm	41	11	36	13
	>25-50 mm	6	1	5	6
	>50-100 mm	3	0	5	4
	>100 mm	0	0	0	2
Induration	Any	32	10	28	23
	1-25 mm	24	9	22	16
	>25-50 mm	7	0	4	0
	>50-100 mm	1	1	2	4
	>100 mm	0	0	0	2
Systemic Adverse Reactions					
Fatigue	Any	37	22	35	20
	Mild	19	17	18	11
	Moderate	14	5	10	7
	Severe	4	0	6	2
Nausea	Any	19	4	18	4
	Mild	12	3	10	3
	Moderate	4	1	5	1
	Severe	4	0	4	0
Myalgia	Any	49	26	48	25
	Mild	21	20	16	14
	Moderate	16	5	19	7
	Severe	12	1	13	4
Arthralgia	Any	13	4	16	4
	Mild	9	3	8	2
	Moderate	3	1	6	2
	Severe	2	0	2	0
Headache	Any	33	20	34	23
	Mild	19	15	21	8
	Moderate	9	4	6	12
	Severe	4	1	6	3

Fever	≥38°C	1	1	5	0
	38.0-38.9°C	1	1	4	0
	39.0-39.9°C	0	0	1	0
	≥40°C	0	0	0	0

Clinicaltrials.gov Identifier NCT01272180.

[a] Erythema and induration: Any (≥1 mm). Pain and systemic reactions: Mild (transient with no limitation in normal daily activity); Moderate (some limitation in normal daily activity); Severe (unable to perform normal daily activity).

[b] Administered 2 months after Dose 1.

Solicited adverse reaction rates were similar among participants aged 11 through 24 years who received BEXSERO in the other 3 clinical studies,[2,3,4] except for severe myalgia which was reported by 3% to 7% of subjects. Severe pain was reported by 8% of university students in the U.K.[3]

Non-serious Adverse Reactions

In the 3 controlled studies[1,2,3] (BEXSERO n = 2,221, control n = 2,204), non-serious unsolicited adverse events that occurred within 7 days of any dose were reported by 439 (20%) participants receiving BEXSERO and 197 (9%) control recipients. Unsolicited adverse reactions that were reported among at least 2% of participants and were more frequently reported in participants receiving BEXSERO than in control recipients were injection site pain, headache, injection site induration unresolved within 7 days, and nasopharyngitis.

Serious Adverse Events

Overall, in clinical studies, among 3,058 participants aged 10 through 25 years who received at least 1 dose of BEXSERO, 66 (2.1%) participants reported serious adverse events at any time during the study. In the 3 controlled studies[1,2,3] (BEXSERO n = 2,716, control n = 2,078), serious adverse events within 30 days after any dose were reported in 23 (0.8%) participants receiving BEXSERO and 10 (0.5%) control recipients.

6.2 Additional Pre-licensure Safety Experience

In response to outbreaks of serogroup B meningococcal disease at 2 universities in the U.S., BEXSERO was administered as a 2-dose series at least 1 month apart. Information on serious adverse events was collected for a period of 30 days after each dose from 15,351 individuals aged 16 through 65 years who received at least 1 dose. Overall, 50 individuals (0.3%) reported serious adverse events, including one reaction considered related to vaccination, a case of anaphylaxis within 30 minutes following vaccination.

6.3 Postmarketing Experience

The following adverse reactions have been identified during postapproval use of BEXSERO. Because these reactions are reported voluntarily from a population of uncertain size, it is not

always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Blood and Lymphatic System Disorders

Lymphadenopathy.

General Disorders and Administration Site Conditions

Injection site reactions (including extensive swelling of the vaccinated limb, blisters at or around the injection site, and injection site nodule which may persist for more than 1 month).

Immune System Disorders

Allergic reactions (including anaphylactic reactions), rash, eye swelling.

Nervous System Disorders

Syncope, vasovagal responses to injection.

7 DRUG INTERACTIONS

Sufficient data are not available to establish the safety and immunogenicity of concomitant administration of BEXSERO with recommended adolescent vaccines.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

There are no adequate and well-controlled studies of BEXSERO in pregnant women in the U.S. Available human data on BEXSERO administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study was performed in female rabbits administered BEXSERO prior to mating and during gestation. The dose was 0.5 mL at each occasion (a single human dose is 0.5 mL). This study revealed no adverse effects on fetal or pre-weaning development due to BEXSERO *(see Data)*.

Data

Animal Data: In a developmental toxicity study, female rabbits were administered BEXSERO by intramuscular injection on Days 29, 15, and 1 prior to mating and on Gestation Days 7 and 20. The total dose was 0.5 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to Postnatal Day 29 were observed. There were no fetal malformations or variations observed.

8.2 Lactation

Risk Summary

It is not known whether the vaccine components of BEXSERO are excreted in human milk. Available data are not sufficient to assess the effects of BEXSERO on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for BEXSERO and any potential adverse effects on the breastfed child from BEXSERO or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of BEXSERO have not been established in children younger than 10 years.

8.5 Geriatric Use

Safety and effectiveness of BEXSERO have not been established in adults older than 65 years.

11 DESCRIPTION

BEXSERO (Meningococcal Group B Vaccine) is a sterile, white, opalescent, suspension for intramuscular injection. Each 0.5-mL dose of BEXSERO is formulated to contain 50 micrograms each of recombinant proteins Neisserial adhesin A (NadA), Neisserial Heparin Binding Antigen (NHBA), and factor H binding protein (fHbp), 25 micrograms of Outer Membrane Vesicles (OMV), 1.5 mg aluminum hydroxide (0.519 mg of Al^{3+}), 3.125 mg sodium chloride, 0.776 mg histidine, and 10 mg sucrose at pH 6.4 – 6.7.

The NadA component is a fragment of the full-length protein derived from *N. meningitidis* strain 2996 (peptide 8 variant 2/3)[5]. The NHBA component is a recombinant fusion protein comprised of NHBA (peptide 2)[5] and accessory protein 953 derived from *N. meningitidis* strains NZ98/254 and 2996, respectively. The fHbp component is a recombinant fusion protein comprised of fHbp (variant 1.1)[5] and the accessory protein 936 derived from *N. meningitidis* strains MC58 and 2996, respectively. These 3 recombinant proteins are individually produced in *Escherichia coli* and purified through a series of column chromatography steps. The OMV antigenic component is produced by fermentation of *N. meningitidis* strain NZ98/254 (expressing outer membrane protein PorA serosubtype P1.4)[6], followed by inactivation of the bacteria by deoxycholate, which also mediates vesicle formation. The antigens are adsorbed onto aluminum hydroxide.

Each dose contains less than 0.01 micrograms kanamycin (by calculation).

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Protection against invasive meningococcal disease is conferred mainly by complement-mediated antibody-dependent killing of *N. meningitidis*. The effectiveness of BEXSERO was assessed by measuring serum bactericidal activity using human complement (hSBA).

NHBA, NadA, fHbp, and PorA are proteins found on the surface of meningococci and contribute to the ability of the bacterium to cause disease. Vaccination with BEXSERO leads to the production of antibodies directed against NHBA, NadA, fHbp, and PorA P1.4 (present in OMV). The susceptibility of serogroup B meningococci to complement-mediated antibody-dependent killing following vaccination with BEXSERO is dependent on both the antigenic similarity of the bacterial and vaccine antigens, as well as the amount of antigen expressed on the surface of the invading meningococci.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

BEXSERO has not been evaluated for carcinogenic or mutagenic potential or impairment of male fertility in animals. Vaccination of female rabbits with BEXSERO had no effect on fertility. *[See Use in Specific Populations (8.1).]*

14 CLINICAL STUDIES

The immunogenicity of BEXSERO following 2 doses was evaluated in individuals aged 11 through 24 years. Serum bactericidal antibodies were measured with hSBA assays using 3 strains selected to measure responses to one of 3 vaccine antigens, either fHbp, NadA, or PorA P1.4, prevalent among strains in the U.S. A suitable strain for assessing bactericidal activity of NHBA-specific antibodies was not available. Studies assessed the proportion of subjects who achieved a 4-fold or greater increase in hSBA titer for each of the 3 strains, and the proportion of subjects with a titer greater than or equal to the lower limit of quantitation (LLOQ) of the assay for all 3 strains (composite response). The LLOQ was defined as the lowest amount of the antibody in a sample that can be reliably quantified. Available data showed that baseline antibody titers across populations vary.

14.1 Immunogenicity

In a clinical trial conducted in Canada and Australia, adolescents aged 11 through 17 years received 2 doses of BEXSERO 1 month apart. The hSBA responses 1 month after the second dose are shown in Table 2.

Table 2. Bactericidal Antibody Response Rates following 2 Doses of BEXSERO Administered 1 Month Apart to Canadian and Australian Adolescents[a]

≥4-Fold hSBA Response 1 Month Post Dose 2[b,c]			
Strain (Antigen)	**n**	**%**	**95% CI**
H44/76 (fHbp)	298	98	95, 99
5/99 (NadA)	299	99	98, 100
NZ98/254 (PorA P1.4)	298	39	33, 44
Composite hSBA Response[c,d]			
Time Point	**n**	**%**	**95% CI**
Baseline (pre-vaccination)	299	0	—
1 Month Post Dose 2	298	63	57, 68

NCT 01423084.

Abbreviations: CI = Confidence interval; hSBA = Serum bactericidal activity measured using human complement; LLOQ = Lower limit of quantitation.

[a] Evaluable Immunogenicity Population (aged 11 through 17 years).

[b] ≥4-fold hSBA response is defined as: a post-vaccination hSBA ≥1:16 for participants with pre-vaccination hSBA <1:4, a post-vaccination titer at least 4-fold the LLOQ for participants with pre-vaccination hSBA ≥1:4 but < LLOQ, and a post-vaccination 4-fold rise for participants with pre-vaccination hSBA ≥LLOQ.

[c] LLOQ = 1:16 for H44/76; 1:16 for 5/99; 1:8 for NZ98/254.

[d] Composite hSBA Response means hSBA ≥LLOQ for all 3 indicator Meningococcal B strains.

In a randomized, controlled clinical trial conducted in the U.K. among university students aged 18 through 24 years, hSBA responses in a subset of participants who received BEXSERO were measured 1 month and 11 months after the second dose (Table 3).

Table 3. Bactericidal Antibody Response Rates following 2 Doses of BEXSERO Administered 1 Month Apart to University Students in the U.K.[a]

≥4-Fold hSBA Response 1 Month Post Dose 2[b,c]			
Strain (Antigen)	**n**	**%**	**95% CI**
H44/76 (fHbp)	148	78	71, 85
5/99 (NadA)	148	94	89, 97
NZ98/254 (PorA P1.4)	147	67	58, 74
Composite hSBA Response[c,d]			
Time Point	**n**	**%**	**95% CI**
Baseline (pre-vaccination)	186	24	18,30
1 Month Post Dose 2	147	88	82,93
11 Months Post Dose 2	136	66	58,72

NCT 01214850.

Abbreviations: CI = Confidence interval; hSBA = Serum bactericidal activity measured using human complement; LLOQ = Lower limit of quantitation.

[a] Evaluable Immunogenicity Population (aged 18 through 24 years).

[b] ≥4-fold hSBA response is defined as: a post-vaccination hSBA ≥1:16 for participants with pre-vaccination hSBA <1:4, a post-vaccination titer at least 4-fold the LLOQ for participants with pre-vaccination hSBA ≥1:4 but <LLOQ, and a post-vaccination 4-fold rise for participants with pre-vaccination hSBA ≥LLOQ.

[c] LLOQ = 1:16 for H44/76; 1:8 for 5/99; 1:16 for NZ98/254.

[d] Composite hSBA Response means hSBA ≥LLOQ for all 3 indicator Meningococcal B strains.

15 REFERENCES

1. NCT01272180 (V102_03).
2. NCT00661713 (V72P10).
3. NCT01214850 (V72_29).
4. NCT01423084 (V72_41).
5. Wang X, et al. *Vaccine.* 2011; 29:4739-4744.
6. Hosking J, et al. *Clin Vaccine Immunol.* 2007;14:1393-1399.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

BEXSERO is supplied as a 0.5-mL suspension in a glass prefilled syringe (packaged without needles). The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex.

Table 4. Product Presentation for BEXSERO

Presentation	Carton NDC Number	Components
Pre-filled syringe Carton of **10** syringes	58160-976-20	0.5-mL single-dose prefilled syringe NDC 58160-976-02

16.2 Storage and Handling

Do not freeze. Discard if the vaccine has been frozen.

Store refrigerated, at 36°F to 46°F (2°C to 8°C).

Protect from light.

Do not use after the expiration date.

17 PATIENT COUNSELING INFORMATION

Give the patient, parent, or guardian the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Inform patients, parents, or guardians about:

- The importance of completing the immunization series.
- Reporting any adverse reactions to their healthcare provider.

Manufactured by **GSK Vaccines, Srl**
Bellaria-Rosia 53018, Sovicille (SI), Italy
U.S. License No. 1617

Distributed by **GlaxoSmithKline**
Research Triangle Park, NC 27709

BXS:6PI

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use TRUMENBA safely and effectively. See full prescribing information for TRUMENBA.

TRUMENBA® (Meningococcal Group B Vaccine)
Suspension for intramuscular injection
Initial U.S. Approval: 2014

----------RECENT MAJOR CHANGES----------
Indications and Usage, removal of statement regarding limitations of two-dose schedule of Trumenba (1) x/2021
Dosage and Administration, Dose and Schedule (2.1) x/2021

----------INDICATIONS AND USAGE----------
Trumenba is indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroup B. Trumenba is approved for use in individuals 10 through 25 years of age. (1)

----------DOSAGE AND ADMINISTRATION----------
- For intramuscular use only. (2)
- **Two-dose schedule:** Administer a dose (0.5 mL) at 0 and 6 months. If the second dose is administered earlier than 6 months after the first dose, a third dose should be administered at least 4 months after the second dose. (2.1)
- **Three-dose schedule:** Administer a dose (0.5 mL) at 0, 1-2, and 6 months. (2.1)

----------DOSAGE FORMS AND STRENGTHS----------
Suspension for intramuscular injection in 0.5 mL single-dose prefilled syringe. (3)

----------CONTRAINDICATIONS----------
Severe allergic reaction (e.g., anaphylaxis) to any component of Trumenba. (4)

---------- WARNINGS AND PRECAUTIONS ----------
Syncope (fainting) can occur in association with administration of injectable vaccines, including Trumenba. Procedures should be in place to avoid injury from fainting. (5.4)

----------ADVERSE REACTIONS----------
The most common solicited adverse reactions in adolescents and young adults were pain at the injection site (≥85%), fatigue (≥60%), headache (≥55%), and muscle pain (≥35%). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer Inc. at 1-800-438-1985 or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS----------
Pediatric Use: Safety and effectiveness have not been established in children <10 years of age. In a clinical study, 90% of infants <12 months of age who were vaccinated with a reduced dosage formulation had fever. (8.4)

See 17 for PATIENT COUNSELING INFORMATION

Revised: x/20xx

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Trumenba is indicated for active immunization to prevent invasive disease caused by *Neisseria meningitidis* serogroup B. Trumenba is approved for use in individuals 10 through 25 years of age.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only.

2.1 Dose and Schedule

Two-dose schedule: Administer a dose (0.5 mL) at 0 and 6 months. If the second dose is administered earlier than 6 months after the first dose, a third dose should be administered at least 4 months after the second dose.

Three-dose schedule: Administer a dose (0.5 mL) at 0, 1-2, and 6 months.

The choice of dosing schedule may depend on the risk of exposure and the patient's susceptibility to meningococcal serogroup B disease.

2.2 Administration

Shake syringe vigorously to ensure that a homogenous white suspension of Trumenba is obtained. Do not use the vaccine if it cannot be re-suspended. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if particulate matter or discoloration is found.

Inject each 0.5 mL dose intramuscularly, using a sterile needle attached to the supplied prefilled syringe. The preferred site for injection is the deltoid muscle of the upper arm. Do not mix Trumenba with any other vaccine in the same syringe.

2.3 Use of Trumenba with other Meningococcal Group B Vaccines

Data are not available on the safety and effectiveness of using Trumenba and other meningococcal group B vaccines interchangeably to complete the vaccination series.

3 DOSAGE FORMS AND STRENGTHS

Trumenba is a suspension for intramuscular injection in 0.5 mL single-dose prefilled syringe.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g. anaphylaxis) to any component of Trumenba *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Allergic Reactions

Epinephrine and other appropriate agents used to manage immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur following administration of Trumenba.

5.2 Altered Immunocompetence

Reduced Immune Response

Some individuals with altered immunocompetence may have reduced immune responses to Trumenba.

Complement Deficiency

Persons with certain complement deficiencies and persons receiving treatment that inhibits terminal complement activation (for example, eculizumab) are at increased risk for invasive disease caused by *N. meningitidis* serogroup B even if they develop antibodies following vaccination with Trumenba *[see Clinical Pharmacology (12)]*.

5.3 Limitation of Vaccine Effectiveness

Vaccination with Trumenba may not protect all vaccine recipients against *N. meningitidis* serogroup B infections.

5.4 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccines, including Trumenba. Procedures should be in place to avoid injury from fainting.

6 ADVERSE REACTIONS

In clinical studies, the most common solicited adverse reactions in adolescents and young adults were pain at the injection site (≥85%), fatigue (≥60%), headache (≥55%), and muscle pain (≥35%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in clinical practice.

The safety of Trumenba was evaluated in 16,284 subjects 10 through 25 years of age in 12 clinical studies (9 randomized controlled and 3 supportive non-controlled studies) conducted in the U.S., Europe, Canada, Chile, and Australia. A total of 11,991 subjects 10 through 18 years of age, and 4,293 subjects 19 through 25 years of age received at least one dose of Trumenba. A total of 5,501 subjects 10 through 25 years of age in the control groups received saline placebo and/or one of the following vaccine(s): Human Papillomavirus Quadrivalent (Types 6, 11, 16, and 18) Vaccine, Recombinant (HPV4) (Merck & Co., Inc.); Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap) (Sanofi Pasteur Ltd.); Meningococcal (Serogroups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine (MenACWY) (Sanofi Pasteur Inc.); a non-U.S. licensed reduced diphtheria toxoid, tetanus toxoid, acellular

pertussis and inactivated polio virus vaccine (dTaP-IPV) (Sanofi Pasteur, Inc.); Hepatitis A Vaccine (HAV) (GlaxoSmithKline Biologicals).

The safety evaluation in the clinical studies included an assessment of: (1) solicited local and systemic reactions, and use of antipyretic medication after each vaccination in an electronic diary maintained by the subject or the subject's parent/legal guardian and (2) spontaneous reports of adverse events (AEs), including serious adverse events (SAEs), throughout the study (day of vaccination through 1 month or 6 months after the last vaccination, depending on the study and safety parameter).

In controlled studies, demographic characteristics were generally similar with regard to gender, race, and ethnicity among subjects who received Trumenba and those who received control. Among participants in clinical trials B1971009 (Study 1009), B1971016 (Study 1016), and B1971057 (Study 1057), 41.3% to 51.5% were male, 76.1% to 87.3% were White, 8.1% to 20.8% were Black or African-American, <2% were Asian, and 5.8% to 17.1% were Hispanic/Latino.

Solicited Local and Systemic Adverse Reactions

Study 1057 was a randomized, observer-blinded, multicenter trial in the U.S. and Europe. In this study, 1057 subjects 10 through 25 years of age received at least 1 dose of Trumenba on a 0- and 6-month schedule. Trumenba was co-administered with Meningococcal (Groups A, C, Y, W-135) Oligosaccharide Diphtheria CRM_{197} Conjugate Vaccine (MenACWY) (GSK Vaccines, SRL) for the first dose.

Study 1009 was a randomized, active-controlled, observer-blinded, multicenter trial in the U.S., Canada, and Europe in which 2,693 subjects 10 through 18 years of age received at least 1 dose of Trumenba on a 0-, 2-, and 6- month schedule. A control group (n=897) received HAV at 0 and 6 months, and saline at 2 months.

Study 1016 was a randomized, placebo-controlled, observer-blinded, multicenter trial in the U.S., Canada, and Europe in which 2,471 subjects 18 through 25 years of age received at least 1 dose of Trumenba and 822 subjects received saline on a 0-, 2,- and 6- month schedule.

Local adverse reactions at the injection site were assessed in the three studies.

Tables 1, 2, and 3 present the percentage and severity of reported local adverse reactions within 7 days following each dose of Trumenba for Study 1057 and following each dose of Trumenba or control (HAV/saline or saline) for Study 1009 and Study 1016, respectively.

In Studies 1009 and 1016, local adverse reactions were reported more frequently following Trumenba compared to control (see Tables 2 and 3, respectively).

Table 1: Percentages of Subjects 10 through 25 Years of Age (Study 1057[a]) Reporting Local Adverse Reactions Within 7 Days After Each Vaccination

	Dose 1	Dose 2
	Trumenba+MenACWY-CRM[b]	Trumenba[b]
Local Reaction	**N=1044**	**N=903**
Pain[c]		
Any[d]	85.0	82.2
Mild	41.2	38.9
Moderate	39.1	37.9
Severe	4.7	5.4

Table 1: Percentages of Subjects 10 through 25 Years of Age (Study 1057[a]) Reporting Local Adverse Reactions Within 7 Days After Each Vaccination		
	Dose 1	**Dose 2**
	Trumenba+MenACWY-CRM[b]	**Trumenba[b]**
Local Reaction	**N=1044**	**N=903**
Redness[e]		
Any[d] (≥ 2.5 cm)	16.9	14.7
Mild	6.8	5.2
Moderate	8.0	8.4
Severe	2.0	1.1
Swelling[e]		
Any[d] (≥ 2.5 cm)	17.0	14.3
Mild	9.8	6.4
Moderate	6.9	7.5
Severe	0.3	0.3

[a] Study 1057: National Clinical Trial (NCT) number NCT03135834.
[b] Trumenba and MenACWY-CRM were administered at 0 month followed by Trumenba alone at 6 months. Local reactions were recorded at the Trumenba injection site only.
[c] Mild (does not interfere with activity); moderate (interferes with activity); severe (prevents daily activity).
[d] "Any" is defined as the cumulative frequency of subjects who reported a reaction as "mild", "moderate", or "severe" within 7 days of vaccination.
[e] Mild (2.5-5.0 cm); moderate (>5.0-10.0 cm); severe (>10.0 cm).

Table 2: Percentages of Subjects 10 through 18 Years of Age (Study 1009[a]) Reporting Local Adverse Reactions Within 7 Days After Each Vaccination						
	Dose 1		**Dose 2**		**Dose 3**	
	Trumenba[b]	**HAV/Saline[b]**	**Trumenba[b]**	**HAV/Saline[b]**	**Trumenba[b]**	**HAV/Saline[b]**
Local Reaction	**N=2681**	**N=890**	**N=2545**	**N=843**	**N=2421**	**N=821**
Pain[c]						
Any[d]	86.7	47.0	77.7	15.2	76.0	34.0
Mild	41.1	36.5	39.4	12.3	34.1	23.8
Moderate	40.7	9.9	33.2	2.7	36.5	9.9
Severe	5.0	0.6	5.1	0.1	5.4	0.4
Redness[e]						
Any[d] (≥ 2.5 cm)	16.2	1.3	12.5	0.6	13.9	1.1
Mild	5.6	1.2	5.2	0.6	4.9	1.0
Moderate	8.8	0.1	6.1	0.0	6.8	0.1
Severe	1.9	0.0	1.1	0.0	2.2	0.0
Swelling[e]						
Any[d] (≥ 2.5 cm)	18.0	2.2	13.9	0.6	15.4	0.9
Mild	8.5	1.8	6.3	0.5	7.9	0.7
Moderate	8.8	0.4	7.3	0.1	6.8	0.1
Severe	0.7	0.0	0.2	0.0	0.7	0.0

[a] Study 1009: NCT01830855.
[b] Trumenba was administered at 0, 2, and 6 months. HAV was administered at 0 and 6 months and saline was administered at 2 months.
[c] Mild (does not interfere with activity); moderate (interferes with activity); severe (prevents daily activity).
[d] "Any" is defined as the cumulative frequency of subjects who reported a reaction as "mild", "moderate", or "severe" within 7 days of vaccination.
[e] Mild (2.5-5.0 cm); moderate (>5.0-10.0 cm); severe (>10.0 cm).

Table 3: Percentages of Subjects 18 through 25 Years of Age (Study 1016[a]) Reporting Local Adverse Reactions Within 7 Days After Each Vaccination						
	Dose 1		Dose 2		Dose 3	
	Trumenba[b]	Saline[b]	Trumenba[b]	Saline[b]	Trumenba[b]	Saline[b]
Local Reaction	**N=2425**	**N=798**	**N=2076**	**N=706**	**N=1823**	**N=624**
Pain[c]						
Any[d]	84.2	11.8	79.3	7.8	80.4	6.7
Mild	42.3	10.7	42.2	6.8	36.1	6.4
Moderate	37.1	1.1	32.7	1.0	38.9	0.3
Severe	4.8	0.0	4.4	0.0	5.3	0.0
Redness[e]						
Any[d] (≥ 2.5 cm)	13.8	0.6	11.8	0.3	17.1	0.2
Mild	5.8	0.5	4.6	0.1	6.2	0.2
Moderate	7.1	0.0	6.3	0.0	8.6	0.0
Severe	0.9	0.1	0.9	0.1	2.3	0.0
Swelling[e]						
Any[d] (≥ 2.5 cm)	15.5	0.6	14.0	0.4	16.6	0.3
Mild	8.5	0.3	7.7	0.3	8.8	0.0
Moderate	6.8	0.3	6.0	0.1	7.2	0.3
Severe	0.2	0.1	0.3	0.0	0.5	0.0

[a] Study 1016: NCT01352845.
[b] Trumenba was administered at 0, 2, and 6 months. Saline was administered at 0, 2, and 6 months.
[c] Mild (does not interfere with activity); moderate (interferes with activity); severe (prevents daily activity).
[d] "Any" is defined as the cumulative frequency of subjects who reported a reaction as "mild", "moderate", or "severe" within 7 days of vaccination.
[e] Mild (2.5-5.0 cm); moderate (>5.0-10.0 cm); severe (>10.0 cm).

In Study 1057, among Trumenba recipients, mean duration of pain was 2.7 days (range 1-17 days) after the first vaccination and 2.7 days (range 1-12 days) after the second vaccination; redness was 2.2 days (range 1-9 days) and 2.4 days (1-11 days), respectively; and swelling was 2.2 days (range 1-17 days) and 2.5 days (range 1-27 days), respectively.

In Study 1009, mean duration of pain was 2.4 to 2.6 days (range 1-17 days), redness was 2.0 to 2.2 days (range 1-12 days) and swelling was 2.0 to 2.1 days (range 1-21 days) for the three-dose series in the Trumenba groups. In Study 1016, mean duration of pain was 2.6 to 2.8 days (range 1-67 days), redness was 2.2 to 2.5 days (range 1-13 days) and swelling was 2.1 to 2.6 days (range 1-70 days) in the Trumenba group.

Tables 4, 5, and 6 present the percentage and severity of reported solicited systemic adverse reactions within 7 days of each dose of Trumenba for Study 1057 and within 7 days of each dose of Trumenba or control (HAV/saline or saline) for Study 1009 and Study 1016, respectively.

Table 4: Percentages of Subjects 10 through 25 Years of Age (Study 1057[a]) Reporting Systemic Adverse Reactions and Use of Antipyretic Medications Within 7 Days After Each Vaccination		
	Dose 1	Dose 2
	Trumenba+MenACWY-CRM[b]	Trumenba[b]
Systemic Reaction	**N=1044**	**N=903**
Fever (≥38°C)		
≥38.0°C	6.7	3.2
38.0°C to <38.5°C	4.0	1.9
38.5°C to <39.0°C	2.1	0.7
39.0°C to ≤40.0°C	0.6	0.7
>40.0°C	0.0	0.0

Table 4: Percentages of Subjects 10 through 25 Years of Age (Study 1057[a]) Reporting Systemic Adverse Reactions and Use of Antipyretic Medications Within 7 Days After Each Vaccination		
	Dose 1	**Dose 2**
	Trumenba+MenACWY-CRM[b]	**Trumenba[b]**
Systemic Reaction	**N=1044**	**N=903**
Vomiting[c]		
Any[d]	3.7	2.8
Mild	2.9	2.0
Moderate	0.9	0.8
Severe	0.0	0.0
Diarrhea[e]		
Any[d]	14.1	10.6
Mild	10.7	7.6
Moderate	3.3	2.5
Severe	0.1	0.4
Headache[f]		
Any[d]	46.5	41.6
Mild	25.1	23.1
Moderate	19.0	16.5
Severe	2.4	2.0
Fatigue[f]		
Any[d]	51.9	45.2
Mild	25.4	23.0
Moderate	23.7	19.2
Severe	2.9	3.0
Chills[f]		
Any[d]	18.5	18.5
Mild	11.5	11.6
Moderate	5.7	6.2
Severe	1.2	0.7
Muscle pain (other than muscle pain at the injection site)[f]		
Any[d]	28.4	21.4
Mild	15.8	11.5
Moderate	11.6	7.8
Severe	1.1	2.1
Joint pain[f]		
Any[d]	19.6	18.7
Mild	10.2	11.2
Moderate	8.6	6.5
Severe	0.8	1.0
Use of antipyretic medication	18.6	14.4

[a] Study 1057: NCT03135834.
[b] Trumenba and MenACWY-CRM were administered at 0 month followed by Trumenba alone at 6 months.
[c] Mild (1-2 times in 24 hours); moderate (>2 times in 24 hours); severe (requires intravenous hydration).
[d] "Any" is defined as the cumulative frequency of subjects who reported a reaction as "mild", "moderate", or "severe" within 7 days of vaccination.
[e] Mild (2-3 loose stools in 24 hours); moderate (4-5 loose stools in 24 hours); severe (6 or more loose stools in 24 hours).
[f] Mild (does not interfere with activity); moderate (some interference with activity); severe (prevents daily routine activity).

Table 5: Percentages of Subjects 10 through 18 Years of Age (Study 1009[a]) Reporting Systemic Adverse Reactions and Use of Antipyretic Medications Within 7 Days After Each Vaccination						
	Dose 1		Dose 2		Dose 3	
	Trumenba[b]	HAV/Saline[b]	Trumenba[b]	HAV/Saline[b]	Trumenba[b]	HAV/Saline[b]
Systemic Reaction	**N=2681**	**N=890**	**N=2545**	**N=843**	**N=2421**	**N=821**
Fever (≥38°C)[c]						
≥38.0°C	6.4	1.9	2.0	1.5	2.7	2.3
38.0°C to <38.5°C	4.0	1.3	1.2	0.7	1.8	1.3
38.5°C to <39.0°C	1.9	0.3	0.7	0.7	0.6	0.4
39.0°C to ≤40.0°C	0.5	0.2	0.1	0.1	0.3	0.5
>40.0°C	0.0	0.0	0.0	0.0	0.0	0.1
Vomiting[d]						
Any[e]	3.7	1.9	2.2	1.4	1.7	2.2
Mild	2.8	1.7	1.7	1.1	1.4	1.7
Moderate	0.9	0.2	0.4	0.4	0.3	0.5
Severe	0.0	0.0	0.0	0.0	0.0	0.0
Diarrhea[f]						
Any[e]	10.6	12.1	7.6	9.1	7.7	7.6
Mild	9.1	10.9	6.2	7.6	6.4	6.2
Moderate	1.3	1.1	1.3	1.2	1.0	1.1
Severe	0.3	0.1	0.1	0.4	0.3	0.2
Headache[g]						
Any[e]	51.8	37.2	37.8	28.1	35.4	24.8
Mild	28.7	24.0	20.2	15.7	18.9	13.5
Moderate	21.0	12.5	16.0	10.9	15.2	10.4
Severe	2.2	0.7	1.7	1.5	1.3	1.0
Fatigue[g]						
Any[e]	54.0	40.3	38.3	26.3	35.9	24.4
Mild	27.8	23.5	20.6	13.2	18.4	13.5
Moderate	23.2	15.2	15.8	11.7	15.2	10.0
Severe	3.0	1.7	1.9	1.4	2.3	0.9
Chills[g]						
Any[e]	25.3	17.2	16.0	10.3	13.1	8.3
Mild	16.2	13.3	10.6	8.1	8.7	6.5
Moderate	8.0	3.5	4.8	1.8	3.8	1.7
Severe	1.2	0.4	0.6	0.5	0.5	0.1
Muscle pain (other than muscle pain at the injection site)[g]						
Any[e]	24.4	19.2	17.8	10.3	17.6	11.1
Mild	13.2	13.5	8.7	5.2	9.5	6.6
Moderate	10.1	5.4	7.9	4.5	7.2	4.3
Severe	1.2	0.3	1.2	0.6	0.8	0.2
Joint pain[g]						
Any[e]	21.9	13.6	16.7	9.1	16.0	8.9
Mild	11.8	8.3	8.4	5.0	8.9	5.5
Moderate	8.7	4.6	7.5	3.4	5.9	3.0
Severe	1.4	0.7	0.8	0.7	1.2	0.4
Use of antipyretic medication	20.7	10.4	13.6	8.9	12.7	6.8

[a] Study 1009: NCT01830855.

[b] Trumenba was administered at 0, 2, and 6 months. HAV was administered at 0 and 6 months and saline was administered at 2 months.

[c] Study 1009: Fever (≥38°C): N=2679, 2540, and 2414 for Trumenba at Dose 1, Dose 2, and Dose 3, respectively; N=890, 840, and 819 for HAV/saline at Dose 1, Dose 2, and Dose 3, respectively.

[d] Mild (1-2 times in 24 hours); moderate (>2 times in 24 hours); severe (requires intravenous hydration).

[e] "Any" is defined as the cumulative frequency of subjects who reported a reaction as "mild", "moderate", or "severe" within 7 days of

Table 5: Percentages of Subjects 10 through 18 Years of Age (Study 1009[a]) Reporting Systemic Adverse Reactions and Use of Antipyretic Medications Within 7 Days After Each Vaccination						
	Dose 1		Dose 2		Dose 3	
	Trumenba[b]	HAV/Saline[b]	Trumenba[b]	HAV/Saline[b]	Trumenba[b]	HAV/Saline[b]
Systemic Reaction	**N=2681**	**N=890**	**N=2545**	**N=843**	**N=2421**	**N=821**

vaccination.

[f] Mild (2-3 loose stools in 24 hours); moderate (4-5 loose stools in 24 hours); severe (6 or more loose stools in 24 hours).

[g] Mild (does not interfere with activity); moderate (interferes with activity); severe (prevents daily activity).

Table 6: Percentages of Subjects 18 through 25 Years of Age (Study 1016[a]) Reporting Systemic Adverse Reactions and Use of Antipyretic Medications Within 7 Days After Each Vaccination						
	Dose 1		Dose 2		Dose 3	
	Trumenba[b]	Saline[b]	Trumenba[b]	Saline[b]	Trumenba[b]	Saline[b]
Systemic Reaction	**N=2425**	**N=798**	**N=2076**	**N=706**	**N=1823**	**N=624**
Fever (≥38°C)[c]						
≥38.0°C	2.4	0.6	1.2	1.0	2.0	0.6
38.0°C to <38.5°C	1.6	0.4	0.7	0.6	1.4	0.5
38.5°C to <39.0°C	0.7	0.0	0.4	0.3	0.4	0.2
39.0°C to ≤40.0°C	0.0	0.3	0.1	0.1	0.1	0.0
>40.0°C	0.0	0.0	0.0	0.0	0.1	0.0
Vomiting[d]						
Any[e]	2.6	2.1	2.1	1.6	2.0	1.4
Mild	2.2	2.1	1.6	1.3	1.8	1.1
Moderate	0.4	0.0	0.5	0.3	0.2	0.3
Severe	0.0	0.0	0.0	0.0	0.0	0.0
Diarrhea[f]						
Any[e]	12.7	11.8	8.6	8.1	7.5	6.9
Mild	10.2	9.8	6.4	4.7	6.1	5.3
Moderate	2.4	1.9	1.7	2.8	1.2	1.3
Severe	0.2	0.1	0.5	0.6	0.2	0.3
Headache[g]						
Any[e]	43.9	36.2	33.1	24.9	32.5	21.6
Mild	24.3	22.1	18.4	13.6	17.6	12.5
Moderate	17.9	13.5	13.3	10.1	13.3	8.3
Severe	1.6	0.6	1.4	1.3	1.6	0.8
Fatigue[g]						
Any[e]	50.9	39.8	39.2	27.3	39.3	24.5
Mild	25.4	23.2	20.6	13.9	18.9	13.1
Moderate	22.1	15.8	16.4	11.5	18.8	9.6
Severe	3.4	0.9	2.2	2.0	1.6	1.8
Chills[g]						
Any[e]	18.1	9.8	12.4	8.5	12.6	6.4
Mild	12.0	8.1	8.1	6.9	7.7	4.3
Moderate	4.9	1.6	3.5	1.6	4.2	2.1
Severe	1.1	0.0	0.8	0.0	0.8	0.0
Muscle pain (other than muscle pain at the injection site)[g]						
Any[e]	25.9	14.5	15.6	8.5	16.9	7.5
Mild	13.0	9.6	7.6	5.8	8.9	4.5
Moderate	11.3	4.4	7.1	2.3	6.8	2.9
Severe	1.6	0.5	0.8	0.4	1.2	0.2

Table 6: Percentages of Subjects 18 through 25 Years of Age (Study 1016[a]) Reporting Systemic Adverse Reactions and Use of Antipyretic Medications Within 7 Days After Each Vaccination						
	Dose 1		Dose 2		Dose 3	
	Trumenba[b]	Saline[b]	Trumenba[b]	Saline[b]	Trumenba[b]	Saline[b]
Systemic Reaction	**N=2425**	**N=798**	**N=2076**	**N=706**	**N=1823**	**N=624**
Joint pain[g]						
Any[e]	19.6	10.9	15.1	6.5	12.6	5.3
Mild	10.3	6.9	8.1	3.7	6.6	2.9
Moderate	7.9	3.5	6.2	2.5	5.4	2.4
Severe	1.4	0.5	0.9	0.3	0.6	0.0
Use of antipyretic medication	13.4	8.9	12.3	7.6	12.8	6.6

[a] Study 1016: NCT01352845.
[b] Trumenba was administered at 0, 2, and 6 months. Saline was administered at 0, 2, and 6 months.
[c] Study 1016: Fever (≥38°C): N=2415, 2067, and 1814 for Trumenba at Dose 1, Dose 2, and Dose 3, respectively; N=796, 705, and 621 for saline at Dose 1, Dose 2, and Dose 3, respectively.
[d] Mild (1-2 times in 24 hours); moderate (>2 times in 24 hours); severe (requires intravenous hydration).
[e] "Any" is defined as the cumulative frequency of subjects who reported a reaction as "mild", "moderate", or "severe" within 7 days of vaccination.
[f] Mild (2-3 loose stools in 24 hours); moderate (4-5 loose stools in 24 hours); severe (6 or more loose stools in 24 hours).
[g] Mild (does not interfere with activity); moderate (interferes with activity); severe (prevents daily activity).

In three early phase studies in which it was solicited, nausea was reported in up to 24% of adolescents. The frequencies of adverse reactions were highest after the first dose regardless of the schedule. After subsequent doses, the frequencies of adverse reactions were similar regardless of dose number and schedule.

Serious Adverse Events

Among the 8 controlled studies investigating the three-dose (0, 1-2, and 6 months) schedule (Trumenba N=13,275, control N=5,501), SAEs were reported by 213 (1.6%) subjects and by 106 (1.9%) subjects who received at least one dose of Trumenba or control, respectively.

Non-serious Adverse Events

Among the 8 controlled studies investigating the three-dose (0, 1-2, and 6 months) schedule (Trumenba N=13,275, control N=5,501), AEs that occurred within 30 days of vaccination were reported in 4,056 (30.6%) subjects who received at least one dose of Trumenba and 1,539 (28.0%) subjects in the control group who received at least one dose. AEs that occurred at a frequency of at least 2% and were more frequently observed in subjects who received Trumenba than subjects in the control group were injection site pain, fever, and headache.

6.2 Postmarketing Experience

The following adverse reactions have been identified during post-approval use of Trumenba. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to product exposure.

Immune System Disorders: Hypersensitivity reactions, including anaphylactic reactions.
Nervous system disorder: Syncope (fainting).

7 DRUG INTERACTIONS

In clinical trials, Trumenba was administered concomitantly with HPV4 in adolescents 11 through 17 years of age and with MenACWY and Tdap in adolescents 10 through 12 years of age *[see Clinical Studies (14) and Adverse Reactions (6)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no adequate and well-controlled studies of Trumenba in pregnant women. Available human data on Trumenba administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

Two developmental toxicity studies were performed in female rabbits administered Trumenba prior to mating and during gestation. The dose was 0.5 mL at each occasion (a single human dose is 0.5 mL). These studies revealed no evidence of harm to the fetus or offspring (until weaning) due to Trumenba [see Animal Data].

Animal Data

Two developmental toxicity studies were performed in female rabbits. Animals were administered Trumenba by intramuscular injection 17 days and 4 days prior to mating and on gestation Days 10 and 24. The dose was 0.5 mL at each occasion (a single human dose is 0.5 mL). No adverse effects on pre-weaning development up to post-natal day 21 were observed. There were no fetal malformations or variations observed due to the vaccine.

8.2 Lactation

Risk Summary

Available data are not sufficient to assess the effects of Trumenba on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Trumenba and any potential adverse effects on the breastfed child from Trumenba or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness have not been established in children <10 years of age. In a clinical study, 90% of infants <12 months of age who were vaccinated with a reduced dosage formulation had fever.

8.5 Geriatric Use

Safety and effectiveness of Trumenba in adults >65 years of age have not been established.

11 DESCRIPTION

Trumenba is a sterile suspension composed of two recombinant lipidated factor H binding protein (fHbp) variants from *N. meningitidis* serogroup B, one from fHbp subfamily A and one from subfamily B (A05 and B01, respectively).[1] The proteins are individually produced in *E. coli*. Production strains are grown in defined fermentation growth media to a specific density. The recombinant proteins are extracted from the production strains and purified through a series of column chromatography steps. Polysorbate 80 (PS80) is added to the drug substances and is present in the final drug product.

Each 0.5 mL dose contains 60 micrograms of each fHbp variant (total of 120 micrograms of protein), 0.018 mg of PS80 and 0.25 mg of Al^{3+} as $AlPO_4$ in 10 mM histidine buffered saline at pH 6.0.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Protection against invasive meningococcal disease is conferred mainly by complement-mediated antibody-dependent killing of *N. meningitidis*. The effectiveness of Trumenba was assessed by measuring serum bactericidal activity using human complement (hSBA).

fHbp is one of many proteins found on the surface of meningococci and contributes to the ability of the bacterium to avoid host defenses. fHbps can be categorized into two immunologically distinct subfamilies, A and B.[1] The susceptibility of serogroup B meningococci to complement-mediated antibody-dependent killing following vaccination with Trumenba is dependent on both the antigenic similarity of the bacterial and vaccine fHbps, as well as the amount of fHbp expressed on the surface of the invading meningococci.

13 NONCLINICAL TOXICOLOGY

Trumenba has not been evaluated for carcinogenic or mutagenic potential or impairment of fertility in males. Vaccination of female rabbits with Trumenba had no effect on fertility *[see Pregnancy (8.1)]*.

14 CLINICAL STUDIES

The immunogenicity of Trumenba described in this section is based on results from four clinical studies:

- Following the two-dose schedule (0 and 6 months) in subjects 10 through 25 years of age in the U.S. and Europe (Study 1057);
- Following the three-dose schedule (0, 2, and 6 months) in subjects 10 through 25 years of age in the U.S., Canada, and Europe (Studies 1009 and 1016); and
- Following the two-dose (0 and 6 months) and three-dose schedules (0, 1-2, and 6 months) in subjects 11 through 18 years of age in Europe (Study 1012).

Serum bactericidal antibodies were measured with hSBA assays that used each of four meningococcal serogroup B strains. These four primary test strains express fHbp variants representing the two subfamilies (A and B) of meningococcal serogroup B strains causing invasive disease in the U.S. and Europe. The studies assessed the proportions of subjects with a 4-fold or greater increase in hSBA titer for each of the four primary strains. The studies also assessed the composite response to the four primary strains combined (proportion of subjects who achieved a hSBA titer greater than or equal to 1:8 [three strains] and 1:16 [one strain]). To assess the effectiveness of the two- and three-dose schedules of Trumenba against diverse meningococcal serogroup B strains, the proportion of subjects achieving a defined hSBA titer (≥LLOQ) following completion of the two- or

three-dose series was evaluated against a panel of 10 additional strains, each expressing a different fHbp variant.

14.1 Immunogenicity

The hSBA responses to each of the primary strains observed after the second dose of Trumenba in Study 1057 are presented in Table 7.

Table 7: Percentages of Subjects 10 through 25 Years of Age With ≥4-fold Rise in hSBA Titer and Composite Response Following Administration of Trumenba on a 0-and 6-Month Schedule for Four Primary Strains (Study 1057)[a,b]

fHbp Variant[c]		N[d]	% (95% CI)[e]
≥4-Fold Increase			
PMB80 (A22)	Dose 2	827	73.8 (70.6, 76.7)
PMB2001 (A56)	Dose 2	823	95.0 (93.3, 96.4)
PMB2948 (B24)	Dose 2	835	67.4 (64.1, 70.6)
PMB2707 (B44)	Dose 2	850	86.4 (83.9, 88.6)
Composite hSBA Response[f]			
	Before Dose 1	799	1.8 (1.0, 2.9)
	Dose 2	814	74.3 (71.2, 77.3)

Abbreviations: CI=confidence interval; fHbp=factor H binding protein; hSBA=serum bactericidal assay using human complement; LLOQ=lower limit of quantitation; LOD=limit of detection.

Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44.

Note: The 4-fold increase is defined as follows: (1) For subjects with a baseline hSBA titer <1:4, a response is defined as an hSBA titer ≥1:16. (2) For subjects with a baseline hSBA titer ≥ LOD and < LLOQ, a response is defined as an hSBA titer ≥4 times the LLOQ. (3) For subjects with a baseline hSBA titer ≥ LLOQ, a response is defined as an hSBA titer ≥4 times the baseline titer.

Note: Pre-specified criteria for assessment of hSBA responses (4-fold rise in titer to each primary test strain, and titer above LLOQ for all four primary test strains) among subjects in the U.S. and Europe were met in this study for all test strains except strain A22. Pre-specified criteria for the lower bound of the 95% CI for 4-fold rise in titer were set at 75%, 85%, 55%, and 60%, respectively, for A22, A56, B24, and B44, and 65% for the composite hSBA response for all four primary test strains.

[a] Evaluable immunogenicity population.

[b] Study 1057: NCT03135834.

[c] For the second dose, serum was obtained approximately 1 month after vaccination.

[d] For ≥4-fold increase, N=number of subjects with valid and determinate hSBA titers for the given strain at both the specified time point and baseline. For composite hSBA response, N=number of subjects with valid and determinate hSBA results on all 4 strains at the given time point. U.S. subjects constituted approximately 80% of the total subjects evaluated for immunogenicity.

[e] Exact 2-sided confidence interval (Clopper-Pearson method) based upon the observed proportion of subjects.

[f] Composite response = hSBA ≥ LLOQ for all 4 primary meningococcal B strains.

The hSBA responses after the second dose of Trumenba in Study 1057 against a panel of 10 additional strains representing the diversity of meningococcal fHbp types prevalent among strains circulating in the US are presented in Table 8.

Table 8. Percentages of Subjects 10 through 25 Years of Age With a hSBA Titer ≥ LLOQ Against 10 Additional Strains Following Administration of Trumenba on a 0- and 6-Month Schedule (Study 1057)[a,b]

fHbp Variant[c]		N[d]	% (95% CI)[e]
PMB3175 (A29)	Before Dose 1	166	4.8 (2.1, 9.3)
	Dose 2	166	95.2 (90.7, 97.9)
PMB3010 (A06)	Before Dose 1	157	5.7 (2.7, 10.6)
	Dose 2	159	89.3 (83.4, 93.6)
PMB3040 (A07)	Before Dose 1	150	32.0 (24.6, 40.1)
	Dose 2	157	96.8 (92.7, 99.0)
PMB824 (A12)	Before Dose 1	154	5.2 (2.3, 10.0)
	Dose 2	157	83.4 (76.7, 88.9)
PMB1672 (A15)	Before Dose 1	166	22.9 (16.7, 30.0)
	Dose 2	165	89.1 (83.3, 93.4)
PMB1989 (A19)	Before Dose 1	167	5.4 (2.5, 10.0)
	Dose 2	167	90.4 (84.9, 94.4)
PMB1256 (B03)	Before Dose 1	172	3.5 (1.3, 7.4)
	Dose 2	164	74.4 (67.0, 80.9)
PMB866 (B09)	Before Dose 1	171	9.9 (5.9, 15.4)
	Dose 2	166	71.1 (63.6, 77.8)
PMB431 (B15)	Before Dose 1	172	6.4 (3.2, 11.2)
	Dose 2	167	85.0 (78.7, 90.1)
PMB648 (B16)	Before Dose 1	172	8.1 (4.5, 13.3)
	Dose 2	164	77.4 (70.3, 83.6)

Abbreviations: CI=confidence interval; fHbp=factor H binding protein; hSBA=serum bactericidal assay using human complement; LLOQ=lower limit of quantitation.

Note: LLOQ = 1:16 for A06, A12, and A19; 1:8 for A07, A15, A29, B03, B09, B15, and B16.

[a] The evaluable immunogenicity population was used for the analysis.

[b] Study 1057: NCT03135834.

[c] For the second dose, serum was obtained approximately 1 month after vaccination.

[d] N=number of subjects with valid and determinate hSBA titers for the given strain. U.S. subjects constituted approximately 80% of the total subjects evaluated for immunogenicity.

[e] Exact 2-sided confidence interval (Clopper and Pearson) based upon the observed proportion of subjects.

The hSBA responses to each of the primary strains observed in U.S. subjects after the third dose of Trumenba are presented for Study 1009 and Study 1016 in Table 9.

Table 9: Percentages of U.S. Subjects 10 through 25 Years of Age With ≥4-fold Rise in hSBA Titer and Composite Response Following Administration of Trumenba on a 0-, 2-, and 6-Month Schedule for Four Primary Strains (Studies 1009 and 1016)[a,b,c,d]

		Study 1009 (10 through 18 Years of Age)		Study 1016 (18 through 25 Years of Age)	
		N[e]	% (95% CI)[f]	N[e]	% (95% CI)[f]
fHbp Variant[g]					
≥4-Fold Increase					
PMB80 (A22)	Dose 3	587	86.2 (83.1, 88.9)	644	81.1 (77.8, 84.0)
PMB2001 (A56)	Dose 3	526	92.0 (89.4, 94.2)	621	90.7 (88.1, 92.8)
PMB2948 (B24)	Dose 3	585	81.9 (78.5, 84.9)	634	83.9 (80.8, 86.7)
PMB2707 (B44)	Dose 3	555	88.3 (85.3, 90.8)	643	79.3 (76.0, 82.4)
Composite hSBA Response[h]					
	Before Dose 1	507	0.6 (0.1, 1.7)	610	3.3 (2.0, 5.0)
	Dose 3	537	85.7 (82.4, 88.5)	625	82.4 (79.2, 85.3)

Abbreviations: CI=confidence interval; fHbp=factor H binding protein; hSBA=serum bactericidal assay using human complement; LLOQ=lower limit of quantitation; LOD=limit of detection.

Note: LLOQ = 1:16 for A22; 1:8 for A56, B24, and B44.

Note: The 4-fold increase is defined as follows: (1) For subjects with a baseline hSBA titer <1:4, a response is defined as an hSBA titer ≥1:16. (2) For subjects with a baseline hSBA titer ≥ LOD and < LLOQ, a response is defined as an hSBA titer ≥4 times the LLOQ. (3) For subjects with a baseline hSBA titer ≥ LLOQ, a response is defined as an hSBA titer ≥4 times the baseline titer.

Note: Pre-specified criteria for assessment of hSBA responses (4-fold rise in titer to each primary test strain, and titer above LLOQ for all four primary test strains) among U.S. subjects were met in these studies. For Study 1009 pre-specified criteria for the lower bound of the 95% CI for 4-fold rise in titer were set at 75%, 85%, 65%, and 60%, respectively, for A22, A56, B24 and B44, and 75% for the composite hSBA response for all four primary test strains. For Study 1016 pre-specified criteria for the lower bound of the 95% CI for 4-fold rise in titer were set at 55%, 85%, 50%, and 60%, respectively, for A22, A56, B24, and B44, and 60% for the composite hSBA response for all four primary test strains.

[a] Evaluable immunogenicity population.

[b] Study 1009: NCT01830855, and Study 1016: NCT01352845.

[c] Study 1009: Group 1 (0, 2, and 6 months).

[d] Study 1016: Group 1 (0, 2, and 6 months).

[e] For ≥4-fold increase, N=number of subjects with valid and determinate hSBA titers for the given strain at both the specified time point and baseline. For composite hSBA response, N=number of subjects with valid and determinate hSBA results on all 4 strains at the given time point.

[f] Exact 2-sided confidence interval (Clopper-Pearson method) based upon the observed proportion of subjects.

[g] For the third dose, serum was obtained approximately 1 month after vaccination.

[h] Composite response = hSBA ≥ LLOQ for all 4 primary meningococcal B strains.

The hSBA responses after the third dose of Trumenba against a panel of 10 additional strains representing the diversity of meningococcal fHbp types prevalent among strains circulating in the U.S. are presented for Study 1009, and Study 1016 in Table10.

Table 10. Percentages of U.S. Subjects 10 through 25 Years of Age With a hSBA Titer ≥ LLOQ Against 10 Additional Strains Following Administration of Trumenba on a 0-, 2-, and 6-Month Schedule (Study 1009 and Study 1016)[a,b]

fHbp Variant[e]		Study 1009 (10 through 18 Years of Age)		Study 1016 (18 through 25 Years of Age)	
		N[c]	% (95% CI)[d]	N[c]	% (95% CI)[d]
PMB3175 (A29)	Before Dose 1	169	11.2 (6.9, 17.0)	160	23.8 (17.4, 31.1)
	Dose 3	176	98.9 (96.0, 99.9)	162	98.8 (95.6, 99.9)
PMB3010 (A06)	Before Dose 1	178	7.9 (4.4, 12.8)	166	10.8 (6.6, 16.6)
	Dose 3	179	97.8 (94.4, 99.4)	164	89.0 (83.2, 93.4)
PMB3040 (A07)	Before Dose 1	170	37.6 (30.3, 45.4)	165	55.8 (47.8, 63.5)
	Dose 3	178	96.1 (92.1, 98.4)	165	95.2 (90.7, 97.9)
PMB824 (A12)	Before Dose 1	180	5.0 (2.3, 9.3)	166	4.8 (2.1, 9.3)
	Dose 3	180	76.1 (69.2, 82.1)	165	66.7 (58.9, 73.8)
PMB1672 (A15)	Before Dose 1	170	15.9 (10.7, 22.3)	159	30.2 (23.2, 38.0)
	Dose 3	166	86.7 (80.6, 91.5)	159	89.9 (84.2, 94.1)
PMB1989 (A19)	Before Dose 1	174	5.7 (2.8, 10.3)	158	23.4 (17.1, 30.8)
	Dose 3	173	91.9 (86.8, 95.5)	163	94.5 (89.8, 97.4)
PMB1256 (B03)	Before Dose 1	183	2.2 (0.6, 5.5)	164	5.5 (2.5, 10.2)
	Dose 3	181	92.3 (87.4, 95.7)	161	84.5 (77.9, 89.7)
PMB866 (B09)	Before Dose 1	180	12.2 (7.8, 17.9)	165	13.9 (9.0, 20.2)
	Dose 3	182	85.7 (79.8, 90.5)	162	72.2 (64.7, 79.0)
PMB431 (B15)	Before Dose 1	180	27.8 (21.4, 34.9)	163	33.1 (26.0, 40.9)
	Dose 3	183	97.3 (93.7, 99.1)	163	95.7 (91.4, 98.3)
PMB648 (B16)	Before Dose 1	180	6.7 (3.5, 11.4)	161	11.8 (7.3, 17.8)
	Dose 3	180	83.9 (77.7, 88.9)	159	72.3 (64.7, 79.1)

Abbreviations: CI=confidence interval; fHbp=factor H binding protein; hSBA=serum bactericidal assay using human complement; LLOQ=lower limit of quantitation.

Note: LLOQ = 1:16 for A06, A12, and A19; 1:8 for A07, A15, A29, B03, B09, B15, and B16.

[a] The evaluable immunogenicity population was used for the analysis.

[b] Study 1009: NCT01830855 and Study 1016 NCT01352845.

[c] N=number of subjects with valid and determinate hSBA titers for the given strain.

[d] Exact 2-sided confidence interval (Clopper and Pearson) based upon the observed proportion of subjects.

[e] For the third dose, serum was obtained approximately 1 month after vaccination.

In Study 1012, Trumenba was administered according to different schedules, including Group 1 (0, 1, and 6 months), Group 2 (0, 2, and 6 months) and Group 3 (0 and 6 months). The hSBA responses observed after the second dose in Groups 1, 2, and 3 and completion of the three-dose series in Group 1 and 2 are presented in Table 11.

Table 11: Percentages of European Subjects 11 through 18 Years of Age With a ≥4-Fold Increase in hSBA Titer and Composite Response[a,b] (Study 1012)

	Group 1	**Group 2**	**Group 3**
	3-Dose Schedule (0, 1, and 6 Months)[c]	**3-Dose Schedule (0, 2, and 6 Months)[d]**	**2-Dose Schedule (0 and 6 Months)[e]**
fHbp Variant[f]	**% (95% CI)[g]**	**% (95% CI)[g]**	**% (95% CI)[g]**
≥4-Fold Increase			
PMB80 (A22)			
Dose 2	58.8 (51.4, 66.0)	72.5 (66.4, 78.0)	82.3 (76.3, 87.3)
Dose 3	77.6 (70.9, 83.4)	87.7 (81.6, 92.3)	NA
PMB2001 (A56)			
Dose 2	87.8 (82.2, 92.2)	90.7 (86.2, 94.1)	90.1 (85.1, 93.8)
Dose 3	91.2 (86.1, 94.9)	93.8 (88.8, 97.0)	NA
PMB2948 (B24)			
Dose 2	51.1 (43.6, 58.5)	54.2 (47.7, 60.7)	64.5 (57.4, 71.1)
Dose 3	74.1 (67.1, 80.2)	78.3 (71.1, 84.4)	NA
PMB2707 (B44)			
Dose 2	48.1 (40.7, 55.6)	53.4 (46.8, 59.9)	66.0 (58.9, 72.6)
Dose 3	80.9 (74.5, 86.2)	78.6 (71.4, 84.7)	NA
Composite Response[h]			
Before Dose 1	4.6 (2.0, 8.8)	2.2 (0.7, 5.0)	1.5 (0.3, 4.4)
Dose 2	52.0 (44.3, 59.7)	52.0 (45.3, 58.6)	72.9 (65.9, 79.1)
Dose 3	80.3 (73.7, 85.9)	81.8 (74.9, 87.4)	NA

Abbreviations: CI=confidence interval; fHbp=factor H binding protein; hSBA=serum bactericidal assay using human complement; LLOQ=lower limit of quantitation; NA=not applicable.

Note: LLOQ = 1:16 for PMB80 (A22) and 1:8 for PMB2001 (A56), PMB2948 (B24), and PMB2707 (B44).

Note: The ≥4-fold increase is defined as follows: (1) For subjects with a baseline hSBA titer <1:4, a ≥4-fold increase was defined as an hSBA titer ≥1:16. (2) For subjects with a baseline hSBA titer ≥ LOD and < LLOQ, a response is defined as an hSBA titer ≥4 times the LLOQ. (3) For subjects with a baseline hSBA titer ≥ LLOQ, a response is defined as an hSBA titer ≥4 times the baseline titer.

[a] Per-schedule Evaluable populations. Dose 2 data include subjects who received two doses, irrespective of whether they received the third dose.

[b] Study1012: NCT01299480.

[c] Group 1 (0, 1, and 6 months). The denominators ranged from 173 to 187 after Dose 2 and 178 to 188 after Dose 3, depending on the strain.

[d] Group 2 (0, 2, and 6 months). The denominators ranged from 229 to 240 after Dose 2 and 159 to 162 after Dose 3, depending on the strain.

[e] Group 3 (0 and 6 months). The denominators ranged from 188 to 203 after Dose 2, depending on the strain.

[f] For the second and third doses, serum was obtained approximately 1 month after vaccination.

[g] Exact 2-sided confidence interval (Clopper and Pearson) based upon the observed proportion of subjects.

[h] Composite response = hSBA ≥LLOQ for all 4 primary meningococcal B strains.

14.2 Concomitant Vaccine Administration

Study B1971011 (Study 1011) evaluated the immunogenicity of concomitantly administered Trumenba and Human Papillomavirus Quadrivalent (Types 6, 11, 16, and 18) Vaccine, Recombinant (HPV4) (Merck & Co, Inc.). U.S. subjects 11 through 17 years of age were randomized into three groups: Group 1 received Trumenba and HPV4 (N=992), Group 2 received Trumenba and saline (N=990), and Group 3 received saline and HPV4 (N=501). All vaccines were administered according to a 0-, 2- and 6-month schedule. Immune responses were evaluated by comparisons of geometric mean titer [GMT] for each HPV type at 1 month after the third HPV4 vaccination (Group 1 vs. Group 3), and hSBA GMTs using two meningococcal serogroup B strains [variants A22 and B24] 1 month after the third Trumenba vaccination (Group 1 vs. Group 2). The noninferiority criteria for the comparisons of GMTs [lower limit of the 2-sided 95% confidence interval (CI) of the GMT ratio (Group 1/Group 3 for HPV and Group 1/Group 2 for meningococcal serogroup B strains) >0.67] were met for three HPV types (6, 11 and 16) and for the meningococcal serogroup B strains tested. For HPV-18, the lower bound of the 95% CI for the GMT ratio was 0.62 at 1 month after the third HPV4 vaccination

Study B1971015 (Study 1015) evaluated the immunogenicity of concomitantly administered Trumenba and Meningococcal (Serogroups A, C, Y and W-135) Polysaccharide Diphtheria Toxoid Conjugate Vaccine (MenACWY) (Sanofi Pasteur Inc.) and Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap) (Sanofi Pasteur Ltd.) vaccines. U.S. subjects 10 through 12 years of age were randomized into three groups: Group 1 received Trumenba at 0, 2, and 6 months, and MenACWY and Tdap were coadministered with the first Trumenba dose (N=883). Group 2 received saline at 0, 2 and 6 months, and MenACWY and Tdap were coadministered with the first saline injection (N=870). Group 3 received Trumenba at 0, 2 and 6 months, and saline was coadministered with the first Trumenba dose (N=875). Immune responses were evaluated by comparisons of GMTs for each of the MenACWY and Tdap antigens 1 month after the first Trumenba vaccination, and hSBA GMTs using two meningococcal serogroup B strains [variants A22 and B24] 1 month after the third Trumenba vaccination. The noninferiority criteria for the comparisons of GMTs [lower limit of the 2-sided 95% CI of the GMT ratio (Group 1/Group 3 for meningococcal serogroup B strains and Group 1/Group 2 for MenACWY and Tdap) >0.67] were met for all antigens.

15 REFERENCES

1. Wang X, et al. Prevalence and genetic diversity of candidate vaccine antigens among invasive *Neisseria meningitidis* isolates in the U.S. Vaccine 2011; 29:4739-4744.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 How Supplied

Trumenba is supplied in the following strengths and package configurations:

Prefilled Syringe, 1 Dose (10 per package) – NDC 0005-0100-10.

Prefilled Syringe, 1 Dose (5 per package) – NDC 0005-0100-05.

Prefilled Syringe, 1 Dose (1 per package) – NDC 0005-0100-02 (This Package Not for Sale).

After shipping, Trumenba may arrive at temperatures between 2°C to 25°C (36°F to 77°F).

The tip cap and rubber plunger of the prefilled syringe are not made with natural rubber latex.

16.2 Storage and Handling

Upon receipt, store refrigerated at 2°C to 8°C (36°F to 46°F).

Store syringes in the refrigerator horizontally (laying flat on the shelf) to minimize the re-dispersion time.

Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

Prior to administration of this vaccine, the healthcare professional should inform the individual, parent, guardian, or other responsible adult of the following:

- The importance of completing the immunization series.
- Report any suspected adverse reactions to a healthcare professional.

Provide the Vaccine Information Statements, which are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by
Wyeth Pharmaceuticals LLC
A subsidiary of Pfizer Inc, Philadelphia, PA 19101

U.S. Govt. License No. 3

LAB-0722-9.4

Pneumococcal (PCV or PPSV)

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PREVNAR 13 safely and effectively. See full prescribing information for PREVNAR 13.

PREVNAR 13 (Pneumococcal 13-valent Conjugate Vaccine [Diphtheria CRM_{197} Protein])
Suspension for intramuscular injection
Initial US Approval: 2010

----------RECENT MAJOR CHANGES----------

Indications and Usage (1.3)		7/2016
Vaccination Schedule for Children Previously Vaccinated With Prevnar Pneumococcal 7-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein) (2.5)	Removal	3/2017
Vaccination Schedule for Adults 18 Years of Age and Older (2.6)		7/2016
Contraindications (4)		7/2016

---------- INDICATIONS AND USAGE ----------
In children 6 weeks through 5 years of age (prior to the 6th birthday), Prevnar 13 is indicated for:

- active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. (1.1)
- active immunization for the prevention of otitis media caused by *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. No otitis media efficacy data are available for serotypes 1, 3, 5, 6A, 7F, and 19A. (1.1)

In children 6 years through 17 years of age (prior to the 18th birthday), Prevnar 13 is indicated for:

- active immunization for the prevention of invasive disease caused by *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. (1.2)

In adults 18 years of age and older, Prevnar 13 is indicated for:

- active immunization for the prevention of pneumonia and invasive disease caused by *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. (1.3)

Limitations of Prevnar 13 Use and Effectiveness

- Prevnar 13 does not protect against disease caused by *S. pneumoniae* serotypes that are not in the vaccine. (1.4)

---------- DOSAGE AND ADMINISTRATION ----------
Children 6 weeks through 5 years: The four-dose immunization series consists of a 0.5 mL intramuscular injection administered at 2, 4, 6, and 12-15 months of age. (2.3)
Children 6 through 17 years of age: a single dose. (2.5)
Adults 18 years and older: a single dose. (2.6)

----------DOSAGE FORMS AND STRENGTHS ----------
0.5 mL suspension for intramuscular injection, supplied in a single-dose prefilled syringe. (3)

----------CONTRAINDICATIONS----------
Severe allergic reaction (e.g., anaphylaxis) to any component of Prevnar 13 or any diphtheria toxoid-containing vaccine. (4)

---------- WARNINGS AND PRECAUTIONS----------
Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including Prevnar 13, to infants born prematurely should be based on consideration of the individual infant's medical status, and the potential benefits and possible risks of vaccination. (5.3)

---------- --ADVERSE REACTIONS----------

- In infants and toddlers vaccinated at 2, 4, 6, and 12-15 months of age in US clinical trials, the most commonly reported solicited adverse reactions (>5%) were irritability (>70%), injection site tenderness (>50%), decreased appetite (>40%), decreased sleep (>40%), increased sleep (>40%), fever (>20%), injection site redness (>20%), and injection site swelling (>20%). (6.1)
- In children aged 5 through 17 years, the most commonly reported solicited adverse reactions (>5%) were injection site tenderness (>80%), injection site redness (>30%), injection site swelling (>30%), irritability (>20%), decreased appetite (>20%), increased sleep (>20%), fever (>5%), and decreased sleep (>5%). (6.1)
- In adults aged 18 years and older, the most commonly reported solicited adverse reactions (>5%) were pain at the injection site (>50%), fatigue (>30%), headache (>20%), muscle pain (>20%), joint pain (>10%), decreased appetite (>10%), injection site redness (>10%), injection site swelling (>10%), limitation of arm movement (>10%), vomiting (>5%), fever (>5%), chills (>5%), and rash (>5%). (6.2)

To report SUSPECTED ADVERSE REACTIONS, contact Wyeth Pharmaceuticals Inc. at 1-800-438-1985 or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

---------- USE IN SPECIFIC POPULATIONS ----------
Pediatric Use: Safety and effectiveness of Prevnar 13 in children below the age of 6 weeks have not been established. (8.4)

See 17 for PATIENT COUNSELING INFORMATION

Revised: 8/2017

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Children 6 Weeks Through 5 Years of Age

In children 6 weeks through 5 years of age (prior to the 6th birthday), Prevnar 13® is indicated for:

- active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.
- active immunization for the prevention of otitis media caused by *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. No otitis media efficacy data are available for serotypes 1, 3, 5, 6A, 7F, and 19A.

1.2 Children 6 Years Through 17 Years of Age

In children 6 years through 17 years of age (prior to the 18th birthday), Prevnar 13 is indicated for:

- active immunization for the prevention of invasive disease caused by *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.

1.3 Adults 18 Years of Age and Older

In adults 18 years of age and older, Prevnar 13 is indicated for:

- active immunization for the prevention of pneumonia and invasive disease caused by *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F.

1.4 Limitations of Prevnar 13 Use and Effectiveness

- Prevnar 13 does not protect against disease caused by *S. pneumoniae* serotypes that are not in the vaccine.

2 DOSAGE AND ADMINISTRATION

2.1 Preparation for Administration

Since this product is a suspension containing an adjuvant, shake vigorously immediately prior to use to obtain a homogenous, white suspension in the vaccine container. Do not use the vaccine if it cannot be resuspended. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration *[see Description (11)]*. This product should not be used if particulate matter or discoloration is found.

Do not mix Prevnar 13 with other vaccines/products in the same syringe.

2.2 Administration Information

For intramuscular injection only.

Each 0.5 mL dose is to be injected intramuscularly using a sterile needle attached to the supplied prefilled syringe. The preferred sites for injection are the anterolateral aspect of the thigh in infants and the deltoid muscle of the upper arm in toddlers, children and adults. The vaccine should not be injected in the gluteal area or areas where there may be a major nerve trunk and/or blood vessel.

2.3 Vaccination Schedule for Infants and Toddlers

Prevnar 13 is to be administered as a four-dose series at 2, 4, 6, and 12-15 months of age.

Table 1: Vaccination Schedule for Infants and Toddlers

Dose	Dose 1[a,b]	Dose 2[b]	Dose 3[b]	Dose 4[c]
Age at Dose	2 months	4 months	6 months	12-15 months

[a] Dose 1 may be given as early as 6 weeks of age.
[b] The recommended dosing interval is 4 to 8 weeks.
[c] The fourth dose should be administered at approximately 12-15 months of age, and at least 2 months after the third dose.

2.4 Vaccination Schedule for Unvaccinated Children 7 Months Through 5 Years of Age

For children 7 months through 5 years of age who have not received Prevnar® or Prevnar 13, the catch-up schedule in Table 2 applies:

Table 2: Vaccination Schedule for Unvaccinated Children 7 Months of Age Through 5 Years of Age

Age at First Dose	Total Number of 0.5 mL Doses
7-11 months of age	3[a]
12-23 months of age	2[b]
24 months through 5 years of age (prior to the 6th birthday)	1

[a] The first 2 doses at least 4 weeks apart; third dose after the one-year birthday, separated from the second dose by at least 2 months.
[b] Two doses at least 2 months apart.

The immune responses induced by this catch-up schedule may result in lower antibody concentrations for some serotypes, compared to antibody concentrations following 4 doses of Prevnar 13 (given at 2, 4, 6, and 12-15 months). In children 24 months through 5 years of age, lower antibody concentrations were observed for some serotypes, compared to antibody concentrations following 3 doses of Prevnar 13 (given at 2, 4, and 6 months).

2.5 Vaccination Schedule for Children 6 Years Through 17 Years of Age

In children 6 years through 17 years of age, Prevnar 13 is administered as single dose. If Prevnar was previously administered, then at least 8 weeks should elapse before receiving Prevnar 13.

2.6 Vaccination Schedule for Adults 18 Years of Age and Older

Prevnar 13 is administered as a single dose.

3 DOSAGE FORMS AND STRENGTHS

Prevnar 13 is a suspension for intramuscular injection available in 0.5 mL single-dose prefilled syringes.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) to any component of Prevnar 13 or any diphtheria toxoid-containing vaccine *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Allergic Reactions

Epinephrine and other appropriate agents used to manage immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur following administration of Prevnar 13.

5.2 Altered Immunocompetence

Individuals with altered immunocompetence, including those at higher risk for invasive pneumococcal disease (e.g., individuals with congenital or acquired splenic dysfunction, HIV infection, malignancy, hematopoietic stem cell transplant, nephrotic syndrome), may have reduced antibody responses to immunization with Prevnar 13 *[see Use in Specific Populations (8.6)]*.

5.3 Apnea in Premature Infants

Apnea following intramuscular vaccination has been observed in some infants born prematurely. Decisions about when to administer an intramuscular vaccine, including Prevnar 13, to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

6 ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse-reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

6.1 Clinical Trials Experience With Prevnar 13 in Children 6 Weeks Through 17 Years of Age

The safety of Prevnar 13 was evaluated in 13 clinical trials in which 4,729 infants (6 weeks through 11 months of age) and toddlers (12 months through 15 months of age) received at least one dose of Prevnar 13 and 2,760 infants and toddlers received at least one dose of Prevnar active control. Safety data for the first three doses are available for all 13 infant studies; dose 4 data are available for 10 studies; and data for the 6-month follow-up are available for 7 studies. The vaccination schedule and concomitant vaccinations used in these infant trials were consistent with country-specific recommendations and local clinical practice. There were no substantive differences in demographic characteristics between the vaccine groups. By race, 84.0% of subjects were White, 6.0% were Black or African-American, 5.8% were Asian and 3.8% were of 'Other' race (most of these being biracial). Overall, 52.3% of subjects were male infants.

Three studies in the US (Studies 1, 2 and 3)[1,2,3] evaluated the safety of Prevnar 13 when administered concomitantly with routine US pediatric vaccinations at 2, 4, 6, and 12-15 months of age. Solicited local and systemic adverse reactions were recorded daily by parents/guardians using an electronic diary for 7 consecutive days following each vaccination. For unsolicited adverse events, study subjects were monitored from administration of the first dose until one month after the infant series, and for one month after the administration of the toddler dose. Information regarding unsolicited and serious adverse events, newly diagnosed chronic medical conditions, and hospitalizations since the last visit were collected during the clinic visit for the fourth-study dose and during a scripted telephone interview 6 months after the fourth-study dose. Serious adverse events were also collected throughout the study period. Overall, the safety data show a similar proportion of Prevnar 13 and Prevnar subjects reporting serious adverse events. Among US study subjects, a similar proportion of Prevnar 13 and Prevnar recipients reported solicited local and systemic adverse reactions as well as unsolicited adverse events.

Serious Adverse Events in All Infant and Toddler Clinical Studies

Serious adverse events were collected throughout the study period for all 13 clinical trials. This reporting period is longer than the 30-day post-vaccination period used in some vaccine trials. The longer reporting period may have resulted in serious adverse events being reported in a higher percentage of subjects than for other vaccines. Serious adverse events reported following vaccination in infants and toddlers occurred in 8.2% among Prevnar 13 recipients and 7.2% among Prevnar recipients. Serious adverse events observed during different study periods for Prevnar 13 and Prevnar respectively were: 1) 3.7% and 3.5% from dose 1 to the blood draw approximately 1 month after the infant series; 2) 3.6% and 2.7% from the blood draw after the infant series to the toddler dose; 3) 0.9% and 0.8% from the toddler dose to the blood draw approximately 1 month after the toddler dose and 4) 2.5% and 2.8% during the 6 month follow-up period after the last dose.

The most commonly reported serious adverse events were in the 'Infections and infestations' system organ class including bronchiolitis (0.9%, 1.1%), gastroenteritis, (0.9%, 0.9%), and pneumonia (0.9%, 0.5%) for Prevnar 13 and Prevnar respectively.

There were 3 (0.063%) deaths among Prevnar 13 recipients, and 1 (0.036%) death in Prevnar recipients, all as a result of sudden infant death syndrome (SIDS). These SIDS rates are consistent with published age specific background rates of SIDS from the year 2000.

Among 6,839 subjects who received at least 1 dose of Prevnar 13 in clinical trials conducted globally, there was 1 hypotonic-hyporesponsive episode adverse reaction reported (0.015%). Among 4,204 subjects who received at least 1 dose of Prevnar in clinical trials conducted globally, there were 3 hypotonic-hyporesponsive episode adverse reactions reported (0.071%). All 4 events occurred in a single clinical trial in Brazil in which subjects received whole cell pertussis vaccine at the same time as Prevnar 13 or Prevnar.

Solicited Adverse Reactions in the Three US Infant and Toddler Studies

A total of 1,907 subjects received at least 1 dose of Prevnar 13 and 701 subjects received at least 1 dose of Prevnar in the three US studies (Studies 1, 2 and 3)[1,2,3]. Most subjects were White (77.3%), 14.2% were Black or African-American, and 1.7% were Asian; 79.1% of subjects were non-Hispanic and non-Latino and 14.6% were Hispanic or Latino. Overall, 53.6% of subjects were male infants.

The incidence and severity of solicited adverse reactions that occurred within 7 days following each dose of Prevnar 13 or Prevnar administered to US infants and toddlers are shown in Tables 3 and 4.

Table 3: Percentage of US Infant and Toddler Subjects Reporting Solicited Local Reactions at the Prevnar 13 or Prevnar Injection Sites Within 7 Days After Each Vaccination at 2, 4, 6, and 12-15 Months of Age[a]

	Dose 1		Dose 2		Dose 3		Dose 4	
Graded Local Reaction	**Prevnar 13** (N^b=1375-1612) %	**Prevnar** (N^b=516-606) %	**Prevnar 13** (N^b=1069-1331) %	**Prevnar** (N^b=405-510) %	**Prevnar 13** (N^b=998-1206) %	**Prevnar** (N^b=348-446) %	**Prevnar 13** (N^b=874-1060) %	**Prevnar** (N^b=283-379) %
Redness[c]								
Any	24.3	26.0	33.3	29.7	37.1	36.6	42.3	45.5
Mild	23.1	25.2	31.9	28.7	35.3	35.3	39.5	42.7
Moderate	2.2	1.5	2.7	2.2	4.6	5.1	9.6	13.4[d]
Severe	0	0	0	0	0	0	0	0
Swelling[c]								
Any	20.1	20.7	25.2	22.5	26.8	28.4	31.6	36.0[d]
Mild	17.2	18.7	23.8	20.5	25.2	27.5	29.4	33.8
Moderate	4.9	3.9	3.7	4.9	3.8	5.8	8.3	11.2[d]
Severe	0	0	0.1	0	0	0	0	0
Tenderness								
Any	62.5	64.5	64.7	62.9	59.2	60.8	57.8	62.5
Interferes with limb movement	10.4	9.6	9.0	10.5	8.4	9.0	6.9	5.7

[a] Data are from three primary US safety studies (the US Phase 2 infant study [National Clinical Trial (NCT) number NCT00205803] Study 1, the US noninferiority study [NCT00373958] Study 2, and the US lot consistency study [NCT00444457] Study 3). All infants received concomitant routine infant immunizations. Concomitant vaccines and pneumococcal conjugate vaccines were administered in different limbs.

[b] Number of subjects reporting Yes for at least 1 day or No for all days.

[c] Diameters were measured in caliper units of whole numbers from 1 to 14 or 14+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of induration and erythema were then characterized as Mild (0.5-2.0 cm), Moderate (2.5-7.0 cm), or Severe (>7.0 cm).

[d] Statistically significant difference $p < 0.05$. No adjustments for multiplicity.

Table 4: Percentage of US Infant and Toddler Subjects Reporting Solicited Systemic Adverse Reactions Within 7 Days After Each Vaccination at 2, 4, 6, and 12-15 Months of Age[a,b]

	Dose 1		Dose 2		Dose 3		Dose 4	
Graded Systemic Events	**Prevnar 13** (N[a]=1360 - 1707) %	**Prevnar** (N[a]=497-640) %	**Prevnar 13** (N[a]=1084-1469) %	**Prevnar** (N[a]=409-555) %	**Prevnar 13** (N[a]=997-1361) %	**Prevnar** (N[a]=354-521) %	**Prevnar 13** (N[a]=850-1227) %	**Prevnar** (N[a]=278-436) %
Fever[c]								
Any	24.3	22.1	36.5	32.8	30.3	31.6	31.9	30.6
Mild	23.6	21.7	34.9	31.6	29.1	30.2	30.3	30.0
Moderate	1.1	0.6	3.4	2.8	4.2	3.3	4.4	4.6
Severe	0.1	0.2	0.1	0.3	0.1	0.7	1.0	0
Decreased appetite	48.3	43.6	47.8	43.6	47.6	47.6	51.0	49.4
Irritability	85.6	83.6	84.8	80.4	79.8	80.8	80.4	77.8
Increased sleep	71.5	71.5	66.6	63.4	57.7	55.2	48.7	55.1
Decreased sleep	42.5	40.6	45.6	43.7	46.5	47.7	45.3	40.3

[a] Number of subjects reporting Yes for at least 1 day or No for all days.
[b] Data are from three primary US safety studies (the US Phase 2 infant study [NCT00205803] Study 1, the US noninferiority study [NCT00373958] Study 2, and the US lot consistency study [NCT00444457] Study 3). All infants received concomitant routine infant immunizations. Concomitant vaccines and pneumococcal conjugate vaccines were administered in different limbs.
[c] Fever gradings: Mild (≥38°C but ≤39°C), Moderate (>39°C but ≤40°C), and Severe (>40°C). No other systemic event other than fever was graded. Parents reported the use of antipyretic medication to treat or prevent symptoms in 62 to 75% of subjects after any of the 4 doses. There were no statistical differences in frequencies of adverse reactions reported between the Prevnar 13 and Prevnar groups.

The incidence rates of any fever (≥38.0°C) were similar on days 1 and 2 following each dose of Prevnar 13 compared to after each dose of Prevnar administered to US infants and toddlers (day 1 = day of vaccination). After dose 1, fever was reported in 11.0-12.7% on day 1 and 6.4-6.8% on day 2. After dose 2, fever was reported in 12.3-13.1% on day 1 and 12.5-12.8% on day 2. After dose 3, fever was reported in 8.0-9.6% on day 1 and 9.1-10.5% on day 2. And after dose 4, fever was reported in 6.3-6.4% on day 1 and 7.3-9.7% on day 2.

Unsolicited Adverse Reactions in the Three US Infant and Toddler Safety Studies

The following were determined to be adverse drug reactions based on experience with Prevnar 13 in clinical trials.

Reactions occurring in greater than 1% of infants and toddlers: diarrhea, vomiting, and rash.

Reactions occurring in less than 1% of infants and toddlers: crying, hypersensitivity reaction (including face edema, dyspnea, and bronchospasm), seizures (including febrile seizures), and urticaria or urticaria-like rash.

Safety Assessments in the Catch-Up Studies in Infants and Children Through 5 Years of Age

In a catch-up study[4] conducted in Poland (Study 4), 354 children (7 months through 5 years of age) receiving at least one dose of Prevnar 13 were also monitored for safety. All subjects in this study were White and non-Hispanic. Overall, 49.6% of subjects were male infants. The incidence and severity of solicited adverse reactions that occurred within 4 days following each dose of Prevnar 13 administered to pneumococcal-vaccine naïve children 7 months through 5 years of age are shown in Tables 5 and 6.

Table 5: Percentage of Subjects 7 Months Through 5 Years of Age Reporting Solicited Local Reactions Within 4 Days After Each Catch-Up Prevnar 13 Vaccination[a]

	7 through 11 months			12 through 23 months		24 months through 5 years
Graded Local Reaction	**Dose 1** N[b]=86 %	**Dose 2** N[b]=86-87 %	**Dose 3** N[b]=78-82 %	**Dose 1** N[b]=108-110 %	**Dose 2** N[b]=98-106 %	**Dose 1** N[b]=147-149 %
Redness[c]						
Any	48.8	46.0	37.8	70.0	54.7	50.0
Mild	41.9	40.2	31.3	55.5	44.7	37.4
Moderate	16.3	9.3	12.5	38.2	25.5	25.7
Severe	0.0	0.0	0.0	0.0	0.0	0.0
Swelling[c]						
Any	36.0	32.2	25.0	44.5	41.0	36.9
Mild	32.6	28.7	20.5	36.7	36.2	28.2
Moderate	11.6	14.0	11.3	24.8	12.1	20.3
Severe	0.0	0.0	0.0	0.0	0.0	0.0
Tenderness						
Any	15.1	15.1	15.2	33.3	43.7	42.3
Interferes with limb movement	1.2	3.5	6.4	0.0	4.1	4.1

[a] Study conducted in Poland (NCT00452452) Study 4.
[b] Number of subjects reporting Yes for at least 1 day or No for all days.
[c] Diameters were measured in caliper units of whole numbers from 1 to 14 or 14+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of redness and swelling were then characterized as Mild (0.5-2.0 cm), Moderate (2.5-7.0 cm), or Severe (>7.0 cm).

Table 6: Percentage of Subjects 7 Months Through 5 Years of Age Reporting Solicited Systemic Adverse Reactions Within 4 Days After Each Catch-Up Prevnar 13 Vaccination[a]

	7 through 11 months			12 through 23 months		24 months through 5 years
Systemic Reaction	**Dose 1** N[b]=86-87 %	**Dose 2** N[b]=86-87 %	**Dose 3** N[b]=78-81 %	**Dose 1** N[b]=108 %	**Dose 2** N[b]=98-100 %	**Dose 1** N[b]=147-148 %
Fever[c]						
Mild	3.4	8.1	5.1	3.7	5.1	0.7
Moderate	1.2	2.3	1.3	0.9	0.0	0.7
Severe	0.0	0.0	0.0	0.0	0.0	0.0
Decreased appetite	19.5	17.2	17.5	22.2	25.5	16.3
Irritability	24.1	34.5	24.7	30.6	34.0	14.3
Increased sleep	9.2	9.3	2.6	13.0	10.1	11.6
Decreased sleep	24.1	18.4	15.0	19.4	20.4	6.8

[a] Study conducted in Poland (NCT00452452) Study 4.
[b] Number of subjects reporting Yes for at least 1 day or No for all days.
[c] Fever gradings: Mild (≥38ºC but ≤39ºC), Moderate (>39ºC but ≤40ºC), and Severe (>40ºC). No other systemic event other than fever was graded.

A US study[5] (Study 5) evaluated the use of Prevnar 13 in children previously immunized with Prevnar. In this open label trial, 596 healthy children 15 through 59 months of age previously vaccinated with at least 3 doses of Prevnar, received 1 or 2 doses of Prevnar 13. Children 15 months through 23 months of age (group 1) received 2 doses, and children 24 months through 59 months of age (group 2) received one dose. Most subjects were White (74.3%), 14.9% were Black or African-American, and 1.2% were Asian; 89.3% of subjects were non-Hispanic and non-Latino and 10.7% were Hispanic or Latino. Overall, 52.2% of subjects were male.

The incidence and severity of solicited adverse reactions that occurred within 7 days following one dose of Prevnar 13 administered to children 15 months through 59 months of age are shown in Tables 7 and 8.

Table 7: Percentage of Subjects 15 Months Through 59 Months of Age, Previously Vaccinated With 3 or 4 Prior Infant Doses of Prevnar, Reporting Solicited Local Reactions Within 7 Days After One Supplemental Prevnar 13 Vaccination[a]

	15 months through 23 months[b]		24 months through 59 months[c]
Graded Local Reaction	**1 dose Prevnar 13 3 prior Prevnar doses** N[d]=67-72 %	**1 dose Prevnar 13 4 prior Prevnar doses** N[d]=154-184 %	**1 dose Prevnar 13 3 or 4 prior Prevnar doses** N[d]=209-238 %
Redness[e]			
Any	26.4	28.2	35.4
Mild	18.8	24.3	31.1
Moderate	11.4	7.5	12.1
Severe	1.5	0.0	0.0
Swelling[e]			
Any	23.9	19.6	20.7
Mild	18.6	16.4	17.2
Moderate	8.8	8.1	7.5
Severe	0.0	0.0	0.0
Tenderness			
Any	48.6	47.3	62.6
Interferes with limb movement	5.9	6.4	10.7

[a] Study conducted in US NCT00761631 (Study 5).
[b] Dose 2 data not shown.
[c] The data for this age group are only represented as a single result as 95% of children received 4 doses of Prevnar prior to enrollment.
[d] Number of subjects reporting Yes for at least 1 day or No for all days.
[e] Diameters were measured in caliper units of whole numbers from 1 to 14 or 14+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of redness and swelling were then characterized as Mild (0.5-2.0 cm), Moderate (2.5-7.0 cm), or Severe (>7.0 cm).

Table 8: Percentage of Subjects 15 Months Through 59 Months of Age, Previously Vaccinated With 3 or 4 Prior Infant Prevnar Doses, Reporting Solicited Systemic Adverse Reactions Within 7 Days After One Supplemental Prevnar 13 Vaccination[a]

	15 through 23 months[b]		24 months through 59 months[c]
Systemic Reaction	**1 dose Prevnar 13 3 prior Prevnar doses** N[d]=66-75 %	**1 dose Prevnar 13 4 prior Prevnar doses** N[d]=154-189 %	**1 dose Prevnar 13 3 or 4 prior Prevnar doses** N[d]=209-236 %
Fever[e]			
Any	19.1	19.9	8.1
Mild	16.2	17.4	7.6
Moderate	6.1	3.9	1.9
Severe	0.0	0.0	0.5
Decreased appetite	44.4	39.3	28.1
Irritability	73.3	65.1	45.8
Increased sleep	35.2	35.3	18.8
Decreased sleep	25.0	29.7	14.8

[a] Study conducted in US NCT00761631 (Study 5).
[b] Dose 2 data not shown.
[c] The data for this age group are only represented as a single result as 95% of children received 4 doses of Prevnar prior to enrollment.
[d] Number of subjects reporting Yes for at least 1 day or No for all days.
[e] Fever gradings: Mild (≥38°C but ≤39°C), Moderate (>39°C but ≤40°C), and Severe (>40°C). No other systemic event other than fever was graded.

Clinical Trials Experience With Prevnar 13 in Children 5 Through 17 Years of Age

In a US study[5] (Study 5), the safety of Prevnar 13 was evaluated in children 5 through 9 years of age previously immunized with at least one dose of Prevnar, and in children 10 through 17 years of age with no prior pneumococcal vaccination. In this open label trial, 592 children, including those with asthma, received a single dose of Prevnar 13. The percentage of children 5 through 9 years of age who received 3 and 4 prior doses of Prevnar was 29.1% and 54.5% respectively.

Most subjects were White (72.8%), 21.8% were Black or African-American, and 1.5% were Asian; 91.4% of subjects were non-Hispanic and non-Latino and 8.6% were Hispanic or Latino. Overall, 51.2% of subjects were male.

The incidence and severity of solicited adverse reactions that occurred within 7 days following one dose of Prevnar 13 administered to children 5 through 17 years of age are shown in Tables 9 and 10.

Table 9: Percentage of Subjects 5 Through 17 Years of Age, Reporting Solicited Local Reactions Within 7 Days After Prevnar 13 Vaccination [a]

	Vaccine Group (as Administered)					
	Prevnar 13 (5 Through 9 Years)			Prevnar 13 (10 Through 17 Years)		
Local Reaction	**N**[b]	**n**[c]	**%**	**N**[b]	**n**[c]	**%**
Redness						
Any	233	100	42.9	232	70	30.2
Mild[d]	226	63	27.9	226	48	21.2
Moderate[d]	218	48	22.0	221	31	14.0
Severe[d]	212	7	3.3	213	4	1.9
Swelling						
Any	226	85	37.6	233	86	36.9
Mild[d]	220	48	21.8	221	50	22.6
Moderate[d]	219	48	21.9	226	48	21.2
Severe[d]	211	7	3.3	214	4	1.9
Tenderness						
Any	265	230	86.8	283	252	89.0
Significant[e]	221	43	19.5	242	106	43.8

[a] Study conducted in US NCT00761631 (Study 5).
[b] N = number of subjects reporting Yes for at least 1 day or No for all days.
[c] n = Number of subjects reporting the specific characteristic.
[d] Mild, 0.5 – 2.0 cm; moderate, 2.5 – 7.0 cm; severe, >7.0 cm.
[e] Significant = present and interfered with limb movement.

Table 10: Percentage of Subjects 5 Through 17 Years of Age, Reporting Solicited Systemic Adverse Reactions Within 7 Days After Prevnar 13 Vaccination[a]

	Vaccine Group (as Administered)					
	Prevnar 13 (5 Through 9 Years)			Prevnar 13 (10 Through 17 Years)		
Systemic Event	N[b]	n[c]	%	N[b]	n[c]	%
Any fever ≥38°C	214	13	6.1	214	12	5.6
Mild[d]	212	9	4.2	214	11	5.1
Moderate[d]	212	5	2.4	212	1	0.5
Severe[d]	210	1	0.5	212	1	0.5
Decreased appetite	227	52	22.9	223	51	22.9
Irritability	234	73	31.2	234	59	25.2
Increased sleep	226	48	21.2	229	61	26.6
Decreased sleep	212	12	5.7	224	42	18.8
Hives (urticaria)	213	4	1.9	214	3	1.4

[a] Study conducted in US NCT00761631 (Study 5).
[b] N = number of subjects reporting Yes for at least 1 day or No for all days.
[c] n = Number of subjects reporting the event.
[d] Fever gradings: Mild (≥38ºC but ≤39ºC), Moderate (>39ºC but ≤40ºC), and Severe (>40ºC). No other systemic event other than fever was graded. Parents reported the use of antipyretic medication to treat or prevent symptoms in 45.1% and 33.1% of subjects 5 through 9 years of age and 10 through 17 years of age, respectively.

6.2 Clinical Trials Experience With Prevnar 13 in Adults ≥18 Years of Age

The safety of Prevnar 13 was assessed in 7 clinical studies (Studies 6-12) [6-12] conducted in the US and Europe which included 91,593 adults (48,806 received Prevnar 13) ranging in age from 18 through 101 years.

The 48,806 Prevnar 13 recipients included 899 adults who were aged 18 through 49 years, 2,616 adults who were aged 50 through 64 years, 45,291 adults aged 65 years and older. Of the 48,806 Prevnar 13 recipients, 46,890 adults had not previously received Pneumovax® 23 (pneumococcal polysaccharide vaccine [23-valent], PPSV23) ("PPSV23 unvaccinated") and 1,916 adults were previously vaccinated ("PPSV23 previously vaccinated") with PPSV23 at least 3 years prior to enrollment.

Safety and Immunogenicity Studies

Safety and immunogenicity of Prevnar 13 is supported by 6 clinical studies. Study 6[6] evaluated the safety and immunogenicity of Prevnar 13 in adults 18 through 64 years of age who had not received a previous dose of pneumococcal vaccine. Adults 18 through 59 years of age received a single dose of Prevnar 13, and adults 60 through 64 years of age received a single dose of Prevnar 13 or PPSV23.

Study 7 was randomized and compared the safety and immunogenicity of Prevnar 13 with PPSV23 as a single dose in adults ≥70 years vaccinated with PPSV23 (≥5 years prior to enrollment).[7] Study 8 was randomized and evaluated the safety and immunogenicity of Prevnar 13 and PPSV23 in different sequential order in PPSV23 naive adults aged 60 through 64 years[8].

One clinical safety study[9] (Study 9) of Prevnar 13, conducted in PPSV23 previously vaccinated (≥3 years prior to enrollment) adults aged ≥68 years was a single arm study. Two studies, one in the US[10] (Study 10) in adults aged 50 through 59 years and the other in Europe[11] (Study 11) in adults aged ≥65 years, evaluated the concomitant administration of Prevnar 13 with inactivated

influenza vaccine, trivalent (Fluarix®, A/H1N1, A/H3N2, and B, Fall 2007/Spring 2008: IIV3) in these two age groups in PPSV23 unvaccinated adults.

The total safety population in the 6 safety and immunogenicity studies was 7,097. In 5 of the 6 safety and immunogenicity studies, more females than males were enrolled (50.2% - 61.8%). Across the 6 studies the racial distribution included: >85% White; 0.2%-10.7% Black or African American; 0%-1.7% Asian; <1% Native Hawaiian or other Pacific Islander; ≤1%, American Indian or Alaskan Native. Ethnicity data were not collected in Study 11; in the 5 other studies 0.6%-4.8% were Hispanic or Latino.

In five studies,[6-8,10,11] subjects with pre-existing underlying diseases were enrolled if the medical condition was stable (did not require a change in therapy or hospitalization for worsening disease for 12 weeks before receipt of study vaccine) except in Study 9 where subjects were enrolled if the medical condition was stable for 6 or more weeks before receipt of study vaccine.

In the 6 safety and immunogenicity studies,[6-11] subjects were excluded from study participation due to prior receipt of diphtheria toxoid-containing vaccines within 6 months of study vaccine. However, the time of prior receipt of a diphtheria toxoid-containing vaccine was not recorded.

Solicited adverse reactions for Prevnar 13 in the safety and immunogenicity studies were monitored by subjects recording local adverse reactions and systemic reactions daily using an electronic diary for 14 consecutive days following vaccination. Unsolicited serious and non-serious adverse events were collected for one month after each vaccination. In addition, serious adverse events were collected for an additional 5 months after each vaccination (at the 6-month follow-up phone contact) in all studies except Study 11.

Following licensure of Prevnar 13 in adults ≥50 years of age, a randomized, double-blind, placebo-controlled US study (Study 13) was conducted to evaluate concomitant administration of Prevnar 13 with inactivated influenza vaccine, quadrivalent (Fluzone® Quadrivalent, A/H1N1, A/H3N2, B/Brisbane, and B/Massachusetts, Fall 2014/Spring 2015: IIV4) in PPSV23 previously vaccinated adults ≥50 years of age. Unsolicited serious and non-serious adverse events were collected as described above for Studies 6-10.

Efficacy Study

Study 12[12] was a randomized double-blind placebo-controlled study conducted in the Netherlands in community-dwelling adults aged 65 years and older with no prior pneumococcal vaccination history. A total of 84,496 subjects received either a single dose of Prevnar 13 (42,240) or placebo* (42,256) in a 1:1 randomization. Among the 84,496 subjects, 58,072 (68.7%) were ≥65 to <75 years of age, 23,481 (27.8%) were ≥75 and <85 years of age, and 2,943 (3.5%) were ≥85 years of age. In the total safety population, more males (55.9%) were enrolled than females. The racial distribution was 98.5% White, 0.3% Black, 0.7% Asian, 0.5% Other, with <0.1% having missing data.

Adults with immunocompromising conditions or receiving immunosuppressive therapy and adults residing in a long-term care facility or requiring semiskilled nursing care were excluded.

* "Placebo" contained 5.0 mM succinate buffer, 0.85% sodium chloride, 0.02% polysorbate 80, and 0.125 mg of aluminum as aluminum phosphate per 0.5-ml dose.

Adults with pre-existing medical conditions, as well as subjects with a history of smoking were eligible for enrollment. In the safety population, 42.3% of subjects had pre-existing medical conditions including heart disease (25.4%), lung disease or asthma (15.1%) and type 1 and type 2 diabetes mellitus (12.5%). Smoking was reported at baseline by 12.3% of the subjects.

For a subset of 2,011 subjects (1,006 Prevnar 13 recipients and 1,005 placebo recipients), solicited adverse reactions were monitored by recording local and systemic events using electronic diaries for 7 days after vaccination; unsolicited adverse events were collected for 28 days after vaccination, and serious adverse events were collected for 6 months after vaccination. For the remaining 41,231 Prevnar 13 and 41,250 placebo vaccinated subjects, serious adverse events were collected for 28 days after vaccination.

Serious Adverse Events in Adult Clinical Studies

Safety and Immunogenicity Studies

Across the 6 safety and immunogenicity studies,[6-11] serious adverse events within 1 month of vaccination were reported after an initial study dose in 0.2%-1.4% of 5,057 subjects vaccinated with Prevnar 13, and in 0.4%-1.7% of 1,124 subjects vaccinated after an initial study dose of PPSV23. From 1 month to 6 months after an initial study dose, serious adverse events were reported in 0.2%-5.8% of subjects vaccinated during the studies with Prevnar 13 and in 2.4%-5.5% of subjects vaccinated with PPSV23. One case of erythema multiforme occurred 34 days after receipt of a second dose of Prevnar 13.

Twelve of 5,667 (0.21%) Prevnar 13 recipients and 4 of 1,391 (0.29 %) PPSV23 recipients died. Deaths occurred between Day 3 and Day 309 after study vaccination with Prevnar 13 or PPSV23. Two of 12 deaths occurred within 30 days of vaccination and both deaths were in subjects >65 years of age. One death due to cardiac failure occurred 3 days after receiving placebo. This subject had received Prevnar 13 and IIV3 one month earlier. The other death was due to peritonitis 20 days after receiving Prevnar 13. The reported causes of the 10 remaining deaths occurring greater than 30 days after receiving Prevnar 13 were cardiac disorders (4), neoplasms (4), *Mycobacterium avium* complex pulmonary infection (1) and septic shock (1).

Efficacy Study

In Study 12[12] (subjects 65 years and older), serious adverse events within 1 month of vaccination were reported in 327 of 42,237 (0.8%) Prevnar 13 recipients (352 events) and in 314 of 42,225 (0.7%) placebo recipients (337 events). In the subset of subjects where serious adverse events were monitored for 6 months, 70 of 1,006 (7%) Prevnar 13 vaccinated subjects (90 events) and 60 of 1,005 (6%) placebo vaccinated subjects (69 events) reported serious adverse events.

During the follow-up period (average of 4 years) for case accumulation there were 3,006 deaths (7.1%) in the Prevnar 13 group and 3,005 deaths (7.1%) in the placebo group. There were 10 deaths (<0.1%) in the Prevnar 13 group and 10 deaths (<0.1%) in the placebo group within 28 days of vaccination. There were 161 deaths (0.4%) in the Prevnar 13 group and 144 deaths

(0.3%) in the placebo group within 29 days – 6 months following vaccination. These data do not provide evidence for a causal relationship between deaths and vaccination with Prevnar 13.

Solicited Adverse Reactions in Adult Clinical Studies

The incidence and severity of solicited adverse reactions that occurred within 7 or 14 days following each dose of Prevnar 13, PPSV23, or placebo administered to adults in 5 studies are shown in Tables 11, 12, 13, and 14.

The commonly reported local adverse reactions after Prevnar 13 vaccination in PPSV23 unvaccinated and PPSV23 previously vaccinated adults were redness, swelling and pain at the injection site, or limitation of arm movement (Tables 11 and 12). The commonly reported systemic adverse reactions in PPSV23 unvaccinated and PPSV23 previously vaccinated adults were fatigue, headache, chills, rash, decreased appetite, or muscle pain and joint pain (Tables 13 and 14).

Table 11 - Percentage of Subjects With Solicited Local Adverse Reactions Within 7 or 14 Days in PPSV23 Unvaccinated Adults[a]

	Study 6				Study 8		Study 12	
Age in Years	**18-49**	**50-59**	**60-64**		**60-64**		**≥65**	
Local Reaction	**Prevnar 13[b]** N[c]=266-787 %	**Prevnar 13[b]** N[c]=152-322 %	**Prevnar 13** N[c]=193-331 %	**PPSV23** N[c]=190-301 %	**Prevnar 13** N[c]=270-370 %	**PPSV23** N[c]=134-175 %	**Prevnar 13** N[c]=886-914 %	**Placebo** N[c]=859-865 %
Redness[d]								
Any	30.5	15.8	20.2	14.2	12.2	11.2	4.9[g]	1.2
Mild	26.4	15.2	15.9	11.2	8.3	9.7	3.7[g]	0.8
Moderate	11.9	5.0	8.6	4.9	6.4	3.9	1.7[g]	0.3
Severe	2.8	0.7	1.7	0.0	1.2	0.8	0.5	0.1
Swelling[d]								
Any	39.4	21.7	19.3	13.1	10.0	10.4	6.8[g]	1.2
Mild	37.2	20.6	15.6	10.1	8.2	6.1	5.5[g]	0.7
Moderate	15.1	4.3	8.2	4.4	3.8	7.6	2.6[g]	0.6
Severe	1.4	0.0	0.6	1.1	0.0	0.0	0.1	0.1
Pain[e]								
Any	96.7	88.8	80.1	73.4	69.2[g]	58.3	36.1[g]	6.1
Mild	93.2	85.9	78.6[g]	68.6	66.1[g]	52.9	32.9[g]	5.6
Moderate	77.1	39.5	23.3	30.0	20.1	21.7	7.7[g]	0.6
Severe	16.0	3.6	1.7	8.6[g]	2.3	0.8	0.3	0.1
Limitation of arm movement[f]								
Any	75.2	40.7	28.5	30.8	23.5	28.2	14.1[g]	3.2
Mild	71.5	38.6	26.9	29.3	22.7	26.1	12.4[g]	2.5
Moderate	18.5	2.9	2.2	3.8	1.2	3.1	1.7[g]	0.5
Severe	15.6	2.9	1.7	4.3	1.1	2.3	1.2	0.7

[a] Studies conducted in US NCT00427895 (Study 6) and NCT00574548 (Study 8) reported local reactions within 14 days. Study conducted in the Netherlands NCT00744263 (Study 12) reported local reactions within 7 days.
[b] Open label administration of Prevnar 13.
[c] Number of subjects with known values (number of subjects reporting yes for at least one day or no for all days).
[d] Diameters were measured in caliper units of whole numbers from 1 to 21 or 21+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of redness and swelling were then characterized as Mild = 2.5 to 5.0 cm, Moderate = 5.1 to 10.0 cm, and Severe is >10.0 cm.
[e] Mild = awareness of symptom but easily tolerated, Moderate = discomfort enough to cause interference with usual activity, Severe = incapacitating with inability to do usual activity.
[f] Mild = some limitation of arm movement, Moderate = unable to move arm above head but able to move arm above shoulder, and Severe = unable to move arm above shoulder.
[g] Statistically significant difference $p < 0.05$. No adjustments for multiplicity.

Table 12 - Percentage of Subjects With Solicited Local Adverse Reactions in PPSV23 Previously Vaccinated Adults[a]

	Study 7		**Study 9**
Age in Years	**≥70**		**≥68**
Local Reaction	**Prevnar 13** N[c]=306-362 %	**PPSV23** N[c]=324-383 %	**Prevnar 13**[b] N[c]=664-777 %
Redness[d]			
Any	10.8	22.2[g]	14.3
Mild	9.5	13.5	12.6
Moderate	4.7	11.5[g]	6.5
Severe	1.7	4.8[g]	1.1
Swelling[d]			
Any	10.4	23.1[g]	12.8
Mild	8.9	14.0[g]	10.9
Moderate	4.0	13.6[g]	5.5
Severe	0.0	4.8[g]	0.6
Pain[e]			
Any	51.7	58.5	51.0
Mild	50.1	54.1	49.4
Moderate	7.5	23.6[g]	9.0
Severe	1.3	2.3	0.2
Limitation of arm movement[f]			
Any	10.5	27.6[g]	16.2
Mild	10.3	25.2[g]	14.8
Moderate	0.3	2.6[g]	1.6
Severe	0.7	3.0[g]	1.6

[a] Study conducted in US and Sweden NCT00546572 (Study 7) reported local reactions within 14 days. Study conducted in US, Sweden and Germany NCT00500266 (Study 9) reported local reactions within 14 days.
[b] Open label administration of Prevnar 13.
[c] Number of subjects with known values.
[d] Diameters were measured in caliper units of whole numbers from 1 to 21 or 21+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of redness and swelling were then characterized as Mild = 2.5 to 5.0 cm, Moderate = 5.1 to 10.0 cm, and Severe is >10.0 cm.
[e] Mild = awareness of symptom but easily tolerated, Moderate = discomfort enough to cause interference with usual activity, Severe = incapacitating with inability to do usual activity.
[f] Mild = some limitation of arm movement, Moderate = unable to move arm above head but able to move arm above shoulder, and Severe = unable to move arm above shoulder.
[g] Statistically significant difference $p < 0.05$. No adjustments for multiplicity.

Table 13 - Percentage of Subjects With Solicited Systemic Events in PPSV23 Unvaccinated Adults[a]

	Study 6				Study 8		Study 12	
Age in Years	**18-49**	**50-59**	**60-64**		**60-64**		**≥65**	
	Prevnar 13[b] N[c]=221-561 %	**Prevnar 13**[b] N[c]=137-248 %	**Prevnar 13** N[c]=174-277 %	**PPSV23** N[c]=176-273 %	**Prevnar 13** N[c]=261-328 %	**PPSV23** N[c]=127-173 %	**Prevnar 13** N[c]=881-896 %	**Placebo** N[c]=860-878 %
Systemic Event								
Fever								
≥38.0°C	7.2	1.5	4.0	1.1	4.2	1.6	2.9[d]	1.3
38.0°C to 38.4°C	4.2	1.5	4.0	1.1	3.8	0.8	1.1	0.6
38.5°C to 38.9°C	1.9	0.0	0.6	0.0	0.8	0.0	0.6	0.2
39.0°C to 40.0°C	1.4	0.0	0.0	0.0	0.4	0.8	0.7	0.2
>40.0°C[e]	0.5	0.0	0.0	0.0	0.0	0.0	0.8	0.3
Fatigue	80.5	63.3	63.2	61.5	50.5	49.1	18.8[d]	14.8
Headache	81.4	65.9	54.0	54.4	49.7	46.1	15.9	14.8
Chills	38.1	19.6	23.5	24.1	19.9	26.9	9.4	8.4
Rash	21.3	14.2	16.5	13.0	8.6	13.4	3.3[d]	0.8
Vomiting	15.0	6.9	3.9	5.4	3.1	3.1	0.3	0.9
Decreased appetite	55.6	25.3	21.3	21.7	14.7	23.0[d]	5.3	3.7
Generalized new muscle pain	82.0	61.8	56.2	57.8	46.9	51.5	18.4[d]	8.4
Generalized aggravated muscle pain	55.9	39.9	32.6	37.3	22.0	32.5[d]	9.1[d]	4.4
Generalized new joint pain	41.7	31.5	24.4	30.1	15.5	23.8[d]	7.4	5.4
Generalized aggravated joint pain	28.6	25.6	24.9	21.4	14.0	21.1	5.2	4.2

[a] Studies conducted in US NCT00427895 (Study 6) and NCT00574548 (Study 8) reported systemic events within 14 days. Study conducted in the Netherlands NCT00744263 (Study 12) reported systemic events within 7 days.

[b] Open label administration of Prevnar 13.

[c] Number of subjects with known values (number of subjects reporting yes for at least one day or no for all days).

[d] Statistically significant difference $p < 0.05$. No adjustments for multiplicity.

[e] Fevers >40.0°C were confirmed to be data entry errors and remain in the table for the following: 1 case in the 18- to 49- year-old cohort (Study 6), and 7 cases in the Prevnar 13 group and 3 cases in placebo group (Study 12). For the other cohorts in Study 6 and for Study 8, data entry errors were removed.

Table 14 - Percentage of Subjects With Systemic Events in PPSV23 Previously Vaccinated Adults[a]

	Study 7		Study 9
Age in Years	**≥70**		**≥68**
	Prevnar 13 N[c]=299-350 %	**PPSV23** N[c]=303-367 %	**Prevnar 13**[b] N[c]=635-733 %
Systemic Event			
Fever			
≥38.0°C	1.0	2.3	1.1
38.0°C to 38.4°C	1.0	2.0	0.8
38.5°C to 38.9°C	0.0	0.0	0.0
39.0°C to 40.0°C	0.0	0.3	0.3
>40.0°C	0.0	0.0	0.0
Fatigue	34.0	43.3[d]	34.4
Headache	23.7	26.0	26.1
Chills	7.9	11.2	7.5
Rash	7.3	16.4[d]	8.4
Vomiting	1.7	1.3	0.9
Decreased appetite	10.4	11.5	11.2
Generalized new muscle pain	36.8	44.7[d]	25.3
Generalized aggravated muscle pain	20.6	27.5[d]	12.3
Generalized new joint pain	12.6	14.9	12.8
Generalized aggravated joint pain	11.6	16.5	9.7

[a] Study conducted in US and Sweden NCT00546572 (Study 7) reported systemic events within 14 days. Study conducted in US, Sweden and Germany NCT00500266 (Study 9) reported systemic events within 14 days.
[b] Open label administration of Prevnar 13.
[c] Number of subjects with known values.
[d] Statistically significant difference p <0.05. No adjustments for multiplicity.

Safety Results from Adult Clinical Study of Concomitant Administration of Prevnar 13 and IIV4 (Fluzone Quadrivalent) (Study 13)

The safety profile of Prevnar 13 when administered concomitantly with seasonal inactivated influenza vaccine, quadrivalent, to PPSV23 previously vaccinated adults ≥50 years of age was generally consistent with the known safety profile of Prevnar 13.

6.3 Post-marketing Experience With Prevnar 13 in Infants and Toddlers

The following adverse events have been reported through passive surveillance since market introduction of Prevnar 13. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine. The following adverse events were included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Prevnar 13 vaccine.

Administration site conditions: Vaccination-site dermatitis, vaccination-site pruritus, vaccination-site urticaria

Blood and lymphatic system disorders: Lymphadenopathy localized to the region of the injection site

Cardiac disorders: Cyanosis

Immune system disorders: Anaphylactic/anaphylactoid reaction including shock

Nervous system disorders: Hypotonia

Skin and subcutaneous tissue disorders: Angioneurotic edema, erythema multiforme

Respiratory: Apnea

Vascular disorders: Pallor

7 DRUG INTERACTIONS

7.1 Concomitant Immunizations

In clinical trials with infants and toddlers, Prevnar 13 was administered concomitantly with the following US-licensed vaccines: Pediarix [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine Combined] (DTaP-HBV-IPV) and ActHIB [Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)] (PRP-T) for the first three doses and with PedvaxHIB [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)] (PRP-OMP), M-M-R II [Measles, Mumps, Rubella Virus Vaccine Live] (MMR) and Varivax [Varicella Virus Vaccine Live], or ProQuad [Measles, Mumps, Rubella and Varicella Virus Vaccine Live] (MMRV) and VAQTA [Hepatitis A vaccine, Inactivated] (HepA) for dose 4 *[see Clinical Studies (14.2) and Adverse Reactions (6.1)]*.

In children and adolescents, data are insufficient to assess the concomitant administration of Prevnar 13 with Human Papillomavirus Vaccine (HPV), Meningococcal Conjugate Vaccine (MCV4) and Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed (Tdap).

In adults, Prevnar 13 was administered concomitantly with US-licensed inactivated influenza vaccines, trivalent and quadrivalent (Studies 10, 11 and 13)*[see Clinical Studies (14.4) and Adverse Reactions (6.2)]*. There are no data on the concomitant administration of Prevnar 13 with diphtheria toxoid-containing vaccines and other vaccines licensed for use in adults 50 years of age and older.

When Prevnar 13 is administered at the same time as another injectable vaccine(s), the vaccines should always be administered with different syringes and given at different injection sites.

Do not mix Prevnar 13 with other vaccines/products in the same syringe.

7.2 Immunosuppressive Therapies

Individuals with impaired immune responsiveness due to the use of immunosuppressive therapy (including irradiation, corticosteroids, antimetabolites, alkylating agents, and cytotoxic agents) may not respond optimally to active immunization.

7.3 Antipyretics

A post-marketing clinical study conducted in Poland using a non-US vaccination schedule (2, 3, 4, and 12 months of age) evaluated the impact of prophylactic oral acetaminophen on antibody responses to Prevnar 13. The data show that 3 doses of acetaminophen (the first dose administered at the time of each vaccination and the subsequent doses at 6 to 8 hour intervals) reduced the antibody response to some serotypes following the third dose of Prevnar 13, compared with responses among infants who received antipyretics only as needed for treatment. Reduced antibody responses were not observed after the fourth dose of Prevnar 13 when acetaminophen was administered prophylactically.

7.4 Prior Vaccination with PPSV23

Prior receipt of PPSV23 within 1 year results in diminished immune responses to Prevnar 13 compared to PPSV23 naïve individuals *[see Clinical Studies (14.3)]*.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. Available data on Prevnar 13 administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study has been performed in female rabbits administered Prevnar 13 prior to mating and during gestation. Each dose was approximately 20 times the human dose. This study revealed no evidence of harm to the fetus due to Prevnar 13 (*see 8.1 Data*).

Data

Animal

In a developmental toxicity study, female rabbits were administered Prevnar 13 by intramuscular injection twice prior to mating (17 days and 3 days prior to mating) and twice during gestation (gestation days 10 and 24), 0.5 mL/rabbit/occasion (each dose approximately 20 times the human dose). No adverse effects on pre-weaning development were observed. There were no vaccine-related fetal malformations or variations.

8.2 Lactation

Risk Summary

Data are not available to assess the effects of Prevnar 13 on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Prevnar 13 and any potential adverse effects on the breastfed child from Prevnar 13 or from the underlying maternal condition. For

preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness of Prevnar 13 in children below the age of 6 weeks have not been established.

8.5 Geriatric Use

Of the total number of Prevnar 13 recipients aged 50 years and older in clinical studies (N=47,907), 94.5% (45,291 of 47,907) were 65 years and older and 30.3 % (14,498 of 47,907) were 75 years and older *[see Clinical Studies (14.1) and (14.3)]*.

8.6 High Risk Populations

Individuals with the diseases or conditions listed below are at increased risk of pneumococcal disease. Immunogenicity and safety data in these populations are limited.

Infants Born Prematurely

Immune responses elicited by Prevnar 13 administered on a US schedule to preterm infants have not been studied. When preterm infants (<37 weeks gestational age, N=100) were administered 4 doses of Prevnar 13 on a non-US schedule, the serotype-specific IgG antibody responses after the third and fourth dose were lower compared to responses among term infants (≥37 weeks gestational age, N=100) for some serotypes; the effectiveness of Prevnar 13 in preterm infants cannot be established from this study.

Children with Sickle Cell Disease

In an open-label, single-arm, descriptive study, 2 doses of Prevnar 13 were administered 6 months apart to children ≥6 to <18 years of age with sickle cell disease who previously received PPSV23 at least 6 months prior to enrollment. Children with a prior history of pneumococcal conjugate vaccination were excluded. For all vaccine serotypes, anti-pneumococcal opsonophagocytic activity (OPA) geometric mean antibody titers (GMTs) were higher after the first dose compared to pre-vaccination (N=95-131); OPA GMTs following the first and second dose were comparable. The effectiveness of Prevnar 13 in this specific population has not been established.

Individuals with Hematopoietic Stem Cell Transplant

In an open-label, single-arm, descriptive study, 4 doses of Prevnar 13 were administered to subjects ≥2 years of age (range 2 to 71 years) who had received an allogeneic hematopoietic stem cell transplant 3 to 6 months prior to enrollment. All subjects had a history of stable engraftment (absolute neutrophil count>1000/µL, platelet count >50,000/µL), and did not have uncontrolled graft versus host disease. The first three doses of Prevnar 13 were administered one month apart, followed by a fourth dose of Prevnar 13 six months after the third dose. Sera were obtained approximately one month after each vaccination. Immune responses (IgG GMCs) after the first

dose of Prevnar 13 were numerically higher for all serotypes compared with baseline. In addition, after each subsequent dose of Prevnar 13, IgG GMCs for all serotypes were numerically higher than responses after the previous dose. A post hoc analysis of the immune responses as measured by OPA antibody assay showed the pattern of functional antibody responses to be consistent with IgG responses for each serotype. The effectiveness of Prevnar 13 in this specific population has not been established.

Individuals with HIV Infection

In an open-label, single-arm, descriptive study, 3 doses of Prevnar 13 were administered 6 months apart to HIV-infected adults ≥18 years of age (median age 48 years), with CD4 counts ≥200 cells/μL and serum HIV RNA titer <50,000 copies/mL. All subjects had been vaccinated previously with PPSV23 at least 6 months prior to enrollment. For all vaccine serotypes anti-pneumococcal OPA GMTs were numerically higher after the first dose compared to pre-vaccination (N=227-253); OPA GMTs following the first, second and third dose were generally comparable. The effectiveness of Prevnar 13 in this specific population has not been established.

In an open-label, single-arm, descriptive study, 3 doses of Prevnar 13 were administered 1 month apart to HIV-infected subjects ≥6 years of age with CD4 counts ≥200 cells/μL, and serum HIV RNA titer <50,000 copies/mL. Subjects had not previously been vaccinated with a pneumococcal vaccine. For all vaccine serotypes anti-pneumococcal OPA GMTs were numerically higher after the first dose compared to pre-vaccination (N=197-257); OPA GMTs following the first, second and third dose were generally comparable. The effectiveness of Prevnar 13 in this specific population has not been established.

11 DESCRIPTION

Prevnar 13, Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein) is a sterile suspension of saccharides of the capsular antigens of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F, individually linked to non-toxic diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified through centrifugation, precipitation, ultrafiltration, and column chromatography. The polysaccharides are chemically activated to make saccharides, which are directly conjugated by reductive amination to the protein carrier CRM_{197}, to form the glycoconjugate. CRM_{197} is a nontoxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (β197) grown in a casamino acids and yeast extract-based medium or in a chemically-defined medium. CRM_{197} is purified through ultrafiltration, ammonium sulfate precipitation, and ion-exchange chromatography. The individual glycoconjugates are purified by ultrafiltration and column chromatography and analyzed for saccharide to protein ratios, molecular size, free saccharide, and free protein.

The individual glycoconjugates are compounded to formulate Prevnar 13. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens and by the saccharide to protein ratios in the individual glycoconjugates. Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 μg of each of *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 23F saccharides, 4.4 μg of 6B saccharides, 34 μg

CRM_{197} carrier protein, 100 µg polysorbate 80, 295 µg succinate buffer and 125 µg aluminum as aluminum phosphate adjuvant.

The tip cap and rubber plunger of the prefilled syringe are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Prevnar 13, comprised of pneumococcal polysaccharides conjugated to a carrier protein (CRM_{197}), elicits a T-cell dependent immune response. Protein carrier-specific T-cells provide the signals needed for maturation of the B-cell response.

Nonclinical and clinical data support opsonophagocytic activity, as measured by opsonophagocytic activity (OPA) antibody assay, as a contributor to protection against pneumococcal disease. The OPA antibody assay provides an in vitro measurement of the ability of serum antibodies to eliminate pneumococci by promoting complement-mediated phagocytosis and is believed to reflect relevant in vivo mechanisms of protection against pneumococcal disease. OPA antibody titers are expressed as the reciprocal of the highest serum dilution that reduces survival of the pneumococci by at least 50%.

In infants that have received Prevnar 13, opsonophagocytic activity correlates well with serotype specific anti-capsular polysaccharide IgG levels as measured by ELISA. A serum anti-capsular polysaccharide antibody concentration of 0.35 µg/mL as measured by ELISA one month after the third dose as a single antibody reference concentration was used to estimate the effectiveness of Prevnar 13 against invasive pneumococcal disease (IPD) in infants and children. The assay used for this determination is a standardized ELISA involving pre-absorption of the test sera with pneumococcal C-polysaccharide and serotype 22F polysaccharide to reduce non-specific background reactivity. The single antibody reference value was based on pooled efficacy estimates from three placebo-controlled IPD efficacy trials with either Prevnar or the investigational 9-valent CRM_{197} conjugate pneumococcal polysaccharide vaccine. This reference concentration is only applicable on a population basis and cannot be used to predict protection against IPD on an individual basis. Functional antibodies elicited by the vaccine (as measured by a dribble opsonophagocytic activity [dOPA] antibody assay) were also evaluated in infants.

In adults, an antipolysaccharide binding antibody IgG level to predict protection against invasive pneumococcal disease or non-bacteremic pneumonia has not been defined. Noninferiority trials for Prevnar 13 were designed to show that functional OPA antibody responses (as measured by a microcolony OPA [mcOPA] antibody assay) for the Prevnar 13 serotypes are noninferior and for some serotypes superior to the common serotypes in the currently licensed pneumococcal polysaccharide vaccine (PPSV23). OPA antibody titers measured in the mcOPA antibody assay cannot be compared directly to titers measured in the dOPA antibody assay.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Prevnar 13 has not been evaluated for the potential to cause carcinogenicity, genotoxicity, or impairment of male fertility. In a study in rabbits, no vaccine-related effects were found regarding reproductive performance including female fertility *[see Use in Specific Populations (*8.1*)]*.

14 CLINICAL STUDIES

14.1 Efficacy Data

Prevnar Efficacy Data

Invasive Pneumococcal Disease (IPD)

Prevnar (Pneumococcal 7-valent Conjugate Vaccine [Diphtheria CRM_{197} Protein]) was licensed in the US for infants and children in 2000, following a randomized, double-blind clinical trial in a multiethnic population at Northern California Kaiser Permanente (NCKP) from October 1995 through August 20, 1998, in which 37,816 infants were randomized to receive either Prevnar or a control vaccine (an investigational meningococcal group C conjugate vaccine [MnCC]) at 2, 4, 6, and 12-15 months of age. In this study, the efficacy of Prevnar against invasive disease due to *S. pneumoniae* in cases accrued during this period was 100% in both the per-protocol and intent-to-treat analyses (95% confidence interval [CI]: 75.4%, 100% and 81.7%, 100%, respectively). Data accumulated through an extended follow-up period to April 20, 1999, resulted in similar efficacy estimates of 97.4% in the per-protocol analysis and 93.9% in the intent-to-treat analysis (95% CI: 82.7%, 99.9% and 79.6%, 98.5%, respectively).

Acute Otitis Media (AOM)

The efficacy of Prevnar against otitis media was assessed in two clinical trials: a trial in Finnish infants at the National Public Health Institute and the efficacy trial in US infants at Northern California Kaiser Permanente (NCKP).

The Finnish Otitis Media (FinOM) trial was a randomized, double-blind trial in which 1,662 infants were equally randomized to receive either Prevnar or a control vaccine Recombivax HB (Hepatitis B vaccine (Recombinant) [Hep B]) at 2, 4, 6, and 12-15 months of age. In this study, conducted between December 1995 and March 1999, parents of study participants were asked to bring their children to the study clinics if the child had respiratory infections or symptoms suggesting acute otitis media (AOM). If AOM was diagnosed, tympanocentesis was performed, and the middle-ear fluid was cultured. If *S. pneumoniae* was isolated, serotyping was performed; the primary endpoint was efficacy against AOM episodes caused by vaccine serotypes in the per-protocol population. In the NCKP trial, the efficacy of Prevnar against otitis media was assessed from the beginning of the trial in October 1995 through April 1998. The otitis media analysis included 34,146 infants randomized to receive either Prevnar (N=17,070), or the control vaccine (N=17,076), at 2, 4, 6, and 12-15 months of age. In this trial, no routine tympanocentesis was performed, and no standard definition of otitis

media was used by study physicians. The primary otitis media endpoint was efficacy against all otitis media episodes in the per-protocol population.

The vaccine efficacy against AOM episodes due to vaccine serotypes assessed in the Finnish trial, was 57% (95% CI: 44%, 67%) in the per-protocol population and 54% (95% CI: 41%, 64%) in the intent-to-treat population. The vaccine efficacy against AOM episodes due to vaccine-related serotypes (6A, 9N, 18B, 19A, 23A), also assessed in the Finnish trial, was 51% (95% CI: 27, 67) in the per-protocol population and 44% (95% CI: 20, 62) in the intent-to-treat population. There was a nonsignificant increase in AOM episodes caused by serotypes unrelated to the vaccine in the per-protocol population, compared to children who received the control vaccine, suggesting that children who received Prevnar appeared to be at increased risk of otitis media due to pneumococcal serotypes not represented in the vaccine. However, vaccination with Prevnar reduced pneumococcal otitis media episodes overall. In the NCKP trial, in which the endpoint was all otitis media episodes regardless of etiology, vaccine efficacy was 7% (95% CI: 4%, 10%) and 6% (95% CI: 4%, 9%), respectively, in the per-protocol and intent-to-treat analyses. Several other otitis media endpoints were also assessed in the two trials.

Recurrent AOM, defined as 3 episodes in 6 months or 4 episodes in 12 months, was reduced by 9% in both the per-protocol and intent-to-treat populations (95% CI: 3%, 15% in per-protocol and 95% CI: 4%, 14% in intent-to-treat) in the NCKP trial; a similar trend was observed in the Finnish trial. The NCKP trial also demonstrated a 20% reduction (95% CI: 2, 35) in the placement of tympanostomy tubes in the per-protocol population and a 21% reduction (95% CI: 4, 34) in the intent-to-treat population. Data from the NCKP trial accumulated through an extended follow-up period to April 20, 1999, in which a total of 37,866 children were included (18,925 in Prevnar group and 18,941 in MnCC control group), resulted in similar otitis media efficacy estimates for all endpoints.

Prevnar 13 Adult Efficacy Data

The efficacy of Prevnar 13 against vaccine-type (VT) pneumococcal community-acquired pneumonia (CAP) and IPD was assessed in a randomized, double-blind, placebo-controlled study conducted over ~ 4 years in the Netherlands[12] (Study 12). A total of 84,496 subjects 65 years and older received a single dose of either Prevnar 13 or placebo in a 1:1 randomization; 42,240 subjects were vaccinated with Prevnar 13 and 42,256 subjects were vaccinated with placebo.

The primary objective was to demonstrate the efficacy of Prevnar 13 in the prevention of a first episode of confirmed VT-CAP (defined as presence of ≥2 specified clinical criteria; chest X-ray consistent with CAP as determined by a central committee of radiologists; and positive VT-specific Urinary Antigen Detection assay (UAD) or isolation of VT S. pneumoniae from blood or other sterile site). The secondary objectives were to demonstrate the efficacy of Prevnar 13 in the prevention of a first episode of 1) confirmed nonbacteremic/noninvasive (NB/NI) VT-CAP (an episode of VT-CAP for which the blood culture result and any other sterile site culture results were negative for S. pneumoniae) and 2) VT-IPD (the presence of *S. pneumoniae* in a sterile site).

Surveillance for suspected pneumonia and IPD began immediately after vaccination and continued through identification of a prespecified number of cases. Subjects who had a CAP or IPD episode with symptom onset less than 14 days after vaccination were excluded from all analyses.

The median duration of follow-up per subject was 3.93 years. Prevnar 13 demonstrated statistically significant vaccine efficacy (VE) in preventing first episodes of VT pneumococcal CAP, nonbacteremic/noninvasive (NB/NI) VT pneumococcal CAP, and VT-IPD (Table 15).

Table 15 - Vaccine Efficacy for the Primary and Secondary Efficacy Endpoints – Per-Protocol Population

		Vaccine Group			
		Prevnar 13	Placebo		
		N=42240	N=42256		
Efficacy Endpoint	**Total Number of Episodes**	**n**	**n**	**VE (%)**	**(95.2% CI)**
Primary endpoint: First case of confirmed VT pneumococcal CAP	139	49	90	45.6	(21.8, 62.5)
Secondary endpoint: First episode of confirmed NB/NI VT pneumococcal CAP	93	33	60	45	(14.2, 65.3)
Secondary endpoint: First episode of VT-IPD	35	7	28	75	(41.1, 90.9)

Abbreviations: CAP = community-acquired pneumonia; CI = confidence interval; NB/NI = nonbacteremic/noninvasive; IPD = invasive pneumococcal disease; VE = vaccine efficacy; VT = vaccine-type.

14.2 Prevnar 13 Clinical Trials in Children 6 Weeks Through 17 Years of Age Infants and Children 6 Weeks Through 17 Months of Age

Prevnar 13 effectiveness against invasive pneumococcal disease was inferred from comparative studies to a US-licensed 7-valent pneumococcal conjugate vaccine, Prevnar, in which Prevnar 13 elicited antipolysaccharide binding and functional OPA antibodies, as measured by ELISA and dOPA assays, respectively. These studies were designed to evaluate immunologic noninferiority of Prevnar 13 to Prevnar.

Clinical trials have been conducted in the US using a 2, 4, 6, and 12-15 month schedule.

The US noninferiority study[2] (Study 2) was a randomized, double-blind, active-controlled trial in which 2 month-old infants were randomly assigned to receive either Prevnar 13 or Prevnar in a 1:1 ratio. The two vaccine groups were well balanced with respect to race, ethnicity, and age and weight at enrollment. Most subjects were White (69.1%), 19.6% were Black or African-American, and 2.4% were Asian; 82.1% of subjects were non-Hispanic and non-Latino and 17.3% were Hispanic or Latino. Overall, 54.0% of subjects were male infants.

In Study 2, immune responses were compared in subjects receiving either Prevnar 13 or Prevnar using a set of noninferiority criteria. Co-primary endpoints included the percentage of subjects with serum pneumococcal anti-capsular polysaccharide IgG ≥0.35 μg/mL measured one month

after the third dose and serum pneumococcal anti-capsular polysaccharide IgG geometric mean concentrations (GMCs) one month after the fourth dose. The assay used for this determination was a standardized ELISA involving pre-absorption of the test sera with pneumococcal C-polysaccharide and serotype 22F polysaccharide to reduce non-specific background reactivity. Responses to the 7 common serotypes in Prevnar 13 and Prevnar recipients were compared directly. Responses to the 6 additional serotypes in Prevnar 13 recipients were each compared to the lowest response observed among the Prevnar serotypes in Prevnar recipients.

Pneumococcal Immune Responses Following Three Doses

In Study 2, the noninferiority criterion for the proportion of subjects with pneumococcal anti-capsular polysaccharide IgG antibody concentrations ≥0.35 μg/mL one month after the third dose was met for 10 of the 13 serotypes. The exceptions were serotypes 6B, 9V, and 3. Although the response to serotypes 6B and 9V did not meet the pre-specified noninferiority criterion, the differences were marginal.

The percentage of infants achieving pneumococcal anti-capsular polysaccharide IgG antibody concentrations ≥0.35 μg/mL one month after the third dose is shown below (Table 16).

Table 16: Percentage of Subjects With Anti-capsular Antibody Concentration ≥0.35 μg/mL One Month After a Three Dose Series Administered at 2, 4 and 6 Months of Age, Study 2[a,b,c,d]

Serotype	Prevnar 13 N=249-252 (95% CI)	Prevnar N=250-252 (95% CI)	Difference in % responders (95% CI)
Prevnar Serotypes			
4	94.4 (90.9, 96.9)	98.0 (95.4, 99.4)	-3.6 (-7.3, -0.1)
6B	87.3 (82.5, 91.1)	92.8 (88.9, 95.7)	-5.5 (-10.9, -0.1)
9V	90.5 (86.2, 93.8)	98.4 (96.0, 99.6)	-7.9 (-12.4, -4.0)
14	97.6 (94.9, 99.1)	97.2 (94.4, 98.9)	0.4 (-2.7, 3.5)
18C	96.8 (93.8, 98.6)	98.4 (96.0, 99.6)	-1.6 (-4.7, 1.2)
19F	98.0 (95.4, 99.4)	97.6 (99.4, 99.1)	0.4 (-2.4, 3.4)
23F	90.5 (86.2, 93.8)	94.0 (90.4, 96.6)	-3.6 (-8.5, 1.2)
Additional Serotypes[e]			
1	95.6 (92.3, 97.8)	e	2.8 (-1.3, 7.2)
3	63.5 (57.1, 69.4)	e	-29.3 (-36.2, -22.4)
5	89.7 (85.2, 93.1)	e	-3.1 (-8.3, 1.9)
6A	96.0 (92.8, 98.1)	e	3.2 (-0.8, 7.6)
7F	98.4 (96.0, 99.6)	e	5.6 (1.9, 9.7)
19A	98.4 (96.0, 99.6)	e	5.6 (1.9, 9.7)

[a] Studies conducted in US NCT00373958 (Study 2).
[b] Evaluable Immunogenicity Population.
[c] Noninferiority was met when the lower limit of the 95% CI for the difference between groups (Prevnar 13 minus Prevnar) was greater than -10%.
[d] Antibody measured by a standardized ELISA involving pre-absorption of the test sera with pneumococcal C-polysaccharide and serotype 22F polysaccharide to reduce non-specific background reactivity.
[e] Comparison for the 6 additional serotypes was to the lowest responder of the 7 common serotypes in Prevnar recipients, which for this analysis was serotype 6B (92.8%; 95% CI: 88.9, 95.7).

Functional dOPA antibody responses were elicited for all 13 serotypes, as shown in Table 17.

Table 17: Pneumococcal dOPA Antibody Geometric Mean Titers One Month After a Three Dose Series Administered at 2, 4 and 6 Months of Age, Study 2[a,b,c]

Serotype	Prevnar 13 N=91-94 (95% CI)	Prevnar N=89-94 (95% CI)
Prevnar Serotypes		
4	359 (276, 468)	536 (421, 681)
6B	1055 (817, 1361)	1514 (1207, 1899)
9V	4035 (2933, 5553)	3259 (2288, 4641)
14	1240 (935, 1646)	1481 (1133, 1934)
18C	276 (210, 361)	376 (292, 484)
19F	54 (40, 74)	45 (34, 60)
23F	791 (605, 1034)	924 (709, 1204)
Additional Serotypes		
1	52 (39, 69)	4 (4, 5)
3	121 (92, 158)	7 (5, 9)
5	91 (67, 123)	4 (4, 4)
6A	980 (783, 1226)	100 (66, 152)
7F	9494 (7339, 12281)	128 (80, 206)
19A	152 (105, 220)	7 (5, 9)

[a] Studies conducted in US NCT00373958 (Study 2).
[b] The dOPA (opsonophagocytic activity) antibody assay measures the ability of immune sera, in conjunction with complement, to mediate the uptake and killing of *S. pneumoniae* by phagocytic cells.
[c] Evaluable Immunogenicity Population.

Pneumococcal Immune Responses Following Four Doses

In Study 2, post-dose 4 antibody concentrations were higher for all 13 serotypes than those achieved after the third dose. The noninferiority criterion for pneumococcal anti-capsular polysaccharide GMCs after 4 doses was met for 12 of the 13 pneumococcal serotypes. The noninferiority criterion was not met for the response to serotype 3 (Table 18).

Table 18: Pneumococcal IgG GMCs (µg/mL) One Month After a Four Dose Series Administered at 2, 4, 6 and 12-15 Months, Study 2[a,b,c,d]

Serotype	Prevnar 13 N=232-236 (95% CI)	Prevnar N=222-223 (95% CI)	GMC Ratio (95% CI)
Prevnar Serotypes			
4	3.73 (3.28, 4.24)	5.49 (4.91, 6.13)	0.68 (0.57, 0.80)
6B	11.53 (9.99, 13.30)	15.63 (13.80, 17.69)	0.74 (0.61, 0.89)
9V	2.62 (2.34, 2.94)	3.63 (3.25, 4.05)	0.72 (0.62, 0.85)
14	9.11 (7.95, 10.45)	12.72 (11.22, 14.41)	0.72 (0.60, 0.86)
18C	3.20 (2.82, 3.64)	4.70 (4.18, 5.28)	0.68 (0.57, 0.81)
19F	6.60 (5.85, 7.44)	5.60 (4.87, 6.43)	1.18 (0.98, 1.41)
23F	5.07 (4.41, 5.83)	7.84 (6.91, 8.90)	0.65 (0.54, 0.78)
Additional Serotypes[e]			
1	5.06 (4.43, 5.80)	e	1.40 (1.17, 1.66)
3	0.94 (0.83, 1.05)	e	0.26 (0.22, 0.30)
5	3.72 (3.31, 4.18)	e	1.03 (0.87, 1.20)
6A	8.20 (7.30, 9.20)	e	2.26 (1.93, 2.65)
7F	5.67 (5.01, 6.42)	e	1.56 (1.32, 1.85)
19A	8.55 (7.64, 9.56)	e	2.36 (2.01, 2.76)

[a] Studies conducted in US NCT00373958 (Study 2).
[b] Evaluable Immunogenicity Population.
[c] Noninferiority was declared if the lower limit of the 2-sided 95% CI for Geometric Mean Ratio (Prevnar 13:Prevnar) was greater than 0.5.
[d] Antibody measured by a standardized ELISA involving pre-absorption of the test sera with pneumococcal C-polysaccharide and serotype 22F polysaccharide to reduce non-specific background reactivity.
[e] Comparison for the 6 additional serotypes was to the lowest responder of the 7 common serotypes in Prevnar recipients, which for this analysis was serotype 9V (3.63; 95% CI 3.25, 4.05).

Following the fourth dose, the functional dOPA antibody response for each serotype was quantitatively greater than the response following the third dose (see Table 19).

Table 19: Pneumococcal dOPA Antibody Geometric Mean Titers One Month After the Fourth Dose-Evaluable Toddler Immunogenicity Population, Study 2[a,b]

Serotype	Prevnar 13 N=88-92 (95% CI)	Prevnar N=92-96 (95% CI)
Prevnar Serotypes		
4	1180 (847, 1643)	1492 (1114, 1999)
6B	3100 (2337, 4111)	4066 (3243, 5098)
9V	11856 (8810, 15955)	18032 (14125, 23021)
14	2002 (1453, 2760)	2366 (1871, 2992)
18C	993 (754, 1308)	1722 (1327, 2236)
19F	200 (144, 276)	167 (121, 230)
23F	2723 (1961, 3782)	4982 (3886, 6387)
Additional Serotypes		
1	164 (114, 237)	5 (4, 6)
3	380 (300, 482)	12 (9, 16)
5	300 (229, 393)	5 (4, 6)
6A	2242 (1707, 2945)	539 (375, 774)
7F	11629 (9054, 14938)	268 (164, 436)
19A	1024 (774, 1355)	29 (19, 44)

[a] Studies conducted in US NCT00373958 (Study 2).
[b] The dOPA (opsonophagocytic activity) antibody assay measures the ability of immune sera, in conjunction with complement, to mediate the uptake and killing of *S. pneumoniae* by phagocytic cells.

Previously Unvaccinated Older Infants and Children 7 Months Through 5 Years of Age

In an open-label descriptive study of Prevnar 13 in Poland[4] (Study 4), children 7 months through 11 months of age, 12 months through 23 months of age and 24 months through 5 years of age (prior to the 6th birthday) who were naïve to pneumococcal conjugate vaccine, were given 3, 2 or 1 dose of Prevnar 13 respectively, according to the age-appropriate schedules in Table 2. Serum IgG concentrations were measured one month after the final dose in each age group and the data are shown in Table 20.

Table 20: Pneumococcal Anti-capsular Polysaccharide IgG Antibody Geometric Mean Concentrations (µg/mL) One Month After the Final Prevnar 13 Catch-Up Dose in Pneumococcal Vaccine Naïve Children 7 Months Through 5 Years of Age by Age Group, Study 4[a,b]

Serotype	3 doses Prevnar 13 7 through 11 months N=83-84 (95% CI)	2 doses Prevnar 13 12 through 23 months N=104-110 (95% CI)	1 dose Prevnar 13 24 months through 5 years N=135-152 (95% CI)
1	2.88 (2.44, 3.39)	2.74 (2.37, 3.16)	1.78 (1.52, 2.08)
3	1.94 (1.68, 2.24)	1.86 (1.60, 2.15)	1.42 (1.23, 1.64)
4	3.63 (3.11, 4.23)	4.28 (3.78, 4.86)	3.37 (2.95, 3.85)
5	2.85 (2.34, 3.46)	2.16 (1.89, 2.47)	2.33 (2.05, 2.64)
6A	3.72 (3.12, 4.45)	2.62 (2.25, 3.06)	2.96 (2.52, 3.47)
6B	4.77 (3.90, 5.84)	3.38 (2.81, 4.06)	3.41 (2.80, 4.16)
7F	5.30 (4.54, 6.18)	5.99 (5.40, 6.65)	4.92 (4.26, 5.68)
9V	2.56 (2.21, 2.96)	3.08 (2.69, 3.53)	2.67 (2.32, 3.07)
14	8.04 (6.95, 9.30)	6.45 (5.48, 7.59)	2.24 (1.71, 2.93)
18C	2.77 (2.39, 3.23)	3.71 (3.29, 4.19)	2.56 (2.17, 3.03)
19A	4.77 (4.28, 5.33)	4.94 (4.31, 5.65)	6.03 (5.22, 6.97)
19F	2.88 (2.35, 3.54)	3.07 (2.68, 3.51)	2.53 (2.14, 2.99)
23F	2.16 (1.82, 2.55)	1.98 (1.64, 2.39)	1.55 (1.31, 1.85)

[a] Studies conducted in Poland NCT00452452 (Study 4).
[b] Open label administration of Prevnar 13.
Note – ClinicalTrials.gov NCT number is as follows: NCT00452452 (Poland).

Children 15 Months Through 59 Months of Age Previously Vaccinated with Prevnar

In an open-label descriptive study in the US[5] (Study 5), children 15 months through 59 months previously vaccinated with 3 or 4 doses of Prevnar, received 2 doses of Prevnar 13 (children >15 through 23 months of age) or 1 dose of Prevnar 13 (children 24 months through 59 months of age). The data following one dose of Prevnar 13 in children 24 months through 59 months of age are shown in Table 21.

Table 21: Pneumococcal Anti-capsular Polysaccharide IgG Antibody Geometric Mean Concentrations (µg/mL) One Month After One Prevnar 13 Catch-Up Dose in Children 24 Through 59 Months of Age With 3 or 4 Prior Doses of Prevnar, US Catch-Up Study 5[a,b]

Serotype	1 dose Prevnar 13 24 months through 59 months N=173-175 (95% CI)
1	2.43 (2.15, 2.75)
3	1.38 (1.17, 1.61)
5	2.13 (1.89, 2.41)
6A	12.96 (11.04, 15.21)
7F	4.22 (3.74, 4.77)
19A	14.18 (12.37, 16.25)

[a] Studies conducted in US NCT00761631 (Study 5).
[b] Open label administration of Prevnar 13.

Children 5 Through 17 Years of Age

In a US study[5] (Study 5), a single dose of Prevnar 13 was administered to children 5 through 9 years of age, who were previously vaccinated with at least one dose of Prevnar, and to pneumococcal vaccine-naïve children 10 through 17 years of age.

In children 5 through 9 years of age, serotype-specific IgG concentrations measured 1 month after vaccination were noninferior (i.e., the lower limit of the 2-sided 95% CI for the geometric mean ratio [GMR] of >0.5) to the corresponding IgG concentrations in toddlers (Study 3) 1 month after a fourth pneumococcal vaccination (after the 4th dose of Prevnar for the 7 common serotypes and after the 4th dose of Prevnar 13 for the 6 additional serotypes) as shown in Tables 22 and 23 respectively.

Table 22: Pneumococcal IgG GMCs (µg/mL) One Month After Vaccination for 7 Common Serotypes, Prevnar 13 in Children 5 through 9 Years of Age in Study 5 Relative to Prevnar in Study 3 (Post-toddler)[a,g,h]

	Vaccine Group (as Enrolled/Randomized)							
	Prevnar 13 5 Through 9 Years (Study 5)			Prevnar Post-Toddler Dose (Study 3)				
Serotype	n[b]	GMC[c]	(95% CI[d])	n[b]	GMC[c]	(95% CI[d])	GMC Ratio[e]	(95% CI[f])
Common								
4	169	8.45	(7.24, 9.87)	173	2.79	(2.45, 3.18)	3.03	(2.48, 3.71)
6B	171	53.56	(45.48, 63.07)	173	9.47	(8.26, 10.86)	5.66	(4.57, 6.99)
9V	171	9.51	(8.38, 10.78)	172	1.97	(1.77, 2.19)	4.83	(4.10, 5.70)
14	169	29.36	(24.78, 34.78)	173	8.19	(7.31, 9.18)	3.58	(2.93, 4.39)
18C	171	8.23	(7.13, 9.51)	173	2.33	(2.05, 2.65)	3.53	(2.91, 4.29)
19F	171	17.58	(14.95, 20.67)	173	3.31	(2.87, 3.81)	5.31	(4.29, 6.58)
23F	169	11.26	(9.79, 12.95)	173	4.49	(3.86, 5.23)	2.51	(2.04, 3.08)

[a] Studies conducted in US NCT00761631 (Study 5) and NCT00444457 (Study 3).
[b] n = Number of subjects with a determinate antibody concentration for the specified serotype.
[c] Geometric mean concentrations (GMCs) were calculated using all subjects with available data for the specified blood draw. GMC after a 4-dose vaccination series with Prevnar (Study 3, post-toddler).
[d] Confidence intervals (CIs) are back transformations of a confidence interval based on the Student t distribution for the mean logarithm of the concentrations.
[e] Ratio of GMCs: Prevnar 13 (Study 5) to Prevnar (Study 3) reference.
[f] CIs for the ratio are back transformations of a confidence interval based on the Student t distribution for the mean difference of the logarithms of the measures [Prevnar 13 (Study 5) – Prevnar (Study 3)].
[g] Evaluable Immunogenicity Population.
[h] Noninferiority was declared if the lower limit of the 2-sided 95% CI for geometric mean ratio was greater than 0.5.

Table 23: Pneumococcal IgG GMCs (µg/mL) One Month After Vaccination for Additional 6 Serotypes, Prevnar 13 in Children 5 through 9 Years of Age in Study 5 Relative to Prevnar 13 in Study 3 (Post-toddler)[a,g,h]

	Vaccine Group (as Enrolled/Randomized)							
	Prevnar 13 5 Through 9 Years (Study 5)			Prevnar 13 Post-Toddler Dose (Study 3)				
Serotype	n[b]	GMC[c]	(95% CI[d])	n[b]	GMC[c]	(95% CI[d])	GMC Ratio[e]	(95% CI[f])
Additional								
1	171	3.57	(3.05, 4.18)	1068	2.90	(2.75, 3.05)	1.23	(1.07, 1.42)
3	171	2.38	(2.07, 2.74)	1065	0.75	(0.72, 0.79)	3.17	(2.78, 3.62)
5	171	5.52	(4.82, 6.32)	1068	2.85	(2.72, 2.98)	1.94	(1.71, 2.20)
6A	169	21.51	(18.15, 25.51)	1063	7.11	(6.78, 7.46)	3.03	(2.64, 3.47)
7F	170	6.24	(5.49, 7.08)	1067	4.39	(4.18, 4.61)	1.42	(1.24, 1.62)
19A	170	17.18	(15.01, 19.67)	1056	8.44	(8.05, 8.86)	2.03	(1.78, 2.32)

[a] Studies conducted in US NCT00761631 (Study 5) and NCT00444457 (Study 3).
[b] n = Number of subjects with a determinate antibody concentration for the specified serotype.
[c] Geometric mean concentrations (GMCs) were calculated using all subjects with available data for the specified blood draw. GMC after a 4-dose vaccination series with Prevnar 13 (Study 3, post-toddler).
[d] Confidence intervals (CIs) are back transformations of a confidence interval based on the Student t distribution for the mean logarithm of the concentrations.
[e] Ratio of GMCs: Prevnar 13 (Study 5) to Prevnar 13 (Study 3).
[f] CIs for the ratio are back transformations of a confidence interval based on the Student t distribution for the mean difference of the logarithms of the measures [Prevnar 13 (Study 5) – Prevnar 13 (Study 3)].
[g] Evaluable Immunogenicity Population.
[h] Noninferiority was declared if the lower limit of the 2-sided 95% CI for geometric mean ratio was greater than 0.5.

In children 10 through 17 years of age OPA GMTs, as measured by the mcOPA assay, 1 month after vaccination were noninferior (i.e., the lower limit of the 2-sided 95% CI for the GMR of

>0.5) to mcOPA GMTs in the 5 through 9 year old group for 12 of 13 serotypes (except for serotype 3), as shown in Table 24.

Table 24: Comparison of Pneumococcal mcOPA GMTs One Month After Vaccination, Prevnar 13, in Children 10 through 17 Years of Age Relative to Prevnar 13 in Children 5 through 9 Years of Age[a,g,h,i]

	Vaccine Group (as Enrolled)							
	Prevnar 13 (10 through 17 Years)			Prevnar 13 (5 through 9 Years)				
Serotype	n[b]	GMT[c]	(95% CI[d])	n[b]	GMT[c]	(95% CI[d])	GMT Ratio[e]	(95% CI[f])
Common								
4	188	6912	(6101, 7831)	181	4629	(4017, 5334)	1.5	(1.24, 1.80)
6B	183	14224	(12316, 16427)	178	14996	(13164, 17083)	0.9	(0.78, 1.15)
9V	186	4485	(4001, 5028)	180	4733	(4203, 5328)	0.9	(0.80, 1.12)
14	187	6894	(6028, 7884)	176	4759	(4120, 5497)	1.4	(1.19, 1.76)
18C	182	6263	(5436, 7215)	175	8815	(7738, 10041)	0.7	(0.59, 0.86)
19F	184	2280	(1949, 2668)	178	1591	(1336, 1893)	1.4	(1.14, 1.81)
23F	187	3808	(3355, 4323)	176	3245	(2819, 3736)	1.2	(0.97, 1.42)
Additional								
1	189	322	(275, 378)	179	191	(165, 221)	1.7	(1.36, 2.10)
3	181	114	(101, 130)	178	203	(182, 226)	0.6	(0.48, 0.67)
5	183	360	(298, 436)	178	498	(437, 568)	0.7	(0.57, 0.91)
6A	182	9928	(8457, 11655)	178	7514	(6351, 8891)	1.3	(1.05, 1.67)
7F	185	6584	(5829, 7436)	178	10334	(9099, 11737)	0.6	(0.53, 0.76)
19A	187	1276	(1132, 1439)	180	1180	(1048, 1329)	1.1	(0.91, 1.28)

[a] Studies conducted in US NCT00761631 (Study 5).
[b] n= Number of subjects with a determinate antibody titer for the specified serotype.
[c] Geometric mean titers (GMTs) were calculated using all subjects with available data for the specified blood draw.
[d] Confidence intervals (CIs) are back transformations of a confidence interval based on the Student t distribution for the mean logarithm of the titers.
[e] Ratio of GMTs: Prevnar 13(10 through 17 years of age) to Prevnar 13 (5 through 9 years of age).
[f] CIs for the ratio are back transformations of a confidence interval based on the Student t distribution for the mean difference of the logarithms of the measures [Prevnar 13(10 through 17 years of age) – Prevnar 13(5 through 9 years of age)] Study 5.
[g] Evaluable Immunogenicity Population.
[h] Noninferiority was declared if the lower limit of the 2-sided 95% CI for geometric mean ratio was greater than 0.5.
[i] Individual mcOPA antibody assay values below the assay LLOQ (lower limit of quantitation) were set at 0.50*LLOQ for the purpose of calculating the mcOPA antibody GMT.

14.3 Prevnar 13 Immunogenicity Clinical Trials in Adults

Six Phase 3 or Phase 4 clinical trials[6-8,10,11,13] were conducted in the US and Europe evaluating the immunogenicity of Prevnar 13 in different adult age groups, in individuals who were either not previously vaccinated with PPSV23 (PPSV23 unvaccinated) or who had received one dose of PPSV23 (PPSV23 previously vaccinated).

Each study included healthy adults and immunocompetent adults with stable underlying conditions including chronic cardiovascular disease, chronic pulmonary disease, renal disorders, diabetes mellitus, chronic liver disease, and medical risk conditions and behaviors (e.g., alcoholism and smoking) that are known to increase the risk of serious pneumococcal pneumonia and invasive pneumococcal disease. A stable medical condition was defined as a medical condition not requiring significant change in therapy (i.e., change to new therapy category due to worsening disease) or hospitalization for worsening disease 6-12 weeks prior to receipt of the study vaccine.

Immune responses elicited by Prevnar 13 and PPSV23 were measured by a mcOPA antibody assay for the 13 pneumococcal serotypes contained in Prevnar 13. Serotype-specific mcOPA antibody GMTs measured 1 month after each vaccination were calculated. For the 12 serotypes in common to both vaccines, noninferiority between vaccines was met if the lower limit of the 2-sided 95% confidence interval (CI) of the GMT ratio (Prevnar 13/PPSV23) was greater than 0.5.

The response to the additional serotype 6A, which is contained in Prevnar 13 but not in PPSV23, was assessed by demonstration of a ≥4-fold increase in the anti-6A mcOPA antibody titer above preimmunization levels. A statistically significantly greater response for Prevnar 13 was defined, for the difference in percentages (Prevnar 13 minus PPSV23) of adults achieving a ≥4-fold increase in anti-6A mcOPA antibody titer, as the lower limit of the 2-sided 95% CI greater than zero. For comparison of mcOPA antibody GMTs, a statistically greater response for serotype 6A was defined as the lower limit of the 2-sided 95% CI of the GMT ratio (Prevnar 13/PPSV23) greater than 2.

Of the 6 Phase 3 or Phase 4 clinical trials, 2 noninferiority trials[6,7] were conducted in which the immune responses to Prevnar 13 were compared with the immune responses to PPSV23; one in PPSV23 unvaccinated adults aged 18 through 64 years[6] (Study 6), and one in PPSV23 previously vaccinated adults aged ≥70 years[7] (Study 7). A third study compared immune responses to a single dose of Prevnar 13 to the response to Prevnar 13 administered one year after a dose of PPSV23 in adults aged 60 through 64 years who were PPSV23 unvaccinated at enrollment[8] (Study 8). The study also compared immune responses of PPSV23 as a single dose to the responses to PPSV23 administered one year after a dose of Prevnar 13. Two studies assessed the concomitant administration of Prevnar 13 with seasonal inactivated Fluarix (IIV3) in the US[10] (Study 10) and Europe[11] (Study 11). One study (Study 13) assessed the concomitant administration of Prevnar 13 with seasonal inactivated Fluzone Quadrivalent (IIV4) in PPSV23 previously vaccinated adults ≥50 years of age in the US.

Overall across the clinical studies evaluating the immunogenicity of Prevnar 13 in adults, persons 18 through 64 years of age responded at least as well as persons 65 years and older, the age group evaluated in a clinical endpoint efficacy trial.

Clinical Trials Conducted in PPSV23 Unvaccinated Adults

In an active-controlled modified[a] double-blind clinical trial[6] (Study 6) of Prevnar 13 in the US, PPSV23 unvaccinated adults aged 60 through 64 years were randomly assigned (1:1) to receive Prevnar 13 or PPSV23. In addition, adults aged 18 through 49 years and 50 through 59 years were enrolled and received one dose of Prevnar 13 (open-label).

[a] Modified double-blind means that the site staff dispensing and administering the vaccine were unblinded, but all other study personnel including the principal investigator and subject were blinded.

In adults aged 60 through 64 years, the mcOPA antibody GMTs elicited by Prevnar 13 were noninferior to those elicited by PPSV23 for the 12 serotypes in common to both vaccines (see Table 24). In addition, the lower limit of the 95% confidence interval for the mcOPA antibody GMT ratio (Prevnar 13/PPSV23) was greater than 1 for 8 of the serotypes in common.

For serotype 6A, which is unique to Prevnar 13, the proportion of subjects with a ≥4-fold increase after Prevnar 13 (88.5%) was statistically significantly greater than after PPSV23 (49.3%) in PPSV23-unvaccinated adults aged 60 through 64 years. OPA antibody GMTs for serotype 6A were statistically significantly greater after Prevnar 13 compared with after PPSV23 (see Table 25).

The mcOPA antibody GMTs elicited by Prevnar 13 in adults aged 50 through 59 years were noninferior to the corresponding mcOPA antibody GMTs elicited by Prevnar 13 in adults aged 60 through 64 years for all 13 serotypes (see Table 25).

In adults aged 18 through 49 years, the mcOPA antibody GMTs elicited by Prevnar 13 were noninferior to those elicited by Prevnar 13 in adults aged 60 through 64 years for all 13 serotypes (see Table 25).

Table 25: mcOPA Antibody GMTs in PPSV23-Unvaccinated Adults Aged 18 Through 49 Years or Aged 50 Through 59 Years Given Prevnar 13 and in Adults Aged 60 Through 64 Years Given Prevnar 13 or PPSV23 (Study 6)[a,b,c,d,e]

	Prevnar 13	Prevnar 13	Prevnar 13	PPSV23	Prevnar 13 18-49 Relative to 60-64 Years	Prevnar 13 50-59 Relative to 60-64 Years	Prevnar 13 Relative to PPSV23, 60-64 Years[g]
	18-49 Years[f] N=836-866	50-59 Years[f] N=350-384	60-64 Years N=359-404	60-64 Years N=367-402			
Serotype	GMT	GMT	GMT	GMT	GMT Ratio (95% CI)	GMT Ratio (95% CI)	GMT Ratio (95% CI)
1	353	211	158	119	2.4 (2.03, 2.87)	1.3 (1.07, 1.65)	1.3 (1.07, 1.65)
3	91	94	96	90	1.0 (0.84, 1.13)	1.0 (0.82, 1.18)	1.1 (0.89, 1.29)
4	4747	2904	2164	1405	2.3 (1.92, 2.76)	1.3 (1.06, 1.70)	1.5 (1.18, 2.00)
5	386	322	236	198	1.9 (1.55, 2.42)	1.4 (1.08, 1.74)	1.2 (0.95, 1.50)
6A[h]	5746	4469	2766	343	2.2 (1.84, 2.67)	1.6 (1.28, 2.03)	8.1 (6.11, 10.67)
6B	9813	3350	2212	998	4.9 (4.13, 5.93)	1.5 (1.20, 1.91)	2.2 (1.70, 2.89)
7F	3249	1807	1535	829	2.9 (2.41, 3.49)	1.2 (0.98, 1.41)	1.9 (1.52, 2.26)
9V	3339	2190	1701	1012	2.9 (2.34, 3.52)	1.3 (1.08, 1.53)	1.7 (1.40, 2.02)
14	2983	1078	733	819	4.9 (4.01, 5.93)	1.5 (1.14, 1.89)	0.9 (0.69, 1.16)
18C	3989	2077	1834	1074	2.3 (1.91, 2.79)	1.1 (0.89, 1.44)	1.7 (1.32, 2.21)
19A	1580	968	691	368	2.3 (2.02, 2.66)	1.4 (1.17, 1.68)	1.9 (1.53, 2.30)
19F	1533	697	622	636	3.0 (2.44, 3.60)	1.1 (0.89, 1.41)	1.0 (0.78, 1.23)
23F	1570	531	404	87	4.2 (3.31, 5.31)	1.3 (0.96, 1.80)	4.6 (3.37, 6.38)

GMT, Geometric Mean Titer.
[a] Study conducted in US NCT00427895 (Study 6).
[b] Noninferiority was defined for the 13 serotypes in adults aged 18 to 49 years, for the 12 common serotypes in adults aged 60 to 64 years and for the 13 serotypes in adults aged 50 to 59 years as the lower limit of the 2-sided 95% CI for GMT ratio greater than 0.5.
[c] mcOPA antibody for the 11 serotypes unique to PPSV23 but not contained in Prevnar 13 were not measured.
[d] Individual mcOPA antibody assay values below the assay LLOQ (lower limit of quantitation) were set at 0.50*LLOQ for the purpose of calculating the mcOPA antibody GMT.
[e] Evaluable Immunogenicity Population.
[f] Open label administration of Prevnar 13.
[g] For serotype 6A, which is unique to Prevnar 13, a statistically significantly greater response was defined for analysis in cohort 1 as the lower limit of the 2-sided 95% CI for the GMT ratio (Prevnar 13/PPSV23) greater than 2.
[h] 6A is a serotype unique to Prevnar 13 but not contained in PPSV23.

Clinical Trials Conducted in PPSV23 Previously Vaccinated Adults

In a Phase 3 active-controlled, modified double-blind clinical trial[7] (Study 7) of Prevnar 13 in the US and Sweden, PPSV23 previously vaccinated adults aged ≥70 years who had received one dose of PPSV23 ≥5 years prior were randomly assigned (1:1) to receive either Prevnar 13 or PPSV23.

The mcOPA antibody GMTs elicited by Prevnar 13 were noninferior to those elicited by PPSV23 for the 12 serotypes in common, when Prevnar 13 or PPSV23 were administered at a minimum of 5 years after a prior dose of PPSV23. In addition, the lower limit of the 95% confidence interval for the mcOPA antibody GMT ratio (Prevnar 13/PPSV23) was greater than 1 for 9 of the serotypes in common.

For serotype 6A, which is unique to Prevnar 13, the proportion of subjects with a ≥4-fold increase in mcOPA antibody titers after Prevnar 13 (71.1%) was statistically significantly greater than after PPSV23 (27.3%) in PPSV23 previously vaccinated adults aged ≥70 years. mcOPA antibody GMTs for serotype 6A were statistically significantly greater after Prevnar 13 compared with after PPSV23.

This clinical trial demonstrated that in adults aged ≥70 years and previously vaccinated with PPSV23 ≥5 years prior, vaccination with Prevnar 13 elicited noninferior immune responses as compared with re-vaccination with PPSV23 (see Table 26).

Table 26: mcOPA Antibody GMTs in PPSV23-Previously Vaccinated Adults Aged ≥70 Years Given Prevnar 13 or PPSV23 (Study 7)[a,b,c,d,e,f]

Serotype	Prevnar 13 N=400-426 GMT	PPSV23 N=395-445 GMT	Prevnar 13 Relative to PPSV23	
			GMT Ratio	(95% CI)
1	93	66	1.4	(1.14, 1.72)
3	59	53	1.1	(0.92, 1.31)
4	613	263	2.3	(1.76, 3.10)
5	100	61	1.6	(1.35, 2.00)
6A[g]	1056	160	6.6	(5.14, 8.49)
6B	1450	565	2.6	(2.00, 3.29)
7F	559	481	1.2	(0.97, 1.39)
9V	622	491	1.3	(1.08, 1.49)
14	355	366	1.0	(0.76, 1.23)
18C	972	573	1.7	(1.33, 2.16)
19A	366	216	1.7	(1.40, 2.07)
19F	422	295	1.4	(1.16, 1.77)
23F	177	53	3.3	(2.49, 4.47)

GMT, Geometric Mean Titer.
[a] Study conducted in US and Sweden NCT00546572 (Study 7).
[b] For the 12 common serotypes, noninferiority was defined as the lower limit of the 2-sided 95% CI for GMT ratio (Prevnar 13/PPSV23) greater than 0.5.
[c] For serotype 6A, which is unique to Prevnar 13, a statistically significantly greater response was defined as the lower limit of the 2-sided 95% CI for the GMT ratio (Prevnar 13/PPSV23) greater than 2.
[d] mcOPA antibody for the 11 serotypes unique to PPSV23 but not contained in Prevnar 13 were not measured.
[e] Individual mcOPA antibody assay values below the assay LLOQ (lower limit of quantitation) were set at 0.50*LLOQ for the purpose of calculating the mcOPA antibody GMT.
[f] Evaluable Immunogenicity Population.
[g] 6A is a serotype unique to Prevnar 13 but not contained in PPSV23.

Clinical Trial of Sequential Vaccination of Prevnar 13 and PPSV23 in PPSV23 Unvaccinated Adults

In a randomized clinical trial conducted in PPSV23-unvaccinated adults 60 through 64 years of age[8] (Study 8), 223 subjects received PPSV23 followed by Prevnar 13 one year later (PPSV23/Prevnar 13), and 478 received only Prevnar 13. mcOPA antibody titers were measured 1 month after vaccination with Prevnar 13 and are shown in Table 26. mcOPA antibody GMTs in those that received Prevnar 13 one year after PPSV23 were diminished when compared to those who received Prevnar 13 alone. Similarly, in exploratory analyses in PPSV23 previously

vaccinated adults ≥70 years of age in Study 7, diminished mcOPA antibody GMTs were observed in those that received Prevnar 13 one year after PPSV23 when compared to those who received Prevnar 13 alone.

Table 27: mcOPA Antibody GMTs for the Prevnar 13 Serotypes in PPSV23 Unvaccinated Adults Aged 60 Through 64 Years Given Prevnar 13 Alone or Prevnar 13 One Year After PPSV23 (Study 8) (PPSV23/Prevnar 13)[a,b,c,d]

	Prevnar 13 N=410-457		PPSV23/Prevnar 13 N=180-196	
Serotype	**GMT**	**(95% CI)**	**GMT**	**(95% CI)**
1	219	(191, 252)	88	(72, 109)
3	78	(69, 88)	54	(45, 65)
4	2590	(2257, 2973)	988	(802, 1218)
5	258	(218, 305)	112	(90, 139)
6A[e]	2947	(2536, 3426)	1210	(962, 1522)
6B	2165	(1845, 2540)	832	(654, 1059)
7F	1518	(1339, 1721)	407	(342, 485)
9V	1279	(1142, 1432)	495	(426, 575)
14	790	(663, 941)	515	(402, 659)
18C	1683	(1437, 1971)	650	(504, 839)
19A	717	(629, 818)	299	(248, 361)
19F	812	(702, 939)	360	(293, 442)
23F	384	(312, 472)	142	(104, 193)

GMT =Geometric Mean Titer.
[a] Study conducted in US NCT00574548 (Study 8).
[b] Evaluable Immunogenicity Population.
[c] mcOPA antibody for the 11 serotypes unique to PPSV23 but not contained in Prevnar 13 were not measured.
[d] Individual mcOPA antibody assay values below the assay LLOQ (lower limit of quantitation) were set at 0.50*LLOQ for the purpose of calculating the mcOPA antibody GMT.
[e] 6A is a serotype unique to Prevnar 13 but not contained in PPSV23.

Also in Study 8, 266 subjects received Prevnar 13 followed by PPSV23 one year later (Prevnar 13/PPSV23). mcOPA antibody GMTs following PPSV23 administered one year after Prevnar 13 (Prevnar 13/PPSV23) were noninferior to those following a single dose of PPSV23 (N=237) for the 12 common serotypes [the lower limit of the 95% CI for the GMT ratio [Prevnar 13/PPSV23 relative to PPSV23] was >0.5] (see Table 27). In Study 6, which was conducted in PPSV23-unvaccinated adults 60 through 64 years of age, 108 subjects received PPSV23 3.5 to 4 years after Prevnar 13 (Prevnar 13/PPSV23) and 414 received a single dose of PPSV23. Higher serotype-specific mcOPA antibody GMT ratios [(Prevnar 13/PPSV23) / PPSV23] were generally observed compared to the one year dosing interval in Study 8.

Table 28: mcOPA Antibody GMTs for the Prevnar 13 Serotypes in PPSV23-Unvaccinated Adults Aged 60 Through 64 Years Given PPSV23 One Year After Prevnar 13 Relative to PPSV23 Alone (Study 8)[a,b,c,d]

	Prevnar 13/PPSV23 N=216-233		PPSV23 N=214-229		GMT Ratio (Prevnar 13/PPSV23) / PPSV23	
Serotype	**GMT**	**95% CI**	**GMT**	**95% CI**	**Ratio**	**95% CI**
1	155	(131, 182)	161	(131, 198)	1.0	(0.74, 1.25)
3	127	(111, 145)	83	(71, 98)	1.5	(1.23, 1.87)
4	1409	(1202, 1651)	1468	(1139, 1893)	1.0	(0.71, 1.29)
5	220	(184, 264)	178	(144, 222)	1.2	(0.93, 1.64)
6A[e]	1366	(1122, 1663)	400	(306, 524)	3.4	(2.45, 4.77)
6B	1345	(1113, 1625)	875	(689, 1111)	1.5	(1.14, 2.08)
7F	748	(653, 857)	719	(598, 865)	1.0	(0.83, 1.31)
9V	848	(731, 984)	824	(694, 977)	1.0	(0.82, 1.29)
14	711	(580, 872)	869	(677, 1115)	0.8	(0.59, 1.13)
18C	1115	(925, 1344)	912	(707, 1177)	1.2	(0.89, 1.67)
19A	471	(408, 543)	390	(318, 477)	1.2	(0.94, 1.55)
19F	819	(697, 963)	626	(504, 779)	1.3	(1.00, 1.71)
23F	216	(169, 277)	84	(62, 114)	2.6	(1.74, 3.79)

GMT =Geometric Mean Titer.
[a] Study conducted in US NCT00574548 (Study 8).
[b]Evaluable Immunogenicity Population.
[c] mcOPA antibody for the 11 serotypes unique to PPSV23 but not contained in Prevnar 13 were not measured.
[d] Individual mcOPA antibody assay values below the assay LLOQ (lower limit of quantitation) were set at 0.50*LLOQ for the purpose of calculating the mcOPA antibody GMT.
[e] 6A is a serotype unique to Prevnar 13 but not contained in PPSV23. Anti-6A mcOPA antibody GMTs were descriptive in nature.

14.4 Concomitant Vaccine Administration

Infants and Toddlers

The concomitant administration of routine US infant vaccines *[see Drug Interactions (7.1)]* with Prevnar 13 was evaluated in two studies: Study 2 *[see Clinical Studies (14.2)]*, Pneumococcal Immune Responses Following Three Doses[2], and the US lot consistency study[3] (Study 3). In Study 3, subjects were randomly assigned to receive one of 3 lots of Prevnar 13 or Prevnar in a 2:2:2:1 ratio. The total number of infants vaccinated was 663[2] (Study 2) and 1699[3] (Study 3). Immune responses to concomitant vaccine antigens were compared in infants receiving Prevnar and Prevnar 13. Responses to diphtheria toxoid, tetanus toxoid, pertussis, polio types 1, 2, and 3, hepatitis B, PRP-T, PRP-OMP, measles, and varicella antigens in Prevnar 13 recipients were similar to those in Prevnar recipients. Based on limited data, responses to mumps and rubella antigens in Prevnar 13 recipients were similar to those in Prevnar recipients.

Adults ≥50 Years of Age

Concomitant Administration with QIV
Prevnar 13 was administered to PPSV23 previously vaccinated adults ≥50 years of age concomitantly with a US-licensed inactivated influenza vaccine, quadrivalent (IIV4) (Fluzone Quadrivalent) for the 2014/2015 influenza season (Study 13) *[see Adverse Reactions (6.2) and Drug Interactions (7.1)]*. One study group received Prevnar 13 and IIV4 concurrently, followed approximately one month later by placebo. A second study group received IIV4 and placebo concurrently, followed approximately one month later by Prevnar 13.

Serotype-specific pneumococcal antibody responses were measured one month after Prevnar 13 vaccination as OPA GMTs. Noninferiority was demonstrated for each pneumococcal serotype if the lower limit of the 2-sided 95% CI for the GMT ratio (Prevnar 13 + IIV4 relative to Prevnar

13 alone) was >0.5. Although OPA antibody responses to Prevnar 13 generally appeared to be slightly lower when Prevnar 13 was administered concomitantly with IIV4 compared to Prevnar 13 administered alone, noninferiority was demonstrated for all Prevnar 13 pneumococcal serotypes evaluated in Study 13.

Strain-specific influenza antibody responses were measured one month after IIV4 as hemagglutinin inhibition assay (HAI) titers. HAI GMTs were evaluated for each IIV4 strain in Study 13. Noninferiority was demonstrated if the lower limit of the 2-sided 95% CI for the HAI GMT ratio (Prevnar 13 + IIV4 relative to IIV4 + Placebo) was >0.5. Noninferiority was demonstrated for each IIV4 vaccine strain evaluated in Study 13.

Concomitant Administration with TIV
Two randomized, double-blind clinical trials evaluated the immunogenicity of Prevnar 13 given with IIV3 (Fall 2007/ Spring 2008 Fluarix, A/H1N1, A/H3N2, and B strains) in PPSV23 unvaccinated adults aged 50 through 59 years[10] (Study 10, conducted in the US) and in adults ≥65 years[11] (Study 11, conducted in Europe). Based on analysis of the primary pre-specified comparison of serotype specific anti-capsular polysaccharide IgG GMCs, noninferiority was met for all serotypes in adults 50-59 years of age and for 12 of 13 serotypes in adults ≥65years of age.

15 REFERENCES

ClinicalTrials.gov identifiers for studies included below:

1. Study 1 NCT00205803
2. Study 2 NCT00373958
3. Study 3 NCT00444457
4. Study 4 NCT00452452
5. Study 5 NCT00761631
6. Study 6 NCT00427895
7. Study 7 NCT00546572
8. Study 8 NCT00574548
9. Study 9 NCT00500266
10. Study 10 NCT00521586
11. Study 11 NCT00492557
12. Study 12 NCT00744263
13. Study 13 NCT02124161

16 HOW SUPPLIED/STORAGE AND HANDLING

Prefilled Syringe, 1 Dose (10 per package) – NDC 0005-1971-02.

Prefilled Syringe, 1 Dose (1 per package) – NDC 0005-1971-05.

After shipping, Prevnar 13 may arrive at temperatures between 2ºC to 25ºC (36ºF to 77ºF).

Upon receipt, store refrigerated at 2ºC to 8ºC (36ºF to 46ºF).

Do not freeze. Discard if the vaccine has been frozen.

Prevnar 13 is stable at temperatures up to 25ºC (77ºF) for 4 days. These data are not recommendations for shipping or storage, but may guide decisions for use in case of temporary temperature excursions.

The tip cap and rubber plunger of the prefilled syringe are not made with natural rubber latex.

17 PATIENT COUNSELING INFORMATION

Prior to administration of this vaccine, inform the individual, parent, guardian, or other responsible adult of the following:

- The potential benefits and risks of immunization with Prevnar 13 *[see Warnings and Precautions (5) and Adverse Reactions (6)]*.
- The importance of completing the immunization series unless contraindicated.
- Any suspected adverse reactions should be reported to their healthcare professional.

Provide the Vaccine Information Statements, which are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

This product's label may have been updated. For current full prescribing information, please visit www.pfizer.com.

US Govt. License No. 3

LAB-0469-17.0
CPT Code 90670

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PREVNAR 20 safely and effectively. See full prescribing information for PREVNAR 20.

PREVNAR 20 (Pneumococcal 20-valent Conjugate Vaccine), suspension for intramuscular injection
Initial U.S. Approval: 2021

---------- INDICATIONS AND USAGE----------
Prevnar 20 is a vaccine indicated for active immunization for the prevention of pneumonia and invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F in adults 18 years of age and older. (1)

This indication for the prevention of pneumonia caused by *S. pneumoniae* serotypes 8, 10A, 11A, 12F, 15B, 22F, and 33F is approved under accelerated approval based on immune responses as measured by opsonophagocytic activity (OPA) assay. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial. (1)

----------DOSAGE AND ADMINISTRATION----------
Adults 18 years of age and older: a single dose (2.3)

---------- DOSAGE FORMS AND STRENGTHS ----------
0.5 mL suspension for intramuscular injection, supplied in a single-dose pre-filled syringe. (3)

---------- CONTRAINDICATIONS ----------
Severe allergic reaction (e.g., anaphylaxis) to any component of Prevnar 20 or to diphtheria toxoid. (4)

---------- ADVERSE REACTIONS ----------
In adults 18 through 59 years of age, the most commonly reported solicited adverse reactions >10% were pain at the injection site (>70%), muscle pain (>50%), fatigue (>40%), headache (>30%), and arthralgia and injection site swelling (>10%). (6)

In adults 60 years of age and older, the most commonly reported solicited adverse reactions >10% were pain at the injection site (>50%), muscle pain and fatigue (>30%), headache (>20%), and arthralgia (>10%). (6)

To report SUSPECTED ADVERSE REACTIONS, contact Pfizer Inc. at 1-800-438-1985 or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: X/202X

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Prevnar 20™ is a vaccine indicated for active immunization for the prevention of pneumonia and invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F in adults 18 years of age and older.

This indication for the prevention of pneumonia caused by *S. pneumoniae* serotypes 8, 10A, 11A, 12F, 15B, 22F, and 33F is approved under accelerated approval based on immune responses as measured by opsonophagocytic activity (OPA) assay *[see Clinical Studies (14.2)]*. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory trial.

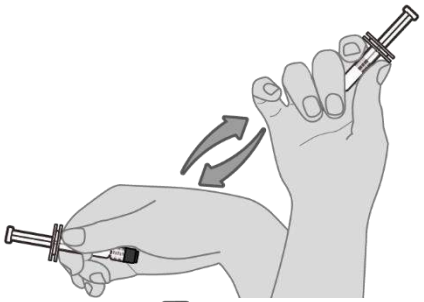
2 DOSAGE AND ADMINISTRATION

For intramuscular administration only.

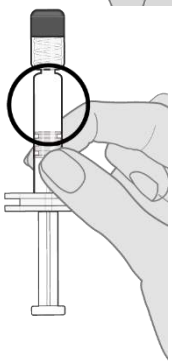
2.1 Preparation

Do not mix Prevnar 20 with other vaccines/products in the same syringe.

Step 1. Resuspend drug product
Hold the pre-filled syringe horizontally between the thumb and the forefinger and shake vigorously until the vaccine is a homogeneous white suspension. Do not use the vaccine if it cannot be re-suspended.

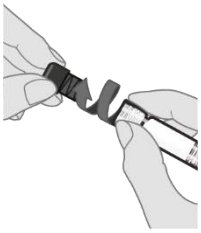


Step 2. Visual inspection
Visually inspect the vaccine for large particulate matter and discoloration prior to administration. Do not use if large particulate matter or discoloration is found. If the vaccine is not a homogeneous suspension, repeat Steps 1 and 2.



Step 3. Remove syringe cap
Remove the syringe cap by slowly turning the cap counterclockwise while holding the Luer lock adapter.

Avoid pressing the syringe plunger rod while removing the syringe cap.



Step 4. Attach a sterile needle
Hold the Luer lock adapter and attach a needle appropriate for intramuscular administration to the pre-filled syringe by turning clockwise.

2.2 Administration

For intramuscular injection only.

Each 0.5 mL dose is to be injected intramuscularly using a sterile needle attached to the supplied pre-filled syringe.

2.3 Vaccination Schedule

Prevnar 20 is administered as a single dose.

3 DOSAGE FORMS AND STRENGTHS

Prevnar 20 is a suspension for intramuscular injection available in a 0.5 mL single-dose pre-filled syringe.

4 CONTRAINDICATIONS

Severe allergic reaction (e.g., anaphylaxis) to any component of Prevnar 20 or to diphtheria toxoid *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate medical treatment and supervision used to manage immediate allergic reactions must be immediately available should an acute anaphylactic reaction occur following administration of Prevnar 20.

5.2 Altered Immunocompetence

Safety and immunogenicity data on Prevnar 20 are not available for individuals in immunocompromised groups and vaccination should be considered on an individual basis.

Based on experience with pneumococcal vaccines, individuals with altered immunocompetence may have reduced immune responses to Prevnar 20.

6 ADVERSE REACTIONS

In adults 18 through 59 years of age, the most commonly reported solicited adverse reactions >10% were pain at the injection site (>70%), muscle pain (>50%), fatigue (>40%), headache (>30%), and arthralgia and injection site swelling (>10%).

In adults 60 years of age and older, the most commonly reported solicited adverse reactions >10% were pain at the injection site (>50%), muscle pain and fatigue (>30%), headache (>20%), and arthralgia (>10%).

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The safety of a single dose of Prevnar 20 in adults 18 years of age and older was evaluated in six randomized, active-controlled, multicenter clinical trials and one open-label, multicenter clinical trial. All of the trials were conducted in the United States and 2 of the trials also enrolled participants (N=172) in Sweden. Across the 7 trials, 6343 adults received Prevnar 20 and 2496 received active control vaccine.

Pneumococcal Vaccine Naïve Adults 18 Years of Age and Older

The safety of Prevnar 20 in adults 18 years of age and older with no history of pneumococcal vaccination was evaluated in five studies (Studies 1-5). In the main cohort of Study 1 (NCT03760146) and in Study 2 (NCT03313037), participants ≥60 years of age and participants 60 through 64 years of age, respectively, received a single dose of Prevnar 20 followed 1 month later with administration of saline placebo or received a single dose of Prevnar 13 followed 1 month later with a dose of PNEUMOVAX® 23 (PPSV23). The 2 other cohorts of Study 1, participants 50 through 59 years of age and participants 18 through 49 years of age, received a single vaccination with Prevnar 20 or Prevnar 13. In Study 3 (NCT03828617), participants 18 through 49 years of age received a single vaccination with Prevnar 20 or Prevnar 13. In Studies 4 (NCT02955160) and 5 (NCT03642847), which were smaller studies conducted early in the clinical development of Prevnar 20, participants 18 through 49 years of age received a single dose of Prevnar 20 or an active control (Tdap or Prevnar 13).

Adults ≥65 Years of Age (Pneumococcal Vaccine Naïve or Previously Immunized with a Pneumococcal Vaccine)

The safety of Prevnar 20 in adults 65 years of age and older with pneumococcal vaccination given as routine care prior to enrollment was assessed in Study 6 (NCT03835975). Participants were enrolled into 1 of 3 cohorts based on their prior pneumococcal vaccination history (PPSV23 only ≥1 to ≤5 years prior to enrollment, Prevnar 13 only ≥6 months prior to enrollment, or Prevnar 13 followed by PPSV23 [with PPSV23 given ≥1 year prior to enrollment]). Participants in 2 of the cohorts received a single vaccination with Prevnar 20 or control pneumococcal vaccine (Prevnar 13), and the other cohort received a single vaccination with Prevnar 20. only.

The safety of Prevnar 20 in adults 65 years of age and older when coadministered with Influenza Vaccine, Adjuvanted (Fluad Quadrivalent) was assessed in Study 7 (NCT 04526574). Randomization was stratified by prior pneumococcal vaccine status (no previous pneumococcal vaccine, receipt of at least 1 dose of PPSV23 only, receipt of at least 1 dose of Prevnar 13 only, or receipt of at least 1 dose each of PPSV23 and Prevnar 13). Participants were randomized in a 1:1 ratio to receive Prevnar 20 concomitantly administered with Fluad Quadrivalent (Group 1) or Fluad Quadrivalent followed approximately one month later by Prevnar 20 (Group 2).

Demographics of Trial Participants

In the three main trials (Studies 1, 3, and 6), participants were predominantly female (52.0% to 65.9%) across groups defined by age and prior pneumococcal vaccination status within the Prevnar 20 and control vaccine groups. Across all 3 trials combined, 59.8% of participants were 60 years of age and older, 6.9% were 50 through 59 years of age, and 33.3% were 18 through 49 years of age. In Studies 1 and 3, participants were 80.7% White, 14.2% Black, 2.1% Asian, and 10.3% Hispanic. In Study 6, participants were predominantly White (92.4%). Participants were primarily from the United States; however a portion of participants 65 years of age and older were enrolled from Sweden in Study 1 (5.7% of participants 60 years of age and older in that study) and also in Study 6 (35.5% of participants with prior PPSV23 only). In Study 7, 54.7% of participants were female. The mean age of participants was 72 years (range 65-103 years). Participants were 90.6% White, 6.9% Black, 1.2% Asian, and 9.4% Hispanic.

In the three main trials, participants with pre-existing underlying diseases were enrolled if the medical condition was stable (did not require a significant change in therapy in the 6 weeks before receipt of study vaccine or any hospitalization for worsening disease within 12 weeks before receipt of study vaccine). In Study 1, approximately one-third of all participants had risk factors that placed them at increased risk for serious pneumococcal disease, including smoking (12.9%), stable medical conditions of chronic cardiovascular disease (5.5%), chronic pulmonary disease including asthma (8.7%), chronic liver disease (0.4%), and diabetes mellitus (13.9%).

Safety Monitoring

Solicited adverse reactions for Prevnar 20 in the three main trials and Study 7 were monitored in participants recording daily into an electronic diary their local adverse reactions for 10 consecutive days and systemic reactions for 7 consecutive days following vaccination. Across all trials, serious and nonserious adverse events were collected for 1 month after each vaccination. Safety follow-up of serious adverse events (SAEs) continued through 6 months after vaccination with Prevnar 20 or Prevnar 13 (or other appropriate control vaccine), as applicable. Newly diagnosed chronic medical conditions occurring within 6 months after vaccination were also collected via telephone contact.

Serious Adverse Events (Studies 1 through 6)

Across studies 1 through 6, performed in adults of all ages, naïve to and with prior pneumococcal vaccination, the proportion of participants reporting 1 or more SAEs within 6 months after vaccination with Prevnar 20 was 1.5% (67 of 4552 participants). This was similar to the proportion of participants with SAEs after vaccination with Prevnar 13 or other applicable control vaccine (1.8%, 44 of 2496). The proportions of participants with SAEs occurring within 1 month after vaccination with Prevnar 20 or with Prevnar 13 or other applicable control vaccine were both 0.4% (19 of 4552 participants and 11 of 2496 participants, respectively). There were no notable patterns or imbalances between vaccine groups for specific categories of serious adverse events that would suggest a causal relationship to Prevnar 20.

Solicited Adverse Reactions

The frequency and severity of the local adverse reactions (redness, swelling, and pain at the injection site) prompted daily in the 10 days after Prevnar 20 vaccination in adults naïve to pneumococcal vaccination (Study 1) and in adults with prior pneumococcal vaccination (Study 6) are shown in Table 1 and Table 2, respectively. The frequency and severity of the systemic adverse reactions (fever, fatigue, headache, muscle pain, and joint pain) prompted daily in the 7 days after Prevnar 20 vaccination in adults naïve to pneumococcal vaccination (Study 1) and in adults with prior pneumococcal vaccination (Study 6) are shown in Table 3 and Table 4, respectively.

Table 1. Percentage of Participants With Solicited Local Adverse Reactions, by Maximum Severity, Within 10 Days After Vaccination in Pneumococcal Vaccine-Naïve Adults - Study 1[a]

	18-49 Years of Age		50-59 Years of Age		≥60 Years of Age	
	Vaccine Group					
	Prevnar 20 (N[b]=335) %	Prevnar 13 (N[b]=112) %	Prevnar 20 (N[b]=331) %	Prevnar 13 (N[b]=111) %	Prevnar 20/Saline (N[b]=1505) %	Prevnar 13/ PPSV23 (N[b]=1483) %
Local Reaction						
Pain at injection site[e]						
Any[d]	81.2	82.1	72.5	69.4	55.4	54.1
Mild	42.7	52.7	53.5	52.3	45.3	44.6
Moderate	38.2	28.6	17.8	16.2	9.9	9.2
Severe	0.3	0.9	1.2	0.9	0.2	0.3
Swelling [c]						
Any (>2.0 cm)[d]	11.6	12.5	8.8	10.8	7.5	8.0
Mild	7.2	8.9	5.7	7.2	4.8	4.9
Moderate	4.5	3.6	3.0	3.6	2.4	2.8
Severe	0	0	0	0	0.3	0.3
Redness [c]						
Any (>2.0 cm)[d]	9.0	9.8	8.2	5.4	7.3	6.2
Mild	3.0	5.4	5.1	2.7	3.7	3.8
Moderate	5.4	4.5	2.7	2.7	2.8	2.2
Severe	0.6	0	0.3	0	0.8	0.2
Any local reaction[f]	81.2	82.1	72.8	70.3	57.4	56.0

a. Study 1 was conducted in the United States and in Sweden (NCT03760146).
b. N = number of participants with any e-diary data reported after vaccination (after Vaccination 1 [Prevnar 20 or Prevnar 13] for Study 1 participants 60 years of age and older). This value is the denominator for the percentage calculations.
c. Diameters were measured in caliper units of whole numbers from 1 to 21 or 21+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of redness and swelling were then characterized as follows: mild is >2.0 to 5.0 cm; moderate is >5.0 to 10.0 cm; severe is >10.0 cm.
d. "Any" includes all participants who reported a reaction as "mild", "moderate", or "severe" during Day 1 to Day 10 after vaccination.
e. Mild = does not interfere with activity; moderate = interferes with activity; severe = prevents daily activity.
f. "Any local reaction" includes all participants who reported any injection site reaction (pain, swelling, or redness) as "mild", "moderate", or "severe" during Day 1 to Day 10 after vaccination.

Table 2. Percentage of Participants With Solicited Local Adverse Reactions, by Maximum Severity, Within 10 Days After Vaccination in Adults 65 Years of Age and Older With Prior Pneumococcal Vaccination – Study 6[a,b]

	Prior Pneumococcal Vaccination Status[c]				
	PPSV23		Prevnar 13		Prevnar 13 and PPSV23
	Vaccine Group				
	Prevnar 20 (N[d]=253) %	Prevnar 13 (N[d]=121) %	Prevnar 20 (N[d]=245) %	PPSV23 (N[d]=126) %	Prevnar 20 (N[d]=125) %
Local Reaction					

Table 2. Percentage of Participants With Solicited Local Adverse Reactions, by Maximum Severity, Within 10 Days After Vaccination in Adults 65 Years of Age and Older With Prior Pneumococcal Vaccination – Study 6[a,b]

	Prior Pneumococcal Vaccination Status[c]				
	PPSV23		Prevnar 13		Prevnar 13 and PPSV23
	Vaccine Group				
	Prevnar 20 (N[d]=253) %	Prevnar 13 (N[d]=121) %	Prevnar 20 (N[d]=245) %	PPSV23 (N[d]=126) %	Prevnar 20 (N[d]=125) %
Pain at the injection site[g]					
Any[f]	50.2	43.0	61.2	56.3	52.8
Mild	45.8	38.8	54.7	40.5	47.2
Moderate	4.3	3.3	6.1	14.3	5.6
Severe	0	0.8	0.4	1.6	0
Swelling [e]					
Any (>2.0 cm)[f]	9.9	6.6	9.4	14.3	4.0
Mild	5.1	6.6	5.7	6.3	1.6
Moderate	3.6	0	3.7	7.1	2.4
Severe	1.2	0	0	0.8	0
Redness [e]					
Any (>2.0 cm)[f]	7.9	2.5	8.6	12.7	4.8
Mild	3.6	1.7	2.9	4.8	1.6
Moderate	3.2	0.8	5.3	7.1	3.2
Severe	1.2	0	0.4	0.8	0
Any local reaction[h]	53.0	43.8	64.1	57.9	54.4

a. Study 6 was conducted in the United States and in Sweden (NCT03835975)
b. Open-label administration of Prevnar 20.
c. Includes participants who previously received either PPSV23 ≥1 to ≤5 years before enrollment (PPSV23), Prevnar 13 ≥6 months before enrollment (Prevnar 13), or Prevnar 13 followed by PPSV23 ≥1 year before enrollment (Prevnar 13 and PPSV23) in the study.
d. N = number of participants with any e-diary data reported after vaccination. This value is the denominator for the percentage calculations.
e. Diameters were measured in caliper units of whole numbers from 1 to 21 or 21+. One caliper unit = 0.5 cm. Measurements were rounded up to the nearest whole number. Intensity of redness and swelling were then characterized as follows: mild is >2.0 to 5.0 cm; moderate is >5.0 to 10.0 cm; severe is >10.0 cm.
f. "Any" includes all participants who reported a reaction as "mild", "moderate", or "severe" during Day 1 to Day 10 after vaccination.
g. Mild = does not interfere with activity; moderate = interferes with activity; severe = prevents daily activity.
h. "Any local reaction" includes all participants who reported any injection site reaction (pain, swelling, or redness) as "mild", "moderate", or "severe" during Day 1 to Day 10 after vaccination.

Table 3. Percentage of Participants With Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Vaccination in Pneumococcal Vaccine-Naïve Adults – Study 1[a]

	18 through 49 Years of Age		50 through 59 Years of Age		≥60 Years of Age	
	Vaccine Group					
	Prevnar 20 (N[b]=335) %	Prevnar 13 (N[b]=112) %	Prevnar 20 (N[b]=331) %	Prevnar 13 (N[b]=111) %	Prevnar 20/Saline (N[b]=1505) %	Prevnar 13/PPSV23 (N[b]=1483) %
Systemic Reaction						
Muscle pain[c]						
Any[d]	66.6	74.1	49.8	49.5	39.1	37.3
Mild	36.4	42.0	33.8	31.5	28.9	26.8
Moderate	29.0	31.3	15.4	17.1	9.8	10.0
Severe	1.2	0.9	0.6	0.9	0.4	0.5
Fatigue[c]						
Any[d]	42.7	43.8	39.3	36.0	30.2	30.7
Mild	18.8	20.5	21.1	18.0	16.1	17.5
Moderate	22.1	19.6	17.2	15.3	12.8	11.9
Severe	1.8	3.6	0.9	2.7	1.2	1.2

Table 3. Percentage of Participants With Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Vaccination in Pneumococcal Vaccine-Naïve Adults – Study 1[a]

	18 through 49 Years of Age		50 through 59 Years of Age		≥60 Years of Age	
	Vaccine Group					
	Prevnar 20 (N[b]=335) %	Prevnar 13 (N[b]=112) %	Prevnar 20 (N[b]=331) %	Prevnar 13 (N[b]=111) %	Prevnar 20/Saline (N[b]=1505) %	Prevnar 13/PPSV23 (N[b]=1483) %
Headache[c]						
Any[d]	38.8	33.9	32.3	36.0	21.5	23.3
Mild	21.5	16.1	20.5	21.6	15.5	17.0
Moderate	14.6	17.0	10.9	13.5	5.4	5.9
Severe	2.7	0.9	0.9	0.9	0.7	0.3
Joint pain[c]						
Any[d]	13.4	17.9	15.4	20.7	12.6	13.7
Mild	6.3	8.9	10.6	12.6	6.9	7.1
Moderate	7.2	8.0	4.8	7.2	5.4	6.3
Severe	0	0.9	0	0.9	0.3	0.2
Fever						
≥38.0°C	1.2	1.8	1.5	0.9	0.9	0.8
≥38.0°C to 38.4°C	0.6	0	0.6	0.9	0.3	0.4
>38.4°C to 38.9°C	0.3	0	0.3	0	0.3	0.2
>38.9°C to 40.0°C	0.3	1.8	0.3	0	0	0
>40.0°C	0	0	0.3	0	0.3	0.2
Any systemic reaction[e]	79.4	83.0	69.5	67.6	55.2	55.4
Use of antipyretic or pain medication[f]	25.7	23.2	24.5	27.9	18.5	20.4

a. Study 1 was conducted in the United States and in Sweden (NCT03760146).
b. N = number of participants with any e-diary data reported after vaccination (after Vaccination 1 [Prevnar 20 or Prevnar 13] for Study 1 participants 60 years of age and older). This value is the denominator for the percentage calculations.
c. Mild = does not interfere with activity; moderate = some interference with activity; severe = prevents daily activity.
d. "Any" includes all participants who reported a reaction as "mild", "moderate", or "severe" during Day 1 to Day 7 after vaccination.
e. "Any systemic reaction" includes all participants who reported any fever ≥38.0°C or any other systemic reaction (fatigue, headache, joint pain, or muscle pain) as "mild", "moderate", or "severe" during Day 1 to Day 7 after vaccination.
f. Severity was not collected for use of antipyretic or pain medication. The numbers listed reflect "yes" responses (i.e., number of reactions reported).

Table 4. Percentage of Participants With Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Vaccination in Adults 65 Years of Age and Older With Prior Pneumococcal Vaccination – Study 6[a,b]

	Prior Pneumococcal Vaccination Status[c]				
	PPSV23		Prevnar 13		Prevnar 13 and PPSV23
	Vaccine Group				
	Prevnar 20 (N[d]=253) %	Prevnar 13 (N[d]=121) %	Prevnar 20 (N[d]=245) %	PPSV23 (N[d]=126) %	Prevnar 20 (N[d]=125) %
Systemic Reaction					
Muscle pain[e]					
Any[f]	32.0	31.4	33.9	46.0	37.6
Mild	26.1	24.0	25.3	31.7	28.0
Moderate	5.5	5.0	8.6	11.9	8.8
Severe	0.4	2.5	0	2.4	0.8
Fatigue[e]					
Any[f]	28.9	22.3	31.0	33.3	32.8
Mild	17.8	9.9	19.6	19.8	19.2
Moderate	11.1	9.9	10.2	13.5	12.0
Severe	0	2.5	1.2	0	1.6

Table 4. Percentage of Participants With Solicited Systemic Reactions, by Maximum Severity, Within 7 Days After Vaccination in Adults 65 Years of Age and Older With Prior Pneumococcal Vaccination – Study 6[a,b]

	Prior Pneumococcal Vaccination Status[c]				
	PPSV23		Prevnar 13		Prevnar 13 and PPSV23
	Vaccine Group				
	Prevnar 20 (N[d]=253) %	Prevnar 13 (N[d]=121) %	Prevnar 20 (N[d]=245) %	PPSV23 (N[d]=126) %	Prevnar 20 (N[d]=125) %
Systemic Reaction					
Headache[e]					
Any[f]	17.8	18.2	13.5	21.4	19.2
Mild	12.6	12.4	9.8	20.6	12.8
Moderate	4.7	5.8	3.7	0.8	5.6
Severe	0.4	0	0	0	0.8
Joint pain[e]					
Any[f]	6.7	10.7	11.8	15.9	16.8
Mild	4.7	5.0	7.8	10.3	12.8
Moderate	2.0	5.0	4.1	5.6	4.0
Severe	0	0.8	0	0	0
Fever					
≥38.0°C	0.8	0	0	1.6	0
≥38.0°C to 38.4°C	0.8	0	0	0.8	0
>38.4°C to 38.9°C	0	0	0	0.8	0
>38.9°C to 40.0°C	0	0	0	0	0
>40.0°C	0	0	0	0	0
Any systemic reaction[g]	51.8	43.8	50.2	59.5	52.8
Use of antipyretic or pain medication[h]	15.8	14.9	17.1	19.8	17.6

a. Study 6 was conducted in the United States and in Sweden (NCT03835975).
b. Open-label administration of Prevnar 20.
c. Includes participants who previously received either PPSV23 ≥1 to ≤5 years before enrollment (PPSV23), Prevnar 13 ≥6 months before enrollment (Prevnar 13), or Prevnar 13 followed by PPSV23 ≥1 year before enrollment (Prevnar 13 and PPSV23) in the study.
d. N = number of participants with any e-diary data reported after vaccination. This value is the denominator for the percentage calculations.
e. Mild = does not interfere with activity; moderate = interferes with activity; severe = prevents daily activity.
f. "Any" includes all participants who reported a reaction as "mild", "moderate", or "severe" during Day 1 to Day 7 after vaccination.
g. "Any systemic reaction" includes all participants who reported any fever ≥38.0°C or any other systemic reaction (fatigue, headache, joint pain, or muscle pain) as "mild", "moderate", or "severe" during Day 1 to Day 7 after vaccination.
h. Severity was not collected for use of antipyretic or pain medication. The numbers listed reflect "yes" responses (i.e., number of reactions reported).

Safety with Concomitant Vaccine Administration in Adults ≥65 years of age

In Study 7, the rates of local reactions at the Prevnar 20 injection site within 10 days after vaccination were similar between participants who received Prevnar 20 and Fluad Quadrivalent concomitantly (Group 1) or separately (Group 2). The rates of systemic reactions within 7 days following administration of Prevnar 20 were generally numerically higher in Group 1 compared to Group 2; however, overall, fever in both groups was uncommon (<1.5%) and other systemic reactions (fatigue, headache, muscle, or joint pain) were primarily mild to moderate (≤0.9% were severe). The proportions of participants with SAEs occurring within 1 month after vaccination with Prevnar 20 were 1.1% for Group 1 and 1.7% in Group 2. No SAEs occurring within 1 month after vaccination with Prevnar 20 were considered related to vaccination.

6.2 Postmarketing Experience With Prevnar 13

The postmarketing safety experience with Prevnar 13 is relevant to Prevnar 20 since the vaccines are manufactured and formulated similarly and contain 13 of the same polysaccharide conjugates. These adverse

reactions are included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Prevnar 13 vaccine in adults. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to product exposure. The following adverse reactions have been spontaneously reported during postapproval use of Prevnar 13 and may also be seen in postmarketing experience with Prevnar 20. Reactions reported in the postmarketing experience and which pertain only to pediatric populations are not included in this listing.

- Immune System Disorders: Anaphylactic/anaphylactoid reaction, including shock
- Skin and Subcutaneous Tissue Disorders: Angioneurotic edema, Erythema multiforme
- Blood and lymphatic system disorders: Lymphadenopathy localized to the region of the injection site
- General Disorders and Administration Site Conditions: Vaccination-site dermatitis, vaccination-site pruritus, vaccination-site urticaria

7 DRUG INTERACTIONS

7.1 Prior Vaccination with PNEUMOVAX 23

Receipt of PPSV23 1 to 5 years prior to Prevnar 20 resulted in diminished OPA geometric mean titers (GMTs) to Prevnar 20 compared to OPA GMTs in recipients who received Prevnar 13 at least 6 months previously, and compared to OPA GMTs in recipients who received Prevnar 13 followed by PPSV23, with the last dose of PPSV23 given at least 1 year prior to Prevnar 20 *[see Clinical Studies (14.2)]*.

7.2 Immunosuppressive Therapies

Individuals with impaired immune responsiveness due to the use of immunosuppressive therapy (including irradiation, corticosteroids, antimetabolites, alkylating agents, and cytotoxic agents) may not respond optimally to Prevnar 20.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no adequate and well-controlled studies of Prevnar 20 in pregnant women. Available data on Prevnar 20 administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

A developmental toxicity study was performed in female rabbits administered Prevnar 20 prior to mating and during gestation. The dose was 0.5 mL at each occasion (a single human dose is 0.5 mL). This study revealed no evidence of harm to the fetus due to Prevnar 20 *(see Data)*.

Data

Animal Data
In a developmental toxicity study, female rabbits were administered Prevnar 20 by intramuscular injection twice prior to mating (17 days and 4 days prior to mating) and twice during gestation (Gestation Days 10 and 24),

0.5 mL/rabbit/occasion (a single human dose). No adverse effects on pre-weaning development were observed. There were no vaccine-related fetal malformations or variations.

8.2 Lactation

Risk Summary

It is not known whether Prevnar 20 is excreted in human milk. Data are not available to assess the effects of Prevnar 20 on the breastfed infant or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for Prevnar 20 and any potential adverse effects on the breastfed child from Prevnar 20 or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

The safety and effectiveness of Prevnar 20 in individuals younger than 18 years of age have not been established.

8.5 Geriatric Use

Of the total number of Prevnar 20 recipients 18 years of age and older evaluated for safety in the 3 main clinical trials (N=4263), 26.7% (n=1138) were 65 years of age and older and 1.7% (n=72) were 80 years of age and older *[see Clinical Studies (14.2)]*.

Prevnar 20 recipients 70 through 79 years of age and ≥80 years of age had lower OPA GMTs for all pneumococcal serotypes compared to Prevnar 20 recipients 18 through 49 years, 50 through 59, and 60 through 64 years of age *[see Clinical Studies (14.1)]*.

11 DESCRIPTION

Prevnar 20, Pneumococcal 20-valent Conjugate Vaccine is a sterile suspension of saccharides of the capsular antigens of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F, individually linked to non-toxic diphtheria CRM_{197} protein. Each serotype is grown in soy peptone broth. The individual polysaccharides are purified by a series of chemical and physical methods. The polysaccharides are chemically activated and then directly conjugated to the carrier protein CRM_{197}, to form the glycoconjugate. CRM_{197} is a non-toxic variant of diphtheria toxin isolated from cultures of *Corynebacterium diphtheriae* strain C7 (β197) grown in a casamino acids and yeast extract-based medium or in a chemically-defined medium. CRM_{197} is purified by a series of chemical and physical methods. The individual glycoconjugates are purified by a series of chemical and physical methods and analyzed for saccharide to protein ratios, molecular size, free saccharide, and free protein.

The individual glycoconjugates are compounded to formulate Prevnar 20. Potency of the formulated vaccine is determined by quantification of each of the saccharide antigens and by the saccharide to protein ratios in the individual glycoconjugates. Each 0.5 mL dose of the vaccine is formulated to contain approximately 2.2 µg of each of *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F saccharides, 4.4 µg of 6B saccharides, 51 µg CRM_{197} carrier protein, 100 µg polysorbate 80, 295 µg succinate buffer, 4.4 mg sodium chloride, and 125 µg aluminum as aluminum phosphate adjuvant.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Protection against pneumococcal disease is conferred mainly by opsonophagocytic killing of *S. pneumoniae*. Prevnar 20 generates functional antibodies as measured by opsonophagocytic activity (OPA).

The effectiveness of Prevnar 20 was assessed by measuring serotype-specific serum OPA of antibodies at 1-month post vaccination.

An opsonic antibody titer that is predictive of protection against invasive pneumococcal disease or pneumococcal pneumonia has not been established.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Prevnar 20 has not been evaluated for the potential to cause carcinogenicity, genotoxicity, or impairment of male fertility. Vaccination of female rabbits with Prevnar 20 had no effect on female fertility *[see Use in Specific Populations (8.1)]*.

14 CLINICAL STUDIES

14.1 Prevnar 13 Adult Efficacy Data

Efficacy and effectiveness of Prevnar 13 are relevant to Prevnar 20, since the vaccines are manufactured similarly and contain 13 of the same polysaccharide conjugates.

The efficacy of Prevnar 13 against vaccine-type (VT) pneumococcal community-acquired pneumonia (CAP) and invasive pneumococcal disease (IPD) was assessed in a randomized, double-blind, placebo-controlled study (Community-Acquired Pneumonia Immunization Trial in Adults [CAPiTA]) conducted over ~4 years in the Netherlands. A total of 84,496 participants 65 years of age and older received a single dose of either Prevnar 13 or placebo in a 1:1 randomization; 42,240 participants were vaccinated with Prevnar 13 and 42,256 participants were vaccinated with placebo. Chronic medical conditions (asthma, diabetes, heart, liver, and/or lung diseases) were reported in 42.3% of study participants at baseline.

The primary objective was to demonstrate the efficacy of Prevnar 13 in the prevention of a first episode of confirmed VT-CAP (defined as presence of ≥2 specified clinical criteria, chest X-ray consistent with CAP as determined by a central committee of radiologists, and positive VT-specific urinary antigen detection assay [UAD] or isolation of VT *S. pneumoniae* from blood or other sterile site). The secondary objectives were to demonstrate the efficacy of Prevnar 13 in the prevention of a first episode of 1) confirmed nonbacteremic/noninvasive (NB/NI) VT-CAP (an episode of VT-CAP for which the blood culture result and any other sterile site culture results were negative for *S. pneumoniae*) and 2) VT-IPD (the presence of *S. pneumoniae* in a sterile site).

Surveillance for suspected pneumonia and IPD began immediately after vaccination and continued through identification of a prespecified number of cases. Participants who had a CAP or IPD episode with symptom onset less than 14 days after vaccination were excluded from all analyses.

The median duration of follow-up per participant was 3.93 years. Prevnar 13 demonstrated statistically significant vaccine efficacy (VE) in preventing first episodes of VT pneumococcal CAP, NB/NI VT pneumococcal CAP, and VT-IPD (see Table 5).

Table 5. Vaccine Efficacy for the Primary and Secondary Efficacy Endpoints – Per-Protocol Population

		Vaccine Group			
		Prevnar 13	Placebo		
		N=42,240	N=42,256		
Efficacy Endpoint	**Total Number of Episodes**	**n**	**n**	**VE (%)**	**(95.2% CI)**
Primary endpoint: First case of confirmed VT pneumococcal CAP	139	49	90	45.6	(21.8, 62.5)
Secondary endpoint: First episode of confirmed NB/NI VT pneumococcal CAP	93	33	60	45	(14.2, 65.3)
Secondary endpoint: First episode of VT-IPD	35	7	28	75	(41.1, 90.9)

Abbreviations: CAP = community-acquired pneumonia; CI = confidence interval; NB/NI = nonbacteremic/noninvasive; IPD = invasive pneumococcal disease; VE = vaccine efficacy; VT = vaccine-type.

14.2 Prevnar 20 Clinical Trials

Immunogenicity of Prevnar 20 in Pneumococcal Vaccine Naïve Adults

Prevnar 20 effectiveness against invasive pneumococcal disease caused by the 20 vaccine serotypes and against pneumonia caused by the 13 serotypes in Prevnar 13 was inferred from comparative immunogenicity to US-licensed pneumococcal vaccines (Prevnar 13 and PPSV23). Study 1, conducted in the United States and Sweden, was designed to evaluate immunologic noninferiority of Prevnar 20 to Prevnar 13 (for the 13 original *S. pneumoniae* serotypes) and PPSV23 (for the 7 new *S. pneumoniae* serotypes) in pneumococcal vaccine naive adults ≥60 years of age. Immune responses elicited by Prevnar 20 and the control pneumococcal vaccines were measured by an OPA assay. OPA assays were used to measure functional antibodies to *S. pneumoniae*.

Study 1 included healthy adults and immunocompetent adults with stable underlying conditions, including chronic cardiovascular disease, chronic pulmonary disease, renal disorders, diabetes mellitus, chronic liver disease, and medical risk conditions and behaviors (e.g., smoking) that are known to increase the risk of serious pneumococcal pneumonia and IPD. A stable medical condition was defined as a medical condition not requiring significant change in therapy in the previous 6 weeks (i.e., change to new therapy category due to worsening disease) or any hospitalization for worsening disease within 12 weeks before receipt of the study vaccine.

Comparison of Immune Responses of Prevnar 20 to Prevnar 13 and PPSV23 in Pneumococcal Vaccine Naïve Adults ≥60 Years of Age

In a randomized, active-controlled, double-blind noninferiority clinical trial (Study 1) of Prevnar 20 in the United States and Sweden, pneumococcal vaccine -naïve adults 18 years of age and older were enrolled into 1 of 3 cohorts based on their age at enrollment and randomized to receive either Prevnar 20 or control. Participants 60 years of age and older were randomly assigned (1:1 ratio) to Prevnar 20 followed 1 month later with saline placebo or to Prevnar 13 followed 1 month later with PPSV23.

Serotype-specific OPA GMTs were measured before the first vaccination and 1 month after each vaccination. Noninferiority of immune responses, OPA GMTs 1 month after vaccination, with Prevnar 20 to a control vaccine for a serotype was declared if the lower bound of the 2 sided 95% CI for the GMT ratio (Prevnar 20/Prevnar 13; Prevnar 20/PPSV23) for that serotype was greater than 0.5.

In adults 60 years of age and older, immune responses to all 13 matched serotypes elicited by Prevnar 20 were noninferior to the immune responses to the serotypes elicited by Prevnar 13 one month after vaccination. Immune responses to 6 out of the 7 additional serotypes induced by Prevnar 20 were noninferior to the immune responses to these same serotypes induced by PPSV23 one month after vaccination. The response to serotype 8 missed the prespecified statistical noninferiority criterion by a small margin (the lower bound of the 2-sided 95% CI for the GMT ratio being 0.49 versus >0.50) (Table 6).

In supportive analyses, 77.8% of participants in the Prevnar 20 group achieved a ≥4-fold rise in serotype 8 OPA titers from before vaccination to 1 month post-vaccination.

Table 6. OPA GMTs 1 Month After Vaccination in Adults 60 Years of Age and Older Given Prevnar 20 Compared to Prevnar 13 for the 13 Matched Serotypes and PPSV23 for the 7 Additional Serotypes (Study 1)[a,b,c,d]

	Prevnar 20 (N = 1157–1430)	**Prevnar 13 (N = 1390–1419)**	**PPSV23 (N = 1201–1319)**	**Vaccine Comparison**
	GMT[e]	**GMT[e]**	**GMT[e]**	**GMT Ratio[e] (95% CI)[e]**
Serotype				
1	123	154		0.80 (0.71, 0.90)
3	41	48		0.85 (0.78, 0.93)
4	509	627		0.81 (0.71, 0.93)
5	92	110		0.83 (0.74, 0.94)
6A	889	1165		0.76 (0.66, 0.88)
6B	1115	1341		0.83 (0.73, 0.95)
7F	969	1129		0.86 (0.77, 0.96)
9V	1456	1568		0.93 (0.82, 1.05)
14	747	747		1.00 (0.89, 1.13)
18C	1253	1482		0.85 (0.74, 0.97)
19A	518	645		0.80 (0.71, 0.90)
19F	266	333		0.80 (0.70, 0.91)

Table 6. OPA GMTs 1 Month After Vaccination in Adults 60 Years of Age and Older Given Prevnar 20 Compared to Prevnar 13 for the 13 Matched Serotypes and PPSV23 for the 7 Additional Serotypes (Study 1)[a,b,c,d]

	Prevnar 20 (N = 1157–1430)	Prevnar 13 (N = 1390–1419)	PPSV23 (N = 1201–1319)	Vaccine Comparison
	GMT[e]	GMT[e]	GMT[e]	GMT Ratio[e] (95% CI)[e]
23F	277	335		0.83 (0.70, 0.97)
Additional Serotypes				
8	466		848	0.55 (0.49, 0.62)
10A	2008		1080	1.86 (1.63, 2.12)
11A	4427		2535	1.75 (1.52, 2.01)
12F	2539		1717	1.48 (1.27, 1.72)
15B	2398		769	3.12 (2.62, 3.71)
22F	3666		1846	1.99 (1.70, 2.32)
33F	5126		3721	1.38 (1.21, 1.57)

Abbreviations: CI = confidence interval; GMT = geometric mean titer; LLOQ = lower limit of quantitation; N = number of participants; OPA = opsonophagocytic activity; PPSV23 = pneumococcal polysaccharide vaccine (23-valent).

a. Study 1 was conducted in the United States and in Sweden (NCT03760146).
b. Noninferiority for a serotype was met if the lower bound of the 2-sided 95% CI for the GMT ratio (ratio of Prevnar 20/comparator) was greater than 0.5 (2-fold criterion for noninferiority).
c. Assay results below the LLOQ were set to 0.5 × LLOQ in the analysis.
d. Evaluable immunogenicity population.
e. GMTs, GMT ratios, and the associated 2-sided CIs were based on analysis of log-transformed OPA titers using a regression model with vaccine group, sex, smoking status, age at vaccination in years, and baseline log-transformed OPA titers.

Immunobridging in Pneumococcal Vaccine Naïve Adults 18 Through 59 Years of Age

In Study 1 (described above), the effectiveness of Prevnar 20 in adults 50 through 59 years of age and in adults 18 through 49 years of age was inferred following comparison of the immune response to each of the 20 vaccine serotypes in each of these age groups to the corresponding serotype-specific immune responses in adults 60 through 64 years of age following Prevnar 20 (immunobridging). In Study 1, pneumococcal vaccine-naïve participants 50 through 59 years of age and 18 through 49 years of age were randomly assigned (3:1 ratio) to receive 1 vaccination with Prevnar 20 or Prevnar 13. Serotype-specific OPA GMTs were measured before vaccination and 1 month after vaccination. A comparative analysis of Prevnar 20 in the younger age group versus Prevnar 20 in adults 60 through 64 years of age for each vaccine serotype was performed to support the indication in adults 18 through 49 years of age and 50 through 59 years of age. Immunobridging was to be declared successful if the lower bound of the 2-sided 95% CI for the GMT ratio (Prevnar 20 in participants 18 through 49 years of age/60 through 64 years of age and in participants 50 through 59 years of age/60 through 64 years of age) for the 20 serotypes was >0.5 (2-fold). Prevnar 20 elicited serotype-specific immune responses to each of the 20 vaccine serotypes in both of the younger age groups that were within 2-fold of the corresponding serotype-specific responses in adults 60 through 64 years of age, when measured 1 month after vaccination (Table 7).

Table 7. Comparisons of OPA GMTs 1 Month After Prevnar 20 in Adults 18 Through 49 or 50 Through 59 Years of Age to Adults 60 Through 64 Years of Age (Study 1)[a,b,c,d]

	18–49 Years (N = 251–317)	60–64 Years (N = 765–941)	18–49 Years Relative to 60–64 Years	50–59 Years (N = 266–320)	60–64 Years (N = 765–941)	50–59 Years Relative to 60–64 Years
	GMT[e]	GMT[e]	GMT Ratio[e] (95% CI)[e]	GMT[e]	GMT[e]	GMT Ratio[e] (95% CI)[e]
Serotype						
1	163	132	1.23 (1.01, 1.50)	136	132	1.03 (0.84, 1.26)
3	42	42	1.00 (0.87, 1.16)	43	41	1.06 (0.92, 1.22)
4	1967	594	3.31 (2.65, 4.13)	633	578	1.10 (0.87, 1.38)
5	108	97	1.11 (0.91, 1.36)	85	97	0.88 (0.72, 1.07)
6A	3931	1023	3.84 (3.06, 4.83)	1204	997	1.21 (0.95, 1.53)
6B	4260	1250	3.41 (2.73, 4.26)	1503	1199	1.25 (1.00, 1.56)
7F	1873	1187	1.58 (1.30, 1.91)	1047	1173	0.89 (0.74, 1.07)
9V	6041	1727	3.50 (2.83, 4.33)	1726	1688	1.02 (0.83, 1.26)
14	1848	773	2.39 (1.93, 2.96)	926	742	1.25 (1.01, 1.54)
18C	4460	1395	3.20 (2.53, 4.04)	1805	1355	1.33 (1.06, 1.68)
19A	1415	611	2.31 (1.91, 2.81)	618	600	1.03 (0.85, 1.25)
19F	655	301	2.17 (1.76, 2.68)	287	290	0.99 (0.80, 1.22)
23F	1559	325	4.80 (3.65, 6.32)	549	328	1.68 (1.27, 2.22)
Additional Serotypes						
8	867	508	1.71 (1.38, 2.12)	487	502	0.97 (0.78, 1.20)
10A	4157	2570	1.62 (1.31, 2.00)	2520	2437	1.03 (0.84, 1.28)
11A	7169	5420	1.32 (1.04, 1.68)	6417	5249	1.22 (0.96, 1.56)
12F	5875	3075	1.91 (1.51, 2.41)	3445	3105	1.11 (0.88, 1.39)
15B	4601	3019	1.52 (1.13, 2.05)	3356	2874	1.17 (0.88, 1.56)
22F	7568	4482	1.69 (1.30, 2.20)	3808	4228	0.90 (0.69, 1.17)

Table 7. Comparisons of OPA GMTs 1 Month After Prevnar 20 in Adults 18 Through 49 or 50 Through 59 Years of Age to Adults 60 Through 64 Years of Age (Study 1)[a,b,c,d]

	18–49 Years (N = 251–317)	60–64 Years (N = 765–941)	18–49 Years Relative to 60–64 Years	50–59 Years (N = 266–320)	60–64 Years (N = 765–941)	50–59 Years Relative to 60–64 Years
	GMT[e]	GMT[e]	GMT Ratio[e] (95% CI)[e]	GMT[e]	GMT[e]	GMT Ratio[e] (95% CI)[e]
33F	7977	5693	1.40 (1.10, 1.79)	5571	5445	1.02 (0.81, 1.30)

Abbreviations: CI = confidence interval; GMT = geometric mean titer; LLOQ = lower limit of quantitation; N = number of participants; OPA = opsonophagocytic activity; PPSV23 = pneumococcal polysaccharide vaccine 23-valent vaccine.

a. Study 1 was conducted in the United States and in Sweden (NCT03760146).
b. Immunobridging for a serotype was met if the lower bound of the 2-sided 95% CI for the GMT ratio (ratio of younger age group/60 through 64 years of age group) was greater than 0.5 (2-fold success criterion).
c. Assay results below the LLOQ were set to 0.5 × LLOQ in the analysis.
d. Evaluable immunogenicity population.
e. GMTs, GMT ratios, and the associated 2-sided CIs were based on analysis of log-transformed OPA titers using a regression model with age group, sex, smoking status, and baseline log-transformed OPA titers. The comparisons between adults 18 through 49 years of age and adults 60 through 64 years of age and between adults 50 through 59 years of age and adults 60 through 64 years of age were based on separate regression models.

Immunogenicity of Prevnar 20 in Adults Previously Vaccinated With Pneumococcal Vaccine

A randomized, open-label clinical trial (Study 6) described immune responses to Prevnar 20 in adults 65 years of age and older previously vaccinated with PPSV23 (≥1 to ≤5 years prior to enrollment), previously vaccinated with Prevnar 13 (≥6 months prior to enrollment), or previously vaccinated with Prevnar 13 followed by PPSV23 (with PPSV23 vaccination ≥1 year prior to enrollment). Participants in this clinical trial previously vaccinated with Prevnar 13 (Prevnar 13 only or followed by PPSV23) were enrolled at sites in the United States and participants previously vaccinated with PPSV23 only were also enrolled from Swedish sites (35.5% in that category). Immune responses elicited by Prevnar 20 were measured by an OPA assay.

OPA GMTs in participants who received PPSV23 1 to 5 years prior to Prevnar 20 were diminished compared to OPA GMTs in participants who received Prevnar 13 at least 6 months previously and compared to OPA GMTs in participants who received Prevnar 13 followed by PPSV23, with the last PPSV23 dose given at least 1 year prior to Prevnar 20.

14.3 Concomitant Vaccine Administration

Clinical Trial in Adults to Assess Prevnar 20 Given With Influenza Vaccine, Adjuvanted (Fluad Quadrivalent)

Study 7 was a double-blind, randomized study conducted in adults 65 years of age and older who had no history of prior pneumococcal vaccination or who had previously received PPSV23 and/or Prevnar 13 at least 6 months prior to enrollment. Study participants were randomized in a 1:1 ratio to receive Prevnar 20 concomitantly administered with Fluad Quadrivalent followed approximately one month later by placebo (Group 1, N=898) or Fluad Quadrivalent concomitantly administered with placebo followed approximately one month later by Prevnar 20 (Group 2, N=898). Pneumococcal serotype-specific OPA GMTs were evaluated 1 month after Prevnar 20 and influenza vaccine strain hemagglutinin inhibition assay (HAI) GMTs were evaluated 1 month after Fluad Quadrivalent. The noninferiority criteria for the comparisons of OPA GMTs (lower limit of the 2-sided 95% CI of the GMT ratio [Group 1/Group 2] >0.5, 2-fold noninferiority criterion) were met for all 20 pneumococcal serotypes in Prevnar 20. The noninferiority criteria for the comparisons of HAI GMTs (lower limit of the 2- sided 95% CI for the GMT ratio [Group 1/Group 2] >0.67, 1.5-fold noninferiority criterion) were also met for all 4 influenza vaccine strains.

16 HOW SUPPLIED/STORAGE AND HANDLING

Pre-filled Syringe, 1 Dose (10 per package) – NDC 0005-2000-10.
Pre-filled Syringe, 1 Dose (1 per package) – NDC 0005-2000-02.

After shipping, Prevnar 20 may arrive at temperatures between 2 ºC to 25 ºC (36 ºF to 77 ºF).

Upon receipt, store refrigerated at 2 ºC to 8 ºC (36 ºF to 46 ºF).

Syringes should be stored in the refrigerator horizontally to minimize the resuspension time.

Do not freeze. Discard if the vaccine has been frozen.

Prevnar 20 should be administered as soon as possible after being removed from refrigeration.

Prevnar 20 can be administered provided total (cumulative multiple excursions) time out of refrigeration (at temperatures between 8 °C and 25 °C) does not exceed 96 hours. Cumulative multiple excursions between 0 °C and 2 °C are also permitted as long as the total time between 0 °C and 2 °C does not exceed 72 hours. These are not, however, recommendations for storage.

The tip cap and plunger stopper of the pre-filled syringe are not made with natural rubber latex.

17 PATIENT COUNSELING INFORMATION

Prior to administration of this vaccine, inform the individual of the following:

- The potential benefits and risks of immunization with Prevnar 20 *[see Warnings and Precautions (5), Adverse Reactions (6)]*.
- Any suspected adverse reactions should be reported to their healthcare professional.

This product's labeling may have been updated. For the most recent prescribing information, please visit www.pfizer.com.

US Govt. License No. 3

LAB-1436-1.4

CPT Code 90677

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use VAXNEUVANCE safely and effectively. See full prescribing information for VAXNEUVANCE.

VAXNEUVANCE™ (Pneumococcal 15-valent Conjugate Vaccine) Suspension for Intramuscular Injection
Initial U.S. Approval: 2021

----------RECENT MAJOR CHANGES ----------

Indications and Usage (1)	06/2022
Dosage and Administration (2.3, 2.4)	06/2022
Warnings and Precautions (5.1, 5.3)	06/2022

----------INDICATIONS AND USAGE ----------

VAXNEUVANCE™ is a vaccine indicated for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F in individuals 6 weeks of age and older. (1)

---------- DOSAGE AND ADMINISTRATION ----------

For intramuscular injection only.
Each dose of VAXNEUVANCE is 0.5 mL. (2.1)
Children: Administer VAXNEUVANCE as a 4-dose series at 2, 4, 6 and 12 through 15 months of age. (2.3)
Adults: Administer VAXNEUVANCE as a single dose in adults 18 years of age and older. (2.3)

---------- DOSAGE FORMS AND STRENGTHS ----------

Suspension for injection (0.5 mL dose), supplied as a single-dose prefilled syringe. (3)

----------CONTRAINDICATIONS ----------

Severe allergic reaction (e.g., anaphylaxis) to any component of VAXNEUVANCE or to diphtheria toxoid. (4)

---------- WARNINGS AND PRECAUTIONS----------

Apnea following intramuscular vaccination has been observed in some infants born prematurely. A decision about when to administer VAXNEUVANCE to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination. (5.3)

---------- ADVERSE REACTIONS ----------

The most commonly reported solicited adverse reactions:

- in children vaccinated with a 4-dose series at 2, 4, 6 and 12 through 15 months of age, provided as a range across the series, were: irritability (57.3% to 63.4%), somnolence (24.2% to 47.5%), injection-site pain (25.9% to 40.3%), fever ≥38.0°C (13.3% to 20.4%), decreased appetite (14.1% to 19.0%), injection-site induration (13.2% to 15.4%), injection-site erythema (13.7% to 21.4%) and injection-site swelling (11.3% to 13.4%). (6.1)
- in children and adolescents 2 through 17 years of age vaccinated with a single dose were: injection-site pain (54.8%), myalgia (23.7%), injection-site swelling (20.9%), injection-site erythema (19.2%), fatigue (15.8%), headache (11.9%) and injection-site induration (6.8%). (6.1)
- in adults 18 through 49 years of age were: injection-site pain (75.8%), fatigue (34.3%), myalgia (28.8%), headache (26.5%), injection-site swelling (21.7%), injection-site erythema (15.1%) and arthralgia (12.7%). (6.1)
- in adults 50 years of age and older were: injection-site pain (66.8%), myalgia (26.9%), fatigue (21.5%), headache (18.9%), injection-site swelling (15.4%), injection-site erythema (10.9%) and arthralgia (7.7%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov .

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 06/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

VAXNEUVANCE™ is indicated for active immunization for the prevention of invasive disease caused by *Streptococcus pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F in individuals 6 weeks of age and older.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Dosage
Each dose of VAXNEUVANCE is 0.5 mL.

2.2 Administration
Hold the prefilled syringe horizontally and shake vigorously immediately prior to use to obtain an opalescent suspension. Do not use the vaccine if it cannot be resuspended. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use if particulate matter or discoloration is observed.

2.3 Vaccination Schedule
Children
Administer VAXNEUVANCE as a 4-dose series at 2, 4, 6 and 12 through 15 months of age (and at least 2 months after the third dose). The first dose may be given as early as 6 weeks of age.

The 4-dose series initiated with a lower valency pneumococcal conjugate vaccine can be completed with VAXNEUVANCE *[see Clinical Studies (14.1)]*.

Adults
Administer VAXNEUVANCE as a single dose in adults 18 years of age and older.

2.4 Catch-Up Vaccination Schedule in Children and Adolescents
Children 7 months through 17 years of age who have never received a pneumococcal conjugate vaccine may receive VAXNEUVANCE according to the schedule in Table 1:

Table 1: Catch-Up Vaccination Schedule for Individuals Initiating Vaccination at 7 Months Through 17 Years of Age

Age at First Dose	Total Number of 0.5 mL Doses
7 through 11 months of age	3*
12 through 23 months of age	2†
2 years through 17 years of age	1

* The first 2 doses are given at least 4 weeks apart; third dose given after the one-year birthday, separated from the second dose by at least 2 months.
† Two doses at least 2 months apart.

Children and Adolescents Previously Vaccinated with a Pneumococcal Conjugate Vaccine
Administer a single dose of VAXNEUVANCE to children and adolescents 2 years through 17 years of age who have received an incomplete series of another pneumococcal conjugate vaccine. At least 2 months should elapse between receipt of the last dose of another pneumococcal conjugate vaccine and administration of VAXNEUVANCE.

3 DOSAGE FORMS AND STRENGTHS

VAXNEUVANCE is a suspension for intramuscular injection supplied in a 0.5 mL single-dose prefilled syringe.

4 CONTRAINDICATIONS

Do not administer VAXNEUVANCE to individuals with a severe allergic reaction (e.g., anaphylaxis) to any component of VAXNEUVANCE or to diphtheria toxoid. *[See Description (11).]*

5 WARNINGS AND PRECAUTIONS

5.1 Management of Allergic Reactions
Appropriate medical treatment to manage allergic reactions must be immediately available in the event an acute anaphylactic reaction occurs following administration of VAXNEUVANCE.

5.2 Altered Immunocompetence
Some individuals with altered immunocompetence, including those receiving immunosuppressive therapy, may have a reduced immune response to VAXNEUVANCE. *[See Use in Specific Populations (8.6).]*

5.3 Apnea in Premature Infants
Apnea following intramuscular vaccination has been observed in some infants born prematurely. A decision about when to administer VAXNEUVANCE to infants born prematurely should be based on consideration of the individual infant's medical status and the potential benefits and possible risks of vaccination.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

The most commonly reported solicited adverse reactions in children vaccinated with a 4-dose series at 2, 4, 6, and 12 through 15 months of age, provided as a range across the series, were: irritability (57.3% to 63.4%), somnolence (24.2% to 47.5%), injection-site pain (25.9% to 40.3%), fever ≥38.0°C (13.3% to 20.4%), decreased appetite (14.1% to 19.0%), injection-site induration (13.2% to 15.4%), injection-site erythema (13.7% to 21.4%) and injection-site swelling (11.3% to 13.4%).

The most commonly reported solicited adverse reactions in children and adolescents 2 through 17 years of age vaccinated with a single dose were: injection-site pain (54.8%), myalgia (23.7%), injection-site swelling (20.9%), injection-site erythema (19.2%), fatigue (15.8%), headache (11.9%) and injection-site induration (6.8%).

The most commonly reported solicited adverse reactions in adults 18 through 49 years of age were: injection-site pain (75.8%), fatigue (34.3%), myalgia (28.8%), headache (26.5%), injection-site swelling (21.7%), injection-site erythema (15.1%) and arthralgia (12.7%).

The most commonly reported solicited adverse reactions in adults 50 years of age and older were: injection-site pain (66.8%), myalgia (26.9%), fatigue (21.5%), headache (18.9%), injection-site swelling (15.4%), injection-site erythema (10.9%) and arthralgia (7.7%).

Clinical Trials Experience in Children 6 Weeks Through 17 Years of Age

Safety Assessment in Children Receiving a 4-Dose Series

The safety of VAXNEUVANCE in healthy infants (6 weeks through 11 months of age) and children (12 months through 15 months of age) was assessed in 4 randomized, double-blind clinical studies (Studies 8-11 (NCT03893448, NCT03620162, NCT03692871 and NCT02987972)) conducted in the Americas, Europe, and Asia Pacific. These studies included 3,349 participants who received at least one dose of a 4-dose series of VAXNEUVANCE, 1,814 participants who received at least one dose of a 4-dose series of Prevnar 13 [Pneumococcal 13-valent Conjugate Vaccine (Diphtheria CRM_{197} Protein)], and 538 participants who received VAXNEUVANCE to complete a 4-dose series of pneumococcal conjugate vaccine initiated with Prevnar 13. In the United States (including Puerto Rico), 2,827 participants received at least one dose of either VAXNEUVANCE or Prevnar 13 and 2,409 participants completed a 4-dose series of either vaccine. Overall, the median age of the participants was 9.0 weeks (6-12 weeks) and 48.6% were female. The racial distribution was as follows: 57.1% were White, 26.4% were Asian, 9.5% were Multi-racial, 4.7% were Black or African American, and 18.8% were of Hispanic or Latino ethnicity. There were no meaningful differences in demographic characteristics across the vaccination groups.

In Studies 8 and 9, Pentacel (Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Inactivated Poliovirus and Haemophilus b Conjugate [Tetanus Toxoid Conjugate] Vaccine) (DTaP-IPV-Hib vaccine) for US participants or a non-US-licensed DTaP-IPV-Hib vaccine for non-US participants was administered concomitantly with VAXNEUVANCE at 2, 4 and 6 months of age. RotaTeq (Rotavirus Vaccine, Live, Oral, Pentavalent) and RECOMBIVAX HB (Hepatitis B Vaccine [Recombinant]) were also administered concomitantly at 2, 4, and 6 months of age. M-M-R II (Measles, Mumps, and Rubella Virus Vaccine Live), VAQTA (Hepatitis A Vaccine, Inactivated), VARIVAX (Varicella Virus Vaccine Live), and Hiberix (Haemophilus b Conjugate Vaccine [Tetanus Toxoid Conjugate]) were administered concomitantly with VAXNEUVANCE at 12 through 15 months of age. Study 9 also evaluated the use of VAXNEUVANCE to complete a pneumococcal conjugate vaccine series initiated with Prevnar 13.

Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination. Study investigators reviewed the VRC with the participant or participant's legal guardian 15 days postvaccination to ensure consistency with protocol definitions. The analyses presented in Tables 2-3 below reflect the information based on the final assessment by the study investigators. Injection-site adverse events and systemic adverse events were solicited on Day 1 through Day 14 postvaccination. Body temperature was solicited on Day 1 through Day 7 postvaccination via rectal or axillary measurement. Unsolicited adverse events were monitored using the VRC through 14 days postvaccination. The duration of the safety follow-up period for serious adverse events following the last study vaccination was 1 month in Study 11 and 6 months in Studies 8-10.

Solicited Adverse Reactions in Children Receiving a 4-Dose Series

Study 8 was a multicenter, double-blind, active comparator-controlled study that assessed the safety of VAXNEUVANCE when administered as a 4-dose series in children (N=858 received VAXNEUVANCE and N=855 received Prevnar 13). The percentage of US participants with solicited adverse reactions that occurred within 14 days following administration of VAXNEUVANCE or Prevnar 13 are shown in Tables 2-3. Solicited adverse reactions following administration of VAXNEUVANCE lasted a median of 1 day with 90.6% of reactions lasting ≤3 days.

Table 2: Percentage of US Participants with Solicited Local Adverse Reactions in Infants at 2, 4, 6 and 12 through 15 Months of Age After Vaccination (Study 8)*

Dose	Dose 1		Dose 2		Dose 3		Dose 4	
	VAXNEUVANCE (%) N=598	Prevnar 13 (%) N=600	VAXNEUVANCE (%) N=584	Prevnar 13 (%) N=570	VAXNEUVANCE (%) N=559	Prevnar 13 (%) N=540	VAXNEUVANCE (%) N=532	Prevnar 13 (%) N=507
Local Reactions†								
Pain‡								
Any	40.3	39.5	32.0	28.8	30.8	26.9	25.9	25.0
Mild	24.1	23.2	18.7	14.7	17.9	16.7	16.9	16.4
Moderate	14.7	15.2	12.5	13.3	12.3	10.0	8.8	8.7
Severe	1.5	1.2	0.9	0.7	0.5	0.2	0.2	0.0
Induration								
Any	14.0	12.7	13.2	16.1	15.4	16.3	13.7	14.6
≤2.5 cm	11.0	10.0	9.1	11.4	10.7	11.5	7.5	8.5
2.6-7.6 cm	2.8	5.4	4.1	4.7	4.7	4.8	6.2	6.1
>7.6 cm	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Erythema								
Any	13.7	14.7	16.4	22.5	20.4	23.9	21.4	24.1
≤2.5 cm	11.0	10.8	12.7	16.7	15.4	17.4	14.7	16.8
2.6-7.6 cm	2.3	3.5	3.8	5.6	4.8	6.5	6.8	7.1
>7.6 cm	0.3	0.2	0.0	0.2	0.2	0.0	0.0	0.2
Swelling								
Any	12.9	12.7	13.2	11.4	13.4	10.4	11.3	10.8
≤2.5 cm	9.5	7.2	8.2	6.5	8.6	5.7	5.8	7.3
2.6-7.6 cm	3.2	5.3	4.8	4.6	4.8	4.4	5.5	3.4
>7.6 cm	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0

* Study 8 (NCT03893448) was a randomized, double-blind, active comparator-controlled clinical study. Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination following each dose. The table represents the final assessment by the study investigators upon review of the VRC 15 days postvaccination, to ensure consistency with protocol definitions.

† Solicited on Day 1 through Day 14 postvaccination following each dose.

‡ Mild: awareness of symptoms, but easily tolerated; moderate: definitely acting like something is wrong; severe: extremely distressed or unable to do usual activities.

N=Number of participants vaccinated, including those with missing solicited adverse event data. The percentage of participants with missing solicited adverse event data, provided as a range across the 4-dose series, was 0.8% to 3.9%.

Table 3: Percentage of US Participants with Solicited Systemic Adverse Reactions in Infants at 2, 4, 6 and 12 through 15 Months of Age After Vaccination (Study 8)*

Dose	Dose 1		Dose 2		Dose 3		Dose 4	
	VAXNEUVANCE (%) N=598	Prevnar 13 (%) N=600	VAXNEUVANCE (%) N=584	Prevnar 13 (%) N=570	VAXNEUVANCE (%) N=559	Prevnar 13 (%) N=540	VAXNEUVANCE (%) N=532	Prevnar 13 (%) N=507
Systemic Reactions†								
Irritability‡								
Any	63.4	67.3	57.4	58.1	59.0	55.4	57.3	56.6
Mild	27.3	29.3	23.6	21.9	30.2	28.9	28.0	26.6
Moderate	31.4	33.0	30.0	33.2	25.0	24.4	26.7	27.4
Severe	4.7	5.0	3.6	3.0	3.8	2.0	2.6	2.6
Somnolence‡								
Any	47.5	52.7	35.6	39.3	31.1	30.2	24.2	29.6
Mild	24.2	29.5	20.2	18.8	19.1	16.3	13.9	17.0
Moderate	21.6	21.8	14.6	19.6	11.4	12.8	10.0	11.8
Severe	1.7	1.3	0.9	0.9	0.5	1.1	0.4	0.8
Decreased appetite‡								
Any	18.2	19.0	19.0	16.0	14.1	17.8	17.5	16.4
Mild	11.0	11.2	12.0	8.2	7.5	11.1	9.2	10.7
Moderate	6.7	7.2	7.0	7.4	6.3	6.5	7.9	5.5
Severe	0.5	0.7	0.0	0.4	0.4	0.2	0.4	0.2
Urticaria‡								
Any	1.2	0.8	1.5	1.4	1.1	1.9	3.4	2.6
Mild	0.8	0.5	1.4	0.7	1.1	1.5	1.7	1.2
Moderate	0.2	0.2	0.2	0.7	0.0	0.2	1.5	1.2
Severe	0.2	0.2	0.0	0.0	0.0	0.2	0.2	0.2
Fever§ ¶								
≥38.0°C	18.4	16.4	20.4	21.7	20.0	20.0	13.3	14.0
≥38.0°C to <39.0°C	17.3	15.7	18.5	18.1	17.2	17.2	12.1	13.2
≥39.0°C to <40.0°C	1.0	0.7	1.6	3.4	2.4	2.5	0.8	0.8
≥40.0°C	0.0	0.0	0.4	0.2	0.4	0.2	0.4	0.0

* Study 8 (NCT03893448) was a randomized, double-blind, active comparator-controlled clinical study. Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination following each dose. The table represents the final assessment by the study investigators upon review of the VRC 15 days postvaccination, to ensure consistency with protocol definitions.
† Solicited on Day 1 through Day 14 postvaccination following each dose.
‡ Mild: awareness of symptoms, but easily tolerated; moderate: definitely acting like something is wrong; severe: extremely distressed or unable to do usual activities.
§ Solicited on Day 1 through Day 7 postvaccination following each dose.
¶ Percentages reflect the number of participants with temperature data.
Following Doses 1-3, rectal temperature measurements were provided for 76.7% to 77.6% of participants and axillary temperature measurements were provided for 22.4% to 23.3% of participants, provided as a range across the doses.
Following Dose 4, rectal temperature measurements were provided for 70.6% of participants and axillary temperature measurements were provided for 29.4% of participants.
N=Number of participants vaccinated, including those with missing solicited adverse event data. The percentage of participants with missing solicited adverse event data, provided as a range across the 4-dose series, was 0.8% to 3.9%.

Across Studies 8-10 (excluding participants in Study 9 who received VAXNEUVANCE to complete a pneumococcal conjugate vaccine series initiated with Prevnar 13), the percentage of participants with fever that occurred within 7 days following administration of VAXNEUVANCE or Prevnar 13 is shown in Table 4.

Table 4: Percentage of Participants with Fever in Infants at 2, 4, 6 and 12 through 15 Months of Age After Vaccination (Studies 8-10)*

Dose	Dose 1		Dose 2		Dose 3		Dose 4	
	VAXNEUVANCE (%) N=2,995	Prevnar 13 (%) N=1,458	VAXNEUVANCE (%) N=2,902	Prevnar 13 (%) N=1,394	VAXNEUVANCE (%) N=2,865	Prevnar 13 (%) N=1,344	VAXNEUVANCE (%) N=2,772	Prevnar 13 (%) N=1,287
Fever†								
≥38.0°C	15.2	12.6	19.2	18.3	17.1	16.4	15.2	13.0
≥38.0°C to <39.0°C	14.4	11.7	17.1	15.8	14.6	14.7	12.7	11.4
≥39.0°C to <40.0°C	0.7	0.9	2.0	2.2	2.3	1.6	1.9	1.4
≥40.0°C	0.0	0.0	0.1	0.3	0.2	0.1	0.5	0.2

* Studies 8-10 (NCT03893448, NCT03620162 and NCT03692871) were randomized, double-blind, active comparator-controlled clinical studies. Licensed pediatric vaccines were administered concomitantly according to the study design or local recommended schedule.
† Solicited on Day 1 through Day 7 postvaccination following each dose.
Following Doses 1-3, rectal temperature measurements were provided for 53.2% to 54.9% of participants and axillary temperature measurements were provided for 45.1% to 46.8% of participants, provided as a range across the doses.
Following Dose 4, rectal temperature measurements were provided for 47.0% of participants and axillary temperature measurements were provided for 53.0% of participants.
N=Number of participants with temperature data.

Unsolicited Adverse Reactions in Children Receiving a 4-Dose Series
Across Studies 8-11 (excluding participants in Study 9 who received VAXNEUVANCE to complete a pneumococcal conjugate vaccine series initiated with Prevnar 13), injection-site urticaria within 14 days following each dose of VAXNEUVANCE occurred in up to 0.6% of children. Participants in these studies may have received either US-licensed or non-US licensed concomitant vaccines according to the local recommended schedule.

Serious Adverse Events in Children Receiving a 4-Dose Series
Among children who received VAXNEUVANCE (N=3,349) or Prevnar 13 (N=1,814) across Studies 8-11 (excluding participants in Study 9 who received VAXNEUVANCE to complete a pneumococcal conjugate vaccine series initiated with Prevnar 13), serious adverse events up to 6 months following vaccination with the 4-dose series were reported by 9.6% of VAXNEUVANCE recipients and by 8.9% of Prevnar 13 recipients. Participants in these studies may have received either US-licensed or non-US licensed concomitant vaccines according to the local recommended schedule.

Up to 30 days following completion of Doses 1 through 3, serious adverse events were reported by 4.8% of VAXNEUVANCE recipients and by 5.0% of Prevnar 13 recipients. An adverse reaction of febrile seizure was reported in a 9 week old female (Study 11) one day after receiving VAXNEUVANCE (Dose 1) and recommended infant vaccines. Up to 30 days following Dose 4, serious adverse events were reported by 1.0% of VAXNEUVANCE recipients and by 0.7% of Prevnar 13 recipients.

There were no notable patterns or numerical imbalances between vaccination groups for specific categories of serious adverse events that would suggest a causal relationship to VAXNEUVANCE.

Safety of VAXNEUVANCE When Used to Complete a 4-Dose Pneumococcal Conjugate Vaccine Series Initiated with Prevnar 13
The safety profile observed when VAXNEUVANCE was used to complete a 4-dose pneumococcal conjugate vaccine series initiated with Prevnar 13 was similar to the safety profile following a complete 4-dose regimen of either VAXNEUVANCE or Prevnar 13 *[see Clinical Studies (14.1)]*.

Safety Assessment in Infants and Children Receiving Catch-Up Vaccination
The safety of VAXNEUVANCE in healthy infants and children 7 months through 17 years of age was assessed in a double-blind, multi-regional, clinical study (Study 12, NCT03885934). Participants were randomized to receive 1 to 3 doses of VAXNEUVANCE (N=303) or Prevnar 13 (N=303), depending on age at enrollment. All infants and children less than 2 years of age were pneumococcal vaccine-naïve. Among 352 children 2 through 17 years of age, 42.9% had a history of previous vaccination with a lower valency

pneumococcal conjugate vaccine. Among participants 7 through 11 months of age, the median age was 8.0 months, 48.4% were female, 82.8% were Asian, 17.2% were White and none were of Hispanic or Latino ethnicity. Among participants 12 through 23 months of age, the median age was 18.0 months, 54.0% were female, 83.3% were Asian, 16.7% were White and 0.8% were of Hispanic or Latino ethnicity. Among participants 2 through 17 years of age, the median age was 4.0 years, 47.7% were female, 66.8% were White, 33.0% were Asian, and none were of Hispanic or Latino ethnicity. The safety assessment was consistent with that used in Studies 8-11, as described above with the exception that in children 3 years of age and older, oral or axillary temperature measurements were obtained. The duration of the safety follow-up period for serious adverse events following the last dose of vaccine within each age cohort was 6 months.

Solicited Adverse Reactions in Children Receiving Catch-Up Vaccination
Among participants 7 through 11 months of age who received 3 doses of VAXNEUVANCE (N=64) or Prevnar 13 (N=64), the percentage of participants reporting solicited local and systemic adverse reactions that occurred within 14 days following any dose (VAXNEUVANCE participants vs. Prevnar 13 participants) were: fever ≥38.0°C (21.9% vs. 14.1%), irritability (32.8% vs. 43.8%), injection-site erythema (28.1% vs. 34.4%), somnolence (21.9% vs. 15.6%), injection-site swelling (18.8% vs. 15.6%), injection-site pain (18.8% vs. 7.8%), injection-site induration (17.2% vs. 14.1%), decreased appetite (15.6% vs. 18.8%) and urticaria (1.6% vs. 4.7%).

Among participants 12 through 23 months of age who received 2 doses of VAXNEUVANCE (N=62) or Prevnar 13 (N=64), the percentage of participants reporting solicited local and systemic adverse reactions that occurred within 14 days following any dose (VAXNEUVANCE participants vs. Prevnar 13 participants) were: fever ≥38.0°C (11.3% vs. 9.4%), irritability (35.5% vs. 21.9%), injection-site pain (33.9% vs. 23.4%), somnolence (24.2% vs. 17.2%), decreased appetite (22.6% vs. 18.8%), injection-site erythema (21.0% vs. 21.9%), injection-site swelling (14.5% vs. 12.5%) and injection-site induration (8.1% vs. 9.4%).

In children 2 through 17 years of age, the percentage of participants with solicited adverse reactions that occurred within 14 days following administration of a single dose of VAXNEUVANCE or Prevnar 13 is shown in Table 5.

Table 5: Percentage of Participants with Solicited Local and Systemic Adverse Reactions in Children and Adolescents 2 Years Through 17 Years of Age Using a Catch Up Vaccination Schedule (Study 12)*

	VAXNEUVANCE (%) N=177	Prevnar 13 (%) N=175
Local Reactions[†]		
Pain[‡]		
Any	54.8	56.6
Moderate	27.7	22.9
Severe	4.5	1.7
Swelling		
Any	20.9	24.0
2.6-7.6 cm	10.2	12.0
>7.6 cm	0.0	0.6
Erythema		
Any	19.2	21.1
2.6-7.6 cm	6.2	7.4
>7.6 cm	1.1	0.6
Induration		
Any	6.8	14.9
2.6-7.6 cm	3.4	5.7
>7.6 cm	0.0	0.0
Systemic Reactions[† ‡]		
Myalgia[§]		
Any	23.7	16.6
Moderate	14.7	6.9
Severe	0.6	0.6
Fatigue[§]		
Any	15.8	17.1
Moderate	6.2	5.7

	VAXNEUVANCE (%) N=177	Prevnar 13 (%) N=175
Severe	2.8	0.6
Headache[§]		
Any	11.9	13.7
Moderate	6.2	8.6
Severe	0.6	0.6
Somnolence[§]		
Any	2.8	2.9
Moderate	1.7	1.1
Severe	0.0	0.6
Irritability[§]		
Any	2.8	4.0
Moderate	0.6	0.6
Severe	0.0	0.0
Decreased appetite[§]		
Any	2.3	2.9
Moderate	0.6	1.7
Severe	0.0	0.0
Urticaria[§]		
Any	1.1	1.1
Moderate	0.0	0.0
Severe	0.0	0.0
Fever[¶ #]		
≥38.0°C	4.0	1.7
≥38.0°C to <39.0°C	2.8	1.7
≥39.0°C to <40.0°C	1.1	0.0
≥40.0°C	0.0	0.0

* Study 12 (NCT03885934) was a randomized, double-blind, active comparator-controlled clinical study. Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination following each dose. The table represents the final assessment by the study investigators upon review of the VRC 15 days postvaccination, to ensure consistency with protocol definitions.

† For all participants, reactions were solicited on Day 1 through Day 14 postvaccination following each dose.

‡ Different systemic adverse events were solicited for participants 2 to <3 years of age than for participants ≥3 to 17 years of age. For participants <3 years of age (VAXNEUVANCE N=32, Prevnar 13 N=28), decreased appetite, irritability, somnolence, and urticaria were solicited from Day 1 through Day 14 following vaccination. For participants ≥3 to 17 years of age, fatigue, headache, myalgia, arthralgia and urticaria were solicited from Day 1 through Day 14 following vaccination; no events of arthralgia were reported in VAXNEUVANCE recipients.

§ Moderate: definitely acting like something is wrong; severe: extremely distressed or unable to do usual activities.

¶ Solicited on Day 1 through Day 7 postvaccination following each dose.

Percentages reflect the number of participants with temperature data.

The percentage of participants 2 to <3 years of age with rectal temperature measurements was 5.0% and with axillary temperature measurements was 95.0%.

The percentage of participants ≥3 to 17 years of age with oral temperature measurements was 65.4% and with axillary temperature measurements was 34.6%.

N=Number of participants vaccinated.

Clinical Trials Experience in Adults

Safety Assessment in Clinical Studies

The safety of VAXNEUVANCE was assessed in 7 randomized, double-blind clinical studies conducted in the Americas, Europe and Asia Pacific, in which 5,630 adults 18 years of age and older received VAXNEUVANCE and 1,808 adults received Prevnar 13. In Studies 1-3 (NCT03950622, NCT03950856, and NCT03480763), a total of 3,032 adults 50 years of age and older with no history of pneumococcal vaccination received VAXNEUVANCE and 1,154 participants received Prevnar 13. In Study 4 (NCT03547167), adults 18 through 49 years of age with no history of pneumococcal vaccination, including individuals with increased risk of developing pneumococcal disease, received VAXNEUVANCE (N=1,134) or Prevnar 13 (N=378), followed by PNEUMOVAX 23 six months later. In Study 5 (NCT02573181), adults 65 years of age and older previously vaccinated with PNEUMOVAX 23 (at least 1 year prior to study entry) received VAXNEUVANCE (N=127) or Prevnar 13 (N=126). In Study 6 (NCT03615482), adults 50 years of age and older received VAXNEUVANCE concomitantly with a seasonal inactivated quadrivalent influenza vaccine (Fluarix Quadrivalent; QIV) (Group 1, N=600) or nonconcomitantly 30 days after QIV (Group 2, N=585). In this study population, 20.9% of individuals had a history of prior vaccination with PNEUMOVAX 23. In Study 7 (NCT03480802), HIV-infected adults 18 years of age and older received VAXNEUVANCE (N=152) or Prevnar 13 (N=150), followed by PNEUMOVAX 23 two months later.

The clinical studies included adults with stable underlying medical conditions (e.g., diabetes mellitus, renal disorders, chronic heart disease, chronic liver disease, chronic lung disease including asthma) and/or behavioral risk factors (e.g., smoking, increased alcohol use) that are known to increase the risk of pneumococcal disease. Overall, the mean age of the participants was 58 years and 54.6% were female. The racial distribution was as follows: 72.3% were White, 9.9% were Asian, 8.1% were American Indian or Alaska Native, 7.4% were Black or African American, and 18.1% were of Hispanic or Latino ethnicity.

In all studies, safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination. Study investigators reviewed the VRC with the participants 15 days postvaccination to ensure consistency with protocol definitions. The analyses presented in Tables 1-3 below reflect the information based on the final assessment by the study investigators. Oral body temperature and injection-site adverse reactions were solicited on Day 1 through Day 5 postvaccination. Systemic adverse reactions were solicited on Day 1 through Day 14 postvaccination. Unsolicited adverse events were reported on Day 1 through Day 14 postvaccination.

The duration of the safety follow-up period for serious adverse events postvaccination with VAXNEUVANCE was 1 month in Study 5; 2 months in Study 7; 6 months in Studies 1, 2, 4 and 6; and 12 months in Study 3.

Solicited Adverse Reactions

The percentage of participants with solicited adverse reactions that occurred within 5 or 14 days following administration of VAXNEUVANCE or Prevnar 13 in 3 studies are shown in Tables 6-8. The majority of solicited adverse reactions lasted ≤3 days.

Table 6: Percentage of Participants with Solicited Local and Systemic Adverse Reactions in Pneumococcal Vaccine-Naïve Adults 50 Years of Age and Older (Study 2)*

	VAXNEUVANCE (%) N=2,103	Prevnar 13 (%) N=230
Local Reactions†		
Pain		
Any	66.8	52.2
Grade 3‡	0.9	0.0
Erythema		
Any	10.9	9.6
>10 cm	0.6	0.4
Swelling		
Any	15.4	14.3
>10 cm	0.2	0.0
Systemic Reactions§		
Fatigue		
Any	21.5	22.2
Grade 3‡	0.7	0.9
Headache		
Any	18.9	18.7
Grade 3‡	0.8	0.0
Myalgia		
Any	26.9	21.7
Grade 3‡	0.4	0.0
Arthralgia		
Any	7.7	5.7
Grade 3‡	0.2	0.0
Fever†¶		
≥38.0°C and <38.5°C	0.6	0.4
≥38.5°C and <39.0°C	0.1	0.0
≥39.0°C	0.0	0.0

* Study 2 (NCT03950856) was a randomized (9:1), double-blind, active comparator-controlled, lot to lot consistency study. Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination. The table represents the final assessment by the study investigators upon review of the VRC 15 days postvaccination, to ensure consistency with protocol definitions.
† Solicited on Day 1 through Day 5 postvaccination.
‡ Any use of narcotic pain reliever or prevents daily activity.
§ Solicited on Day 1 through Day 14 postvaccination.
¶ Percentages are based on the number of participants with temperature data.
N=Number of participants vaccinated.

Table 7: Percentage of Participants with Solicited Local and Systemic Adverse Reactions in Pneumococcal Vaccine-Naïve Adults 18 to 49 Years of Age With or Without Risk Factors for Pneumococcal Disease (Study 4)*

	VAXNEUVANCE (%) N=1,134	Prevnar 13 (%) N=378
Local Reactions†		
Pain		
Any	75.8	68.8
Grade 3‡	1.1	1.6
Erythema		
Any	15.1	14.0
>10 cm	0.5	0.3
Swelling		
Any	21.7	22.2
>10 cm	0.4	0.5
Systemic Reactions§		
Fatigue		
Any	34.3	36.8
Grade 3‡	1.0	0.8
Headache		
Any	26.5	24.9
Grade 3‡	0.8	0.5
Myalgia		
Any	28.8	26.5
Grade 3‡	0.3	0.5
Arthralgia		
Any	12.7	11.6
Grade 3‡	0.4	0.0
Fever†¶		
≥38.0°C and <38.5°C	1.0	0.3
≥38.5°C and <39.0°C	0.3	0.0
≥39.0°C	0.2	0.0

* Study 4 (NCT03547167) was a randomized (3:1), double-blind, descriptive study. Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination. The table represents the final assessment by the study investigators upon review of the VRC 15 days postvaccination, to ensure consistency with protocol definitions.
† Solicited on Day 1 through Day 5 postvaccination.
‡ Any use of narcotic pain reliever or prevents daily activity.
§ Solicited on Day 1 through Day 14 postvaccination.
¶ Percentages are based on the number of participants with temperature data.
N=Number of participants vaccinated.

Table 8: Percentage of Participants with Solicited Local and Systemic Adverse Reactions in Adults 65 Years of Age and Older with Previous Pneumococcal Vaccination (Study 5)*

	VAXNEUVANCE (%) N=127	Prevnar 13 (%) N=126
Local Reactions†		
Pain		
Any	55.1	44.4
Grade 3‡	0.8	0.0
Erythema		
Any	7.9	7.1
>10 cm	0.8	0.0
Swelling		
Any	14.2	6.3
>10 cm	0.0	0.0
Systemic Reactions§		
Fatigue		
Any	18.1	19.0
Grade 3‡	0.0	0.0
Headache		
Any	13.4	15.9
Grade 3‡	0.0	0.0
Myalgia		
Any	15.7	11.1
Grade 3‡	0.8	0.0
Arthralgia		
Any	5.5	8.7
Grade 3‡	0.0	0.0
Fever†¶		
≥38.0°C and <38.5°C	1.6	0.0
≥38.5°C and <39.0°C	0.0	0.0
≥39.0°C	0.0	0.0

* Study 5 (NCT02573181) was a randomized, double-blind, descriptive study. Safety was monitored using a Vaccination Report Card (VRC) for up to 14 days postvaccination. The table represents the final assessment by the study investigators upon review of the VRC 15 days postvaccination, to ensure consistency with protocol definitions.
† Solicited on Day 1 through Day 5 postvaccination.
‡ Any use of narcotic pain reliever or prevents daily activity.
§ Solicited on Day 1 through Day 14 postvaccination.
¶ Percentages are based on the number of participants with temperature data.
N=Number of participants vaccinated.

Unsolicited Adverse Reactions
Across all studies, injection-site pruritus was reported to occur in up to 2.8% of adults vaccinated with VAXNEUVANCE.

Serious Adverse Events
Across all studies, among participants 18 years of age and older who received VAXNEUVANCE (excluding those who received QIV concomitantly; N=5,030) or Prevnar 13 (N=1,808), serious adverse events within 30 days postvaccination were reported by 0.4% of VAXNEUVANCE recipients and by 0.7% of Prevnar 13 recipients. In a subset of these studies, among those who received VAXNEUVANCE (N=4,751) and Prevnar 13 (N=1,532), serious adverse events within 6 months postvaccination were reported by 2.5% of VAXNEUVANCE recipients and by 2.4% of Prevnar 13 recipients.

There were no notable patterns or numerical imbalances between vaccination groups for specific categories of serious adverse events that would suggest a causal relationship to VAXNEUVANCE.

Safety with Concomitant Influenza Vaccine Administration
The safety profile was similar when VAXNEUVANCE was administered with or without inactivated quadrivalent influenza vaccine.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

There are no adequate and well-controlled studies of VAXNEUVANCE in pregnant women. Available data on VAXNEUVANCE administered to pregnant women are insufficient to inform vaccine-associated risks in pregnancy.

Developmental toxicity studies have been performed in female rats administered a human dose of VAXNEUVANCE on four occasions; twice prior to mating, once during gestation and once during lactation. These studies revealed no evidence of harm to the fetus due to VAXNEUVANCE *[see Animal Data below]*.

Data

Animal Data

Developmental toxicity studies have been performed in female rats. In these studies, female rats received a human dose of VAXNEUVANCE by intramuscular injection on day 28 and day 7 prior to mating, and on gestation day 6 and on lactation day 7. No vaccine related fetal malformations or variations were observed. No adverse effect on pup weight up to post-natal day 21 was noted.

8.2 Lactation

Risk Summary

Human data are not available to assess the impact of VAXNEUVANCE on milk production, its presence in breast milk, or its effects on the breastfed child. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VAXNEUVANCE and any potential adverse effects on the breastfed child from VAXNEUVANCE or from the underlying maternal condition. For preventive vaccines, the underlying condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

The safety and effectiveness of VAXNEUVANCE have been established in individuals 6 weeks through 17 years of age *[see Adverse Reactions (6.1) and Clinical Studies (14.1)].* The safety and effectiveness of VAXNEUVANCE in individuals younger than 6 weeks of age have not been established.

8.5 Geriatric Use

Of the 4,389 individuals aged 50 years and older who received VAXNEUVANCE, 2,478 (56.5%) were 65 years and older, and 479 (10.9%) were 75 years and older *[see Adverse Reactions (6.1) and Clinical Studies (14.1)].* Overall, there were no clinically meaningful differences in the safety profile or immune responses observed in older individuals (65 to 74 years and 75 years of age and older) when compared to younger individuals.

8.6 Individuals at Increased Risk for Pneumococcal Disease

Infants Born Prematurely

The safety and immunogenicity of VAXNEUVANCE were evaluated in preterm infants (<37 weeks gestation at birth) who were randomized to receive a complete 4-dose series of either VAXNEUVANCE (N=142) or Prevnar 13 (N=144) within Study 8, Study 9, and Study 10. Participants in these studies may have received either US-licensed or non-US licensed concomitant vaccines according to the local recommended schedule. In descriptive analyses, serotype-specific immunoglobulin G (IgG) and opsonophagocytic activity (OPA) responses at 30 days postdose 3, predose 4 and at 30 days postdose 4 were numerically similar between vaccination groups for the 13 shared serotypes and higher in VAXNEUVANCE for the 2 unique serotypes. The safety profile of VAXNEUVANCE was similar to the safety profile of Prevnar 13. In addition, the immune responses and safety profile in preterm infants receiving a 4-dose series of VAXNEUVANCE were similar to those observed in term infants in these studies. The effectiveness of VAXNEUVANCE in infants born prematurely has not been established.

Children with Sickle Cell Disease

In a double-blind, descriptive study (Study 13, NCT03731182), the safety and immunogenicity of VAXNEUVANCE were evaluated in children 5 through 17 years of age with sickle cell disease. Participants were randomized 2:1 to receive a single dose of VAXNEUVANCE (N=70) or Prevnar 13 (N=34). Immune responses were assessed by serotype-specific IgG GMCs and OPA GMTs at 30 days postvaccination for all 15 serotypes contained in VAXNEUVANCE. For all vaccine serotypes included in VAXNEUVANCE, serotype-specific IgG GMCs and OPA GMTs were higher following vaccination compared to pre-vaccination. IgG GMCs and OPA GMTs were numerically similar between the two vaccination groups for the 13 shared serotypes and higher in VAXNEUVANCE for serotypes 22F and 33F. The safety profile of VAXNEUVANCE was similar to the safety profile of Prevnar 13. The effectiveness of VAXNEUVANCE in children with sickle cell disease has not been established.

Individuals with HIV Infection

Children with HIV Infection

In a double-blind, descriptive study (Study 14, NCT03921424), the safety and immunogenicity of VAXNEUVANCE were evaluated in HIV-infected children 6 through 17 years of age, with CD4+ T-cell count ≥200 cells per microliter and plasma HIV RNA value <50,000 copies/mL. Participants were randomized to receive a single dose of VAXNEUVANCE (N=203) or Prevnar 13 (N=204), followed by PNEUMOVAX 23 two months later. For all vaccine serotypes included in VAXNEUVANCE, serotype-specific IgG GMCs and OPA GMTs were higher following vaccination compared to pre-vaccination. Serotype-specific IgG GMCs and OPA GMTs were numerically similar for the 13 shared serotypes and higher for the 2 unique serotypes (22F and 33F) at 30 days following vaccination with VAXNEUVANCE or Prevnar 13 and were numerically similar for all 15 serotypes contained in VAXNEUVANCE at 30 days following subsequent vaccination with PNEUMOVAX 23. The safety profile of VAXNEUVANCE was similar to the safety profile of Prevnar 13. The effectiveness of VAXNEUVANCE in HIV-infected children has not been established.

Adults with HIV Infection

In a double-blind, descriptive study (Study 7), the safety and immunogenicity of VAXNEUVANCE were evaluated in pneumococcal vaccine-naïve HIV-infected adults 18 years of age and older, with CD4+ T-cell count ≥50 cells per microliter and plasma HIV RNA value <50,000 copies/mL. Participants were randomized to receive VAXNEUVANCE (N=152) or Prevnar 13 (N=150), followed by PNEUMOVAX 23 two months later *[see Adverse Reactions (6.1)]*. Anti-pneumococcal opsonophagocytic activity (OPA) geometric mean antibody titers (GMTs) were higher after administration of VAXNEUVANCE, compared to pre-vaccination, for the 15 serotypes contained in VAXNEUVANCE. After sequential administration with PNEUMOVAX 23, OPA GMTs observed at 30 days after PNEUMOVAX 23 vaccination were numerically similar between the two vaccination groups for all 15 serotypes contained in VAXNEUVANCE. The safety profile of VAXNEUVANCE was similar to the safety profile of Prevnar 13. The effectiveness of VAXNEUVANCE in HIV-infected adults has not been established.

11 DESCRIPTION

VAXNEUVANCE (Pneumococcal 15-valent Conjugate Vaccine) is a sterile suspension of purified capsular polysaccharides from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F individually conjugated to CRM_{197}. Each pneumococcal capsular polysaccharide is activated via sodium metaperiodate oxidation and then individually conjugated to CRM_{197} carrier protein via reductive amination. CRM_{197} is a non-toxic variant of diphtheria toxin (originating from *Corynebacterium diphtheriae* C7) expressed recombinantly in *Pseudomonas fluorescens*.

Each of the fifteen serotypes is manufactured independently using the same manufacturing steps with slight variations to accommodate for differences in strains, polysaccharides and process stream properties. Each *S. pneumoniae* serotype is grown in media containing yeast extract, dextrose, salts and soy peptone. Each polysaccharide is purified by a series of chemical and physical methods. Then each polysaccharide is chemically activated and conjugated to the carrier protein CRM_{197} to form each glycoconjugate. CRM_{197} is isolated from cultures grown in a glycerol-based, chemically-defined, salt medium and purified by chromatography and ultrafiltration. The final vaccine is prepared by blending the fifteen glycoconjugates with aluminum phosphate adjuvant in a final buffer containing histidine, polysorbate 20 and sodium chloride.

Each 0.5 mL dose contains 2.0 mcg each of *S. pneumoniae* polysaccharide serotypes 1, 3, 4, 5, 6A, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F, and 4.0 mcg of polysaccharide serotype 6B, 30 mcg of CRM_{197} carrier protein, 1.55 mg L-histidine, 1 mg of polysorbate 20, 4.50 mg sodium chloride, and 125 mcg of aluminum as aluminum phosphate adjuvant. VAXNEUVANCE does not contain any preservatives.

The tip cap and plunger stopper of the prefilled syringe are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Protection against invasive disease is conferred mainly by antibodies (Immunoglobulin G [IgG] directed against capsular polysaccharides) and opsonophagocytic activity (OPA) against *S. pneumoniae*. VAXNEUVANCE induces IgG antibodies and OPA against the serotypes contained in the vaccine.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

VAXNEUVANCE has not been evaluated for carcinogenic or mutagenic potential or for impairment of male fertility in animals. VAXNEUVANCE administered to female rats had no effect on fertility *[see Use in Specific Populations (8.1)]*.

14 CLINICAL STUDIES

Immune responses elicited by VAXNEUVANCE and Prevnar 13 in children were measured by a pneumococcal electrochemiluminescence (Pn ECL) assay for total IgG and a multiplexed opsonophagocytic assay (MOPA) for opsonophagocytic killing for the 15 pneumococcal serotypes contained in VAXNEUVANCE postdose 3, predose 4 and postdose 4. In children, a serotype-specific Immunoglobulin G (IgG) antibody level corresponding to ≥0.35 mcg/mL using the WHO enzyme linked immunosorbent assay (ELISA) has been used as the threshold value for the clinical evaluation of pneumococcal conjugate vaccines. Immune responses elicited by VAXNEUVANCE and Prevnar 13 in adults were measured by MOPA and Pn ECL assays for the 15 pneumococcal serotypes contained in VAXNEUVANCE pre- and post-vaccination.

14.1 Clinical Trials in Children

Children Receiving a 4-Dose Series

In a double-blind, active comparator-controlled study (Study 8), participants were randomized to receive VAXNEUVANCE (N=860) or Prevnar 13 (N=860) in a 4-dose series; the first 3 doses were administered to infants at 2, 4, and 6 months of age and the fourth dose was administered to children 12 through 15 months of age. Pentacel (US participants) or a non-US-licensed DTaP-IPV-Hib vaccine (non-US participants), RECOMBIVAX HB, and RotaTeq were administered concomitantly with each of the 3 infant doses. VAQTA, M-M-R II, VARIVAX, and Hiberix were administered concomitantly with the fourth dose. *[See Adverse Reactions (6.1) and Clinical Studies (14.3).]*

Study 8 assessed serotype-specific IgG response rates, IgG geometric mean concentrations (GMCs), and opsonophagocytic activity (OPA) geometric mean titers (GMTs), for all 15 serotypes contained in VAXNEUVANCE. At 30 days postdose 3, VAXNEUVANCE was noninferior to Prevnar 13 for the 13 shared serotypes, as assessed by the proportion of participants meeting the serotype-specific IgG threshold value of ≥0.35 mcg/mL (response rate). VAXNEUVANCE was noninferior for the 2 unique vaccine serotypes, as assessed by the IgG response rates for serotypes 22F and 33F compared with the response rate for serotype 6B (the lowest response rate for any of the shared serotypes in Prevnar 13 among US participants, excluding serotype 3) at 30 days postdose 3 (Table 9).

Table 9: Proportions of US Participants with IgG Response Rates ≥0.35 mcg/mL at 30 Days Following Dose 3 in Infants Administered VAXNEUVANCE at 2, 4 and 6 Months of Age (Study 8)

Pneumococcal Serotype	VAXNEUVANCE (n=452-455)	Prevnar 13 (n=426-430)	Percentage Point Difference (VAXNEUVANCE – Prevnar 13) (95% CI)* †
	Observed Response Percentage	Observed Response Percentage	
Serotype			
1	93.8	98.6	-4.8 (-7.5, -2.4)
3	93.1	74.0	19.1 (14.4, 24.0)
4	94.7	98.1	-3.4 (-6.1, -1.0)
5	93.4	96.0	-2.6 (-5.7, 0.3)
6A	92.7	99.3	-6.6 (-9.4, -4.2)
6B	86.7	89.9	-3.2 (-7.5, 1.1)
7F	98.7	100.0	-1.3 (-2.9, -0.4)
9V	96.7	97.2	-0.5 (-2.9, 1.9)
14	97.8	98.1	-0.3 (-2.4, 1.7)
18C	96.2	98.1	-1.9 (-4.3, 0.3)
19A	97.4	99.8	-2.4 (-4.3, -1.0)
19F	98.5	100.0	-1.5 (-3.2, -0.6)
23F	89.8	91.4	-1.5 (-5.4, 2.4)
Additional Serotypes			
22F	98.0	‡	8.1 (5.1, 11.5)
33F	84.8	‡	-5.1 (-9.5, -0.7)

* CIs are based on the Miettinen & Nurminen method.

† A conclusion of non-inferiority of VAXNEUVANCE to Prevnar 13 is based on the lower bound of the 2-sided 95% CI for the difference in percentages (VAXNEUVANCE - Prevnar 13) being >-10 percentage points.

‡ A conclusion of non-inferiority of VAXNEUVANCE to Prevnar 13 is based on the comparison of the response rate for the 2 additional serotypes to the lowest responding Prevnar 13 serotype (serotype 6B), excluding serotype 3.

n=Number of participants contributing to the analysis.

CI=Confidence interval; IgG=Immunoglobulin G.

At 30 days postdose 3, serotype-specific IgG GMCs in the VAXNEUVANCE group were noninferior to Prevnar 13 for 12 of the 13 shared serotypes, except for serotype 6A. The IgG response to serotype 6A missed the prespecified noninferiority criterion by a small margin (the lower bound of the 2-sided 95% CI for the GMC ratio [VAXNEUVANCE/Prevnar 13] being 0.48 versus >0.5). VAXNEUVANCE was noninferior to Prevnar 13 for the 2 unique serotypes, as assessed by serotype-specific IgG GMCs for serotypes 22F and 33F compared with the IgG GMCs for serotype 4 (the lowest IgG GMC for any of the shared serotypes in Prevnar 13 among US participants, excluding serotype 3) (Table 10).

Table 10: Serotype-Specific IgG GMCs at 30 Days Following Dose 3 in US Infants Administered VAXNEUVANCE at 2, 4 and 6 Months of Age (Study 8)

Pneumococcal Serotype	VAXNEUVANCE (n=452-455)	Prevnar 13 (n=426-430)	GMC Ratio* (VAXNEUVANCE/Prevnar 13) (95% CI)* †
	GMC	GMC	
Serotype			
1	1.02	1.54	0.66 (0.61, 0.73)
3	0.96	0.56	1.70 (1.54, 1.86)
4	1.07	1.11	0.97 (0.89, 1.06)
5	1.29	1.69	0.76 (0.68, 0.85)
6A	1.33	2.48	0.53 (0.48, 0.60)
6B	1.42	1.58	0.90 (0.76, 1.06)
7F	2.17	2.83	0.77 (0.70, 0.84)
9V	1.47	1.48	1.00 (0.90, 1.10)
14	4.17	5.57	0.75 (0.66, 0.85)
18C	1.29	1.55	0.83 (0.76, 0.91)
19A	1.39	1.88	0.74 (0.67, 0.82)
19F	1.82	2.33	0.78 (0.72, 0.85)
23F	1.09	1.23	0.89 (0.79, 1.01)
Additional Serotypes			
22F	4.01	‡	3.63 (3.26, 4.04)
33F	1.38	‡	1.25 (1.09, 1.44)

* GMC ratio and CI are calculated using the t-distribution with the variance estimate from a serotype-specific linear model utilizing the natural log-transformed antibody concentrations as the response and a single term for vaccination group.

† A conclusion of non-inferiority of VAXNEUVANCE to Prevnar 13 is based on the lower bound of the 2-sided 95% CI for the GMC ratio (VAXNEUVANCE/Prevnar 13) being >0.5.

‡ A conclusion of non-inferiority of VAXNEUVANCE to Prevnar 13 is based on the comparison of the GMC for the 2 additional serotypes to the lowest responding Prevnar 13 serotype (serotype 4), excluding serotype 3.

n=Number of participants contributing to the analysis.

CI=Confidence interval; GMC=Geometric mean concentration (mcg/mL); IgG=Immunoglobulin G.

At 30 days postdose 4, serotype-specific IgG GMCs for VAXNEUVANCE were noninferior to Prevnar 13 for all 13 shared serotypes (the lower bound of the 2-sided 95% CI for the GMC ratio [VAXNEUVANCE/Prevnar 13] being >0.5) and for the 2 unique serotypes 22F and 33F as assessed by the IgG GMCs for serotypes 22F and 33F compared with the IgG GMCs for serotype 4 (the lowest IgG GMC for any of the shared serotypes in Prevnar 13 among US participants, excluding serotype 3) (Table 11).

Table 11: Serotype-Specific IgG GMCs at 30 Days Following Dose 4 in US Infants Administered VAXNEUVANCE at 2, 4, 6 and 12 to 15 Months of Age (Study 8)

Pneumococcal Serotype	VAXNEUVANCE (n=466-470)	Prevnar 13 (n=443-447)	GMC Ratio* (VAXNEUVANCE/Prevnar 13) (95% CI)* †
	GMC	GMC	
Serotype			
1	1.21	1.82	0.66 (0.60, 0.73)
3	0.91	0.63	1.43 (1.30, 1.57)
4	1.07	1.42	0.76 (0.68, 0.84)
5	2.21	3.47	0.64 (0.57, 0.71)
6A	3.56	5.93	0.60 (0.54, 0.67)
6B	4.70	6.07	0.77 (0.69, 0.87)
7F	3.22	4.65	0.69 (0.62, 0.77)
9V	2.18	2.86	0.76 (0.69, 0.84)
14	5.09	6.21	0.82 (0.72, 0.93)
18C	2.37	2.59	0.92 (0.82, 1.02)
19A	3.86	4.93	0.78 (0.71, 0.86)
19F	3.32	4.02	0.83 (0.75, 0.91)
23F	1.85	2.88	0.64 (0.57, 0.72)
Additional Serotypes			
22F	6.76	‡	4.77 (4.28, 5.32)
33F	3.80	‡	2.68 (2.40, 3.00)

* GMC ratios and CIs are calculated using the t-distribution with the variance estimate from a serotype-specific linear model utilizing the natural log-transformed antibody concentrations as the response and a single term for vaccination group.

† A conclusion of non-inferiority of VAXNEUVANCE to Prevnar 13 is based on the lower bound of the 2-sided 95% CI for the GMC ratio (VAXNEUVANCE/Prevnar 13) being >0.5.

‡ A conclusion of non-inferiority of VAXNEUVANCE to Prevnar 13 is based on the comparison of the GMC for the 2 additional serotypes to the lowest responding Prevnar 13 serotype (serotype 4), excluding serotype 3.

n=Number of participants contributing to the analysis.

CI=Confidence interval; GMC=Geometric mean concentration (mcg/mL); IgG=Immunoglobulin G.

Additionally, IgG response rates and IgG GMCs at 30 days postdose 3 and IgG GMCs at 30 days postdose 4 were statistically significantly greater for VAXNEUVANCE compared to Prevnar 13 for serotype 3 and the 2 unique serotypes (22F, 33F).

Serotype-specific OPA GMTs and response rates at 30 days postdose 3 and OPA GMTs at 30 days postdose 4 were descriptively evaluated in a subset of participants in Study 8. Serotype specific OPA GMTs and response rates were numerically similar across groups for the 13 shared serotypes and higher in the VAXNEUVANCE group for the 2 unique serotypes.

Children Receiving VAXNEUVANCE to Complete a 4-Dose Series Initiated with Prevnar 13

In a double-blind, active comparator-controlled, descriptive study (Study 9), participants were randomized in a 1:1:1:1:1 ratio to one of five vaccination groups. Two vaccination groups received a 4-dose series composed entirely of either VAXNEUVANCE (N=180) or Prevnar 13 (N=179). The remaining 3 study groups received either 1, 2, or 3 doses of Prevnar 13 followed by VAXNEUVANCE to complete the 4-dose series (N=180, 180, and 181, respectively). Participants also received other pediatric vaccines concomitantly *[see Adverse Reactions (6.1) and Clinical Studies (14.3)]*. Serotype-specific IgG GMCs for the 13 shared serotypes at 30 days postdose 4 were numerically similar for participants completing the vaccination series with VAXNEUVANCE compared to participants who received a complete series with Prevnar 13.

Children and Adolescents Receiving Catch-Up Vaccination

In a double-blind, active comparator-controlled, descriptive study (Study 12), participants were enrolled in three age cohorts (7 through 11 months of age, 12 through 23 months of age, and 2 through 17 years of age) and randomized to receive VAXNEUVANCE (N=303) or Prevnar 13 (N=303). Children in the two

youngest age cohorts were pneumococcal vaccine-naïve at enrollment. Children in the oldest age cohort (2 through 17 years of age) were either pneumococcal vaccine naïve, not fully vaccinated, or had completed a dosing regimen with a lower valency pneumococcal conjugate vaccine (excluding Prevnar 13). Participants who were pneumococcal vaccine-naïve at enrollment received 1 to 3 doses of VAXNEUVANCE or Prevnar 13, depending on age at enrollment and according to the schedule shown in Table 1. All participants 2 through 17 years of age received one dose of VAXNEUVANCE. Catch-up vaccination with VAXNEUVANCE elicited immune responses, as assessed by serotype-specific IgG GMCs at 30 days following the last dose of vaccine, in children 7 months through 17 years of age that were numerically similar to Prevnar 13 for the shared serotypes and higher than Prevnar 13 for the unique serotypes 22F and 33F. Within each age cohort, serotype-specific IgG GMCs at 30 days following the last dose of vaccine were numerically similar between the vaccination groups for the 13 shared serotypes and higher in VAXNEUVANCE for the 2 unique serotypes.

14.2 Clinical Trials in Pneumococcal Vaccine-Naïve Adults

Study 1

Study 1 assessed serotype-specific opsonophagocytic activity (OPA) responses for each of the 15 serotypes contained in VAXNEUVANCE at 30 days postvaccination in a double-blind, active comparator-controlled study that enrolled pneumococcal vaccine-naïve participants 50 years of age and older. Participants were randomized to receive either VAXNEUVANCE (N=604) or Prevnar 13 (N=601) at sites in USA, Canada, Spain, Taiwan, and Japan. The mean age of participants was 66 years and 57.3% were female. The racial distribution was as follows: 67.7% were White, 25.1% were Asian, 6.1% were Black or African American and 22.0% were of Hispanic or Latino ethnicity.

Table 12 summarizes the OPA geometric mean antibody titers (GMTs) at 30 days postvaccination for the 15 serotypes contained in VAXNEUVANCE. The study demonstrated that VAXNEUVANCE is noninferior to Prevnar 13 for the 13 shared serotypes and induces statistically significantly greater OPA GMTs compared to Prevnar 13 for shared serotype 3 and for the 2 unique serotypes (22F, 33F).

Table 12: Serotype-Specific OPA GMTs in Pneumococcal Vaccine-Naïve Adults 50 Years of Age and Older (Study 1)

Pneumococcal Serotype	VAXNEUVANCE (N = 602)		Prevnar 13 (N = 600)		GMT Ratio* (VAXNEUVANCE/Prevnar 13) (95% CI)*
	n	GMT*	n	GMT*	
Serotype†					
1	598	257	598	321	0.80 (0.66, 0.97)
3‡	598	215	598	133	1.62 (1.40, 1.87)
4	598	1109	598	1633	0.68 (0.57, 0.80)
5	598	445	598	560	0.79 (0.64, 0.98)
6A	596	5371	596	5276	1.02 (0.85, 1.22)
6B	598	3984	598	3179	1.25 (1.04, 1.51)
7F	596	4575	596	5830	0.78 (0.68, 0.90)
9V	598	1809	597	2193	0.83 (0.71, 0.96)
14	598	1976	598	2619	0.75 (0.64, 0.89)
18C	598	2749	598	2552	1.08 (0.91, 1.27)
19A	598	3177	597	3921	0.81 (0.70, 0.94)
19F	598	1688	598	1884	0.90 (0.77, 1.04)
23F	598	2029	598	1723	1.18 (0.96, 1.44)
Additional Serotypes§					
22F	594	2381	585	73	32.52 (25.87, 40.88)
33F	598	8010	597	1114	7.19 (6.13, 8.43)

* GMTs, GMT ratio, and 95% CI are estimated from a cLDA model.

† Non-inferiority for the 13 shared serotypes was met if the lower bound of the 95% CI for the GMT ratio (VAXNEUVANCE/Prevnar 13) was > 0.5.

‡ Statistically significantly greater OPA GMT for serotype 3 was based on the lower bound of the 95% CI for the estimated GMT ratio (VAXNEUVANCE/Prevnar 13) > 1.2.

§ Statistically significantly greater OPA GMTs for serotypes 22F and 33F was based on the lower bound of the 95% CI for the estimated GMT ratio (VAXNEUVANCE/Prevnar 13) > 2.0.

N=Number of participants randomized and vaccinated; n=Number of participants contributing to the analysis that had at least one pre-dose OPA measurement (VAXNEUVANCE, n=537-597; Prevnar 13, n=545-595) or post-dose OPA measurement (VAXNEUVANCE, n=568-580; Prevnar 13, n=528-574).

CI=confidence interval; cLDA=constrained longitudinal data analysis; GMT=geometric mean titer; OPA=opsonophagocytic activity.

Study 3

In a double-blind, active comparator-controlled, descriptive study (Study 3), pneumococcal vaccine-naïve adults 50 years of age and older were randomized to receive either VAXNEUVANCE (N=327) or Prevnar 13 (N=325), followed by PNEUMOVAX 23 one year later.

Following vaccination with PNEUMOVAX 23, OPA GMTs were numerically similar between the two vaccination groups for the 15 serotypes in VAXNEUVANCE.

Study 4

In a double-blind, descriptive study (Study 4), adults 18 through 49 years of age, including individuals with increased risk of developing pneumococcal disease, were randomized to receive VAXNEUVANCE (N=1,135) or Prevnar 13 (N=380), followed by PNEUMOVAX 23 six months later *[see Adverse Reactions (6.1)]*. Among those who received VAXNEUVANCE, 620 participants had one risk factor and 228 participants had two or more risk factors for pneumococcal disease.

Table 13 presents OPA GMTs in the overall study population for each of the 15 serotypes 30 days following vaccination with VAXNEUVANCE or Prevnar 13.

Table 13: Serotype-Specific OPA GMTs in Pneumococcal Vaccine-Naïve Adults 18 through 49 Years of Age With or Without Risk Factors for Pneumococcal Disease (Study 4)

Pneumococcal Serotype	VAXNEUVANCE (N = 1,133)			Prevnar 13 (N = 379)		
	n	Observed GMT	95% CI*	n	Observed GMT	95% CI*
Serotype						
1	1004	267	(242, 295)	337	267	(220, 324)
3	990	198	(184, 214)	336	150	(129, 173)
4	1001	1401	(1294, 1517)	338	2568	(2268, 2908)
5	1003	560	(508, 618)	339	731	(613, 873)
6A	994	12763	(11772, 13838)	333	11313	(9739, 13141)
6B	999	10164	(9486, 10891)	338	6958	(5987, 8086)
7F	1004	5725	(5382, 6090)	338	7583	(6762, 8503)
9V	1000	3353	(3132, 3590)	339	3969	(3541, 4449)
14	1001	5245	(4860, 5660)	339	5863	(5191, 6623)
18C	999	5695	(5314, 6103)	339	3050	(2685, 3465)
19A	1001	5335	(4985, 5710)	339	5884	(5221, 6632)
19F	1003	3253	(3051, 3468)	339	3272	(2949, 3631)
23F	1001	4828	(4443, 5247)	337	3876	(3323, 4521)
Additional Serotypes						
22F	991	3939	(3654, 4246)	317	291	(221, 383)
33F	999	11734	(10917, 12612)	334	2181	(1826, 2606)

* The within-group 95% CIs are obtained by exponentiating the CIs of the mean of the natural log values based on the t-distribution.
N=Number of participants randomized and vaccinated; n=Number of participants contributing to the analysis.
CI=confidence interval; GMT=geometric mean titer; OPA=opsonophagocytic activity.

Following vaccination with PNEUMOVAX 23, the OPA GMTs for the 15 serotypes in VAXNEUVANCE were numerically similar among subjects who had received VAXNEUVANCE or Prevnar 13 for the first vaccination.

14.3 Concomitant Vaccination

Children

In Study 8, the concomitant administration of Pentacel with each of the 3 infant doses of either VAXNEUVANCE (N=598) or Prevnar 13 (N=601) was evaluated 30 days following the third dose; concomitant administration of single doses of VAQTA, M-M-R II, VARIVAX and Hiberix with the fourth dose of either VAXNEUVANCE or Prevnar 13 was evaluated 30 days following vaccination. There was no evidence that VAXNEUVANCE, as compared to Prevnar 13, interfered with the immune responses to these concomitantly administered vaccines. The immune responses to the antigens in Pentacel following completion of the 4-dose series were not evaluated.

In Study 9, the concomitant administration of RECOMBIVAX HB with either VAXNEUVANCE (N=124) or Prevnar 13 (N=266) was evaluated 30 days following the third dose of pneumococcal conjugate vaccine. Most infants (97.2%) received a birth dose of hepatitis B vaccine, followed by two doses of RECOMBIVAX HB administered concomitantly with VAXNEUVANCE or Prevnar 13. There was no evidence that VAXNEUVANCE, as compared to Prevnar 13, interfered with the immune response to RECOMBIVAX HB.

Adults

In a double-blind, randomized study (Study 6), adults 50 years of age and older were randomized to receive VAXNEUVANCE concomitantly administered with a seasonal inactivated quadrivalent influenza vaccine (Fluarix Quadrivalent; QIV) (Group 1, N=600) or VAXNEUVANCE 30 days after receiving QIV (Group 2, N=600) *[see Adverse Reactions (6.1)]*. Pneumococcal vaccine serotype OPA GMTs were evaluated 30 days after VAXNEUVANCE and influenza vaccine strain hemagglutinin inhibition assay (HAI) GMTs were evaluated 30 days after QIV. The noninferiority criteria for the comparisons of GMTs [lower limit of the

2-sided 95% confidence interval (CI) of the GMT ratio (Group 1/Group 2) >0.5] were met for the 15 pneumococcal serotypes in VAXNEUVANCE and for the 4 influenza vaccine strains tested.

16 HOW SUPPLIED/STORAGE AND HANDLING

VAXNEUVANCE is supplied as follows:
Carton of one 0.5 mL single-dose prefilled Luer Lock syringes with tip caps. NDC 0006-4329-02
Carton of ten 0.5 mL single-dose prefilled Luer Lock syringes with tip caps. NDC 0006-4329-03

Store refrigerated at 2°C to 8°C (36°F to 46°F).
Do not freeze. Protect from light.

The tip cap and plunger stopper of the prefilled syringe are not made with natural rubber latex.

17 PATIENT COUNSELING INFORMATION

Advise the patient, parent or guardian to read the FDA-approved patient labeling (Patient Information).

Discuss the following with the patient, parent or guardian:

- Provide the required vaccine information to the patient, parent or guardian.
- Inform the patient, parent or guardian of the benefits and risks associated with vaccination.
- Inform the patient, parent or guardian that vaccination with VAXNEUVANCE may not protect all vaccine recipients.
- Discuss the importance of completing the vaccination series unless contraindicated.
- Instruct the patient, parent or guardian to report any serious adverse reactions to their healthcare provider who in turn should report such events to the vaccine manufacturer or the U.S. Department of Health and Human Services through the Vaccine Adverse Event Reporting System (VAERS), 1-800-822-7967, or report online at www.vaers.hhs.gov .

Manufactured by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

U.S. license number 0002

For patent information: www.merck.com/product/patent/home.html

uspi-v114-i-2206r001

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PNEUMOVAX 23 safely and effectively. See full prescribing information for PNEUMOVAX 23.

PNEUMOVAX® 23 (pneumococcal vaccine polyvalent)
Sterile, Liquid Vaccine for Intramuscular or Subcutaneous Injection
Initial U.S. Approval: 1983

----------INDICATIONS AND USAGE----------

PNEUMOVAX 23 is a vaccine indicated for active immunization for the prevention of pneumococcal disease caused by the 23 serotypes contained in the vaccine (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F). (1.1)

PNEUMOVAX 23 is approved for use in persons 50 years of age or older and persons aged ≥2 years who are at increased risk for pneumococcal disease. (1.1, 14.1)

----------DOSAGE AND ADMINISTRATION----------

Single 0.5-mL dose of PNEUMOVAX 23 administered intramuscularly or subcutaneously only. (2.2)

----------DOSAGE FORMS AND STRENGTHS----------

Clear, sterile solution supplied in a (0.5-mL dose) single-dose vial and a single-dose, prefilled syringe. (3)

----------CONTRAINDICATIONS----------

Severe allergic reaction (e.g., anaphylaxis) to any component of PNEUMOVAX 23. (4.1)

----------WARNINGS AND PRECAUTIONS----------

- Use caution and appropriate care for individuals with severely compromised cardiovascular and/or pulmonary function in whom a systemic reaction would pose a significant risk. (5.2)

----------ADVERSE REACTIONS----------

The most common adverse reactions, reported in >10% of subjects vaccinated with PNEUMOVAX 23 for the first time in a clinical trial, were: injection-site pain/soreness/tenderness (60.0%), injection-site swelling/induration (20.3%), headache (17.6%), injection-site erythema (16.4%), asthenia and fatigue (13.2%), and myalgia (11.9%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

----------DRUG INTERACTIONS----------

In a randomized clinical study, a reduced immune response to ZOSTAVAX® as measured by gpELISA was observed in individuals who received concurrent administration of PNEUMOVAX 23 and ZOSTAVAX compared with individuals who received these vaccines 4 weeks apart. Consider administration of the two vaccines separated by at least 4 weeks. (7.1, 14.3)

----------USE IN SPECIFIC POPULATIONS----------

Pediatrics: PNEUMOVAX 23 is not approved for use in children younger than 2 years of age because children in this age group do not develop an effective immune response to capsular types contained in the polysaccharide vaccine. (8.4)

Geriatrics: For subjects aged 65 years or older in a clinical study systemic adverse reactions, determined by the investigator to be vaccine-related, were higher following revaccination (33.1%) than following initial vaccination (21.7%). Routine revaccination of immunocompetent persons previously vaccinated with a 23-valent vaccine, is not recommended. (8.5)

Immunocompromised Individuals: Response to vaccination may be diminished. (5.4, 8.6)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 04/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

1.1 Indications and Use

PNEUMOVAX® 23 is a vaccine indicated for active immunization for the prevention of pneumococcal disease caused by the 23 serotypes contained in the vaccine (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A,

12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F). PNEUMOVAX 23 is approved for use in persons 50 years of age or older and persons aged ≥2 years who are at increased risk for pneumococcal disease.

1.2 Limitations of Use

PNEUMOVAX 23 will not prevent disease caused by capsular types of pneumococcus other than those contained in the vaccine.

2 DOSAGE AND ADMINISTRATION

For intramuscular or subcutaneous injection only.

2.1 Preparation

- Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. If either of these two conditions exists, the vaccine should not be administered.
- Do not mix PNEUMOVAX 23 with other vaccines in the same syringe or vial.
- Use a separate sterile syringe and needle for each individual patient to prevent transmission of infectious agents from one person to another.

  Single-Dose Vial

  Withdraw 0.5 mL from the vial using a sterile needle and syringe free of preservatives, antiseptics, and detergents.

  Single-Dose, Prefilled Syringe

  The package does not contain a needle. Attach a sterile needle to the prefilled syringe by twisting in a clockwise direction until the needle fits securely on the syringe.

2.2 Administration

Administer PNEUMOVAX 23 intramuscularly or subcutaneously into the deltoid muscle or lateral mid-thigh. Do not inject intravascularly or intradermally.

Single-Dose Vial

Administer a single 0.5-mL dose of PNEUMOVAX 23 using a sterile needle and syringe. Discard vial after use.

Single-Dose, Prefilled Syringe

Administer the entire contents of the single-dose, prefilled syringe per standard protocol using a sterile needle. Discard syringe after use.

2.3 Revaccination

The Advisory Committee on Immunization Practices (ACIP) has recommendations for revaccination against pneumococcal disease for persons at high risk who were previously vaccinated with PNEUMOVAX 23. Routine revaccination of immunocompetent persons previously vaccinated with a 23-valent vaccine, is not recommended.

3 DOSAGE FORMS AND STRENGTHS

PNEUMOVAX 23 is a clear, sterile solution supplied in a (0.5-mL dose) single-dose vial and a single-dose, prefilled syringe. *[See Description (11) and How Supplied/Storage and Handling (16).]*

4 CONTRAINDICATIONS

4.1 Hypersensitivity

Do not administer PNEUMOVAX 23 to individuals with a history of anaphylactic/anaphylactoid or severe allergic reaction to any component of the vaccine. *[See Description (11).]*

5 WARNINGS AND PRECAUTIONS

5.1 Persons with Moderate or Severe Acute Illness

Defer vaccination with PNEUMOVAX 23 in persons with moderate or severe acute illness.

5.2 Persons with Severely Compromised Cardiovascular or Pulmonary Function

Caution and appropriate care should be exercised in administering PNEUMOVAX 23 to individuals with severely compromised cardiovascular and/or pulmonary function in whom a systemic reaction would pose a significant risk.

5.3 Use of Antibiotic Prophylaxis

This vaccine does not replace the need for penicillin (or other antibiotic) prophylaxis against pneumococcal infection. In patients who require penicillin (or other antibiotic) prophylaxis against pneumococcal infection, such prophylaxis should not be discontinued after vaccination with PNEUMOVAX 23.

5.4 Persons with Altered Immunocompetence

Persons who are immunocompromised, including persons receiving immunosuppressive therapy, may have a diminished immune response to PNEUMOVAX 23. *[See Use in Specific Populations (8.6).]*

5.5 Persons with Chronic Cerebrospinal Fluid Leakage

PNEUMOVAX 23 may not be effective in preventing pneumococcal meningitis in patients who have chronic cerebrospinal fluid (CSF) leakage resulting from congenital lesions, skull fractures, or neurosurgical procedures.

6 ADVERSE REACTIONS

The most common adverse reactions, reported in >10% of subjects vaccinated with PNEUMOVAX 23 for the first time in a clinical trial, were: injection-site pain/soreness/tenderness (60.0%), injection-site swelling/induration (20.3%), headache (17.6%), injection-site erythema (16.4%), asthenia/fatigue (13.2%), and myalgia (11.9%). *[See Adverse Reactions (6.1).]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

Primary Vaccination and Revaccination with PNEUMOVAX 23 in Adults 50 Years of Age or Older

In a randomized, double-blind, placebo-controlled crossover clinical trial, subjects were enrolled in four different cohorts defined by age (50-64 years of age and ≥65 years of age) and vaccination status (no pneumococcal vaccination or receipt of a pneumococcal polysaccharide vaccine 3-5 years prior to the study). Subjects in each cohort were randomized to receive intramuscular injections of PNEUMOVAX 23 followed by placebo (saline containing 0.25% phenol), or placebo followed by PNEUMOVAX 23, at 30-day (±7 days) intervals. The safety of an initial vaccination (first dose) was compared to revaccination (second dose) with PNEUMOVAX 23 for 14 days following each vaccination.

All 1008 subjects (average age, 67 years; 49% male and 51% female; 91% Caucasian, 4.7% African-American, 3.5% Hispanic, and 0.8% Other) received placebo injections.

Initial vaccination was evaluated in a total of 444 subjects (average age 65 years; 32% male and 68% female; 93% Caucasian, 3.2% African-American, 3.4% Hispanic, and 1.1% Other).

Revaccination was evaluated in 564 subjects (average age 69 years; 53% male and 47% female; 90% Caucasian, 3.5% Hispanic, 6.0% African-American, and 0.5% Other).

Serious Adverse Experiences

In this study, 10 subjects had serious adverse experiences within 14 days of vaccination: 6 who received PNEUMOVAX 23 and 4 who received placebo. Serious adverse experiences within 14 days after PNEUMOVAX 23 included angina pectoris, heart failure, chest pain, ulcerative colitis, depression, and headache/tremor/stiffness/sweating. Serious adverse experiences within 14 days after placebo included myocardial infarction complicated with heart failure, alcohol intoxication, angina pectoris, and edema/urinary retention/heart failure/diabetes.

Five subjects reported serious adverse experiences that occurred outside the 14-day follow-up window: 3 who received PNEUMOVAX 23 and 2 who received placebo. Serious adverse experiences after PNEUMOVAX 23 included cerebrovascular accident, lumbar radiculopathy, and pancreatitis/myocardial infarction resulting in death. Serious adverse experiences after placebo included heart failure and motor vehicle accident resulting in death.

Solicited and Unsolicited Reactions

Table 1 presents the adverse event rates for all solicited and unsolicited reactions reported in ≥1% in any group in this study, without regard to causality.

The most common local adverse reactions reported at the injection site after initial vaccination with PNEUMOVAX 23 were pain/tenderness/soreness (60.0%), swelling/induration (20.3%), and erythema

(16.4%). The most common systemic adverse experiences were headache (17.6%), asthenia/fatigue (13.2%), and myalgia (11.9%).

The most common local adverse reactions reported at the injection site after revaccination with PNEUMOVAX 23 were pain/soreness/tenderness (77.2%), swelling (39.8%), and erythema (34.5%). The most common systemic adverse reactions with revaccination were headache (18.1%), asthenia/fatigue (17.9%), and myalgia (17.3%). All of these adverse reactions were reported at a rate lower than 10% after receiving a placebo injection.

Table 1: Incidence of Injection-Site and Systemic Complaints in Adults ≥50 Years of Age Receiving Their First (Initial) or Second (Revaccination) Dose of PNEUMOVAX 23 (Pneumococcal Polysaccharide Vaccine, 23 Valent) or Placebo Occurring at ≥1% in Any Group

	PNEUMOVAX 23 Initial Vaccination N=444	PNEUMOVAX 23 Revaccination* N=564	Placebo Injection† N=1008
Number Followed for Safety	438	548	984‡
	AE Rate	AE Rate	AE Rate
Injection-Site Complaints			
Solicited Events			
Pain/Soreness/Tenderness	60.0%	77.2%	7.7%
Swelling/Induration	20.3%	39.8%	2.8%
Erythema	16.4%	34.5%	3.3%
Unsolicited Events			
Ecchymosis	0%	1.1%	0.3%
Pruritus	0.2%	1.6%	0.0%
Systemic Complaints			
Solicited Events			
Asthenia/Fatigue	13.2%	17.9%	6.7%
Chills	2.7%	7.8%	1.8%
Myalgia	11.9%	17.3%	3.3%
Headache	17.6%	18.1%	8.9%
Unsolicited Events			
Fever§	1.4%	2.0%	0.7%
Diarrhea	1.1%	0.7%	0.5%
Dyspepsia	1.1%	1.1%	0.9%
Nausea	1.8%	1.8%	0.9%
Back Pain	0.9%	0.9%	1.0%
Neck Pain	0.7%	1.5%	0.2%
Upper Respiratory Infection	1.8%	2.6%	1.8%
Pharyngitis	1.1%	0.4%	1.3%

*Subjects receiving their second dose of pneumococcal polysaccharide vaccine as PNEUMOVAX 23 approximately 3-5 years after their first dose.
†Subjects receiving placebo injection from this study combined over periods.
‡The number of subjects receiving placebo followed for injection-site complaints. The corresponding number of subjects followed for systemic complaints was 981.
§Fever events include subjects who felt feverish in addition to subjects with elevated temperature.

In this clinical study an increased rate of local reactions was observed with revaccination at 3-5 years following initial vaccination.

For subjects aged 65 years or older, injection-site adverse reaction rate was higher following revaccination (79.3%) than following initial vaccination (52.9%). The proportion of subjects reporting

injection site discomfort that interfered with or prevented usual activity or injection site induration ≥4 inches was higher following revaccination (30.6%) than following initial vaccination (10.4%). Injection site reactions typically resolved by 5 days following vaccination.

For subjects aged 50-64 years, the injection-site adverse reaction rate for revaccinees and initial vaccinees was similar (79.6% and 72.8% respectively).

The rate of systemic adverse reactions was similar among both initial vaccinees and revaccinees within each age group. The rate of vaccine-related systemic adverse reactions was higher following revaccination (33.1%) than following initial vaccination (21.7%) in subjects 65 years of age or older, and was similar following revaccination (37.5%) and initial vaccination (35.5%) in subjects 50-64 years of age. The most common systemic adverse reactions reported after PNEUMOVAX 23 were as follows: asthenia/fatigue, myalgia and headache.

Regardless of age, the observed increase in post vaccination use of analgesics (≤13% in the revaccinees and ≤4% in the initial vaccinees) returned to baseline by day 5.

Sequential Administration of Prevnar 13 and PNEUMOVAX 23

In a randomized, double-blind, placebo-controlled, multicenter study, healthy adults, 50 years of age and older, received Prevnar 13 followed by PNEUMOVAX 23 either 8 weeks later (Group 1) or 26 weeks later (Group 2). Placebo was administered instead of PNEUMOVAX 23 at 26 weeks (Group 1) or 8 weeks (Group 2). Solicited injection site adverse reactions were evaluated during Days 1 through 5 postvaccination. Solicited systemic adverse reactions and any other adverse reactions were evaluated during Days 1 through 14 postvaccination, and any serious adverse events (SAEs) were collected throughout the study period (through Week 30). *[See Clinical Studies (14.2).]*

Overall, subjects were a mean age of 64.2 years (range: 50 to 97 years). There were more females (n=219, 54.8%) than males (n=181, 45.3%). By race, 84.8% of subjects were White, 9.3% were Black or African-American, and 6.1% were other racial groups; the majority of subjects were not Hispanic or Latino (n=322, 80.5%).

Serious Adverse Reactions

There were 24 SAEs reported in 20 subjects (n=9 [4.5%] Group 1; n=11 [5.5%] Group 2). No SAEs were considered related to vaccination.

Solicited Adverse Reactions

Solicited injection site adverse reactions that occurred during Days 1 through 5 postvaccination with PNEUMOVAX 23, solicited systemic adverse reactions that occurred during Days 1 through 14, and fever that occurred during Days 1 through 5 postvaccination with PNEUMOVAX 23 are presented in Table 2. In this study, 81.4% of subjects in Group 1 and 64.0% of subjects in Group 2 reported at least 1 injection site adverse reaction from Days 1 through 5 postvaccination with PNEUMOVAX 23, and 64.9% of subjects in Group 1 and 54.9% of subjects in Group 2 reported at least 1 systemic adverse reaction from Days 1 through 14 postvaccination with PNEUMOVAX 23.

Table 2: Rates (%) of Solicited Injection Site Reactions Occurring on Days 1 to 5 After PNEUMOVAX 23 and Solicited Systemic Adverse Reactions Occurring on Days 1 to 14 After PNEUMOVAX 23

	Group 1* (Prevnar 13 -> PNEUMOVAX 23 -> Placebo)		Group 2† (Prevnar 13 -> Placebo -> PNEUMOVAX 23)	
	n	(%)	n	(%)
Injection Site Adverse Reactions				
Subjects in population with follow-up	188		164	
Any injection site reaction	153	(81.4)	105	(64.0)
Any Injection site pain‡	149	(79.3)	105	(64.0)
Mild	72	(38.3)	65	(39.6)
Moderate	65	(34.6)	36	(22.0)
Severe§	12	(6.4)	4	(2.4)
Any Injection site swelling	95	(50.5)	48	(29.3)
0 to <2.5 cm	28	(14.9)	19	(11.6)
≥2.5 to <5.1 cm	20	(10.6)	9	(5.5)
≥5.1 to <7.6 cm	20	(10.6)	10	(6.1)
≥7.6 to <10.2 cm	15	(8.0)	2	(1.2)
≥10.2 cm§	12	(6.4)	8	(4.9)
Any Injection site erythema	78	(41.5)	48	(29.3)
0 to <2.5 cm	26	(13.8)	20	(12.2)
≥2.5 to <5.1 cm	12	(6.4)	13	(7.9)
≥5.1 to <7.6 cm	12	(6.4)	6	(3.7)
≥7.6 to <10.2 cm	7	(3.7)	3	(1.8)
≥10.2 cm	19	(10.1)	6	(3.7)
Unknown [missing data]	2	(1.1)	0	(0.0)
Systemic Adverse Reactions				
Subjects in population with follow-up	188		164	
Any systemic adverse reaction	122	(64.9)	90	(54.9)
Myalgia	93	(49.5)	70	(42.7)
Fatigue	59	(31.4)	45	(27.4)
Headache	46	(24.5)	30	(18.3)
Arthralgia	37	(19.7)	25	(15.2)
Subjects with temperature data¶	185		161	
Temperature ≥ 100.4°F	1	(0.5)	0	(0.0)

Every subject is counted a single time for each applicable row and column.
A specific adverse reaction appears in this table only if its incidence in one or more of the columns meets the incidence criterion in the table title, after rounding.
*Group 1: 8-week interval between Prevnar 13 and PNEUMOVAX 23.
†Group 2: 26-week interval between Prevnar 13 and PNEUMOVAX 23.
‡Pain was characterized as mild, moderate or severe. (Mild: awareness of sign or symptom, but easily tolerated. Moderate: discomfort enough to cause interference with usual activity. Severe: incapacitating with inability to work or do usual activity).
§One Group 1 subject with severe pain and swelling greater than 10.2 cm after receipt of PNEUMOVAX 23, went to the Emergency Room for medical attention.
¶Percentages are calculated based on number of subjects with temperature data. Oral temperature was solicited on Days 1 to 5 after PNEUMOVAX 23 vaccination.

6.2 Post-Marketing Experience

The following list of adverse reactions includes those identified during post approval use of PNEUMOVAX 23. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or their causal relationship to product exposure.

General disorders and administration site conditions
- Cellulitis
- Malaise
- Fever (>102°F)
- Warmth at the injection site
- Decreased limb mobility
- Peripheral edema in the injected extremity
- Injection-site necrosis

Digestive System
- Nausea
- Vomiting

Hematologic/Lymphatic
- Lymphadenitis
- Lymphadenopathy
- Thrombocytopenia in patients with stabilized idiopathic thrombocytopenic purpura
- Hemolytic anemia in patients who have had other hematologic disorders
- Leukocytosis

Hypersensitivity reactions including
- Anaphylactoid reactions
- Serum Sickness
- Angioneurotic edema

Musculoskeletal System
- Arthralgia
- Arthritis

Nervous System
- Paresthesia
- Radiculoneuropathy
- Guillain-Barré syndrome
- Febrile convulsion

Skin
- Rash
- Urticaria
- Cellulitis-like reactions
- Erythema multiforme

Investigations
- Increased serum C-reactive protein

7 DRUG INTERACTIONS

7.1 Concomitant Administration with Other Vaccines

In a randomized clinical study, a reduced immune response to ZOSTAVAX® as measured by gpELISA was observed in individuals who received concurrent administration of PNEUMOVAX 23 and ZOSTAVAX compared with individuals who received these vaccines 4 weeks apart. Consider administration of the two vaccines separated by at least 4 weeks. *[See Clinical Studies (14.3).]*

Limited safety and immunogenicity data from clinical trials are available on the concurrent administration of PNEUMOVAX 23 and vaccines other than ZOSTAVAX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Available human data from clinical trials of PNEUMOVAX 23 in pregnancy have not established the presence or absence of a vaccine-associated risk.

Developmental toxicity studies have not been conducted with PNEUMOVAX 23 in animals.

8.2 Lactation

Risk Summary

It is not known whether PNEUMOVAX 23 is excreted in human milk. Data are not available to assess the effects of PNEUMOVAX 23 on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for PNEUMOVAX 23 and any potential adverse effects on the breastfed child from PNEUMOVAX 23 or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to the disease prevented by the vaccine.

8.4 Pediatric Use

PNEUMOVAX 23 is not approved for use in children less than 2 years of age. Children in this age group do not develop an effective immune response to the capsular types contained in this polysaccharide vaccine.

The ACIP has recommendations for use of PNEUMOVAX 23 in children 2 years of age or older, who have previously received pneumococcal vaccines, and who are at increased risk for pneumococcal disease.

8.5 Geriatric Use

In one clinical trial of PNEUMOVAX 23, conducted post-licensure, a total of 629 subjects who were aged ≥65 years and 201 subjects who were aged ≥75 years were enrolled.

In this trial, the safety of PNEUMOVAX 23 in adults 65 years of age and older (N=629) was compared to the safety of PNEUMOVAX 23 in adults 50 to 64 years of age (N=379). The subjects in this study had underlying chronic illness but were in stable condition; at least 1 medical condition at enrollment was reported by 86.3% of subjects who were 50 to 64 years old, and by 96.7% of subjects who were 65 to 91 years old. The rate of vaccine-related systemic adverse experiences was higher following revaccination (33.1%) than following primary vaccination (21.7%) in subjects ≥65 years of age, and was similar following revaccination (37.5%) and primary vaccination (35.5%) in subjects 50 to 64 years of age.

Since elderly individuals may not tolerate medical interventions as well as younger individuals, a higher frequency and/or a greater severity of reactions in some older individuals cannot be ruled out.

Post-marketing reports have been received in which some elderly individuals had severe adverse experiences and a complicated clinical course following vaccination. Some individuals with underlying medical conditions of varying severity experienced local reactions and fever associated with clinical deterioration requiring hospital care.

8.6 Immunocompromised Individuals

Persons who are immunocompromised, including persons receiving immunosuppressive therapy, may have a diminished immune response to PNEUMOVAX 23.

11 DESCRIPTION

PNEUMOVAX 23 (Pneumococcal Vaccine Polyvalent) is a sterile, liquid vaccine consisting of a mixture of purified capsular polysaccharides from *Streptococcus pneumoniae* types (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F).

PNEUMOVAX 23 is a clear, colorless solution. Each 0.5-mL dose of vaccine contains 25 micrograms of each polysaccharide type in isotonic saline solution containing 0.25% phenol as a preservative. The vaccine is used directly as supplied. No dilution or reconstitution is necessary.

The vial stoppers, syringe plunger stopper and syringe tip cap are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

PNEUMOVAX 23 induces type-specific antibodies that enhance opsonization, phagocytosis, and killing of pneumococci by leukocytes and other phagocytic cells. The levels of antibodies that correlate with protection against pneumococcal disease have not been clearly defined.

14 CLINICAL STUDIES

14.1 Effectiveness

The protective efficacy of pneumococcal vaccines containing six (types 1, 2, 4, 8, 12F, and 25) or twelve (types 1, 2, 3, 4, 6A, 8, 9N, 12F, 25, 7F, 18C, and 46) capsular polysaccharides was investigated in two controlled studies in South Africa in male novice gold miners ranging in age from 16 to 58 years, in whom there was a high attack rate for pneumococcal pneumonia and bacteremia. In both studies, participants in the control groups received either meningococcal polysaccharide serogroup A vaccine or saline placebo. In both studies, attack rates for vaccine type pneumococcal pneumonia were observed for the period from 2 weeks through about 1 year after vaccination. Protective efficacy was 76% and 92%, respectively, for the 6- and 12-valent vaccines, for the capsular types represented.

Three similar studies in South African young adult male novice gold miners were carried out by Dr. R. Austrian and associates using similar pneumococcal vaccines prepared for the National Institute of Allergy and Infectious Diseases, with pneumococcal vaccines containing a 6-valent formulation (types 1, 3, 4, 7, 8, and 12) or a 13-valent formulation (types 1, 2, 3, 4, 6, 7, 8, 9, 12, 14, 18, 19, and 25) capsular polysaccharides. The reduction in pneumococcal pneumonia caused by the capsular types contained in the vaccines was 79%. Reduction in type-specific pneumococcal bacteremia was 82%.

A prospective study in France found a pneumococcal vaccine containing fourteen (types 1, 2, 3, 4, 6A, 7F, 8, 9N, 12F, 14, 18C, 19F, 23F, and 25) capsular polysaccharides to be 77% (95%CI: 51% to 89%) effective in reducing the incidence of pneumonia among male and female nursing home residents with a mean age of 74 (standard deviation of 4 years).

In a study using a pneumococcal vaccine containing eight (types 1, 3, 6, 7, 14, 18, 19, and 23) capsular polysaccharides, vaccinated children and young adults aged 2 to 25 years who had sickle cell disease, congenital asplenia, or undergone a splenectomy experienced significantly less bacteremic pneumococcal disease than patients who were not vaccinated.

In the United States, one post-licensure randomized controlled trial, in the elderly or patients with chronic medical conditions who received a 14-valent pneumococcal polysaccharide vaccine (types 1, 2, 3, 4, 6A, 8, 9N, 12F, 14, 19F, 23F, 25, 7F, and 18C), did not support the efficacy of the vaccine for nonbacteremic pneumonia.

A retrospective cohort analysis study based on the U.S. Centers for Disease Control and Prevention (CDC) pneumococcal surveillance system, showed 57% (95%CI: 45% to 66%) overall protective effectiveness against invasive infections caused by serotypes included in PNEUMOVAX 23 in persons ≥6 years of age, 65 to 84% effectiveness among specific patient groups (e.g., persons with diabetes mellitus, coronary vascular disease, congestive heart failure, chronic pulmonary disease, and anatomic asplenia) and 75% (95%CI: 57% to 85%) effectiveness in immunocompetent persons aged ≥65 years of age. Vaccine effectiveness could not be confirmed for certain groups of immunocompromised patients.

14.2 Immunogenicity

The levels of antibodies that correlate with protection against pneumococcal disease have not been clearly defined.

Antibody responses to most pneumococcal capsular types are generally low or inconsistent in children less than 2 years of age.

Sequential Administration of Prevnar 13 and PNEUMOVAX 23

In a randomized, double-blind, placebo-controlled, multicenter study, healthy adults, 50 years of age and older, received Prevnar 13 followed by PNEUMOVAX 23 either 8 weeks later (Group 1) or 26 weeks later (Group 2). Four hundred subjects were randomized 1:1 into Group 1 or Group 2, all of whom were initially vaccinated with Prevnar 13; of these, 188 subjects received PNEUMOVAX 23 (Group 1) and 185 subjects received placebo (Group 2) at Week 8, and 172 subjects received placebo (Group 1) and 164 subjects received PNEUMOVAX 23 (Group 2) at Week 26.

Opsonophagocytic activity (OPA) titers were measured at prevaccination, at Week 12 and at Week 30 for the 12 shared serotypes contained in both PNEUMOVAX 23 and Prevnar 13 (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F), 2 of the 11 serotypes unique to PNEUMOVAX 23 (22F and 33F), and 1 serotype unique to Prevnar 13 (6A). OPA testing was performed on evaluable serum samples from all subjects at baseline (Day 1) and Week 12, and on sera from a random subset of subjects (approximately 50% of subjects) at Week 30. Estimated GMTs, GMT ratio, and 95% confidence intervals were obtained from a constrained Longitudinal Data Analysis model {1}.

For each of the shared serotypes, Week 12 OPA geometric mean titers (GMTs) in Group 1 were noninferior to those of Group 2, as the lower bounds of the 95% CIs for the OPA GMT ratios were >0.5 for all 12 shared serotypes. For serotypes 22F and 33F, OPA GMTs in Group 1 at Week 12 were superior to those of Group 2 at Week 12, as the lower bounds of the 95% CIs for the OPA GMT ratios were >2.0 for both serotypes.

The OPA GMTs to the 12 shared serotypes and 2 unique serotypes (22F and 33F) when measured 4 weeks after dosing with PNEUMOVAX 23 were generally similar between Group 1 (Week 12) and Group 2 (Week 30 subset).

14.3 Concomitant Administration with Other Vaccines

In a double-blind, controlled clinical trial, 473 adults, 60 years of age or older, were randomized to receive ZOSTAVAX and PNEUMOVAX 23 concomitantly (N=237), or PNEUMOVAX 23 alone followed 4 weeks later by ZOSTAVAX alone (N=236). At four weeks postvaccination, the varicella-zoster virus (VZV) antibody levels following concomitant use were significantly lower than the VZV antibody levels following nonconcomitant administration (GMTs of 338 vs. 484 gpELISA units/mL, respectively; GMT ratio = 0.70 (95% CI: [0.61, 0.80]).

Limited safety and immunogenicity data from clinical trials are available on the concurrent administration of PNEUMOVAX 23 and vaccines other than ZOSTAVAX.

15 REFERENCES

1. Liang KY, Zeger S. Longitudinal data analysis of continuous and discrete responses for pre-post designs. Sankhyā: The Indian Journal of Statistics (Series B) 2000; 62: 134-148.

16 HOW SUPPLIED/STORAGE AND HANDLING

PNEUMOVAX 23 is supplied as follows:

NDC 0006-4943-00 — a box of 10 single-dose vials, color coded with a purple cap and stripe on the vial labels and cartons.

NDC 0006-4837-03 — a box of 10 single-dose, pre-filled Luer-Lok™ syringes with tip caps, color coded with a violet plunger rod and purple stripe on the syringe labels and cartons.

Storage and Handling

- Store at 2-8°C (36-46°F).
- All vaccine must be discarded after the expiration date.

The vial stoppers, syringe plunger stopper and syringe tip cap are not made with natural rubber latex.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

- Inform the patient, parent or guardian of the benefits and risks associated with vaccination.
- Tell the patient, parent or guardian that vaccination with PNEUMOVAX 23 may not offer 100% protection from pneumococcal infection.
- Provide the patient, parent or guardian with the vaccine information statements required by the National Childhood Vaccine Injury Act of 1986, with each immunization.
- Instruct the patient, parent or guardian to report any serious adverse reactions to their health care provider who in turn should report such events to the vaccine manufacturer or the U.S. Department of Health and Human Services through the Vaccine Adverse Event Reporting System (VAERS), 1-800-822-7967, or report online at www.vaers.hhs.gov.

Manuf. and Dist. by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

For patent information: www.merck.com/product/patent/home.html

uspi-v110-i-2104r044

Polio

AHFS Category: 80:12 **IPV**

Poliovirus Vaccine Inactivated

IPOL®

Rx only

DESCRIPTION

IPOL®, Poliovirus Vaccine Inactivated, produced by Sanofi Pasteur SA, is a sterile suspension of three types of poliovirus: Type 1 (Mahoney), Type 2 (MEF-1), and Type 3 (Saukett). IPOL vaccine is a highly purified, inactivated poliovirus vaccine with enhanced potency. Each of the three strains of poliovirus is individually grown in vero cells, a continuous line of monkey kidney cells cultivated on microcarriers. (1) (2) The cells are grown in Eagle MEM modified medium, supplemented with newborn calf bovine serum tested for adventitious agents prior to use, originated from countries free of bovine spongiform encephalopathy. For viral growth, the culture medium is replaced by M-199, without calf bovine serum. This culture technique and improvements in purification, concentration, and standardization of poliovirus antigen produce a more potent and consistent immunogenic vaccine than the inactivated poliovirus vaccine (IPV) available in the US prior to 1988. (3) (4)

After clarification and filtration, viral suspensions are concentrated by ultrafiltration, and purified by three liquid chromatography steps; one column of anion exchanger, one column of gel filtration, and again one column of anion exchanger. After re-equilibration of the purified viral suspension with Medium M-199 and adjustment of the antigen titer, the monovalent viral suspensions are inactivated at +37°C for at least 12 days with 1:4000 formalin.

Each dose (0.5 mL) of trivalent vaccine is formulated to contain 40 D antigen units of Type 1, 8 D antigen units of Type 2, and 32 D antigen units of Type 3 poliovirus. For each lot of IPOL vaccine, D-antigen content is determined *in vitro* using the D-antigen ELISA assay. IPOL vaccine is produced from vaccine concentrates diluted with M-199 medium. Also present are 0.5% of 2-phenoxyethanol and a maximum of 0.02% of formaldehyde per dose as preservatives. Neomycin, streptomycin, and polymyxin B are used in vaccine production; and, although purification procedures eliminate measurable amounts, less than 5 ng neomycin, 200 ng streptomycin, and 25 ng polymyxin B per dose may still be present. The residual calf bovine serum albumin is less than 50 ng/dose in the final vaccine.

The vaccine is clear and colorless and should be administered intramuscularly or subcutaneously.

The vial stopper is not made with natural rubber latex.

CLINICAL PHARMACOLOGY

Poliomyelitis is caused by poliovirus Types 1, 2, or 3. It is primarily spread by the fecal-oral route of transmission but may also be spread by the pharyngeal route.

Approximately 90% to 95% of poliovirus infections are asymptomatic. Nonspecific illness with low-grade fever and sore throat (minor illness) occurs in 4% to 8% of infections. Aseptic meningitis occurs in 1% to 5% of patients a few days after the minor illness has resolved. Rapid onset of asymmetric acute flaccid paralysis occurs in 0.1% to 2% of infections, and residual

paralytic disease involving motor neurons (paralytic poliomyelitis) occurs in approximately 1 per 1,000 infections. (5)

Prior to the introduction of inactivated poliovirus vaccines in 1955, large outbreaks of poliomyelitis occurred each year in the United States (US). The annual incidence of paralytic disease of 11.4 cases/100,000 population declined to 0.5 cases by the time oral poliovirus vaccine (OPV) was introduced in 1961. Incidence continued to decline thereafter to a rate of 0.002 to 0.005 cases per 100,000 population. Of the 127 cases of paralytic poliomyelitis reported in the US between 1980 and 1994, six were imported cases (caused by wild polioviruses), two were "indeterminate" cases, and 119 were vaccine associated paralytic poliomyelitis (VAPP) cases associated with the use of live, attenuated oral poliovirus vaccine (OPV). (6) An all IPV schedule was adopted in 1999 to eliminate VAPP cases. (7)

Poliovirus Vaccine Inactivated induces the production of neutralizing antibodies against each type of virus which are related to protective efficacy. Antibody response in most children was induced after receiving fewer doses (8) of IPV vaccine than the vaccine available in the United States prior to 1988.

Studies in developed (8) and developing (9), (10) countries with a similar enhanced IPV manufactured by the same process as IPOL vaccine in primary monkey kidney cells have shown a direct relationship exists between the antigenic content of the vaccine, the frequency of seroconversion, and resulting antibody titer. Approval in the US was based upon demonstration of immunogenicity and safety in US children. (11)

In the US, 219 infants received three doses of a similar enhanced IPV at two, four, and eighteen months of age manufactured by the same process as IPOL vaccine except the cell substrate for IPV was using primary monkey kidney cells. Seroconversion to all three types of poliovirus was demonstrated in 99% of these infants after two doses of vaccine given at 2 and 4 months of age. Following the third dose of vaccine at 18 months of age, neutralizing antibodies were present at a level of ≥1:10 in 99.1% of children to Type 1 and 100% of children to Types 2 and 3 polioviruses. (3)

IPOL vaccine was administered to more than 700 infants between 2 to 18 months of age during three clinical studies conducted in the US using IPV only schedules and sequential IPV-OPV schedules. (12) (13) Seroprevalence rates for detectable serum neutralizing antibody (DA) at a ≥1:4 dilution were 95% to 100% (Type 1); 97% to 100% (Type 2) and 96% to 100% (Type 3) after two doses of IPOL vaccine depending on studies.

Table 1: US Studies with IPOL Vaccine Administered Using IPV Only or Sequential IPV-OPV Schedules

Age (months) for				Post Dose 2				Post Dose 3				Pre Booster				Post Booster			
2	4	6	12 to 18		Type 1	Type 2	Type 3		Type 1	Type 2	Type 3		Type 1	Type 2	Type 3		Type 1	Type 2	Type 3
Dose 1	Dose 2	Dose 3	Booster	N*	%DA†	%DA	%DA	N*	%DA	%DA	%DA	N*	%DA	%DA	%DA	N*	%DA	%DA	%DA
STUDY 1 (11) ‡																			
I(s)	I(s)	NA§	I(s)	56	97	100	97		–	–	–	53	91	97	93	53	97	100	100
O	O	NA	O	22	100	100	100		–	–	–	22	78	91	78	20	100	100	100
I(s)	O	NA	O	17	95	100	95		–	–	–	17	95	100	95	17	100	100	100
I(s)	I(s)	NA	O	17	100	100	100		–	–	–	16	100	100	94	16	100	100	100
STUDY 2 (10) ¶																			
I(c)	I(c)	NA	I(s)	94	98	97	96		–	–	–	100	92	95	88	97	100	100	100
I(s)	I(s)	NA	I(s)	68	99	100	99		–	–	–	72	100	100	94	75	100	100	100
I(c)	I(c)	NA	O	75	95	99	96		–	–	–	77	86	97	82	78	100	100	97
I(s)	I(s)	NA	O	101	99	99	95		–	–	–	103	99	97	89	107	100	100	100
STUDY 3 (10) ¶																			
I(c)	I(c)	I(c)	O	91	98	99	100	91	100	100	100	41	100	100	100	40	100	100	100
I(c)	I(c)	O	O	96	100	98	99	94	100	100	99	47	100	100	100	45	100	100	100
I(c)	I(c)	I(c) + O	O	91	96	97	100	85	100	100	100	47	100	100	100	46	100	100	100

* N = Number of children from whom serum was available

† Detectable antibody (neutralizing titer ≥1:4)

‡ IPOL vaccine given subcutaneously

§ NA – No poliovirus vaccine administered

¶ IPOL vaccine given intramuscularly

I IPOL vaccine given either separately in association with DTP in two sites (s) or combined (c) with DTP in a dual chambered syringe

O OPV

In one study, (13) the persistence of DA in infants receiving two doses of IPOL vaccine at 2 and 4 months of age was 91% to 100% (Type 1), 97% to 100% (Type 2), and 93% to 94% (Type 3) at twelve months of age. In another study, (12) 86% to 100% (Type 1), 95% to 100% (Type 2), and 82% to 94% (Type 3) of infants still had DA at 18 months of age.

In trials and field studies conducted outside the US, IPOL vaccine, or a combination vaccine containing IPOL vaccine and DTP, was administered to more than 3,000 infants between 2 to 18 months of age using IPV only schedules and immunogenicity data are available from 1,485 infants. After two doses of vaccine given during the first year of life, seroprevalence rates for detectable serum neutralizing antibody (neutralizing titer ≥1:4) were 88% to 100% (Type 1); 84% to 100% (Type 2) and 94% to 100% (Type 3) of infants, depending on studies. When three doses were given during the first year of life, post-dose 3 DA ranged between 93% to 100% (Type 1); 89% to 100% (Type 2) and 97% to 100% (Type 3) and reached 100% for Types 1, 2, and 3 after the fourth dose given during the second year of life (12 to 18 months of age). (14)

In infants immunized with three doses of an unlicensed combination vaccine containing IPOL vaccine and DTP given during the first year of life, and a fourth dose given during the second year of life, the persistence of detectable neutralizing antibodies was 96%, 96%, and 97% against poliovirus Types 1, 2, and 3, respectively, at six years of age. DA reached 100% for all types after a booster dose of IPOL vaccine combined with DTP vaccine. (11) A survey of Swedish children and young adults given a Swedish IPV only schedule demonstrated persistence of detectable serum neutralizing antibody for at least 10 years to all three types of poliovirus. (15)

IPV is able to induce secretory antibody (IgA) produced in the pharynx and gut and reduces pharyngeal excretion of poliovirus Type 1 from 75% in children with neutralizing antibodies at levels less than 1:8 to 25% in children with neutralizing antibodies at levels more than 1:64. (4) (14) (16) (17) (18) (19) (20) (21) (22) There is also evidence of induction of herd immunity with IPV, (15) (23) (24) (25) (26) and that this herd immunity is sufficiently maintained in a population vaccinated only with IPV. (26)

VAPP has not been reported in association with administration of IPOL vaccine. (27) It is expected that an IPV only schedule will eliminate the risk of VAPP in both recipients and contacts compared to a schedule that included OPV. (7)

INDICATIONS AND USAGE

IPOL vaccine is indicated for active immunization of infants (as young as 6 weeks of age), children, and adults for the prevention of poliomyelitis caused by poliovirus Types 1, 2, and 3. (28)

INFANTS, CHILDREN AND ADOLESCENTS

General Recommendations

It is recommended that all infants (as young as 6 weeks of age), unimmunized children, and adolescents not previously immunized be vaccinated routinely against paralytic poliomyelitis. (29) Following the eradication of poliomyelitis caused by wild poliovirus from the Western

Hemisphere (including North and South America) (30), an IPV-only schedule was recommended to eliminate VAPP. (7)

All children should receive four doses of IPV at ages 2, 4, 6 to 18 months, and 4 to 6 years. OPV is no longer available in the US and is not recommended for routine immunization. (7)

Previous clinical poliomyelitis (usually due to only a single poliovirus type) or incomplete immunization with OPV are not contraindications to completing the primary series of immunization with IPOL vaccine.

Children Incompletely Immunized

Children of all ages should have their immunization status reviewed and be considered for supplemental immunization as follows for adults. Time intervals between doses longer than those recommended for routine primary immunization do not necessitate additional doses as long as a final total of four doses is reached (see **DOSAGE AND ADMINISTRATION** section).

ADULTS

General Recommendations

Routine primary poliovirus vaccination of adults (generally those 18 years of age or older) residing in the US is not recommended. Unimmunized adults who are potentially exposed to wild poliovirus and have not been adequately immunized should receive polio vaccination in accordance with the schedule given in the **DOSAGE AND ADMINISTRATION** section. (28)

Persons with previous wild poliovirus disease who are incompletely immunized or unimmunized should be given additional doses of IPOL vaccine if they fall into one or more categories listed.

The following categories of adults are at an increased risk of exposure to wild polioviruses: (28) (31)

• Travelers to regions or countries where poliomyelitis is endemic or epidemic.

• Healthcare workers in close contact with patients who may be excreting polioviruses.

• Laboratory workers handling specimens that may contain polioviruses.

• Members of communities or specific population groups with disease caused by wild polioviruses.

IMMUNODEFICIENCY AND ALTERED IMMUNE STATUS

IPOL vaccine should be used in all patients with immunodeficiency diseases and members of such patients' households when vaccination of such persons is indicated. This includes patients with asymptomatic HIV infection, AIDS or AIDS-Related Complex, severe combined immunodeficiency, hypogammaglobulinemia, or agammaglobulinemia; altered immune states due to diseases such as leukemia, lymphoma, or generalized malignancy; or an immune system compromised by treatment with corticosteroids, alkylating drugs, antimetabolites or radiation. Immunogenicity of IPOL vaccine in individuals receiving immunoglobulin could be impaired, and patients with an altered immune state may or may not develop a protective response against paralytic poliomyelitis after administration of IPV. (32)

As with any vaccine, vaccination with IPOL vaccine may not protect 100% of individuals.

Use with other vaccines: refer to **DOSAGE AND ADMINISTRATION** section for this information.

CONTRAINDICATIONS

IPOL vaccine is contraindicated in persons with a history of hypersensitivity to any component of the vaccine, including 2-phenoxyethanol, formaldehyde, neomycin, streptomycin, and polymyxin B.

No further doses should be given if anaphylaxis or anaphylactic shock occurs within 24 hours of administration of one dose of vaccine.

Vaccination of persons with an acute, febrile illness should be deferred until after recovery; however, minor illness, such as mild upper respiratory infection, with or without low grade fever, are not reasons for postponing vaccine administration.

WARNINGS

Neomycin, streptomycin, polymyxin B, 2-phenoxyethanol, and formaldehyde are used in the production of this vaccine. Although purification procedures eliminate measurable amounts of these substances, traces may be present (see **DESCRIPTION** section), and allergic reactions may occur in persons sensitive to these substances (see **CONTRAINDICATIONS** section).

Systemic adverse reactions reported in infants receiving IPV concomitantly at separate sites or combined with DTP have been similar to those associated with administration of DTP alone. (11) Local reactions are usually mild and transient in nature.

Although no causal relationship between IPOL vaccine and Guillain-Barré Syndrome (GBS) has been established, (28) GBS has been temporally related to administration of another inactivated poliovirus vaccine. Deaths have been reported in temporal association with the administration of IPV (see **ADVERSE REACTIONS** section).

PRECAUTIONS

GENERAL

Prior to an injection of any vaccine, all known precautions should be taken to prevent adverse reactions. This includes a review of the patient's history with respect to possible sensitivity to the vaccine or similar vaccines.

Healthcare providers should question the patient, parent or guardian about reactions to a previous dose of this product, or similar product.

Epinephrine injection (1:1000) and other appropriate agents should be available to control immediate allergic reactions.

Healthcare providers should obtain the previous immunization history of the vaccinee, and inquire about the current health status of the vaccinee.

Immunodeficient patients or patients under immunosuppressive therapy may not develop a protective immune response against paralytic poliomyelitis after administration of IPV.

Administration of IPOL vaccine is not contraindicated in individuals infected with HIV. (33) (34) (35)

Special care should be taken to ensure that the injection does not enter a blood vessel.

Syncope (fainting) has been reported following vaccination with IPOL. Procedures should be in place to avoid injury from fainting.

INFORMATION FOR PATIENTS

Patients, parents, or guardians should be instructed to report any serious adverse reactions to their healthcare provider.

The healthcare provider should inform the patient, parent, or guardian of the benefits and risks of the vaccine.

The healthcare provider should inform the patient, parent, or guardian of the importance of completing the immunization series.

The healthcare provider should provide the Vaccine Information Statements (VISs) which are required to be given with each immunization.

DRUG INTERACTIONS

There are no known interactions of IPOL vaccine with drugs or foods. Concomitant administration of other parenteral vaccines, with separate syringes at separate sites, is not contraindicated. The first two doses of IPOL vaccine may be administered at separate sites using separate syringes concomitantly with DTaP, acellular pertussis, *Haemophilus influenzae* type b (Hib), and hepatitis B vaccines. From historical data on the antibody responses to diphtheria, tetanus, acellular pertussis, Hib, or hepatitis B vaccines used concomitantly or in combination with IPOL vaccine, no interferences have been observed on the immunological end points accepted for clinical protection. (11) (16) (36) (See **DOSAGE AND ADMINISTRATION** section.)

If IPOL vaccine has been administered to persons receiving immunosuppressive therapy, an adequate immunologic response may not be obtained. (See **PRECAUTIONS** – **GENERAL** section.)

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

Long-term studies in animals to evaluate carcinogenic potential or impairment of fertility have not been conducted.

PREGNANCY

Animal reproduction studies have not been conducted with IPOL vaccine. It is also not known whether IPOL vaccine can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. IPOL vaccine should be given to a pregnant woman only if clearly needed.

NURSING MOTHERS

It is not known whether IPOL vaccine is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when IPOL vaccine is administered to a nursing woman.

PEDIATRIC USE

SAFETY AND EFFECTIVENESS OF IPOL VACCINE IN INFANTS BELOW SIX WEEKS OF AGE HAVE NOT BEEN ESTABLISHED. (12) (20) (See **DOSAGE AND ADMINISTRATION** section.)

In the US, infants receiving two doses of IPV at 2 and 4 months of age, the seroprevalence to all three types of poliovirus was demonstrated in 95% to 100% of these infants after two doses of vaccine. (12) (13)

ADVERSE REACTIONS

Body System As A Whole

In earlier studies with the vaccine grown in primary monkey kidney cells, transient local reactions at the site of injection were observed. (3) Erythema, induration and pain occurred in 3.2%, 1% and 13%, respectively, of vaccinees within 48 hours post-vaccination. Temperatures of ≥39°C (≥102°F) were reported in 38% of vaccinees. Other symptoms included irritability, sleepiness,

fussiness, and crying. Because IPV was given in a different site but concurrently with Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed (DTP), these systemic reactions could not be attributed to a specific vaccine. However, these systemic reactions were comparable in frequency and severity to that reported for DTP given alone without IPV. (12) Although no causal relationship has been established, deaths have occurred in temporal association after vaccination of infants with IPV. (37)

Four additional US studies using IPOL vaccine in more than 1,300 infants, (12) between 2 to 18 months of age administered with DTP at the same time at separate sites or combined have demonstrated that local and systemic reactions were similar when DTP was given alone.

Table 2 (12): Percentage of Infants Presenting with Local or Systemic Reactions at 6, 24, and 48 Hours of Immunization with IPOL Vaccine Administered Intramuscularly Concomitantly at Separate Sites with Sanofi* Whole-Cell DTP Vaccine at 2 and 4 Months of Age and with Sanofi Acellular Pertussis Vaccine (Tripedia®) at 18 Months of Age

	AGE AT IMMUNIZATION								
REACTION	**2 Months (n=211)**			**4 Months (n=206)**			**18 Months† (n=74)**		
	6 Hrs.	**24 Hrs.**	**48 Hrs.**	**6 Hrs.**	**24 Hrs.**	**48 Hrs.**	**6 Hrs.**	**24 Hrs.**	**48 Hrs.**
Local, IPOL vaccine alone‡									
Erythema >1"	0.5%	0.5%	0.5%	1.0%	0.0%	0.0%	1.4%	0.0%	0.0%
Swelling	11.4%	5.7%	0.9%	11.2%	4.9%	1.9%	2.7%	0.0%	0.0%
Tenderness	29.4%	8.5%	2.8%	22.8%	4.4%	1.0%	13.5%	4.1%	0.0%
Systemic§									
Fever >102.2°F	1.0%	0.5%	0.5%	2.0%	0.5%	0.0%	0.0%	0.0%	4.2%
Irritability	64.5%	24.6%	17.5%	49.5%	25.7%	11.7%	14.7%	6.7%	8.0%
Tiredness	60.7%	31.8%	7.1%	38.8%	18.4%	6.3%	9.3%	5.3%	4.0%
Anorexia	16.6%	8.1%	4.3%	6.3%	4.4%	2.4%	2.7%	1.3%	2.7%
Vomiting	1.9%	2.8%	2.8%	1.9%	1.5%	1.0%	1.3%	1.3%	0.0%
Persistent Crying	Percentage of infants within 72 hours after immunization was 0.0% after dose one, 1.4% after dose two, and 0.0% after dose three.								

* Sanofi Pasteur Inc. formerly known as Aventis Pasteur Inc.

† Children who have been vaccinated with Tripedia vaccine.

‡ Data are from the IPOL vaccine administration site, given intramuscularly.

§ The adverse reaction profile includes the concomitant use of Sanofi whole-cell DTP vaccine or Tripedia vaccine with IPOL vaccine. Rates are comparable in frequency and severity to that reported for whole-cell DTP given alone.

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Digestive System

Anorexia and vomiting occurred with frequencies not significantly different as reported when DTP was given alone without IPV or OPV. (12)

Nervous System

Although no causal relationship between IPOL vaccine and GBS has been established, (28) GBS has been temporally related to administration of another inactivated poliovirus vaccine.

Post-marketing Experience

The following adverse events have been identified during postapproval use of IPOL vaccine. Because these events are reported voluntarily from a population of uncertain size, it may not be possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure. Adverse events were included based on one or more of the following factors: severity, frequency of reporting or strength of evidence for a causal relationship.

- ***Blood and lymphatic system disorders:*** lymphadenopathy
- ***General disorders and administration site conditions:*** agitation, injection site reaction including injection site rash and mass
- ***Immune system disorders:*** type I hypersensitivity including allergic reaction, anaphylactic reaction, and anaphylactic shock
- ***Musculoskeletal and connective tissue disorders:*** arthralgia, myalgia
- ***Nervous system disorders:*** convulsion, febrile convulsion, headache, paresthesia, somnolence, syncope

- ***Skin and subcutaneous tissue disorders:*** rash, urticaria

Reporting of Adverse Events

The National Vaccine Injury Compensation Program, established by the National Childhood Vaccine Injury Act of 1986, requires physicians and other healthcare providers who administer vaccines to maintain permanent vaccination records and to report occurrences of certain adverse events to the US Department of Health and Human Services. Reportable events include those listed in the Act for each vaccine and events specified in the package insert as contraindications to further doses of that vaccine. (38) (39) (40)

Reporting by parents or guardians of all adverse events after vaccine administration should be encouraged. Adverse events following immunization with vaccine should be reported by healthcare providers to the US Department of Health and Human Services (DHHS) Vaccine Adverse Event Reporting System (VAERS). Reporting forms and information about reporting requirements or completion of the form can be obtained from VAERS through a toll-free number 1-800-822-7967. (38) (39) (40)

Healthcare providers also should report these events to the Pharmacovigilance Department, Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 or call 1-800-822-2463.

DOSAGE AND ADMINISTRATION

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. The vial and its packaging should be inspected prior to use for evidence of leakage or a faulty seal. If evidence of such defects are observed, the vaccine should not be used. Do not remove the vial stopper or the metal seal holding it in place.

After preparation of the injection site, using a suitable sterile needle and aseptic technique, immediately administer IPOL vaccine intramuscularly or subcutaneously. In infants and small children, the mid-lateral aspect of the thigh is the preferred site. In older children and adults, IPOL vaccine should be administered intramuscularly or subcutaneously in the deltoid area. IPOL should not be combined through reconstitution or mixed with any other vaccine.

To help avoid HIV (AIDS), HBV (Hepatitis), and other infectious diseases due to accidental needlesticks, contaminated needles should not be recapped or removed, unless there is no alternative or that such action is required by a specific medical procedure.

Care should be taken to avoid administering the injection into or near blood vessels and nerves. If blood or any suspicious discoloration appears in the syringe, do not inject but discard contents and repeat procedures using a new dose of vaccine administered at a different site.

DO NOT ADMINISTER VACCINE INTRAVENOUSLY.

Children

The primary series of IPOL vaccine consists of three 0.5 mL doses administered intramuscularly or subcutaneously, preferably eight or more weeks apart and usually at ages 2, 4, and 6 to 18 months. Under no circumstances should the vaccine be given more frequently than four weeks apart. The first immunization may be administered as early as six weeks of age. For this series, a booster dose of IPOL vaccine is administered at 4 to 6 years of age. (41)

Use with Other Vaccines

From historical data on the antibody responses to diphtheria, tetanus, whole-cell or acellular pertussis, Hib, or hepatitis B vaccines used concomitantly with IPOL vaccine, no interferences have been observed on the immunological end points accepted for clinical protection. (11) (16) (36) (See **DRUG INTERACTIONS** section.)

If the third dose of IPOL vaccine is given between 12 to 18 months of age, it may be desirable to administer this dose with Measles, Mumps, and Rubella (MMR) vaccine and/or other vaccines using separate syringes at separate sites, (28) but no data on the immunological interference between IPOL vaccine and these vaccines exist.

Use in Previously Vaccinated Children

Children and adolescents with a previously incomplete series of polio vaccine should receive sufficient additional doses of IPOL vaccine to complete the series.

Interruption of the recommended schedule with a delay between doses does not interfere with the final immunity. There is no need to start the series over again, regardless of the time elapsed between doses.

The need to routinely administer additional doses is unknown at this time. (28)

Adults

Unvaccinated Adults

A primary series of IPOL vaccine is recommended for unvaccinated adults at increased risk of exposure to poliovirus. While the responses of adults to primary series have not been studied, the recommended schedule for adults is two 0.5 mL doses given at a 1 to 2 month interval and a third 0.5 mL dose given 6 to 12 months later. If less than 3 months but more than 2 months are available before protection is needed, three doses of IPOL vaccine should be given at least 1 month apart. Likewise, if only 1 or 2 months are available, two 0.5 mL doses of IPOL vaccine should be given at least 1 month apart. If less than 1 month is available, a single 0.5 mL dose of IPOL vaccine is recommended. (28)

Incompletely Vaccinated Adults

Adults who are at an increased risk of exposure to poliovirus and who have had at least one dose of OPV, fewer than three doses of conventional IPV or a combination of conventional IPV or OPV totaling fewer than three doses should receive at least one 0.5 mL dose of IPOL vaccine. Additional doses needed to complete a primary series should be given if time permits. (28)

Completely Vaccinated Adults

Adults who are at an increased risk of exposure to poliovirus and who have previously completed a primary series with one or a combination of polio vaccines can be given a 0.5 mL dose of IPOL vaccine.

The preferred injection site of IPOL vaccine for adults is in the deltoid area.

HOW SUPPLIED

Multi-dose vial , 5mL: NDC 49281-860-78. Supplied as package: NDC 49281-860-10.

STORAGE

The vaccine is stable if stored in the refrigerator at 2°C to 8°C (35°F to 46°F). The vaccine must not be frozen.

Protect from light.

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Product Information as of May 2022

Manufactured by:

Sanofi Pasteur SA

Marcy L'Etoile France

US Govt License #1724

Distributed by:

Sanofi Pasteur Inc.

Swiftwater PA 18370 USA

1-800-VACCINE (1-800-822-2463)

Rotavirus

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ROTARIX safely and effectively. See full prescribing information for ROTARIX.

ROTARIX (Rotavirus Vaccine, Live, Oral)
Suspension, for oral use
Initial U.S. Approval: 2008

----------RECENT MAJOR CHANGES----------
Dosage and Administration (2.1, 2.2, 2.3) 11/2022

---------- INDICATIONS AND USAGE----------
ROTARIX is a vaccine indicated for the prevention of rotavirus gastroenteritis caused by G1 and non-G1 types (G3, G4, and G9). ROTARIX is approved for use in infants 6 weeks and up to 24 weeks of age. (1)

----------DOSAGE AND ADMINISTRATION----------
FOR ORAL USE ONLY.
ROTARIX is supplied as either:
- Vial and oral dosing applicator presentation: The vial contains the lyophilized vaccine component and the oral dosing applicator contains the diluent. The contents of the vial must be reconstituted with the diluent to form ROTARIX prior to administration (2.1), or
- Oral dosing applicator only presentation: The oral dosing applicator contains ROTARIX and does NOT require reconstitution or dilution before use. (2.1)

Schedule
- Administer first dose to infants beginning at 6 weeks of age. (2.3)
- Administer second dose after an interval of at least 4 weeks and up to 24 weeks of age. (2.3)

---------- DOSAGE FORMS AND STRENGTHS----------
Suspension for oral use.
- Vial and oral dosing applicator presentation: a single dose is 1 mL. (3)
- Oral dosing applicator only presentation: a single dose is 1.5 mL. (3)

---------- CONTRAINDICATIONS ----------
- A demonstrated history of hypersensitivity to the vaccine or any component of the vaccine. (4.1, 11)
- History of uncorrected congenital malformation of the gastrointestinal tract that would predispose the infant to intussusception. (4.2)
- History of intussusception. (4.3)
- History of Severe Combined Immunodeficiency Disease (SCID). (4.4, 6.2)

---------- WARNINGS AND PRECAUTIONS ----------
- The tip caps of the prefilled oral dosing applicators contain natural rubber latex which may cause allergic reactions. (5.1)
- Administration of ROTARIX in infants suffering from acute diarrhea or vomiting should be delayed. Safety and effectiveness of ROTARIX in infants with chronic gastrointestinal disorders have not been evaluated. (5.2)
- Safety and effectiveness of ROTARIX in infants with known primary or secondary immunodeficiencies have not been established. (5.3)
- In a postmarketing study, cases of intussusception were observed in temporal association within 31 days following the first dose of ROTARIX, with a clustering of cases in the first 7 days. (5.5, 6.2)

---------- ADVERSE REACTIONS ----------
Common (≥5%) solicited adverse reactions included fussiness/irritability, cough/runny nose, fever, loss of appetite, and vomiting. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 11/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ROTARIX is indicated for the prevention of rotavirus gastroenteritis caused by G1 and non-G1 types (G3, G4, and G9) when administered as a 2-dose series *[see Clinical Studies (14.3)]*. ROTARIX is approved for use in infants 6 weeks and up to 24 weeks of age.

2 DOSAGE AND ADMINISTRATION

For oral use only.

2.1 ROTARIX Presentations

ROTARIX is supplied in two presentations, a vial and oral dosing applicator presentation and an oral dosing applicator only presentation.

Vial and Oral Dosing Applicator Presentation

The vial contains the lyophilized vaccine component, and the oral dosing applicator contains the diluent. The contents of the vial must be reconstituted with the diluent to form ROTARIX prior to administration.

Oral Dosing Applicator Only Presentation

The oral dosing applicator only presentation contains ROTARIX and does NOT require reconstitution or dilution before use.

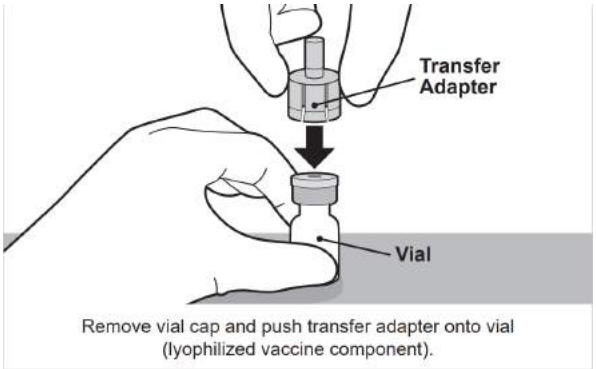
2.2 Preparation and Administration

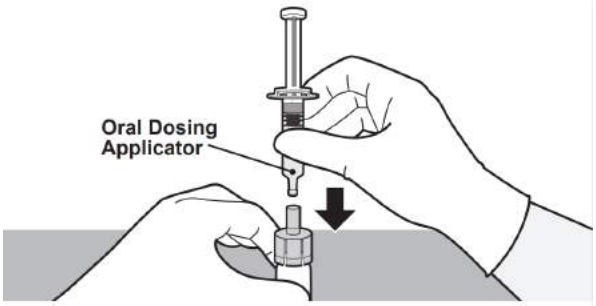
Vial and Oral Dosing Applicator Presentation

Use accompanying diluent to reconstitute the lyophilized vaccine component to form ROTARIX. After reconstitution, each dose of 1 mL is administered orally.

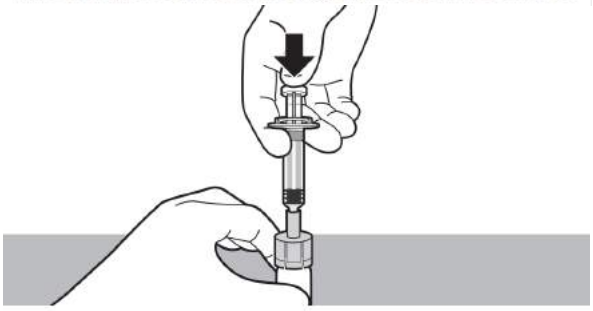
See Figure 1 for preparation and administration steps.

Figure 1. Preparation and Administration steps of Vial and Oral Dosing Applicator Presentation

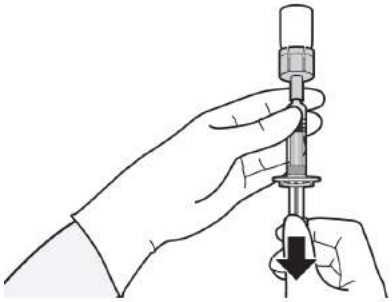




Shake diluent in oral dosing applicator. The diluent appears white and turbid. Connect oral dosing applicator to transfer adapter.

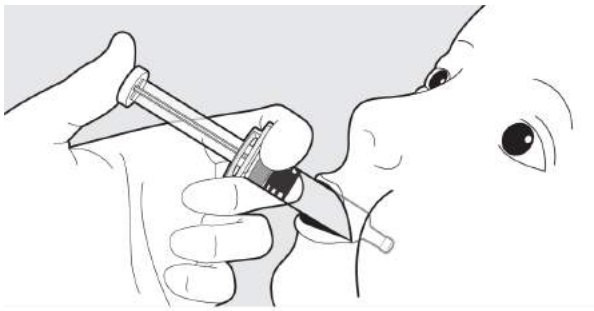


Push plunger of oral dosing applicator to transfer diluent into vial. After reconstitution, vaccine will appear white and turbid.

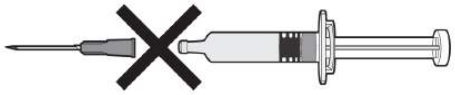


Withdraw vaccine into oral dosing applicator.

Twist to remove oral dosing applicator.



Oral administration **only.**



Do not use a needle with ROTARIX.
Do not inject.

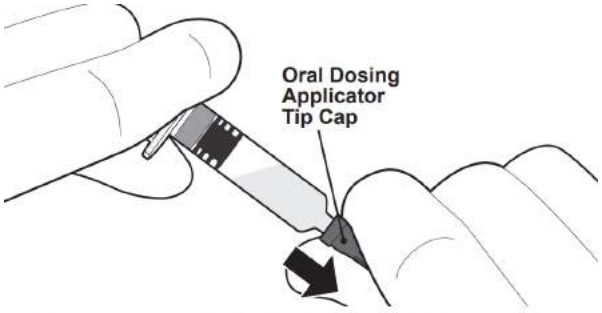
For **oral** administration only. Have the infant seated in a reclining position. Place the oral dosing applicator towards the inner cheek. Administer the entire contents of the oral dosing applicator into the infant's mouth.

Oral Dosing Applicator Only Presentation

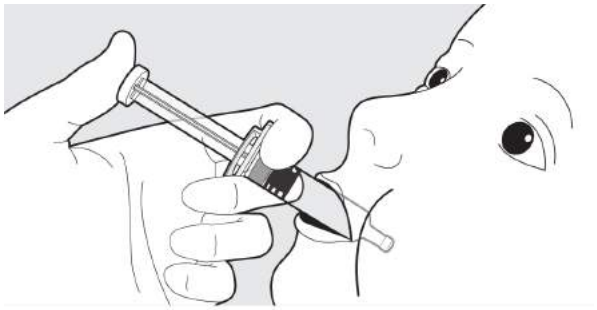
The ROTARIX oral dosing applicator only presentation does NOT require reconstitution or dilution before use. Each dose of 1.5 mL is administered orally.

See Figure 2 for preparation and administration steps.

Figure 2. Preparation and Administration Steps of Oral Dosing Applicator Only Presentation

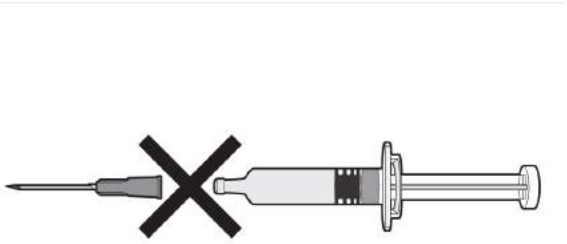


Remove the protective Tip Cap from the oral dosing applicator.
Vaccine appears clear and colorless.



Oral administration **only.**

For **oral administration only**. Have the infant seated in a reclining position. Place the oral dosing applicator towards the inner cheek. Administer the entire contents of the oral dosing applicator into the infant's mouth.



Do not use a needle with ROTARIX.
Do not inject.

2.3 Dosing and Schedule

The vaccination series consists of two doses administered **orally**. The first dose should be administered to infants beginning at 6 weeks of age. There should be an interval of at least 4 weeks between the first and second dose. The 2-dose series should be completed by 24 weeks of age.

Safety and effectiveness have not been evaluated if ROTARIX were administered for the first dose and another rotavirus vaccine were administered for the second dose or vice versa.

In the event that the infant spits out or regurgitates most of the vaccine dose, a single replacement dose may be considered at the same vaccination visit.

2.4 Infant Feeding

Breastfeeding was permitted in clinical studies. There was no evidence to suggest that breastfeeding reduced the protection against rotavirus gastroenteritis afforded by ROTARIX. There are no restrictions on the infant's liquid consumption, including breast milk, either before or after vaccination with ROTARIX.

3 DOSAGE FORMS AND STRENGTHS

Suspension for oral use

- Vial and oral dosing applicator presentation: after reconstitution a single dose is 1 mL.
- Oral dosing applicator only presentation: a single dose is 1.5 mL.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A demonstrated history of hypersensitivity to any component of the vaccine.

Infants who develop symptoms suggestive of hypersensitivity after receiving a dose of ROTARIX should not receive further doses of ROTARIX.

4.2 Gastrointestinal Tract Congenital Malformation

Infants with a history of uncorrected congenital malformation of the gastrointestinal tract (such as Meckel's diverticulum) that would predispose the infant for intussusception should not receive ROTARIX.

4.3 History of Intussusception

Infants with a history of intussusception should not receive ROTARIX *[see Warnings and Precautions (5.5)]*. In postmarketing experience, intussusception resulting in death following a second dose has been reported following a history of intussusception after the first dose *[see Adverse Reactions (6.2)]*.

4.4 Severe Combined Immunodeficiency Disease

Infants with Severe Combined Immunodeficiency Disease (SCID) should not receive ROTARIX. Postmarketing reports of gastroenteritis, including severe diarrhea and prolonged shedding of vaccine virus, have been reported in infants who were administered live, oral rotavirus vaccines and later identified as having SCID *[see Adverse Reactions (6.2)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Latex

The tip caps of the prefilled oral dosing applicators contain natural rubber latex which may cause allergic reactions.

5.2 Gastrointestinal Disorders

Administration of ROTARIX should be delayed in infants suffering from acute diarrhea or vomiting.

Safety and effectiveness of ROTARIX in infants with chronic gastrointestinal disorders have not been evaluated. *[See Contraindications (4.2).]*

5.3 Altered Immunocompetence

Safety and effectiveness of ROTARIX in infants with known primary or secondary immunodeficiencies, including infants with human immunodeficiency virus (HIV), infants on

immunosuppressive therapy, or infants with malignant neoplasms affecting the bone marrow or lymphatic system have not been established.

5.4 Shedding and Transmission

Rotavirus shedding in stool occurs after vaccination with peak excretion occurring around Day 7 after Dose 1.

One clinical trial demonstrated that vaccinees transmit vaccine virus to healthy seronegative contacts *[see Clinical Pharmacology (12.2)]*.

The potential for transmission of vaccine virus following vaccination should be weighed against the possibility of acquiring and transmitting natural rotavirus. Caution is advised when considering whether to administer ROTARIX to individuals with immunodeficient close contacts, such as individuals with malignancies, primary immunodeficiency or receiving immunosuppressive therapy.

5.5 Intussusception

Following administration of a previously licensed oral live rhesus rotavirus-based vaccine, an increased risk of intussusception was observed.[1] The risk of intussusception with ROTARIX was evaluated in a pre-licensure randomized, placebo-controlled safety study (including 63,225 infants) conducted in Latin America and Finland. No increased risk of intussusception was observed in this clinical trial following administration of ROTARIX when compared with placebo.* *[See Adverse Reactions (6.1).]*

In a postmarketing, observational study conducted in Mexico, cases of intussusception were observed in temporal association within 31 days following the first dose of ROTARIX, with a clustering of cases in the first 7 days. *[See Adverse Reactions (6.2).]*

Other postmarketing observational studies conducted in Brazil and Australia also suggest an increased risk of intussusception within the first 7 days following the second dose of ROTARIX.[2,3] *[See Adverse Reactions (6.2).]*

In worldwide passive postmarketing surveillance, cases of intussusception have been reported in temporal association with ROTARIX *[see Adverse Reactions (6.2)]*.

5.6 Post-Exposure Prophylaxis

Safety and effectiveness of ROTARIX when administered after exposure to rotavirus have not been evaluated.

*"The placebo had the same constituents as the active vaccine but without the vaccine virus."
Reference: https://www.nejm.org/doi/10.1056/NEJMoa052434

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

There are two formulations of ROTARIX: a reconstituted lyophilized formulation (supplied in a vial and oral dosing applicator presentation) and a liquid formulation (supplied in an oral dosing applicator only presentation) *[see Description (11)]*. Safety data accrued with each formulation is relevant to the other because each contains the same live, attenuated rotavirus strain and is manufactured using a similar process.

Common (≥5%) solicited adverse reactions included fussiness/irritability, cough/runny nose, fever, loss of appetite, and vomiting.

Solicited adverse reactions, unsolicited adverse events, serious adverse events (SAEs), and cases of intussusception were collected in 7 clinical studies (Studies 1 to 7; NCT00729001, NCT00385320, NCT00429481, NCT00757770, NCT00140686, NCT00169455, NCT00137930). Cases of intussusception and SAEs were collected in an additional large safety study (Study 8; NCT00140673) that compared ROTARIX (reconstituted lyophilized formulation) to placebo. Solicited adverse reactions, unsolicited adverse events, and SAEs were collected in 3 clinical studies (Studies 9 to 11; NCT02914184, NCT03207750, NCT03954743) that compared the two ROTARIX formulations.

Clinical Trials Experience with ROTARIX (Reconstituted Lyophilized Formulation)

Studies 1 to 8 evaluated a total of 71,209 infants who received ROTARIX (n = 36,755) or placebo (n = 34,454). The racial distribution for these studies was as follows: Hispanic 73.4%, White 16.2%, Black 1.0%, and other 9.4%; 51% were male.

Solicited Adverse Reactions: In 7 clinical studies (Studies 1 to 7), detailed safety information was collected by parents/guardians for 8 consecutive days following vaccination with ROTARIX (i.e., day of vaccination and the next 7 days). A diary card was completed to record fussiness/irritability, cough/runny nose, the infant's temperature, loss of appetite, vomiting, or diarrhea on a daily basis during the first week following each dose of ROTARIX or placebo. Adverse reactions among recipients of ROTARIX and placebo occurred at similar rates (Table 1).

Table 1. Solicited Adverse Reactions within 8 Days Following Doses 1 and 2 of ROTARIX or Placebo (Total Vaccinated Cohort)

Adverse Reaction	Dose 1		Dose 2	
	ROTARIX n = 3,284 %	Placebo n = 2,013 %	ROTARIX n = 3,201 %	Placebo n = 1,973 %
Fussiness/irritability[a]	52	52	42	42
Cough/runny nose[b]	28	30	31	33
Fever[c]	25	33	28	34
Loss of appetite[d]	25	25	21	21
Vomiting	13	11	8	8
Diarrhea	4	3	3	3

Total vaccinated cohort = All vaccinated infants for whom safety data were available.
n = Number of infants for whom at least one symptom sheet was completed.
[a] Defined as crying more than usual.
[b] Data not collected in 1 of 7 studies; Dose 1: ROTARIX n = 2,583; placebo n = 1,897; Dose 2: ROTARIX n = 2,522; placebo n = 1,863.
[c] Defined as temperature ≥100.4°F (≥38.0°C) rectally or ≥99.5°F (≥37.5°C) orally.
[d] Defined as eating less than usual.

Unsolicited Adverse Reactions: Infants were monitored for unsolicited serious and non-SAEs that occurred in the 31-day period following vaccination in 7 clinical studies (Studies 1 to 7). The following adverse reactions occurred at a statistically higher incidence (95% Confidence Interval [CI] of Relative Risk [RR] excluding 1) among recipients of ROTARIX (n = 5,082) as compared with placebo recipients (n = 2,902): irritability (ROTARIX 11.4%, placebo 8.7%) and flatulence (ROTARIX 2.2%, placebo 1.3%).

Serious Adverse Reactions: Infants were monitored for SAEs that occurred in the 31-day period following vaccination in 8 clinical studies (Studies 1 to 8). Serious adverse reactions occurred in 1.7% of recipients of ROTARIX (n = 36,755) as compared with 1.9% of placebo recipients (n = 34,454). Among placebo recipients, diarrhea (placebo 0.07%, ROTARIX 0.02%), dehydration (placebo 0.06%, ROTARIX 0.02%), and gastroenteritis (placebo 0.3%, ROTARIX 0.2%) occurred at a statistically higher incidence (95% CI of RR excluding 1) as compared with recipients of ROTARIX.

Deaths: During the entire course of 8 clinical studies (Studies 1 to 8), there were 68 (0.19%) deaths following administration of ROTARIX (n = 36,755) and 50 (0.15%) deaths following placebo administration (n = 34,454). The most commonly reported cause of death following vaccination was pneumonia, which was observed in 19 (0.05%) recipients of ROTARIX and 10 (0.03%) placebo recipients (RR: 1.74, 95% CI: 0.76, 4.23).

Intussusception: In a controlled safety study (Study 8) conducted in Latin America and Finland, the risk of intussusception was evaluated in 63,225 infants (31,673 received ROTARIX and 31,552 received placebo). Infants were monitored by active surveillance including independent, complementary methods (prospective hospital surveillance and parent reporting at scheduled study visits) to identify potential cases of intussusception within 31 days after vaccination and, in a subset of 20,169 infants (10,159 received ROTARIX and 10,010 received placebo), up to one year after the first dose.

No increased risk of intussusception following administration of ROTARIX was observed within a 31-day period following any dose, and rates were comparable to the placebo group after a median of 100 days (Table 2). In a subset of 20,169 infants (10,159 received ROTARIX and 10,010 received placebo) followed up to one year after Dose 1, there were 4 cases of intussusception with ROTARIX compared with 14 cases of intussusception with placebo (RR: 0.28 [95% CI: 0.10, 0.81]). All of the infants who developed intussusception recovered without sequelae.

Table 2. Intussusception and Relative Risk with ROTARIX Compared with Placebo

Confirmed Cases of Intussusception	ROTARIX n = 31,673	Placebo n = 31,552
Within 31 days following diagnosis after any dose	6	7
Relative Risk (95% CI)	0.85 (0.30, 2.42)	
Within 100 days following Dose 1[a]	9	16
Relative Risk (95% CI)	0.56 (0.25, 1.24)	

CI = Confidence Interval.

[a] Median duration after Dose 1 (follow-up visit at 30 to 90 days after Dose 2).

Among vaccine recipients, there were no confirmed cases of intussusception within the 0- to 14-day period after the first dose (Table 3), which was the period of highest risk for the previously licensed oral live rhesus rotavirus-based vaccine.[1]

Table 3. Intussusception Cases by Day Range in Relation to Dose

	Dose 1		Dose 2		Any Dose	
Day Range	ROTARIX n = 31,673	Placebo n = 31,552	ROTARIX n = 29,616	Placebo n = 29,465	ROTARIX n = 31,673	Placebo n = 31,552
0-7	0	0	2	0	2	0
8-14	0	0	0	2	0	2
15-21	1	1	2	1	3	2
22-30	0	1	1	2	1	3
Total (0-30)	1	2	5	5	6	7

Kawasaki Disease – Results from Controlled and Uncontrolled Clinical Studies: Kawasaki disease has been reported in 18 (0.035%) recipients of ROTARIX and 9 (0.021%) placebo recipients from 16 completed or ongoing clinical trials (Studies 1 to 8; Studies 12 to 14, NCT00425737, NCT00346892, NCT00139347; Studies 15 to 17, NCT00197210 for the 3 studies; Studies 18 and 19, NCT00334607, NCT00382772). Of the 27 cases, 5 occurred following ROTARIX in clinical trials that were either not placebo-controlled or 1:1 randomized. In placebo-controlled trials, Kawasaki disease was reported in 17 recipients of ROTARIX and 9 placebo recipients (RR: 1.71 [95% CI: 0.71, 4.38]). Three of the 27 cases were reported within 30 days post-vaccination: 2 cases (ROTARIX = 1, placebo = 1) were from placebo-controlled trials (RR: 1.00 [95% CI: 0.01, 78.35]) and one case following ROTARIX was from a non–placebo-controlled trial. Among recipients of ROTARIX, the time of onset after study dose ranged 3 days to 19 months.

Clinical Trials Comparing the Two ROTARIX Formulations

The safety of the ROTARIX liquid formulation was evaluated in 3 randomized clinical studies (Studies 9 to 11). A total of 4,223 infants received ROTARIX (liquid formulation, n = 2,507; reconstituted lyophilized formulation, n = 1,716). The racial distribution for these 3 studies was as follows: Asian 24.4%, White 63.2%, Black or African American 5.1%, and other 7.3%; 49.7% were male.

In Study 10, a concomitant vaccine administration study conducted in United States, 1,272 infants received ROTARIX (liquid formulation, n = 632; reconstituted lyophilized formulation, n = 640). The racial distribution for this study was as follows: Asian 3.3%, White 73.8%, Black or African American 11.9%, and other 10.9%; 51.5% were male.

In Studies 9 to 11, solicited general adverse reactions (cough/runny nose, diarrhea, fever, irritability/fussiness, loss of appetite and vomiting) were recorded by the parent on diary cards during the 8 days after each vaccination (day of vaccination and 7 following days). Unsolicited adverse events were assessed within 31 days following each vaccination (day of vaccination and 30 following days). SAEs were assessed through 6 months after the last dose.

Solicited Adverse Reactions: In Study 10, solicited adverse reactions among recipients of the two ROTARIX formulations are presented in the Table 4:

Table 4. Solicited Adverse Reactions within 8 Days Following Doses 1 and 2 of ROTARIX (Liquid or Reconstituted Lyophilized Formulation), Study 10 Total Vaccinated Cohort

	Dose 1				Dose 2			
	ROTARIX (Liquid Formulation) n = 632		ROTARIX (Reconstituted Lyophilized Formulation) n = 640		ROTARIX (Liquid Formulation) n = 607		ROTARIX (Reconstituted Lyophilized Formulation) n = 609	
Adverse Reactions	**Overall%**	**G3%**	**Overall%**	**G3%**	**Overall%**	**G3%**	**Overall%**	**G3%**
Fussiness/irritability[a]	70.9	9.2	71.6	8.8	72.5	13.7	70.1	11.8
Cough/runny nose[b]	27.2	0.5	28.1	1.3	36.9	3.8	36.5	3.6
Fever[c]	5.7	0.3	5.0	0.3	10.5	0.3	12.3	0.7
Loss of appetite[d]	32.3	0.2	33.4	0.9	29.5	1.2	29.2	1.8
Vomiting[e]	17.4	2.8	16.4	3.4	13.7	4.0	12.8	3.3
Diarrhea[f]	6.2	0.2	5.6	0.8	5.6	0.5	4.3	0.3

G3 = Grade 3.

Total vaccinated cohort = All vaccinated infants for whom safety data were available.

n = Number of infants who received the specified dose.

[a] Overall: Defined as crying more than usual. Grade 3: Crying that could not be comforted/prevented normal activity.

[b] Grade 3 cough/runny nose: cough/runny nose that prevented daily activity.

[c] Overall: Defined as temperature ≥100.4°F (≥38.0°C). Grade 3: Defined as temperature >103.1 F (>39.5°C).

[d] Overall: Defined as eating less than usual. Grade 3: Defined as not eating at all.

[e] Overall: Defined as 1 or more episodes of forceful emptying of partially digested stomach contents ≥1 hour after feeding within a day. Grade 3: ≥3 episodes of vomiting/day.

[f] Overall: Defined as passage of 3 or more looser than normal stools within a day. Grade 3: ≥6 looser than normal stools/day.

Unsolicited Adverse Events: Infants were monitored for unsolicited serious and non-SAEs that occurred in the 31-day period following vaccination in Studies 9 to 11. There were no notable differences in the occurrence and frequency of unsolicited adverse events between the groups.

Serious Adverse Events: During the entire course of Studies 9 to 11, SAEs occurred in 4.7% of recipients of ROTARIX liquid formulation (n = 2,507) as compared with 4.4% of ROTARIX reconstituted lyophilized formulation recipients (n = 1,716).

During the entire course of Studies 9 to 11, there was 1 fatal SAE (with diagnosis of sudden infant death syndrome) following administration of ROTARIX liquid formulation (Study 10). The SAE was assessed as not causally related to the vaccination.

Among participants in Studies 9 to 11, 2 intussusception cases were reported. One subject from Study 10 experienced intussusception 8 days after receiving the second dose of ROTARIX (reconstituted lyophilized formulation). The event was considered possibly related to ROTARIX. One subject from Study 9 experienced intussusception 133 days after receiving the second dose of ROTARIX (liquid formulation); the event was not considered related to ROTARIX. Both subjects were hospitalized, and the outcome of intussusception was reported as resolved.

6.2 Postmarketing Experience

The temporal association between vaccination with ROTARIX and intussusception was evaluated in a hospital-based active surveillance study that identified infants with intussusception at participating hospitals in Mexico. Using a self-controlled case series method,[4] the incidence of intussusception during the first 7 days after receipt of ROTARIX and during the 31-day period after receipt of ROTARIX was compared with a control period. The control period was from birth to one year, excluding the pre-defined risk period (first 7 days or first 31 days post-vaccination, respectively).

Over a 2-year period, the participating hospitals provided health services to approximately 1 million infants under 1 year of age. Among 750 infants with intussusception, the relative incidence of intussusception in the 31-day period after the first dose of ROTARIX compared with the control period was 1.96 (95.5% CI: 1.46, 2.63)]; the relative incidence of intussusception in the first 7 days after the first dose of ROTARIX compared with the control period was 6.07 (95.5% CI: 4.20, 8.63).

The Mexico study did not take into account all medical conditions that may predispose infants to intussusception. The results may not be generalizable to U.S. infants who have a lower background rate of intussusception than Mexican infants. However, if a temporal increase in the risk for intussusception following ROTARIX similar in magnitude to that observed in the Mexico study does exist in U.S. infants, it is estimated that approximately 1 to 3 additional cases of intussusception hospitalizations would occur per 100,000 vaccinated infants in the U.S. within 7 days following the first dose of ROTARIX. In the first year of life, the background rate of intussusception hospitalizations in the U.S. has been estimated to be approximately 34 per 100,000 infants.[5]

Other postmarketing observational studies conducted in Brazil and Australia also suggest an increased risk of intussusception within the first 7 days following the second dose of ROTARIX.[2,3]

Worldwide passive postmarketing surveillance data suggest that most cases of intussusception reported following ROTARIX occur in the 7-day period after the first dose.

The following adverse reactions have been identified during post-approval use of ROTARIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccination.

Gastrointestinal Disorders

Intussusception (including death), recurrent intussusception (including death), hematochezia, gastroenteritis with vaccine viral shedding in infants with SCID.

Blood and Lymphatic System Disorders

Idiopathic thrombocytopenic purpura.

Vascular Disorders

Kawasaki disease.

General Disorders and Administration Site Conditions

Maladministration.

7 DRUG INTERACTIONS

7.1 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to ROTARIX. *[See Warnings and Precautions (5.3).]*

8 USE IN SPECIFIC POPULATIONS

8.4 Pediatric Use

Safety and effectiveness of ROTARIX in infants younger than 6 weeks or older than 24 weeks of age have not been evaluated.

The effectiveness of ROTARIX in pre-term infants has not been established. Safety data are available in pre-term infants (ROTARIX = 134, placebo = 120) with a reported gestational age ≤36 weeks. These pre-term infants were followed for SAEs up to 30 to 90 days after Dose 2. SAEs were observed in 5.2% of recipients of ROTARIX as compared with 5.0% of placebo recipients. No deaths or cases of intussusception were reported in this population.

11 DESCRIPTION

ROTARIX (Rotavirus Vaccine, Live, Oral), for oral administration, is a live, attenuated rotavirus vaccine derived from the human 89-12 strain which belongs to G1P[8] type. The rotavirus vaccine strain is propagated on Vero cells.

There are two formulations of ROTARIX: a reconstituted lyophilized formulation (supplied in a vial and oral dosing applicator presentation) and a liquid formulation (supplied in an oral dosing applicator only presentation).

Formulation for the Vial and Oral Dosing Applicator Presentation

After reconstitution of the lyophilized vaccine component, each 1 mL dose of the ROTARIX formulation for the vial and oral dosing applicator presentation contains at least $10^{6.0}$ median Cell Culture Infective Dose ($CCID_{50}$) of live, attenuated rotavirus.

The lyophilized vaccine component of this ROTARIX formulation contains amino acids, dextran, Dulbecco's Modified Eagle Medium (DMEM), sorbitol, and sucrose. DMEM contains the following ingredients: sodium chloride, potassium chloride, magnesium sulfate, ferric (III) nitrate, sodium dihydrogen phosphate, sodium pyruvate, D-glucose, concentrated vitamin solution, L-cystine, L-tyrosine, amino acids solution, L-glutamine, calcium chloride, and sodium hydrogenocarbonate.

In the manufacturing process, porcine-derived materials are used. Porcine circovirus type 1 (PCV-1) is present in this ROTARIX formulation. PCV-1 is not known to cause disease in humans.

The liquid diluent contains calcium carbonate, sterile water, and xanthan. The diluent includes an antacid component (calcium carbonate) to protect the vaccine during passage through the stomach and prevent its inactivation due to the acidic environment of the stomach.

This ROTARIX formulation is available in single-dose vials of lyophilized vaccine, accompanied by a prefilled oral dosing applicator of liquid diluent *[see How Supplied/Storage and Handling (16)]*. The tip caps of the prefilled oral dosing applicators contain natural rubber latex; the vial stoppers are not made with natural rubber latex.

This ROTARIX formulation contains no preservatives.

Formulation for the Oral Dosing Applicator Only Presentation

Each 1.5 mL dose of the ROTARIX formulation for the oral dosing applicator only presentation contains at least $10^{6.0}$ $CCID_{50}$ of live, attenuated rotavirus.

This ROTARIX formulation contains disodium adipate, Dulbecco's Modified Eagle Medium (DMEM), sucrose, and sterile water. DMEM contains the following ingredients: sodium chloride, potassium chloride, magnesium sulfate, ferric (III) nitrate, sodium dihydrogen phosphate, sodium pyruvate, D-glucose, concentrated vitamin solution, L-cystine, L-tyrosine, amino acids solution, L-glutamine, calcium chloride, and sodium hydrogenocarbonate.

This ROTARIX formulation contains an antacid component (disodium adipate) to protect the vaccine during passage through the stomach and prevent its inactivation due to the acidic environment of the stomach.

This ROTARIX formulation is available in single-dose prefilled oral dosing applicator *[see How Supplied/Storage and Handling (16)]*. The tip caps of the prefilled oral dosing applicators contain natural rubber latex.

This ROTARIX formulation contains no preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The exact immunologic mechanism by which ROTARIX protects against rotavirus gastroenteritis is unknown *[see Clinical Studies (14.4)]*. ROTARIX contains a live, attenuated human rotavirus that replicates in the small intestine and induces immunity.

12.2 Pharmacodynamics

Shedding and Transmission

A prospective, randomized, double-blind, placebo-controlled study was performed in the Dominican Republic in twins within the same household to assess whether transmission of vaccine virus occurs from a vaccinated infant to a non-vaccinated infant (Study 20; NCT00396630). One hundred pairs of healthy twins 6 to 14 weeks of age (gestational age ≥32 weeks) were randomized with one twin to receive ROTARIX (n = 100) and the other twin to receive placebo (n = 100). Twenty subjects in each arm were excluded for reasons such as having rotavirus antibody at baseline. Stool samples were collected on the day of or 1 day prior to each dose, as well as 3 times weekly for 6 consecutive weeks after each dose of ROTARIX or placebo. Transmission was defined as presence of the vaccine virus strain in any stool sample from a twin receiving placebo.

Transmitted vaccine virus was identified in 15 of 80 twins receiving placebo (18.8% [95% CI: 10.9, 29.0]). Median duration of the rotavirus shedding was 10 days in twins who received ROTARIX as compared with 4 days in twins who received placebo in whom the vaccine virus was transmitted. In the 15 twins who received placebo, no gastrointestinal symptoms related to transmitted vaccine virus were observed.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

ROTARIX has not been evaluated for carcinogenic or mutagenic potential, or for impairment of fertility.

14 CLINICAL STUDIES

There are two formulations of ROTARIX: a reconstituted lyophilized formulation (supplied in a vial and oral dosing applicator presentation) and a liquid formulation (supplied in an oral dosing applicator only presentation) *[see Description (11)]*. Efficacy was evaluated using the

reconstituted lyophilized formulation *[see Clinical Studies (14.1, 14.2, 14.3)]*. These data are relevant to the liquid formulation because both formulations contain the same live, attenuated rotavirus strain and are manufactured using a similar process.

14.1 Efficacy Studies

The data demonstrating the efficacy of ROTARIX in preventing rotavirus gastroenteritis come from 24,163 infants randomized in two placebo-controlled studies conducted in 17 countries in Europe and Latin America (Studies 5 and 8). In these studies, oral polio vaccine (OPV) was not coadministered; however, other routine childhood vaccines could be concomitantly administered. Breastfeeding was permitted in both studies.

A randomized, double-blind, placebo-controlled study was conducted in 6 European countries (Study 5). A total of 3,994 infants were enrolled to receive ROTARIX (n = 2,646) or placebo (n = 1,348). Vaccine or placebo was given to healthy infants as a 2-dose series with the first dose administered orally from 6 through 14 weeks of age followed by one additional dose administered at least 4 weeks after the first dose. The 2-dose series was completed by 24 weeks of age. For both vaccination groups, 98.3% of infants were White and 53% were male.

The clinical case definition of rotavirus gastroenteritis was an episode of diarrhea (passage of 3 or more loose or watery stools within a day), with or without vomiting, where rotavirus was identified in a stool sample. Severity of gastroenteritis was determined by a clinical scoring system, the Vesikari scale, assessing the duration and intensity of diarrhea and vomiting, the intensity of fever, use of rehydration therapy, or hospitalization for each episode. Scores range from 0 to 20, where higher scores indicate greater severity. An episode of gastroenteritis with a score of 11 or greater was considered severe.[6]

The primary efficacy endpoint was prevention of any grade of severity of rotavirus gastroenteritis caused by naturally occurring rotavirus from 2 weeks after the second dose through one rotavirus season (according to protocol, ATP). Other efficacy evaluations included prevention of severe rotavirus gastroenteritis, as defined by the Vesikari scale, and reductions in hospitalizations due to rotavirus gastroenteritis and all-cause gastroenteritis regardless of presumed etiology. Analyses were also done to evaluate the efficacy of ROTARIX against rotavirus gastroenteritis among infants who received at least one vaccination (total vaccinated cohort, TVC).

Efficacy of ROTARIX against any grade of severity of rotavirus gastroenteritis through one rotavirus season was 87.1% (95% CI: 79.6, 92.1); TVC efficacy was 87.3% (95% CI: 80.3, 92.0). Efficacy against severe rotavirus gastroenteritis through one rotavirus season was 95.8% (95% CI: 89.6, 98.7); TVC efficacy was 96.0% (95% CI: 90.2, 98.8) (Table 5). The protective effect of ROTARIX against any grade of severity of rotavirus gastroenteritis observed immediately following Dose 1 administration and prior to Dose 2 was 89.8% (95% CI: 8.9, 99.8).

Efficacy of ROTARIX in reducing hospitalizations for rotavirus gastroenteritis through one rotavirus season was 100% (95% CI: 81.8, 100); TVC efficacy was 100% (95% CI: 81.7, 100) (Table 5). ROTARIX reduced hospitalizations for all-cause gastroenteritis regardless of presumed etiology by 74.7% (95% CI: 45.5, 88.9).

Table 5. Efficacy Evaluation of ROTARIX through One Rotavirus Season

	According to Protocol[a]		Total Vaccinated Cohort[b]	
Infants in Cohort	**ROTARIX n = 2,572**	**Placebo n = 1,302**	**ROTARIX n = 2,646**	**Placebo n = 1,348**
Gastroenteritis cases				
Any severity	24	94	26	104
Severe[c]	5	60	5	64
Efficacy estimate against RV GE				
Any severity	87.1%[d]		87.3%[d]	
(95% CI)	(79.6, 92.1)		(80.3, 92.0)	
Severe[c]	95.8%[d]		96.0%[d]	
(95% CI)	(89.6, 98.7)		(90.2, 98.8)	
Cases of hospitalization due to RV GE	0	12	0	12
Efficacy in reducing hospitalizations due to RV GE	100%[d]		100%[d]	
(95% CI)	(81.8, 100)		(81.7, 100)	

RV GE = Rotavirus gastroenteritis; CI = Confidence Interval.

[a] ATP analysis includes all infants in the efficacy cohort who received two doses of vaccine according to randomization.

[b] TVC analysis includes all infants in the efficacy cohort who received at least one dose of vaccine or placebo.

[c] Severe gastroenteritis defined as ≥11 on the Vesikari scale.

[d] Statistically significant versus placebo (P <0.001).

A randomized, double-blind, placebo-controlled study was conducted in 11 countries in Latin America and Finland (Study 8). A total of 63,225 infants received ROTARIX (n = 31,673) or placebo (n = 31,552). An efficacy subset of these infants consisting of 20,169 infants from Latin America received ROTARIX (n = 10,159) or placebo (n = 10,010). Vaccine or placebo was given to healthy infants as a 2-dose series with the first dose administered orally from 6 through 13 weeks of age followed by one additional dose administered at least 4 weeks after the first dose. The 2-dose series was completed by 24 weeks of age. For both vaccination groups, the racial distribution of the efficacy subset was as follows: Hispanic 85.8%, White 7.9%, Black 1.1%, and other 5.2%; 51% were male.

The clinical case definition of severe rotavirus gastroenteritis was an episode of diarrhea (passage of 3 or more loose or watery stools within a day), with or without vomiting, where rotavirus was identified in a stool sample, requiring hospitalization and/or rehydration therapy equivalent to World Health Organization (WHO) plan B (oral rehydration therapy) or plan C (intravenous rehydration therapy) in a medical facility.

The primary efficacy endpoint was prevention of severe rotavirus gastroenteritis caused by naturally occurring rotavirus from 2 weeks after the second dose through one year (ATP). Analyses were done to evaluate the efficacy of ROTARIX against severe rotavirus gastroenteritis among infants who received at least one vaccination (TVC). Reduction in hospitalizations due to rotavirus gastroenteritis was also evaluated (ATP).

Efficacy of ROTARIX against severe rotavirus gastroenteritis through one year was 84.7% (95% CI: 71.7, 92.4); TVC efficacy was 81.1% (95% CI: 68.5, 89.3) (Table 6).

Efficacy of ROTARIX in reducing hospitalizations for rotavirus gastroenteritis through one year was 85.0% (95% CI: 69.6, 93.5); TVC efficacy was 80.8% (95% CI: 65.7, 90.0) (Table 6).

Table 6. Efficacy Evaluation of ROTARIX through One Year

	According to Protocol[a]		**Total Vaccinated Cohort[b]**	
Infants in Cohort	**ROTARIX n = 9,009**	**Placebo n = 8,858**	**ROTARIX n = 10,159**	**Placebo n = 10,010**
Gastroenteritis cases				
Severe	12	77	18	94
Efficacy estimate against RV GE				
Severe	84.7%[c]		81.1%[c]	
(95% CI)	(71.7, 92.4)		(68.5, 89.3)	
Cases of hospitalization due to RV GE	9	59	14	72
Efficacy in reducing hospitalizations due to RV GE	85.0%[c]		80.8%[c]	
(95% CI)	(69.6, 93.5)		(65.7, 90.0)	

RV GE = Rotavirus gastroenteritis; CI = Confidence Interval.

[a] ATP analysis includes all infants in the efficacy cohort who received two doses of vaccine according to randomization.

[b] TVC analysis includes all infants in the efficacy cohort who received at least one dose of vaccine or placebo.

[c] Statistically significant versus placebo (P <0.001).

14.2 Efficacy through Two Rotavirus Seasons

The efficacy of ROTARIX persisting through two rotavirus seasons was evaluated in two studies.

In the European study (Study 5), the efficacy of ROTARIX against any grade of severity of rotavirus gastroenteritis through two rotavirus seasons was 78.9% (95% CI: 72.7, 83.8). Efficacy in preventing any grade of severity of rotavirus gastroenteritis cases occurring only during the second season post-vaccination was 71.9% (95% CI: 61.2, 79.8). The efficacy of ROTARIX against severe rotavirus gastroenteritis through two rotavirus seasons was 90.4% (95% CI: 85.1, 94.1). Efficacy in preventing severe rotavirus gastroenteritis cases occurring only during the second season post-vaccination was 85.6% (95% CI: 75.8, 91.9).

The efficacy of ROTARIX in reducing hospitalizations for rotavirus gastroenteritis through two rotavirus seasons was 96.0% (95% CI: 83.8, 99.5).

In the Latin American study (Study 8), the efficacy of ROTARIX against severe rotavirus gastroenteritis through two years was 80.5% (95% CI: 71.3, 87.1). Efficacy in preventing severe rotavirus gastroenteritis cases occurring only during the second year post-vaccination was 79.0% (95% CI: 66.4, 87.4). The efficacy of ROTARIX in reducing hospitalizations for rotavirus gastroenteritis through two years was 83.0% (95% CI: 73.1, 89.7).

The efficacy of ROTARIX beyond the second season post-vaccination was not evaluated.

14.3 Efficacy against Specific Rotavirus Types

The type-specific efficacy against any grade of severity and severe rotavirus gastroenteritis caused by G1P[8], G3P[8], G4P[8], G9P[8], and combined non-G1 (G2, G3, G4, G9) types was statistically significant through one year. Additionally, type-specific efficacy against any grade of severity and severe rotavirus gastroenteritis caused by G1P[8], G2P[4], G3P[8], G4P[8], G9P[8], and combined non-G1 (G2, G3, G4, G9) types was statistically significant through two years (Table 7).

Table 7. Type-Specific Efficacy of ROTARIX against Any Grade of Severity and Severe Rotavirus Gastroenteritis (According to Protocol)

Type Identified[a]	Through One Rotavirus Season			Through Two Rotavirus Seasons		
	Number of Cases			Number of Cases		
	ROTARIX n = 2,572	Placebo n = 1,302	% Efficacy (95% CI)	ROTARIX n = 2,572	Placebo n = 1,302	% Efficacy (95% CI)
Any Grade of Severity						
G1P[8]	4	46	95.6%[b] (87.9, 98.8)	18	89[c,d]	89.8%[b] (82.9, 94.2)
G2P[4]	3	4[c]	NS	14	17[c]	58.3%[b] (10.1, 81.0)
G3P[8]	1	5	89.9%[b] (9.5, 99.8)	3	10	84.8%[b] (41.0, 97.3)
G4P[8]	3	13	88.3%[b] (57.5, 97.9)	6	18	83.1%[b] (55.6, 94.5)
G9P[8]	13	27	75.6%[b] (51.1, 88.5)	38	71[d]	72.9%[b] (59.3, 82.2)
Combined non-G1 (G2, G3, G4, G9, G12) types[e]	20	49	79.3%[b] (64.6, 88.4)	62	116	72.9%[b] (62.9, 80.5)
Severe						
G1P[8]	2	28	96.4%[b] (85.7, 99.6)	4	57	96.4%[b] (90.4, 99.1)
G2P[4]	1	2[c]	NS	2	7[c]	85.5%[b] (24.0, 98.5)
G3P[8]	0	5	100%[b] (44.8, 100)	1	8	93.7%[b] (52.8, 99.9)
G4P[8]	0	7	100%[b] (64.9, 100)	1	11	95.4%[b] (68.3, 99.9)
G9P[8]	2	19	94.7%[b] (77.9, 99.4)	13	44[d]	85.0%[b] (71.7, 92.6)
Combined non-G1 (G2, G3, G4, G9, G12) types[e]	3	33	95.4%[b] (85.3, 99.1)	17	70	87.7%[b] (78.9, 93.2)

CI = Confidence Interval; NS = Not significant.

[a] Statistical analyses done by G type; if more than one rotavirus type was detected from a rotavirus gastroenteritis episode, the episode was counted in each of the detected rotavirus type categories.

[b] Statistically significant versus placebo (P <0.05).

[c] The P genotype was not typeable for one episode.

[d] P[8] genotype was not detected in one episode.
[e] Two cases of G12P[8] were isolated in the second season (one in each group).

14.4 Immunogenicity

A relationship between antibody responses to rotavirus vaccination and protection against rotavirus gastroenteritis has not been established. Seroconversion was defined as the appearance of anti-rotavirus IgA antibodies (concentration ≥20 U/mL) post-vaccination in the serum of infants previously negative for rotavirus.

In 2 safety and efficacy studies (Studies 5 and 8), one to two months after a 2-dose series, 86.5% of 787 recipients of ROTARIX reconstituted lyophilized formulation seroconverted compared with 6.7% of 420 placebo recipients, and 76.8% of 393 recipients of ROTARIX reconstituted lyophilized formulation seroconverted compared with 9.7% of 341 placebo recipients, respectively.

Immunogenicity of a 2-dose series of ROTARIX liquid formulation was evaluated in Study 9. This study compared seroconversion rates and Geometric Mean Concentrations (GMCs) following administration of the ROTARIX liquid formulation (n = 984) or the ROTARIX reconstituted lyophilized formulation (n = 329). The primary analyses demonstrated non-inferiority of IgA seroconversion rates and GMCs at 1 to 2 months post-vaccination for the ROTARIX liquid formulation group compared to the ROTARIX reconstituted lyophilized formulation group (Lower Limit of the 95% CI for the difference in seroconversion rates ≥-10%; Lower Limit of the 95% CI for the GMC ratio ≥0.67).

In Study 10, 3 months after a 2-dose series, the percentage of subjects with anti-rotavirus IgA antibodies (concentration ≥20 U/mL) was comparable after administration of the ROTARIX liquid formulation (76.3% of 417) and the ROTARIX reconstituted lyophilized formulation (78.9% of 426).

14.5 Concomitant Vaccine Administration

In clinical trials, ROTARIX was administered concomitantly with U.S.-licensed and non–U.S.-licensed vaccines. In a U.S. concomitant vaccine administration study (Study 18) using ROTARIX (reconstituted lyophilized formulation) in 484 infants, there was no evidence of interference in the immune responses to any of the antigens when PEDIARIX [Diphtheria and Tetanus Toxoids and Acellular Pertussis Adsorbed, Hepatitis B (Recombinant) and Inactivated Poliovirus Vaccine], a U.S.-licensed 7-valent pneumococcal conjugate vaccine (Wyeth Pharmaceuticals Inc.), and a U.S.-licensed Haemophilus b conjugate vaccine (Sanofi Pasteur SA) were concomitantly administered with ROTARIX as compared with separate administration of ROTARIX.

In a concomitant vaccine administration study (Study 10) in 1,272 infants, non-inferiority was demonstrated regarding the immune response to each of the antigens in PEDIARIX, HIBERIX [Haemophilus b Conjugate Vaccine (Tetanus Toxoid Conjugate)], and a U.S.-licensed, 13-valent

pneumococcal conjugate vaccine (Pfizer Inc.) when concomitantly administered with the ROTARIX liquid formulation as compared to when concomitantly administered with the ROTARIX reconstituted lyophilized formulation.

15 REFERENCES

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2. Carlin JB, Macartney KK, Lee KJ, et al. Intussusception Risk and Disease Prevention Associated with Rotavirus Vaccines in Australia's National Immunization Program. *CID.* 2013;57(10):1427-1434.
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4. Farrington CP, Whitaker HJ, Hocine MN, et al. Case series analysis for censored, perturbed, or curtailed post-event exposures. *Biostatistics.* 2009;10(1):3–16.
5. Tate JE, Simonsen L, Viboud C, et al. Trends in intussusception hospitalizations among US infants, 1993–2004: implications for monitoring the safety of the new rotavirus vaccination program. *Pediatrics.* 2008;121:e1125-e1132.
6. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for severity of diarrheal episodes. *Scand J Infect Dis.* 1990;22:259-267.

16 HOW SUPPLIED/STORAGE AND HANDLING

16.1 ROTARIX Vial and Oral Dosing Applicator Presentation

The ROTARIX vial and oral dosing applicator presentation is supplied as single-dose vials of lyophilized vaccine component, accompanied by a prefilled oral dosing applicator of liquid diluent (1 mL) with a plunger stopper, and a transfer adapter for reconstitution.

Supplied as an outer package of 10 doses (NDC 58160-854-52) containing:

- Inner package of 10 vials of lyophilized vaccine component (NDC 58160-851-10)
  - Single vial of lyophilized vaccine component (NDC 58160-851-01)
- Oral dosing applicator of diluent (NDC 58160-853-02) (10 applicators)

Storage before Reconstitution

- Lyophilized vaccine component in vials: Store refrigerated at 2° to 8°C (36° to 46°F). **Protect vials from light.**
- Diluent in oral dosing applicators: Store refrigerated at 2° to 8°C (36° to 46°F) or at a controlled room temperature up to 25°C (77°F). **Do not freeze. Discard if the diluent has been frozen.**

Storage after Reconstitution

ROTARIX (vial and oral dosing applicator presentation) should be administered within 24 hours of reconstitution. After reconstitution, store refrigerated at 2° to 8°C (36° to 46°F) or at a controlled room temperature up to 25°C (77°F). Discard the reconstituted vaccine in biological waste container if not used within 24 hours. **Do not freeze. Discard if the reconstituted vaccine has been frozen.**

16.2 ROTARIX Oral Dosing Applicator Only Presentation

ROTARIX oral dosing applicator only presentation is supplied as a single, 1.5-mL dose in a prefilled oral dosing applicator with a plunger stopper (NDC 58160-740-02) in a carton of 10 (NDC 58160-740-21).

Storage

Store refrigerated at 2° to 8°C (36° to 46°F). **Do not freeze. Discard if the vaccine has been frozen. Keep in original package to protect from light.**

17 PATIENT COUNSELING INFORMATION

See FDA-approved patient labeling (Patient Information). Patient labeling is provided as a tear-off leaflet at the end of this full prescribing information.

Provide the following information to the parent or guardian:

- Inform of the potential benefits and risks of immunization with ROTARIX, and of the importance of completing the immunization series.
- Inform about the potential for adverse reactions that have been temporally associated with administration of ROTARIX or other vaccines containing similar components.
- Instruct to immediately report any signs and/or symptoms of intussusception to their healthcare provider.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 to be given prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by **GlaxoSmithKline Biologicals**
Rixensart, Belgium, U.S. License 1617
Distributed by **GlaxoSmithKline**
Durham, NC 27701

RTX:19PI

DETACH HERE AND GIVE LEAFLET TO PATIENT

PATIENT INFORMATION

ROTARIX (ROW-tah-rix)

Rotavirus Vaccine, Live, Oral

What is ROTARIX?

ROTARIX is a vaccine that protects your baby from a kind of virus (called a rotavirus) that can cause bad diarrhea and vomiting. Rotavirus can cause diarrhea and vomiting that is so bad that your baby can lose too much body fluid and need to go to the hospital.

Rotavirus vaccine is a liquid that is given to your baby by mouth. It is not an injection.

Who should not take ROTARIX?

Your baby should not get ROTARIX if:

- he or she has had an allergic reaction after getting a dose of ROTARIX.
- he or she is allergic to any of the ingredients of this vaccine. A list of ingredients can be found at the end of this leaflet.
- a doctor has told you that your baby's digestive system has a defect (is not normal).
- he or she has a history of a serious problem called intussusception that happens when a part of the intestine gets blocked or twisted.
- he or she has Severe Combined Immunodeficiency Disease (SCID), a severe problem with his/her immune system.

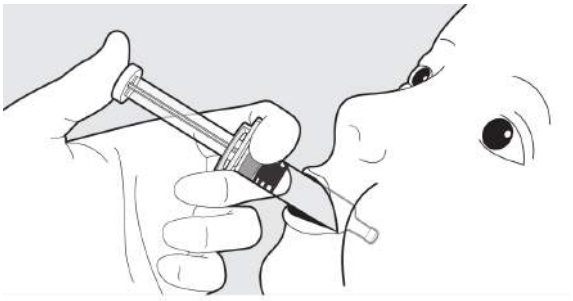
Tell your doctor if your baby:

- is allergic to latex.
- has problems with his/her immune system.
- has cancer.
- will be in close contact with someone who has problems with his/her immune system or is getting treated for cancer, as the spread of vaccine virus to non-vaccinated contacts could occur. Hand washing is recommended after diaper changes to help prevent the spread of vaccine virus.

If your baby has been having diarrhea and vomiting, your doctor may want to wait before giving your baby a dose of ROTARIX.

How is ROTARIX given?

ROTARIX is a liquid that is dropped into your baby's mouth and swallowed.



Oral administration **only.**

Your baby will get the first dose at around 6 weeks old.

The second dose will be at least 4 weeks after the first dose (before 6 months old).
Be sure to plan the time for your baby's second dose with the doctor because it is important that your baby gets both doses of ROTARIX before your baby is 6 months old.
The doctor may decide to give your baby other vaccines at the same time as ROTARIX.
Your baby can be fed normally after getting ROTARIX.

What are possible side effects of ROTARIX?

The most common side effects of ROTARIX are:

- crying
- fussiness
- cough
- runny nose
- fever
- loss of appetite
- vomiting.

Call your doctor right away or go to the emergency department if your baby has any of these problems after getting ROTARIX, even if it has been several weeks since the last vaccine dose because these may be signs of a serious problem called intussusception:

- bad vomiting
- bad diarrhea
- bloody bowel movement
- high fever
- severe stomach pain (if your baby brings his/her knees to his/her chest while crying or screaming).

Studies showed an increased risk of intussusception after the first and second dose of vaccine, especially in the first 7 days.
Since FDA approval, reports of infants with intussusception have been received by Vaccine Adverse Event Reporting System (VAERS). Intussusception occurred days and sometimes weeks after vaccination. Some infants needed hospitalization, surgery on their intestines, or a special enema to treat this problem. Death due to intussusception has occurred.
Other reported side effects include: Kawasaki disease (a serious condition that can affect the heart; symptoms may include fever, rash, red eyes, red mouth, swollen glands, swollen hands and feet, and, if not treated, death can occur).
Talk to your baby's doctor if your baby has any problems that concern you.

What are the ingredients in ROTARIX?

There are two formulations of Rotarix, one that requires your vaccination provider to mix a freeze-dried component with a liquid component and one that is provided as a liquid that does not require mixing. Your provider can tell you the formulation your baby will receive.

Formulation that requires mixing

This formulation of ROTARIX contains weakened human rotavirus.
This formulation of ROTARIX also contains calcium carbonate, dextran, sorbitol, sucrose, amino acids, xanthan, Dulbecco's Modified Eagle Medium (DMEM), and sterile water. The ingredients of DMEM are as

follows: sodium chloride, potassium chloride, magnesium sulphate, ferric (III) nitrate, sodium dihydrogen phosphate, sodium pyruvate, D-glucose, concentrated vitamin solution, L-cystine, L-tyrosine, amino acids solution, L-glutamine, calcium chloride, and sodium hydrogenocarbonate.
Porcine circovirus type 1 (PCV-1), a virus found in pigs, is present in this formulation of ROTARIX. PCV-1 is not known to cause disease in humans.
This formulation of ROTARIX contains no preservatives.
The tip cap of the dropper used to give your baby ROTARIX contains latex.

Formulation that does not require mixing

This formulation of ROTARIX contains weakened human rotavirus.
This formulation of ROTARIX also contains disodium adipate, sucrose, Dulbecco's Modified Eagle Medium (DMEM), and sterile water. The ingredients of DMEM are as follows: sodium chloride, potassium chloride, magnesium sulphate, ferric (III) nitrate, sodium dihydrogen phosphate, sodium pyruvate, D-glucose, concentrated vitamin solution, L-cystine, L-tyrosine, amino acids solution, L-glutamine, calcium chloride, and sodium hydrogenocarbonate.
This formulation of ROTARIX contains no preservatives.
The tip cap of the dropper used to give your baby ROTARIX contains latex.

Manufactured by **GlaxoSmithKline Biologicals**
Rixensart, Belgium, U.S. License 1617
Distributed by **GlaxoSmithKline**
Durham, NC 27701

RTX:15PIL

This Patient Information has been approved by the U.S. Food and Drug Administration. Revised: 11/2022

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use RotaTeq safely and effectively. See full prescribing information for RotaTeq.

RotaTeq® (Rotavirus Vaccine, Live, Oral, Pentavalent)
Oral Solution
Initial U.S. Approval: 2006

---------- INDICATIONS AND USAGE ----------
RotaTeq is a vaccine indicated for the prevention of rotavirus gastroenteritis caused by types G1, G2, G3, G4, and G9. (1)

RotaTeq is approved for use in infants 6 weeks to 32 weeks of age. (1)

----------DOSAGE AND ADMINISTRATION----------
- FOR ORAL USE ONLY. NOT FOR INJECTION. (2)
- The vaccination series consists of three ready-to-use liquid doses of RotaTeq administered orally starting at 6 to 12 weeks of age, with the subsequent doses administered at 4- to 10-week intervals. The third dose should not be given after 32 weeks of age. (2)

----------DOSAGE FORMS AND STRENGTHS----------
2 mL solution for oral administration of 5 live human-bovine reassortant rotaviruses which contains a minimum of 2.0 – 2.8 x 10^6 infectious units (IU) per reassortant dose, depending on the reassortant, and not greater than 116 x 10^6 IU per aggregate dose. (3)

---------- CONTRAINDICATIONS ----------
- A demonstrated history of hypersensitivity to the vaccine or any component of the vaccine. (4.1)
- History of Severe Combined Immunodeficiency Disease (SCID). (4.2, 6.2)
- History of intussusception. (4.3)

----------WARNINGS AND PRECAUTIONS----------
- No safety or efficacy data are available from clinical trials regarding the administration of RotaTeq to infants who are potentially immunocompromised (e.g., HIV/AIDS). (5.2)
- In a post-marketing study, cases of intussusception were observed in temporal association within 21 days following the first dose of RotaTeq, with a clustering of cases in the first 7 days. (5.3, 6.2)
- No safety or efficacy data are available for the administration of RotaTeq to infants with a history of gastrointestinal disorders (e.g., active acute gastrointestinal illness, chronic diarrhea, failure to thrive, history of congenital abdominal disorders, and abdominal surgery). (5.4)
- Vaccine virus transmission from vaccine recipient to non-vaccinated contacts has been reported. Caution is advised when considering whether to administer RotaTeq to individuals with immunodeficient contacts. (5.5)

---------- ADVERSE REACTIONS ----------
Most common adverse events included diarrhea, vomiting, irritability, otitis media, nasopharyngitis, and bronchospasm. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., Kenilworth, NJ, USA (herein after MSD) at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov .[4]

----------USE IN SPECIFIC POPULATIONS----------
Pediatric Use: Safety and efficacy have not been established in infants less than 6 weeks of age or greater than 32 weeks of age. Data are available from clinical studies to support the use of RotaTeq in:
- Pre-term infants according to their age in weeks since birth
- Infants with controlled gastroesophageal reflux disease. (8.4)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 04/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

RotaTeq® is indicated for the prevention of rotavirus gastroenteritis in infants and children caused by types G1, G2, G3, G4, and G9 when administered as a 3-dose series to infants between the ages of 6 to 32 weeks. The first dose of RotaTeq should be administered between 6 and 12 weeks of age *[see Dosage and Administration (2)]*.

2 DOSAGE AND ADMINISTRATION

FOR ORAL USE ONLY. NOT FOR INJECTION.

The vaccination series consists of three ready-to-use liquid doses of RotaTeq administered orally starting at 6 to 12 weeks of age, with the subsequent doses administered at 4- to 10-week intervals. The third dose should not be given after 32 weeks of age *[see Clinical Studies (14)]*.

There are no restrictions on the infant's consumption of food or liquid, including breast milk, either before or after vaccination with RotaTeq.

Do not mix the RotaTeq vaccine with any other vaccines or solutions. Do not reconstitute or dilute *[see Dosage and Administration (2.2)]*.

For storage instructions *[see How Supplied/Storage and Handling (16.1)]*.

Each dose is supplied in a container consisting of a squeezable plastic dosing tube with a twist-off cap, allowing for direct oral administration. The dosing tube is contained in a pouch *[see Dosage and Administration (2.2)]*.

2.1 Use with Other Vaccines

In clinical trials, RotaTeq was administered concomitantly with other licensed pediatric vaccines *[see Adverse Reactions (6.1), Drug Interactions (7.1), and Clinical Studies (14)]*.

2.2 Instructions for Use

To administer the vaccine:

Tear open the pouch and remove the dosing tube.

Clear the fluid from the dispensing tip by holding tube vertically and tapping cap.

Open the dosing tube in 2 easy motions:

1. Puncture the dispensing tip by screwing cap ***clockwise*** until it becomes tight.

2. Remove cap by turning it ***counterclockwise***.

Administer dose by gently squeezing liquid into infant's mouth toward the inner cheek until dosing tube is empty. (A residual drop may remain in the tip of the tube.)

If for any reason an incomplete dose is administered (e.g., infant spits or regurgitates the vaccine), a replacement dose is not recommended, since such dosing was not studied in the clinical trials. The infant should continue to receive any remaining doses in the recommended series.

Discard the empty tube and cap in approved biological waste containers according to local regulations.

3 DOSAGE FORMS AND STRENGTHS

RotaTeq, 2 mL for oral use, is a ready-to-use solution of live reassortant rotaviruses, containing G1, G2, G3, G4 and P1A[8] which contains a minimum of 2.0 – 2.8 x 10^6 infectious units (IU) per individual reassortant dose, depending on the reassortant and not greater than 116 x 10^6 IU per aggregate dose.

Each dose is supplied in a container consisting of a squeezable plastic dosing tube with a twist-off cap, allowing for direct oral administration. The dosing tube is contained in a pouch.

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A demonstrated history of hypersensitivity to any component of the vaccine.

Infants who develop symptoms suggestive of hypersensitivity after receiving a dose of RotaTeq should not receive further doses of RotaTeq.

4.2 Severe Combined Immunodeficiency Disease

Infants with Severe Combined Immunodeficiency Disease (SCID) should not receive RotaTeq. Post-marketing reports of gastroenteritis, including severe diarrhea and prolonged shedding of vaccine virus, have been reported in infants who were administered RotaTeq and later identified as having SCID *[see Adverse Reactions (6.2)]*.

4.3 History of Intussusception

Infants with a history of intussusception should not receive RotaTeq.

5 WARNINGS AND PRECAUTIONS

5.1 Managing Allergic Reactions

Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of the vaccine.

5.2 Immunocompromised Populations

No safety or efficacy data are available from clinical trials regarding the administration of RotaTeq to infants who are potentially immunocompromised including:

- Infants with blood dyscrasias, leukemia, lymphomas of any type, or other malignant neoplasms affecting the bone marrow or lymphatic system.
- Infants on immunosuppressive therapy (including high-dose systemic corticosteroids). RotaTeq may be administered to infants who are being treated with topical corticosteroids or inhaled steroids.

- Infants with primary and acquired immunodeficiency states, including HIV/AIDS or other clinical manifestations of infection with human immunodeficiency viruses; cellular immune deficiencies; and hypogammaglobulinemic and dysgammaglobulinemic states. There are insufficient data from the clinical trials to support administration of RotaTeq to infants with indeterminate HIV status who are born to mothers with HIV/AIDS.
- Infants who have received a blood transfusion or blood products, including immunoglobulins within 42 days.

Vaccine virus transmission from vaccine recipient to non-vaccinated contacts has been reported *[see Warnings and Precautions (5.5)].*

5.3 Intussusception

Following administration of a previously licensed live rhesus rotavirus reassortant vaccine, an increased risk of intussusception was observed.[1]

In a post-marketing observational study in the US cases of intussusception were observed in temporal association within 21 days following the first dose of RotaTeq, with a clustering of cases in the first 7 days. *[See Adverse Reactions (6.2).]*

In worldwide passive post-marketing surveillance, cases of intussusception have been reported in temporal association with RotaTeq. *[See Adverse Reactions (6.2).]*

5.4 Gastrointestinal Illness

No safety or efficacy data are available for administration of RotaTeq to infants with a history of gastrointestinal disorders including infants with active acute gastrointestinal illness, infants with chronic diarrhea and failure to thrive, and infants with a history of congenital abdominal disorders, and abdominal surgery. Caution is advised when considering administration of RotaTeq to these infants.

5.5 Shedding and Transmission

Shedding of vaccine virus was evaluated among a subset of subjects in the Rotavirus Efficacy and Safety Trial (Study 006, also known as REST) 4 to 6 days after each dose and among all subjects who submitted a stool antigen rotavirus positive sample at any time. RotaTeq was shed in the stools of 32 of 360 [8.9%, 95% CI (6.2%, 12.3%)] vaccine recipients tested after dose 1; 0 of 249 [0.0%, 95% CI (0.0%, 1.5%)] vaccine recipients tested after dose 2; and in 1 of 385 [0.3%, 95% CI (<0.1%, 1.4%)] vaccine recipients after dose 3. In phase 3 studies, shedding was observed as early as 1 day and as late as 15 days after a dose. Transmission of vaccine virus was not evaluated in phase 3 studies.

Transmission of vaccine virus strains from vaccinees to non-vaccinated contacts has been observed post-marketing.

The potential risk of transmission of vaccine virus should be weighed against the risk of acquiring and transmitting natural rotavirus.

Caution is advised when considering whether to administer RotaTeq to individuals with immunodeficient close contacts such as:

- Individuals with malignancies or who are otherwise immunocompromised;
- Individuals with primary immunodeficiency; or
- Individuals receiving immunosuppressive therapy.

5.6 Febrile Illness

Febrile illness may be reason for delaying use of RotaTeq except when, in the opinion of the physician, withholding the vaccine entails a greater risk. Low-grade fever (<100.5°F [38.1°C]) itself and mild upper respiratory infection do not preclude vaccination with RotaTeq.

5.7 Incomplete Regimen

The clinical studies were not designed to assess the level of protection provided by only one or two doses of RotaTeq.

5.8 Limitations of Vaccine Effectiveness

RotaTeq may not protect all vaccine recipients against rotavirus.

5.9 Post-Exposure Prophylaxis

No clinical data are available for RotaTeq when administered after exposure to rotavirus.

6 ADVERSE REACTIONS

6.1 Clinical Studies Experience

71,725 infants were evaluated in 3 placebo-controlled clinical trials including 36,165 infants in the group that received RotaTeq and 35,560 infants in the group that received placebo.* Parents/guardians

*"Placebo" is not defined in this clinical trial or associated publications. In another RotaTeq trial, "placebo" is described: "The placebo was identical to the vaccine except that it did not contain the rotavirus reassortants or trace trypsin". Reference: https://pubmed.ncbi.nlm.nih.gov/17200266/

were contacted on days 7, 14, and 42 after each dose regarding intussusception and any other serious adverse events. The racial distribution was as follows: White (69% in both groups); Hispanic-American (14% in both groups); Black (8% in both groups); Multiracial (5% in both groups); Asian (2% in both groups); Native American (RotaTeq 2%, placebo 1%); and Other (<1% in both groups). The gender distribution was 51% male and 49% female in both vaccination groups.

Because clinical trials are conducted under conditions that may not be typical of those observed in clinical practice, the adverse reaction rates presented below may not be reflective of those observed in clinical practice.

Serious Adverse Events

Serious adverse events occurred in 2.4% of recipients of RotaTeq when compared to 2.6% of placebo recipients within the 42-day period of a dose in the phase 3 clinical studies of RotaTeq. The most frequently reported serious adverse events for RotaTeq compared to placebo were:

bronchiolitis (0.6% RotaTeq vs. 0.7% Placebo),
gastroenteritis (0.2% RotaTeq vs. 0.3% Placebo),
pneumonia (0.2% RotaTeq vs. 0.2% Placebo),
fever (0.1% RotaTeq vs. 0.1% Placebo), and
urinary tract infection (0.1% RotaTeq vs. 0.1% Placebo).

Deaths

Across the clinical studies, 52 deaths were reported. There were 25 deaths in the RotaTeq recipients compared to 27 deaths in the placebo recipients. The most commonly reported cause of death was sudden infant death syndrome, which was observed in 8 recipients of RotaTeq and 9 placebo recipients.

Intussusception

In Study 006, 34,837 vaccine recipients and 34,788 placebo recipients were monitored by active surveillance to identify potential cases of intussusception at 7, 14, and 42 days after each dose, and every 6 weeks thereafter for 1 year after the first dose.

For the primary safety outcome, cases of intussusception occurring within 42 days of any dose, there were 6 cases among RotaTeq recipients and 5 cases among placebo recipients (see Table 1). The data did not suggest an increased risk of intussusception relative to placebo.

Table 1: Confirmed cases of intussusception in recipients of RotaTeq as compared with placebo recipients during Study 006

	RotaTeq (n=34,837)	Placebo (n=34,788)
Confirmed intussusception cases within 42 days of any dose	6	5
Relative risk (95% CI) *	1.6 (0.4, 6.4)	
Confirmed intussusception cases within 365 days of dose 1	13	15
Relative risk (95% CI)	0.9 (0.4, 1.9)	

* Relative risk and 95% confidence interval based upon group sequential design stopping criteria employed in Study 006.

Among vaccine recipients, there were no confirmed cases of intussusception within the 42-day period after the first dose, which was the period of highest risk for the rhesus rotavirus-based product (see Table 2).

Table 2: Intussusception cases by day range in relation to dose in Study 006

	Dose 1		Dose 2		Dose 3		Any Dose	
Day Range	RotaTeq	Placebo	RotaTeq	Placebo	RotaTeq	Placebo	RotaTeq	Placebo
1-7	0	0	1	0	0	0	1	0
1-14	0	0	1	0	0	1	1	1
1-21	0	0	3	0	0	1	3	1
1-42	0	1	4	1	2	3	6	5

All of the children who developed intussusception recovered without sequelae with the exception of a 9-month-old male who developed intussusception 98 days after dose 3 and died of post-operative sepsis. There was a single case of intussusception among 2,470 recipients of RotaTeq in a 7-month-old male in the phase 1 and 2 studies (716 placebo recipients).

Hematochezia

Hematochezia reported as an adverse experience occurred in 0.6% (39/6,130) of vaccine and 0.6% (34/5,560) of placebo recipients within 42 days of any dose. Hematochezia reported as a serious adverse experience occurred in <0.1% (4/36,150) of vaccine and <0.1% (7/35,536) of placebo recipients within 42 days of any dose.

Seizures

All seizures reported in the phase 3 trials of RotaTeq (by vaccination group and interval after dose) are shown in Table 3.

Table 3: Seizures reported by day range in relation to any dose in the phase 3 trials of RotaTeq

Day range	1-7	1-14	1-42
RotaTeq	10	15	33
Placebo	5	8	24

Seizures reported as serious adverse experiences occurred in <0.1% (27/36,150) of vaccine and <0.1% (18/35,536) of placebo recipients (not significant). Ten febrile seizures were reported as serious adverse experiences, 5 were observed in vaccine recipients and 5 in placebo recipients.

Kawasaki Disease

In the phase 3 clinical trials, infants were followed for up to 42 days of vaccine dose. Kawasaki disease was reported in 5 of 36,150 vaccine recipients and in 1 of 35,536 placebo recipients with unadjusted relative risk 4.9 (95% CI 0.6, 239.1).

Most Common Adverse Events

Solicited Adverse Events

Detailed safety information was collected from 11,711 infants (6,138 recipients of RotaTeq) which included a subset of subjects in Study 006 and all subjects from Studies 007 and 009 (Detailed Safety Cohort). A Vaccination Report Card was used by parents/guardians to record the child's temperature and any episodes of diarrhea and vomiting on a daily basis during the first week following each vaccination. Table 4 summarizes the frequencies of these adverse events and irritability.

Table 4: Solicited adverse experiences within the first week after doses 1, 2, and 3 (Detailed Safety Cohort)

Adverse experience	Dose 1		Dose 2		Dose 3	
	RotaTeq	Placebo	RotaTeq	Placebo	RotaTeq	Placebo
Elevated temperature*	n=5,616 17.1%	n=5,077 16.2%	n=5,215 20.0%	n=4,725 19.4%	n=4,865 18.2%	n=4,382 17.6%
Vomiting	n=6,130 6.7%	n=5,560 5.4%	n=5,703 5.0%	n=5,173 4.4%	n=5,496 3.6%	n=4,989 3.2%
Diarrhea	10.4%	9.1%	8.6%	6.4%	6.1%	5.4%
Irritability	7.1%	7.1%	6.0%	6.5%	4.3%	4.5%

* Temperature ≥100.5°F [38.1°C] rectal equivalent obtained by adding 1 degree F to otic and oral temperatures and 2 degrees F to axillary temperatures

Other Adverse Events

Parents/guardians of the 11,711 infants were also asked to report the presence of other events on the Vaccination Report Card for 42 days after each dose.

Fever was observed at similar rates in vaccine (N=6,138) and placebo (N=5,573) recipients (42.6% vs. 42.8%). Adverse events that occurred at a statistically higher incidence (i.e., 2-sided p-value <0.05) within the 42 days of any dose among recipients of RotaTeq as compared with placebo recipients are shown in Table 5.

Table 5: Adverse events that occurred at a statistically higher incidence within 42 days of any dose among recipients of RotaTeq as compared with placebo recipients

Adverse event	RotaTeq N=6,138 n (%)	Placebo N=5,573 n (%)
Diarrhea	1,479 (24.1%)	1,186 (21.3%)
Vomiting	929 (15.2%)	758 (13.6%)
Otitis media	887 (14.5%)	724 (13.0%)
Nasopharyngitis	422 (6.9%)	325 (5.8%)
Bronchospasm	66 (1.1%)	40 (0.7%)

Safety in Pre-Term Infants

RotaTeq or placebo was administered to 2,070 pre-term infants (25 to 36 weeks gestational age, median 34 weeks) according to their age in weeks since birth in Study 006. All pre-term infants were followed for serious adverse experiences; a subset of 308 infants was monitored for all adverse experiences. There were 4 deaths throughout the study, 2 among vaccine recipients (1 SIDS and 1 motor

vehicle accident) and 2 among placebo recipients (1 SIDS and 1 unknown cause). No cases of intussusception were reported. Serious adverse experiences occurred in 5.5% of vaccine and 5.8% of placebo recipients. The most common serious adverse experience was bronchiolitis, which occurred in 1.4% of vaccine and 2.0% of placebo recipients. Parents/guardians were asked to record the child's temperature and any episodes of vomiting and diarrhea daily for the first week following vaccination. The frequencies of these adverse experiences and irritability within the week after dose 1 are summarized in Table 6.

Table 6: Solicited adverse experiences within the first week of doses 1, 2, and 3 among pre-term infants

	Dose 1		Dose 2		Dose 3	
Adverse event	RotaTeq	Placebo	RotaTeq	Placebo	RotaTeq	Placebo
	N=127	N=133	N=124	N=121	N=115	N=108
Elevated temperature*	18.1%	17.3%	25.0%	28.1%	14.8%	20.4%
	N=154	N=154	N=137	N=137	N=135	N=129
Vomiting	5.8%	7.8%	2.9%	2.2%	4.4%	4.7%
Diarrhea	6.5%	5.8%	7.3%	7.3%	3.7%	3.9%
Irritability	3.9%	5.2%	2.9%	4.4%	8.1%	5.4%

* Temperature ≥100.5°F [38.1°C] rectal equivalent obtained by adding 1 degree F to otic and oral temperatures and 2 degrees F to axillary temperatures

6.2 Post-Marketing Experience

The following adverse events have been identified during post-approval use of RotaTeq from reports to the Vaccine Adverse Event Reporting System (VAERS).

Reporting of adverse events following immunization to VAERS is voluntary, and the number of doses of vaccine administered is not known; therefore, it is not always possible to reliably estimate the adverse event frequency or establish a causal relationship to vaccine exposure using VAERS data.

In post-marketing experience, the following adverse events have been reported following the use of RotaTeq:

Immune system disorders:

Anaphylactic reaction

Gastrointestinal disorders:

Intussusception (including death)

Hematochezia

Gastroenteritis with vaccine viral shedding in infants with Severe Combined Immunodeficiency Disease (SCID)

Skin and subcutaneous tissue disorders:

Urticaria

Angioedema

Infections and infestations:

Kawasaki disease

Transmission of vaccine virus strains from vaccine recipient to non-vaccinated contacts.

Post-Marketing Observational Safety Surveillance Studies

The temporal association between vaccination with RotaTeq and intussusception was evaluated in the Post-licensure Rapid Immunization Safety Monitoring (PRISM) program[2], an electronic active surveillance program comprised of 3 US health insurance plans.

More than 1.2 million RotaTeq vaccinations (507,000 of which were first doses) administered to infants 5 through 36 weeks of age were evaluated. From 2004 through 2011, potential cases of intussusception in either the inpatient or emergency department setting and vaccine exposures were identified through electronic procedure and diagnosis codes. Medical records were reviewed to confirm intussusception and rotavirus vaccination status.

The risk of intussusception was assessed using self-controlled risk interval and cohort designs, with adjustment for age. Risk windows of 1-7 and 1-21 days were evaluated. Cases of intussusception were observed in temporal association within 21 days following the first dose of RotaTeq, with a clustering of cases in the first 7 days. Based on the results, approximately 1 to 1.5 excess cases of intussusception occur per 100,000 vaccinated US infants within 21 days following the first dose of RotaTeq. In the first year of life, the background rate of intussusception hospitalizations in the US has been estimated to be approximately 34 per 100,000 infants.[3]

In an earlier prospective post-marketing observational cohort study conducted using a large US medical claims database, the risks of intussusception or Kawasaki disease resulting in emergency department visits or hospitalizations during the 30 days following any dose of vaccine were analyzed among 85,150 infants receiving one or more doses of RotaTeq from February 2006 through March 2009. Medical charts were reviewed to confirm these diagnoses. Evaluation included concurrent (n = 62,617) and historical (n=100,000 from 2001-2005) control groups of infants who received diphtheria, tetanus and acellular pertussis vaccine (DTaP) but not RotaTeq.

Confirmed intussusception cases in the RotaTeq group were compared with those in the concurrent DTaP control group and in the historical control group. The data were analyzed post-dose 1 and post any dose, in both 7 day and 30 day risk windows. A statistically significant increased risk of intussusception after RotaTeq vaccination was not observed.

One confirmed case of Kawasaki disease (23 days post-dose 3) was identified among infants vaccinated with RotaTeq and one confirmed case of Kawasaki disease (22 days post-dose 2) was identified among concurrent DTaP controls (relative risk = 0.7; 95% CI: 0.01-55.56).

In addition, general safety was monitored by electronic search of the automated records database for all emergency department visits and hospitalizations in the 30-day period after each dose of RotaTeq compared with: 1) days 31-60 after each dose of RotaTeq (self-matched controls) and 2) the 30-day period after each dose of DTaP vaccine (historical control subset from 2004-2005, n=40,000). In safety analyses which evaluated multiple follow-up windows after vaccination (days: 0-7, 1-7, 8-14 and 0-30), no safety concerns were identified for infants vaccinated with RotaTeq when compared with self-matched controls and the historical control subset.

Reporting Adverse Events

Parents or guardians should be instructed to report any adverse reactions to their health care provider.

Health care providers should report all adverse events to the U.S. Department of Health and Human Services' Vaccine Adverse Events Reporting System (VAERS).

VAERS accepts all reports of suspected adverse events after the administration of any vaccine, including but not limited to the reporting of events required by the National Childhood Vaccine Injury Act of 1986. For information or a copy of the vaccine reporting form, call the VAERS toll-free number at 1-800-822-7967 or report online to www.vaers.hhs.gov .[4]

7 DRUG INTERACTIONS

Immunosuppressive therapies including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to vaccines.

7.1 Concomitant Vaccine Administration

In clinical trials, RotaTeq was administered concomitantly with diphtheria and tetanus toxoids and acellular pertussis (DTaP), inactivated poliovirus vaccine (IPV), H. influenzae type b conjugate (Hib), hepatitis B vaccine, and pneumococcal conjugate vaccine *[see Clinical Studies (14)]*. The safety data available are in the ADVERSE REACTIONS section *[see Adverse Reactions (6.1)]*. There was no evidence for reduced antibody responses to the vaccines that were concomitantly administered with RotaTeq.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

RotaTeq is not approved for individuals 32 weeks of age and older. No human or animal data are available to assess vaccine-associated risks in pregnancy.

8.2 Lactation

No human or animal data are available to assess the impact of RotaTeq on milk production, its presence in breast milk, or its effect on the breastfed infant.

8.4 Pediatric Use

Safety and effectiveness of RotaTeq have not been established in infants less than 6 weeks of age or greater than 32 weeks of age.

Data are available from clinical studies to support the use of RotaTeq in pre-term infants according to their age in weeks since birth *[see Adverse Reactions (6.1)].*

Data are available from clinical studies to support the use of RotaTeq in infants with controlled gastroesophageal reflux disease.

10 OVERDOSAGE

There have been post-marketing reports of infants who received more than one dose or a replacement dose of RotaTeq after regurgitation *[see Dosage and Administration (2.2)]*. In limited post-marketing experience of reported overdosage, the adverse events reported after incorrect administration of higher than recommended doses of RotaTeq were similar to adverse events observed with the approved dosage and schedule.

11 DESCRIPTION

RotaTeq is a live, oral pentavalent vaccine that contains 5 live reassortant rotaviruses. The rotavirus parent strains of the reassortants were isolated from human and bovine hosts. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (type P7) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein, P1A (genotype P[8]), herein referred to as type P1A[8], from the human rotavirus parent strain and the outer capsid protein of type G6 from the bovine rotavirus parent strain (see Table 7).

Table 7

Name of Reassortant	Human Rotavirus Parent Strains and Outer Surface Protein Compositions	Bovine Rotavirus Parent Strain and Outer Surface Protein Composition	Reassortant Outer Surface Protein Composition (Human Rotavirus Component in Bold)	Minimum Dose Levels (10^6 infectious units)
G1	WI79 – G1P1A[8]	WC3 - G6, P7[5]	**G1**P7[5]	2.2
G2	SC2 – G2P2[6]		**G2**P7[5]	2.8
G3	WI78 – G3P1A[8]		**G3**P7[5]	2.2
G4	BrB – G4P2[6]		**G4**P7[5]	2.0
P1A[8]	WI79 – G1P1A[8]		G6**P1A[8]**	2.3

The reassortants are propagated in Vero cells using standard cell culture techniques in the absence of antifungal agents.

The reassortants are suspended in a buffered stabilizer solution. Each vaccine dose contains sucrose, sodium citrate, sodium phosphate monobasic monohydrate, sodium hydroxide, polysorbate 80, cell culture media, and trace amounts of fetal bovine serum. RotaTeq contains no preservatives.

RotaTeq is a pale yellow clear liquid that may have a pink tint.

The plastic dosing tube and cap do not contain latex.

12 CLINICAL PHARMACOLOGY

Rotavirus is a leading cause of severe acute gastroenteritis in infants and young children, with over 95% of these children infected by the time they are 5 years old.[5] The most severe cases occur among infants and young children between 6 months and 24 months of age.[6]

12.1 Mechanism of Action

The exact immunologic mechanism by which RotaTeq protects against rotavirus gastroenteritis is unknown *[see Clinical Studies (14.6)]*. RotaTeq is a live viral vaccine that replicates in the small intestine and induces immunity.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

RotaTeq has not been evaluated for its carcinogenic or mutagenic potential or its potential to impair fertility.

14 CLINICAL STUDIES

Overall, 73,086 infants were randomized in 4 placebo-controlled, phase 3 studies conducted in 12 countries on 4 continents. The data demonstrating the efficacy of RotaTeq in preventing rotavirus gastroenteritis come from 7,744 of these infants from the US (including Navajo and White Mountain

Apache Nations), Finland, and Japan who were enrolled in 3 of these studies: Study 006, Study 007, and Study 029. A fourth trial, Study 009, provided clinical evidence supporting the consistency of manufacture and contributed data to the overall safety evaluation.

The racial distribution of the efficacy subset was as follows: White (RotaTeq 61%, placebo 62%); Hispanic-American (RotaTeq 9%, placebo 8%); Black (2% in both groups); Multiracial (4% in both groups); Asian (10% in both groups); Native American (13% in both groups); and Other (<1% in both groups). The gender distribution was 52% male and 48% female in both vaccination groups.

The efficacy evaluations in these studies included: 1) Prevention of any grade of severity of rotavirus gastroenteritis; 2) Prevention of severe rotavirus gastroenteritis, as defined by a clinical scoring system; and 3) Reduction in hospitalizations due to rotavirus gastroenteritis.

The vaccine was given as a three-dose series to healthy infants with the first dose administered between 6 and 12 weeks of age and followed by two additional doses administered at 4- to 10-week intervals. The age of infants receiving the third dose was 32 weeks of age or less. Oral polio vaccine administration was not permitted; however, other childhood vaccines could be concomitantly administered. Breast-feeding was permitted in all studies.

The case definition for rotavirus gastroenteritis used to determine vaccine efficacy required that a subject meet both of the following clinical and laboratory criteria: (1) greater than or equal to 3 watery or looser-than-normal stools within a 24-hour period and/or forceful vomiting; and (2) rotavirus antigen detection by enzyme immunoassay (EIA) in a stool specimen taken within 14 days of onset of symptoms. The severity of rotavirus acute gastroenteritis was determined by a clinical scoring system that took into account the intensity and duration of symptoms of fever, vomiting, diarrhea, and behavioral changes.

The primary efficacy analyses included cases of rotavirus gastroenteritis caused by types G1, G2, G3, G4 (and G types containing P1A8 (in Study 029 only)) that occurred at least 14 days after the third dose through the first rotavirus season post vaccination.

Analyses were also done to evaluate the efficacy of RotaTeq against rotavirus gastroenteritis caused by any of types G1, G2, G3, and G4 (and G types containing P1A8 (in Study 029 only)) at any time following the first dose through the first rotavirus season postvaccination among infants who received at least one vaccination (Intent-to-treat, ITT).

14.1 Rotavirus Efficacy and Safety Trial (Study 006)

Primary efficacy against any grade of severity of rotavirus gastroenteritis caused by naturally occurring types G1, G2, G3, or G4 through the first rotavirus season after vaccination was 74.0% (95% CI: 66.8, 79.9) and the ITT efficacy was 60.0% (95% CI: 51.5, 67.1). Primary efficacy against severe rotavirus gastroenteritis caused by naturally occurring types G1, G2, G3, or G4 through the first rotavirus season after vaccination was 98.0% (95% CI: 88.3, 100.0), and ITT efficacy was 96.4% (95% CI: 86.2, 99.6). See Table 8.

Table 8: Efficacy of RotaTeq against any grade of severity of and severe* G1-4 rotavirus gastroenteritis through the first rotavirus season postvaccination in Study 006

	Per Protocol		Intent-to-Treat†	
	RotaTeq	Placebo	RotaTeq	Placebo
Subjects vaccinated	2,834	2,839	2,834	2,839
	Gastroenteritis cases			
Any grade of severity	82	315	150	371
Severe*	1	51	2	55
Efficacy estimate % and (95% confidence interval)				
Any grade of severity	74.0 (66.8, 79.9)		60.0 (51.5, 67.1)	
Severe*	98.0 (88.3, 100.0)		96.4 (86.2, 99.6)	

* Severe gastroenteritis defined by a clinical scoring system based on the intensity and duration of symptoms of fever, vomiting, diarrhea, and behavioral changes

† ITT analysis includes all subjects in the efficacy cohort who received at least one dose of vaccine.

The efficacy of RotaTeq against severe disease was also demonstrated by a reduction in hospitalizations for rotavirus gastroenteritis among all subjects enrolled in Study 006. RotaTeq reduced hospitalizations for rotavirus gastroenteritis caused by types G1, G2, G3, and G4 through the first two years after the third dose by 95.8% (95% CI: 90.5, 98.2). The ITT efficacy in reducing hospitalizations was 94.7% (95% CI: 89.3, 97.3) as shown in Table 9.

Table 9: Efficacy of RotaTeq in reducing G1-4 rotavirus-related hospitalizations in Study 006

	Per Protocol		Intent-to-Treat*	
	RotaTeq	Placebo	RotaTeq	Placebo
Subjects vaccinated	34,035	34,003	34,035	34,003
Number of hospitalizations	6	144	10	187
Efficacy estimate % and (95% confidence interval)	95.8 (90.5, 98.2)		94.7 (89.3, 97.3)	

* ITT analysis includes all subjects who received at least one dose of vaccine.

14.2 Study 007

Primary efficacy against any grade of severity of rotavirus gastroenteritis caused by naturally occurring types G1, G2, G3, or G4 through the first rotavirus season after vaccination was 72.5% (95% CI: 50.6, 85.6) and the ITT efficacy was 58.4% (95% CI: 33.8, 74.5). Primary efficacy against severe rotavirus gastroenteritis caused by naturally occurring types G1, G2, G3, or G4 through the first rotavirus season after vaccination was 100% (95% CI: 13.0, 100.0) and ITT efficacy against severe rotavirus disease was 100% (95% CI: 30.2, 100.0) as shown in Table 10.

Table 10: Efficacy of RotaTeq against any grade of severity of and severe* G1-4 rotavirus gastroenteritis through the first rotavirus season postvaccination in Study 007

	Per Protocol		Intent-to-Treat†	
	RotaTeq	Placebo	RotaTeq	Placebo
Subjects vaccinated	650	660	650	660
Gastroenteritis cases				
Any grade of severity	15	54	27	64
Severe*	0	6	0	7
Efficacy estimate % and (95% confidence interval)				
Any grade of severity	72.5 (50.6, 85.6)		58.4 (33.8, 74.5)	
Severe*	100.0 (13.0, 100.0)		100.0 (30.2, 100.0)	

* Severe gastroenteritis defined by a clinical scoring system based on the intensity and duration of symptoms of fever, vomiting, diarrhea, and behavioral change

† ITT analysis includes all subjects in the efficacy cohort who received at least one dose of vaccine.

14.3 Multiple Rotavirus Seasons

The efficacy of RotaTeq through a second rotavirus season was evaluated in a single study (Study 006). Efficacy against any grade of severity of rotavirus gastroenteritis caused by rotavirus types G1, G2, G3, and G4 through the two rotavirus seasons after vaccination was 71.3% (95% CI: 64.7, 76.9). The efficacy of RotaTeq in preventing cases occurring only during the second rotavirus season postvaccination was 62.6% (95% CI: 44.3, 75.4). The efficacy of RotaTeq beyond the second season postvaccination was not evaluated.

14.4 Rotavirus Gastroenteritis Regardless of Type

The rotavirus types identified in the efficacy subset of Study 006 and Study 007 were G1P1A[8]; G2P1[4]; G3P1A[8]; G4P1A[8]; and G9P1A[8].

In Study 006, the efficacy of RotaTeq against any grade of severity of naturally occurring rotavirus gastroenteritis regardless of type was 71.8% (95% CI: 64.5, 77.8) and efficacy against severe rotavirus disease was 98.0% (95% CI: 88.3, 99.9). The ITT efficacy starting at dose 1 was 50.9% (95% CI: 41.6, 58.9) for any grade of severity of rotavirus disease and was 96.4% (95% CI: 86.3, 99.6) for severe rotavirus disease.

In Study 007, the primary efficacy of RotaTeq against any grade of severity of rotavirus gastroenteritis regardless of type was 72.7% (95% CI: 51.9, 85.4) and efficacy against severe rotavirus disease was 100% (95% CI: 12.7, 100). The ITT efficacy starting at dose 1 was 48.0% (95% CI: 21.6, 66.1) for any grade of severity of rotavirus disease and was 100% (95% CI: 30.4, 100.0) for severe rotavirus disease.

14.5 Rotavirus Gastroenteritis by Type

The efficacy against any grade of severity of rotavirus gastroenteritis by type was evaluated in Study 006 and Study 029. The efficacy cohort analysis from Study 006 is shown in Table 11.

Table 11: Type-specific efficacy of RotaTeq against any grade of severity of rotavirus gastroenteritis among infants in Study 006 efficacy cohort through the first rotavirus season postvaccination (Per Protocol)

	Number of cases		
Type identified by PCR	RotaTeq (N=2,834)	Placebo (N=2,839)	% Efficacy (95% Confidence Interval)
G1P1A[8]	72	286	74.9 (67.3, 80.9)
G2P1[4]	6	17	63.4 (2.6, 88.2)
G3P1A[8]	1	6	NS
G4P1A[8]	3	6	NS
G9P1A[8]	1	3	NS
Unidentified*	11	15	NS

N=number vaccinated
NS=not significant

* Includes rotavirus antigen-positive samples in which the specific type could not be identified by PCR

Additional analyses were conducted to evaluate efficacy in the prevention of rotavirus gastroenteritis due to G9P1A[8].

- In Study 029 (a Phase 3 randomized, blinded, placebo-controlled study conducted in Japan), efficacy on the pre-specified primary endpoint (rotavirus gastroenteritis caused by G1, G2, G3, G4, and G-serotypes associated with serotype P1A[8] (e.g., G9)) was 74.5% (95% CI: 39.9, 90.6). G9P1A[8]-associated gastroenteritis was observed in 0/356 and 5/354 subjects in the RotaTeq and placebo groups, respectively (100% (95% CI: -9.0, 100)).
- In a post hoc analysis of health care utilization data from 68,038 infants (RotaTeq 34,035 and placebo 34,003) in Study 006, using a case definition that included culture confirmation, hospitalization and emergency departments visits for rotavirus gastroenteritis, cases due to G9P1A[8] were reduced (RotaTeq 0 cases: placebo 14 cases) by 100% (95% CI: 69.6, 100.0).

14.6 Immunogenicity

A relationship between antibody responses to RotaTeq and protection against rotavirus gastroenteritis has not been established. In phase 3 studies, 92.9% to 100% of 439 recipients of RotaTeq achieved a 3-fold or more rise in serum anti-rotavirus IgA after a three-dose regimen when compared to 12.3%-20.0% of 397 placebo recipients.

15 REFERENCES

1. Murphy TV, Gargiullo PM, Massoudi MS et al. Intussusception among infants given an oral rotavirus vaccine. N Engl J Med 2001;344:564-572.
2. Yih WK, Lieu TA, Kulldorff M, et al. Intussusception risk after rotavirus vaccination in US infants. Mini-Sentinel. www.mini-sentinel.org.
3. Tate JE, Simonsen L, Viboud C, et al. Trends in intussusception hospitalizations among US infants, 1993-2004: implications for monitoring the safety of the new rotavirus vaccination program. Pediatrics 2008;121(5):e1125-e1132.
4. Centers for Disease Control and Prevention. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP). MMWR 2002;51(RR-2):1-35.
5. Parashar UD et al. Global illness and deaths caused by rotavirus disease in children. Emerg Infect Dis 2003;9(5):565-572.
6. Parashar UD, Holman RC, Clarke MJ, Bresee JS, Glass RI. Hospitalizations associated with rotavirus diarrhea in the United States, 1993 through 1995: surveillance based on the new ICD-9-CM rotavirus-specific diagnostic code. J Infect Dis 1998;177:13-7.

16 HOW SUPPLIED/STORAGE AND HANDLING

RotaTeq, 2 mL, a solution for oral use, is a pale yellow clear liquid that may have a pink tint. It is supplied as follows:

NDC 0006-4047-41 package of 10 individually pouched single-dose tubes.

NDC 0006-4047-20 package of 25 individually pouched single-dose tubes.

The plastic dosing tube and cap do not contain latex.

16.1 Storage and Handling

Store and transport refrigerated at 2-8°C (36-46°F). RotaTeq should be administered as soon as possible after being removed from refrigeration. For information regarding stability under conditions other than those recommended, call 1-800-637-2590.

Protect from light.
RotaTeq should be discarded in approved biological waste containers according to local regulations.
The product must be used before the expiration date.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Parents or guardians should be given a copy of the required vaccine information and be given the "Patient Information" appended to this insert. Parents and/or guardians should be encouraged to read the patient information that describes the benefits and risks associated with the vaccine and ask any questions they may have during the visit *[see Warnings and Precautions (5) and Patient Information].*

Distributed by: Merck Sharp & Dohme Corp.
Whitehouse Station, NJ, USA

For patent information: www.msd.com/research/patent

uspi-v260-os-2204r025

Smallpox

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all information needed to use ACAM2000 safely and effectively. See full prescribing information for ACAM2000.

ACAM2000, (Smallpox (Vaccinia) Vaccine, Live)

Lyophilized preparation for percutaneous scarification

Initial U.S. Approval: 2007

WARNING:

See full prescribing information for complete boxed warning

- **Myocarditis and pericarditis (suspect cases observed at a rate of 5.7 per 1000 primary vaccinees (95% CI: 1.9-13.3)), encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia, generalized vaccinia, severe vaccinial skin infections, erythema multiforme major (including STEVENS-JOHNSON SYNDROME), eczema vaccinatum resulting in permanent sequelae or death, ocular complications, blindness and fetal death, have occurred following either primary vaccination or revaccination with live vaccinia virus smallpox vaccines. These risks are increased in certain individuals and may result in severe disability, permanent neurological sequelae and/or death [see Warnings and Precautions (5)].**

----------INDICATIONS AND USAGE----------

ACAM2000® is indicated for active immunization against smallpox disease for persons determined to be at high risk for smallpox infection.

----------DOSAGE AND ADMINISTRATION----------

- Administer ACAM2000 only after being trained on the safe and effective administration of the vaccine by the percutaneous route (scarification). (2.3)
- A droplet of ACAM2000 is administered by the percutaneous route (scarification) using 15 jabs of a bifurcated needle. ACAM2000 should not be injected by the intradermal, subcutaneous, intramuscular, or intravenous route. (2.3)
- The droplet (0.0025 mL) of reconstituted vaccine is picked up with a bifurcated needle by dipping needle into ACAM2000 vial. (2.3)
- See full prescribing information for instructions for vaccine preparation (2.2), administration including provision of the Medication Guide to vaccinees and instruction to vaccinees about vaccination site care, (2.3) and interpretation of response to vaccination. (2.4)
- Re-vaccination may be recommended (e.g. every 3 years). (2.5)

----------DOSAGE FORMS AND STRENGTHS----------

- Lyophilized powder reconstituted with packaged diluent. After reconstitution, each vial has approximately 100 doses of 0.0025 mL of live vaccinia virus containing 2.5 – 12.5 x 10^5 plaque forming units. (3)

----------CONTRAINDICATIONS----------

- Individuals with severe immunodeficiency who are not expected to benefit from the vaccine. These individuals may include persons who are undergoing bone marrow transplantation or persons with primary or acquired immunodeficiency states who require isolation (4).

-- ----------WARNINGS AND PRECAUTIONS----------

- Myocarditis and/or pericarditis, ischemic heart disease and non-ischemic dilated cardiomyopathy. (5.1, 5.2)
- Encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia (vaccinia necrosum), generalized vaccinia, severe vaccinial skin infections, erythema multiforme major (including Stevens-Johnson syndrome), eczema vaccinatum, fetal vaccinia and fetal death. (5.1)
- Ocular vaccinia and blindness. (5.3)
- These risks, including risks of severe disability and/or death, are increased in vaccinees with:
  - Cardiac disease (5.2).
  - Eye disease treated with topical steroids. (5.3)
  - Congenital or acquired immune deficiency disorders. (5.4)
  - History or presence of eczema and other skin conditions. (5.5)
  - Infants < 12 months of age. (5.6)
  - Pregnancy (5.7)
- ACAM2000 is a live vaccinia virus that can be transmitted to persons who have close contact with the vaccinee and the risks in contacts are the same as those stated for vaccinees. (5.10)

----------ADVERSE REACTIONS----------

Common adverse events include inoculation site signs and symptoms, lymphadenitis, and constitutional symptoms, such as malaise, fatigue, fever, myalgia, and headache (6.1). These adverse events are less frequent in revaccinated persons than persons receiving the vaccine for the first time.

Inadvertent inoculation at other sites is the most frequent complication of vaccinia vaccination. The most common sites involved are the face, nose, mouth, lips, genitalia and anus.

Self-limited skin rashes not associated with vaccinia replication in skin, including urticaria and folliculitis, may occur following vaccination.

To report SUSPECTED ADVERSE REACTIONS, contact Emergent BioSolutions at 1-877-246-8472 or VAERS at 800-822-7967 and https://vaers.hhs.gov.

----------USE IN SPECIFIC POPULATIONS----------

- ACAM2000 may rarely cause fetal infection, usually resulting in stillbirth or death. (8.1)
- ACAM2000 live vaccinia virus may be transmitted from a lactating mother to her infant causing complications in the infant from inadvertent inoculation. (8.3)
- ACAM2000 may be associated with an increased risk of serious complications in children, especially in infants younger than 12 months. (8.4)

See 17 for PATIENT COUNSELING INFORMATION and MEDICATION GUIDE

Revised: [03/2018]

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the Full Prescribing Information are not listed.

FULL PRESCRIBING INFORMATION

WARNING:

- Suspected cases of myocarditis and/or pericarditis have been observed in healthy adult primary vaccinees (at an approximate rate of 5.7 per 1000, 95% CI: 1.9-13.3) receiving ACAM2000 [see Warnings and Precautions (5.1)].
- Encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia, generalized vaccinia, severe vaccinial skin infections, erythema multiforme major (including STEVENS-JOHNSON SYNDROME), eczema vaccinatum resulting in permanent sequelae or death, ocular complications, blindness, and fetal death have occurred following either primary vaccination or revaccination with live vaccinia virus smallpox vaccines [see Warnings and Precautions (5)].
- These risks are increased in vaccinees with the following conditions and may result in severe disability, permanent neurological sequelae and/or death:
  - Cardiac disease or a history of cardiac disease
  - Eye disease treated with topical steroids
  - Congenital or acquired immune deficiency disorders, including those taking immunosuppressive medications
  - Eczema and persons with a history of eczema or other acute or chronic exfoliative skin conditions
  - Infants less than 12 months of age
  - Pregnancy

ACAM2000 is a live vaccinia virus that can be transmitted to persons who have close contact with the vaccinee and the risks in contacts are the same as those for the vaccinee.

The risk for experiencing serious vaccination complications must be weighed against the risks for experiencing a potentially fatal smallpox infection.

1 INDICATIONS AND USAGE

ACAM2000® is indicated for active immunization against smallpox disease for persons determined to be at high risk for smallpox infection.

2 DOSAGE AND ADMINISTRATION

Administer ACAM2000 only after being trained on the safe and effective administration of the vaccine by the percutaneous route (scarification). ACAM2000 should not be injected by the intradermal, subcutaneous, intramuscular, or intravenous route. Provide each patient with the FDA-approved Medication Guide prior to administering the vaccine.

2.1 Instructions for Vaccine Preparation

2.1.1 Reconstitution

ACAM2000 is reconstituted by addition of 0.3 mL of diluent to the vial containing lyophilized vaccine. **Note: this 0.3 mL of diluent is not the entire content of the diluent vial.** ACAM2000 should only be reconstituted with 0.3 mL of the diluent provided. The vaccine vial should be removed from cold storage and brought to room temperature before reconstitution. The flip cap seals of the vaccine and diluent vials are removed, and each rubber stopper is wiped with an isopropyl alcohol swab and allowed to dry thoroughly. Using aseptic technique and a sterile 1 mL syringe fitted with a 25 gauge x 5/8" needle (provided), draw up 0.3 mL of diluent and transfer the entire content of the syringe to the vaccine vial. Gently swirl to mix but try not to get product on the rubber stopper. The reconstituted vaccine should be a clear to slightly hazy, colorless to straw-colored liquid free from extraneous matter. Reconstituted vaccine should be inspected visually for particulate matter and discoloration prior to administration. If particulate matter or discoloration is observed, the vaccine should not be used and the vial should be disposed safely. [See Preparation / Handling Precautions and Instructions for Disposal (2.2)]

2.1.2 Storage following Reconstitution

After reconstitution, ACAM2000 vaccine may be administered within 6 to 8 hours if kept at room temperature (20-25°C, 68-77°F). Unused, reconstituted ACAM2000 vaccine may be stored in a refrigerator (2-8°C, 36-46°F) up to 30 days, after which it should be discarded as a biohazardous material. [See Preparation / Handling Precautions and Instructions for Disposal (2.2)] Exposure of reconstituted vaccine to room temperature during vaccination sessions should be minimized by placing it in refrigerator or on ice between patient administrations.

2.2 Preparation / Handling Precautions and Instructions for Disposal

Personnel preparing and administering the vaccine should wear surgical or protective gloves and avoid contact of vaccine with skin, eyes or mucous membranes.

The vaccine vial, its stopper, the diluent syringe, the vented needle used for reconstitution, the bifurcated needle used for administration, and any gauze or cotton that came in contact with the vaccine should be discarded in leak-proof, puncture-proof biohazard containers. These containers should then be disposed of appropriately.

2.3 Vaccination Instructions

The reconstituted vaccine should be brought to room temperature prior to administration. Before administration, the vial contents should be visually examined to verify the absence of particulates and gently swirled, without allowing the product to contact the rubber stopper, if necessary to re-dissolve any precipitates that might have formed.

The site of vaccination is the upper arm over the insertion of the deltoid muscle.

No skin preparation should be performed unless the skin at the intended site of vaccination is obviously dirty, in which case an alcohol swab(s) may be used to clean the area. If alcohol is used, the skin must be allowed to dry thoroughly to prevent inactivation of the live vaccine virus by the alcohol.

Remove the vaccine vial cap. Remove bifurcated needle from individual wrapping. Submerge bifurcated end of needle in reconstituted vaccine solution. The needle will pick up a droplet of vaccine (0.0025 mL) within the fork of the bifurcation. Use aseptic technique, i.e., do not insert the upper part of the needle that has been in contact with fingers into the vaccine vial, and never re-dip the needle into the vaccine vial if the needle has touched skin.

Deposit the droplet of vaccine onto clean, dry skin of the arm prepared for vaccination. The needle is held between thumb and first finger perpendicular to the skin. The wrist of the hand holding the needle of the vaccinator rests against the patient's arm. Rapidly make 15 jabs of the needle perpendicular to the skin through the vaccine droplet to puncture the skin, within a diameter of about 5 mm. The jabs should be vigorous enough so that a drop of blood appears at the vaccination site.

Any excess droplets of vaccine and blood should be wiped off the skin using a dry gauze pad and discarded in a biohazard container. Discard the needle in a biohazard sharps container. Close the vaccine vial by reinserting the rubber cap and return to a refrigerator or place on ice unless it will be used immediately to vaccinate another subject. [See Storage Following Reconstitution (2.1.2)]

Cover the vaccination site loosely with a gauze bandage, using first aid adhesive tape to keep it in place. This bandage provides a barrier to protect against spread of the vaccinia virus. If the vaccinee is involved in direct patient care, the gauze should be covered with a semipermeable (semiocclusive) dressing as an additional barrier. A semipermeable dressing is one that allows for the passage of air but does not allow for the passage of fluids.

Wash hands with soap and warm water or with alcohol-based hand rubs such as gels or foams after direct contact with the vaccination site, the bandage or clothes, towels or sheets that might be contaminated with virus from the vaccination site. This is vital in order to remove any virus from your hands and prevent contact spread.

Put the contaminated bandages in a sealed plastic bag and throw them away in the trash.

Wash separately clothing, towels, bedding or other items that may have come in direct contact with the vaccination site or drainage from the site, using hot water with detergent and/or bleach. Wash hands afterwards.

Don't use a bandage that blocks air from the vaccination site. This may cause the skin at the vaccination site to soften and wear away. Use loose gauze secured with medical tape to cover the site.

Don't put salves or ointments on the vaccination site.

2.4 Instructions for Interpreting Vaccination Response

2.4.1 Primary Vaccinees

In an individual vaccinated for the first time (primary vaccination), the expected response to vaccination is the development of a major cutaneous reaction (characterized by a pustule) at the site of inoculation. The lesion evolves gradually, with appearance of a papule at the site of vaccination after 2-5 days. The papule becomes vesicular, then pustular, and reaches its maximum size at 8-10 days after vaccination. The pustule dries and forms a scab, which usually separates within 14-21 days, leaving a pitted scar. (See Figure 1) Formation of a major cutaneous reaction by day 6-8 is evidence of a successful 'take' and acquisition of protective immunity. An equivocal reaction is any reaction that is not a major reaction, and indicates a non-take (vaccination failure) due to impotent vaccine or inadequate vaccination technique.

2.4.2 Previously Vaccinated Individuals (Revaccination)

Successful vaccination in an individual previously exposed to vaccine is confirmed when a major cutaneous reaction [See Primary Vaccinees (2.4.1) and Figure 1] is observed 6 to 8 days post-vaccination. However any prior vaccination may modify (reduce) the cutaneous response upon revaccination (Figure 2) such that the absence of a cutaneous response does not necessarily indicate vaccination failure. Previously vaccinated individuals who do not have a cutaneous response on revaccination do not require revaccination to try to elicit a cutaneous response.

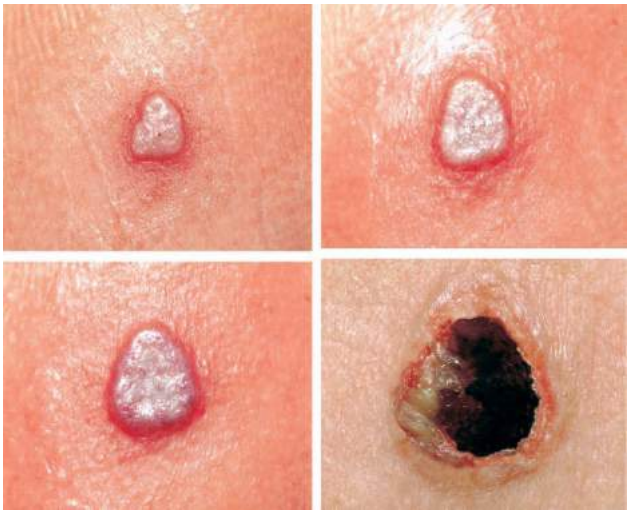
2.4.3 Vaccination Failures

Individuals who are not successfully vaccinated (i.e., vaccination failures) after primary vaccination may be revaccinated again in an attempt to achieve a satisfactory take. The vaccination procedures should be checked, and vaccination repeated with vaccine from another vial or vaccine lot, employing the same technique described in 2.3 [See Vaccination Instructions (2.3)].

If a repeat vaccination is conducted using vaccine from another vial or vaccine lot fails to produce a major reaction, healthcare providers should consult the Centers for Disease Control and Prevention (CDC) Emergency Operations Center (EOC) at 770-488-7100and their state or local health department before giving another vaccination.

Figure 1: Progression of major cutaneous reaction after primary vaccination[1]

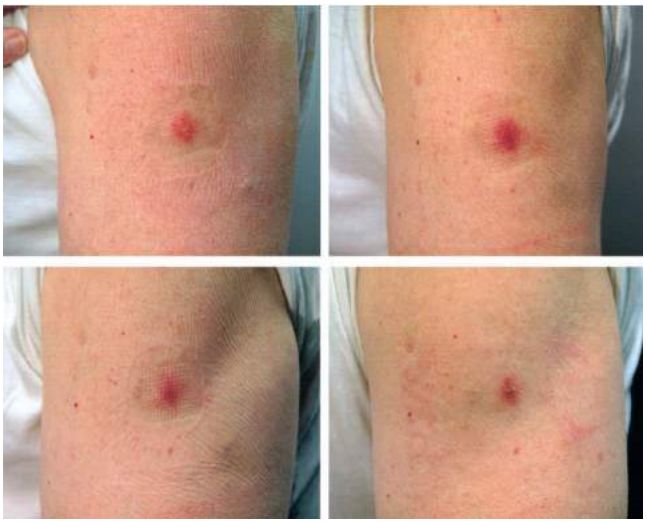
Day 5 **Day 8**



Day 10 **Day 14**

Figure 2: Progression of major cutaneous reaction after revaccination[1]

Day 3 **Day 7**



Day 10 **Day 14**

2.5 Booster Schedule

Persons at continued high risk of exposure to smallpox (e.g., research laboratory workers handling variola virus) should receive repeat ACAM2000 vaccination every three years.

2.6 Smallpox Vaccination Recommendations from US Government Agencies

Additional information may be obtained from U.S. Department of Defense (http://www.esd.whs.mil/Portals/54/Documents/DD/issuances/dodd/620503p.pdf) and U.S. Centers for Disease Control and Prevention (CDC) about smallpox vaccination (https://www.cdc.gov/smallpox/clinicians/vaccination.html).

3 DOSAGE FORMS AND STRENGTHS

After reconstitution of the lyophilized preparation, each vial has approximately 100 doses of 0.0025 mL of vaccinia virus (live) containing 2.5-12.5x10^5 plaque forming units / dose.

4 CONTRAINDICATIONS

There are very few absolute contraindications to this vaccine for those who are at high risk for smallpox. The risk for experiencing serious vaccination complications must be weighed against the risks for experiencing a potentially fatal smallpox infection. See *Warnings and Precautions* (5) for persons who are at higher risk of experiencing serious vaccination complications.

Severe Immune Deficiency

Severe localized or systemic infection with vaccinia (progressive vaccinia) may occur in persons with weakened immune systems. Individuals with severe immunodeficiency who are not expected to benefit from the vaccine should not receive ACAM2000. These individuals may include individuals who are undergoing bone marrow transplantation or individuals with primary or acquired immunodeficiency who require isolation.

5 WARNINGS AND PRECAUTIONS

Persons at greatest risk of experiencing serious vaccination complications are often those at greatest risk for death from smallpox. The risk for experiencing serious vaccination complications must be weighed against the risks for experiencing a potentially fatal smallpox infection.

5.1 Serious Complications and Death

Serious complications that may follow either primary live vaccinia smallpox vaccination or revaccination include: myocarditis and/or pericarditis, encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia (vaccinia necrosum), generalized vaccinia, severe vaccinial skin infections, erythema multiforme major (including Stevens-Johnson syndrome), eczema vaccinatum, blindness, and fetal death in pregnant women. These complications may rarely lead to severe disability, permanent neurological sequelea and death. Based on clinical trials, symptoms of suspected myocarditis or pericarditis (such as chest pain, raised troponin/cardiac enzymes, or ECG abnormalities) occur in 5.7 per 1000 primary vaccinations. This finding includes cases of acute symptomatic or asymptomatic myocarditis or pericarditits or both. Historically, death following vaccination

with live vaccinia virus is a rare event; approximately 1 death per million primary vaccinations and 1 death per 4 million revaccinations have occurred after vaccination with live vaccinia virus. Death is most often the result of sudden cardiac death, postvaccinial encephalitis, progressive vaccinia, or eczema vaccinatum. Death has also been reported in unvaccinated contacts accidentally infected by individuals who have been vaccinated.

5.1.1 Incidence of Serious Complications in 1968 US Surveillance Studies

Estimates of the risks of occurrence of serious complications after primary vaccination and revaccination, based on safety surveillance studies conducted when live vaccinia virus smallpox vaccine (i.e., New York City Board of Health strain, Dryvax®) was routinely recommended, are as follows:

Table 1A - Rates of reported complications[(a)] associated with primary vaccinia vaccinations (cases/million vaccinations)[(b)]

Age (yrs)	<1	1-4	5-19	≥20	Overall rates[(h)]
Inadvertent inoculation[(c)] §	507.0	577.3	371.2	606.1	**529.2**
Generalized vaccinia	394.4	233.4	139.7	212.1	**241.5**
Eczema vaccinatum	14.1	44.2	34.9	30.3	**38.5**
Progressive vaccinia[(d)]	--[(g)]	3.2	--[(g)]	--[(g)]	**1.5**
Post-vaccinial encephalitis	42.3	9.5	8.7	--[(g)]	**12.3**
Death[(e)]	5	0.5	0.5	unknown	--
Total[(f)]	1549.3	1261.8	855.9	1515.2	**1253.8**

a. See article for descriptions of complications.
b. Adapted from Lane JM, Ruber FL, Neff JM, Millar JD. Complications of smallpox vaccination, 1968: results of ten statewide surveys. J Infect Dis. 1970; 122:303-309.
c. Referenced as accidental implantation.
d. Referenced as vaccinia necrosum.
e. Death from all complications.
f. Rates of overall complications by age group include complications not provided in this table, including severe local reactions, bacterial superinfection of the vaccination site, and erythema multiforme.
g. No instances of this complication were identified during the 1968 10 state survey.
h. Overall rates for each complication include persons of unknown age.

Table 1B - Rates of reported serious complications[(a)] associated with vaccinia revaccinations (cases/million vaccinations)[(b)]

Age (yrs)	<1	1-4	5-19	≥20	Overall rates[(b)]
Inadvertent inoculation[(c)]	[(g)]	109.1	47.7	25.0	**42.1**
Generalized vaccinia	[(g)]	[(g)]	9.9	9.1	**9.0**
Eczema vaccinatum	[(g)]	[(g)]	2.0	4.5	**3.0**
Progressive vaccinia[(d)]	[(g)]	[(g)]	[(g)]	6.8	**3.0**
Post-vaccinial encephalitis	[(g)]	[(g)]	[(g)]	4.5	**2.0**
Death[(e)]	--	--	--	--	--
Total[(f)]	[(g)]	200.0	85.5	113.6	**108.2**

See Table 1A for explanation of footnotes.

5.1.2 Incidence of Serious Complications and Emergence of Myocarditis and/or Pericarditis in 2002-2005

Data on the incidence of adverse events among U.S. military personnel and civilian first responders vaccinated with Dryvax®, a licensed live vaccinia virus smallpox vaccine, during vaccination programs initiated in December 2002 are shown below in Table 2. The incidence of preventable adverse events (eczema vaccinatum, contact transmission, and auto-inoculation) were notably lower in these programs when compared with data collected in the 1960s; presumably because of better vaccination screening procedures and routine use of protective bandages over the inoculation site. Myocarditis and pericarditis were not commonly reported following smallpox vaccination in the 1960s, but emerged as a more frequent event based on more active surveillance in the military and civilian programs.

Table 2 - Serious adverse events in 2002-2005 [5]

Adverse event	N[a]	Incidence/ million	N[b]	Incidence/million
Myo/pericarditis	86	117.71	21	519.52
Post-vaccinal encephalitis	1	1.37	1	24.74
Eczema vaccinatum	0	0.00	0	0.00
Generalized vaccinia	43	58.86	3	74.22
Progressive vaccinia	0	0.00	0	0.00
Fetal vaccinia	0	0.00	0	0.00
Contact transmission	52	71.18	0	0.00
Auto-inoculation (non-ocular)	62	84.86	20	494.78
Ocular vaccinia	16	21.90	3	74.22

a. Department of Defense program (n=730,580) as of Jan05 where 71% primary vaccination; 89% male; median age 28.5 yr
b. Department of Health and Human Services program (n=40, 422) as of Jan04 where 36% primary vaccination; 36% male; median age 47.1 yr

5.1.3 Myocarditis and Pericarditis in the ACAM2000 Clinical Trial Experience

In clinical trials involving 2983 subjects who received ACAM2000 and 868 subjects who received Dryvax®, ten (10) cases of suspected myocarditis [0.2% (7 of 2983) ACAM2000 subjects and 0.3% (3 of 868) Dryvax® subjects] were identified. The mean time to onset of suspected myocarditis and/or pericarditis from vaccination was 11 days, with a range of 9 to 20 days. All subjects who experienced these cardiac events were naïve to vaccinia. Of the 10 subjects, 2 were hospitalized. None of the remaining 8 cases required hospitalization or treatment with medication. Of the 10 cases, 8 were sub-clinical and were detected only by ECG abnormalities with or without associated elevations of cardiac troponin I. All cases resolved by 9 months, with the exception of one female subject in the Dryvax® group, who had persistent borderline abnormal left ventricular ejection fraction on echocardiogram. The best estimate of risk for myocarditis and pericarditis is derived from the Phase 3 ACAM2000 clinical trials where there was active monitoring for potential of myocarditis and pericarditis. Among vaccinees naïve to vaccinia, 8 cases of suspected myocarditis and pericarditis were identified across both treatment groups, for a total incidence rate of 6.9 per 1000 vaccinees (8 of 1,162). The rate for the ACAM2000 treatment group were similar: 5.7 (95% CI: 1.9-13.3) per 1000 vaccinees (5 of 873 vaccinees) and for the Dryvax® group 10.4 (95% CI: 2.1-30.0) per 1000 vaccinees (3 of 289 vaccinees). No cases of myocarditis and/or pericarditis were identified in 1819 previously vaccinated subjects. The long-term outcome of myocarditis and pericarditis following ACAM2000 vaccination is currently unknown.

5.2 Cardiac Disease

Ischemic cardiac events, including fatalities, have been reported following smallpox vaccination; the relationship of these events, if any, to vaccination has not been established. In addition, cases of non-ischemic, dilated cardiomyopathy have been reported following smallpox vaccination; the relationship of these cases to smallpox vaccination is unknown.

There may be increased risks of adverse events with ACAM2000 in persons with known cardiac disease, including those diagnosed with previous myocardial infarction, angina, congestive heart failure, cardiomyopathy, chest

pain or shortness of breath with activity, stroke or transient ischemic attack, or other heart conditions. In addition, subjects who have been diagnosed with 3 or more of the following risk factors for ischemic coronary disease: 1) high blood pressure; 2) elevated blood cholesterol; 3) diabetes mellitus or high blood sugar; 4) first degree relative (for example mother, father, brother, or sister) who had a heart condition before the age of 50; or 5) smoke cigarettes may have increased risks.

5.3 Ocular Complications and Blindness

Accidental infection of the eye (ocular vaccinia) may result in ocular complications including keratitis, corneal scarring and blindness. Patients who are using corticosteroid eye drops may be at increased risk of ocular complications with ACAM2000.

5.4 Presence of Congenital or Acquired Immune Deficiency Disorders

Severe localized or systemic infection with vaccinia (progressive vaccinia) may occur in persons with weakened immune systems, including patients with leukemia, lymphoma, organ transplantation, generalized malignancy, HIV/AIDS, cellular or humoral immune deficiency, radiation therapy, or treatment with antimetabolites, alkylating agents, high-dose corticosteroids (>10 mg prednisone/day or equivalent for ≥2 weeks), or other immunomodulatory drugs. The vaccine is contraindicated in individuals with severe immunodeficiency [See Contraindications (4)]. Vaccinees with close contacts who have these conditions may be at increased risk because live vaccinia virus can be shed and be transmitted to close contacts.

5.5 History or Presence of Eczema and Other Skin Conditions

Persons with eczema of any description such as, atopic dermatitis, neurodermatitis, and other eczematous conditions, regardless of severity of the condition, or persons who have a history of these conditions at any time in the past, are at higher risk of developing eczema vaccinatum. Vaccinees with close contacts who have eczematous conditions, may be at increased risk because live vaccinia virus can shed and be transmitted to these close contacts. Vaccinees with other active acute, chronic or exfoliative skin disorders (including burns, impetigo, varicella zoster, acne vulgaris with open lesions, Darier's disease, psoriasis, seborrheic dermatitis, erythroderma, pustular dermatitis, etc.), or vaccinees with household contacts having such skin disorders might also be at higher risk for eczema vaccinatum.

5.6 Infants (< 12 months of Age) and Children

ACAM2000 has not been studied in infants or children. The risk of serious adverse events following vaccination with live vaccinia virus is higher in infants. Vaccinated persons who have close contact with infants, e.g., breastfeeding, must take precautions to avoid inadvertent transmission of ACAM2000 live vaccinia virus to infants.

5.7 Pregnancy

ACAM2000 has not been studied in pregnant women. Live vaccinia virus vaccines can cause fetal vaccinia and fetal death. If ACAM2000 is administered during pregnancy, the vaccinee should be apprised of the potential hazard to the fetus [See Use in Specific Populations (8.1)]. Pregnant women who are close contacts of vaccinees may be at increased risk because live vaccinia virus can shed and be transmitted to close contacts.

5.8 Allergy to ACAM2000 Smallpox Vaccine or its Components

ACAM2000 contains neomycin and polymyxin B. Persons allergic to these components may be at higher risk for adverse events after vaccination.

5.9 Management of Smallpox Vaccine Complications

The CDC can assist physicians in the diagnosis and management of patients with suspected complications of vaccinia (smallpox) vaccination. Vaccinia Immune Globulin (VIG) is indicated for certain complications of vaccination live vaccinia virus smallpox vaccine. If VIG and/or other antivirals are needed or additional information is required, physicians should contact the CDC EOC at 770-488-7100, Monday through Friday 8 AM to 4:30 PM Eastern Standard Time; at other times call (404) 639-2888.

5.10 Prevention of Transmission of Live Vaccinia Virus

The most important measure to prevent inadvertent auto-inoculation and contact transmission from vaccinia vaccination is thorough hand washing after changing the bandage or after any other contact with the vaccination site.

Individuals susceptible to adverse effects of vaccinia virus, i.e., those with cardiac disease, eye disease, immunodeficiency states, including HIV infection, eczema, pregnant women and infants, should be identified and measures should be taken to avoid contact between those individuals and persons with active vaccination lesions.

Recently vaccinated healthcare workers should avoid contact with patients, particularly those with immunodeficiencies, until the scab has separated from the skin at the vaccination site. However, if continued contact with patients is unavoidable, vaccinated healthcare workers should ensure the vaccination site is well covered and follow good hand-washing technique. In this setting, a more occlusive dressing may be used. Semipermeable polyurethane dressings are effective barriers to shedding of vaccinia. However, exudate may accumulate beneath the dressing, and care must be taken to prevent viral spread when the dressing is changed. In addition, accumulation of fluid beneath the dressing may increase skin maceration at the vaccination site. Accumulation of exudate may be decreased by first covering the vaccination with dry gauze, then applying the dressing over the gauze. The dressing should be changed every 1-3 days [See Self Inoculation and Spread to Close Contacts (17.3) and Care of the Vaccination Site and Potentially Contaminated Materials (17.4)].

5.11 Blood and Organ Donation

Blood and organ donation should be avoided for at least 30 days following vaccination with ACAM2000.

5.12 Limitations of Vaccine Effectiveness

ACAM2000 smallpox vaccine may not protect all persons exposed to smallpox.

6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections of the labeling:

- Encephalitis, encephalomyelitis, encephalopathy, progressive vaccinia (vaccinia necrosum), generalized vaccinia, severe vaccinial skin infections, erythema multiforme major (including Stevens-Johnson syndrome) and eczema vaccinatum. Severe disability, permanent neurological sequelae, and/or death may occur. Death of unvaccinated individuals who have contact with vaccinated individuals. [See Warnings and Precautions (5.1)].
- Myocarditis and/or pericarditis, ischemic heart disease and non-ischemic, dilated cardiomyopathy [See Warnings and Precautions (5.1)].
- Ocular complications and blindness [See Warnings and Precautions (5.3)].

6.1 Overall Adverse Reaction Profile

Information regarding the safety of ACAM2000 has been derived from three sources: 1) ACAM2000 clinical trial experience (Phase 1, 2 and 3 clinical trials), 2) data compiled during the era of routine smallpox vaccination using other NYCBH vaccinia vaccines and 3) adverse event data obtained during military and civilian smallpox vaccination programs (2002-2005) that used Dryvax®, a licensed live vaccinia virus smallpox vaccine.

- General Disorders and Administrative Site Conditions: In the ACAM2000 clinical studies 97% and 92% of vaccinia-naïve and previously vaccinated subjects, respectively, experienced one or more adverse event. Common events included injection site reactions (erythema, pruritus, pain and swelling) and constitutional symptoms (fatigue, malaise, feeling hot, rigors and exercise tolerance decreased). Across all ACAM2000 studies 10% of vaccinia-naïve and 3% of previously vaccinated subjects experienced at least one severe adverse event (defined as interfering with normal daily activities).
- Nervous System Disorder: Overall, 50% and 34% of vaccinia-naïve subjects and previously vaccinated subjects, respectively, reported headaches in ACAM2000 studies. There have been reports of headache following smallpox vaccination which required hospitalization. Although <1% of the subjects in the ACAM2000 program experienced severe headaches, none required hospitalization.

  Neurological adverse events assessed among the 2002 - 2005 military (n=590,400) and DHHS (n=64,600) programs temporally associated with smallpox vaccination included headache (95 cases), non-serious limb paresthesias (17 cases) or pain (13 cases) and dizziness or vertigo (13 cases). Serious neurologic adverse events included 13 cases of suspected meningitis, 3 cases of suspected encephalitis or myelitis, 11 cases of Bell palsy, 9 seizures (including 1 death), and 3 cases of Guillain-Barre syndrome. Among these 39 events, 27 (69%) occurred in primary vaccinees and all but 2 occurred within 12 days of vaccination. There have also been cases of photophobia following smallpox vaccination, some of which required hospitalization.
- Musculoskeletal and Connective Tissue Disorders: Across all ACAM2000 studies, severe, vaccine-related myalgia was seen in 1% of vaccinia-naïve subjects and <1% of previously vaccinated subjects.

Other adverse events included back pain, arthralgia and pain in extremity and none occurred with a frequency of more than 2% in either the vaccinia-naïve or previously vaccinated populations.

- Blood and Lymphatic System Disorders: The only adverse event occurring at ≥5% in the ACAM2000 studies were lymph node pain and lymphadenopathy. The incidence of severe lymph node pain and lymphadenopathy was <1%.

- Gastrointestinal (GI) Disorders: Commonly reported GI disorders among ACAM2000-treated subjects included nausea and diarrhea (14%), constipation (6%), and vomiting (4%). Severe abdominal pain, nausea, vomiting, constipation diarrhea and toothache accounted for all the severe adverse events reported and occurred in <1% of subjects.

- Skin and Subcutaneous Tissue Disorders: Erythema and rash were noted in 18% and 8% of subjects respectively. In ACAM2000 subjects 1% of vaccinia-naïve and <1% of previously vaccinated subjects experienced at least one severe adverse event. With the exception of one case of contact dermatitis and one case of urticaria, erythema and rash accounted for all severe events.

  Generalized rashes (erythematous, papulovesicular, urticarial, folliculitis, nonspecific) are not uncommon following smallpox vaccination and are presumed to be hypersensitivity reactions occurring among persons without underlying illnesses. These rashes are generally self-limited and require little or no therapy, except among patients whose conditions appear to be toxic or who have serious underlying illnesses.

  Inadvertent inoculation at other body sites is the most frequent complication of vaccinia vaccination, usually resulting from autoinoculation of the vaccine virus transferred from the site of vaccination. The most common sites involved are the face, nose, mouth, lips, genitalia and anus. Accidental infection of the eye (ocular vaccinia) may result in ocular complications including, but not limited to, keratitis, corneal scarring and blindness.

  Major cutaneous reactions at the site of inoculation, characterized by large area of erythema and induration and streaking inflammation of draining lymphatics may resemble cellulitis. Benign and malignant lesions have been reported to occur at the smallpox vaccination site.

6.2 ACAM2000 Clinical Trial Experience

Two randomized, controlled, multi-center Phase 3 trials enrolled 2244 subjects that received ACAM2000 and 737 that received a comparison licensed live vaccinia virus vaccine, Dryvax®. Study 1 was conducted in male (66% and 63% for ACAM2000 and Dryvax®, respectively) and female (34% and 37% for ACAM2000 and Dryvax®, respectively) subjects who previously had not been vaccinated with smallpox vaccine (i.e., vaccinia-naïve subjects). The majority of subjects were Caucasian (76% and 71% for ACAM2000 and Dryvax®, respectively) and the mean age was 23 in both groups with an age range from 18-30 years. Study 2 was conducted in male (50% and 48% for ACAM2000 and Dryvax®, respectively) and female (50% and 52% for ACAM2000 and Dryvax®, respectively) subjects who had been vaccinated with smallpox vaccine >10 years previously (i.e., previously vaccinated subjects). The majority of subjects were Caucasian (78% for both groups) and the mean age was 49 years in both groups with an age range of 31 to 84 years.

6.2.1 Common Adverse Events Reported in ACAM2000 Clinical Program

Adverse events reported by ≥5% of subjects in either the ACAM2000 or the comparison treatment group during Phase 3 studies are presented by type of adverse events, by baseline vaccination status (vaccinia-naïve versus previously vaccinated) and by treatment group. Severe vaccine-related adverse events, defined as interfering with normal daily activities, in vaccinia-naïve subjects were reported by 10% of subjects in the ACAM2000 group and 13% in the comparison group. In the previously vaccinated subjects, the incidence of severe vaccine-related adverse events was 4% for the ACAM2000 groups and 6% for the comparison group.

Table 3 - Adverse Events Reported by ≥5% of Subjects in ACAM2000 or Dryvax®

	ACAM2000 N=873[b] n (%)	Dryvax® N=289[b] n (%)	ACAM2000 N=1371[c] n (%)	Dryvax® N=448[c] n (%)
At least 1 adverse event	**864 (99)**	**288 (100)**	**1325 (97)**	**443 (99)**
Blood and lymphatic system disorders	**515 (59)**	**204 (71)**	**302 (22)**	**133 (30)**
Lymph node pain[a]*	494 (57)	199 (69)	261 (19)	119 (27)
Lymphadenopathy	72 (8)	35 (12)	78 (6)	29 (6)
Gastrointestinal disorders	**273 (31)**	**91 (31)**	**314 (23)**	**137 (31)**
Nausea[a]	170 (19)	65 (22)	142 (10)	63 (14)
Diarrhea[a]	144 (16)	34 (12)	158 (12)	77 (17)
Constipation[a]	49 (6)	9 (3)	88 (6)	31 (7)
Vomiting[a]	42 (5)	10 (3)	40 (3)	18 (4)
General disorders and administration site conditions	**850 (97)**	**288 (100)**	**1280 (93)**	**434 (97)**
Injection site pruritus[a]	804 (92)	277 (96)	1130 (82)	416 (93)
Injection site erythema[a]	649 (74)	229 (79)	841 (61)	324 (72)
Injection site pain[a]	582 (67)	208 (72)	505 (37)	209 (47)
Fatigue[a]	423 (48)	161 (56)	468 (34)	184 (41)
Injection site swelling	422 (48)	165 (57)	384 (28)	188 (42)
Malaise[a]	327 (37)	122 (42)	381 (28)	147 (33)
Feeling hot[a]	276 (32)	97 (34)	271 (20)	114 (25)
Rigors[a]	185 (21)	66 (23)	171 (12)	76 (17)
Exercise tolerance decreased[a]	98 (11)	35 (12)	105 (8)	50 (11)
Musculoskeletal and connective tissue disorders	**418 (48)**	**153 (53)**	**418 (30)**	**160 (36)**
Myalgia[a]	404 (46)	147 (51)	374 (27)	148 (33)
Nervous system disorders	444 (51)	151 (52)	453 (33)	174 (39)
Headache[a]	433 (50)	150 (52)	437 (32)	166 (37)
Respiratory, thoracic, and mediastinal disorders	**134 (15)**	**40 (14)**	**127 (9)**	**42 (9)**
Dyspnea[a]	39 (4)	16 (6)	41 (3)	18 (4)
Skin and subcutaneous tissue disorders	**288 (33)**	**103 (36)**	**425 (31)**	**139 (31)**
Erythema[a]	190 (22)	69 (24)	329 (24)	107 (24)
Rash[a]	94 (11)	30 (10)	80 (6)	29 (6)

a. Event was listed on a checklist included in subject diaries; therefore should be considered solicited. In addition to events listed above the following were also included as part of the checklist: chest pain and heart palpitations, but these events did not occur in ≥5% of subjects.

b. Study 1 Vaccinia Naïve Subjects
Study 2 Previously Vaccinated Subjects

7 DRUG INTERACTIONS

7.1 Simultaneous Administration with Other Vaccines

There are no data evaluating the simultaneous administration of ACAM2000 with other vaccines.

7.2 Interference with Laboratory Tests

ACAM2000 may induce false-positive tests for syphilis. Positive RPR tests results should be confirmed using a more specific test, such as the FTA assay.

ACAM2000 may induce temporary false-negative results for the tuberculin skin test (purified protein derivative [PPD]) and possibly, blood tests for tuberculosis. Tuberculin testing should be delayed if possible for 1 month following smallpox vaccination.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Category D

ACAM2000 has not been studied in pregnant women. Live vaccinia virus vaccines can cause fetal harm when administered to a pregnant woman. Congenital infection, principally occurring during the first trimester, has been observed after vaccination with live vaccinia smallpox vaccines, although the risk may be low. Generalized vaccinia of the fetus, early delivery of a stillborn infant, or a high risk of perinatal death has been reported.

The only setting in which vaccination of pregnant women should be considered is when exposure to smallpox is considered likely. If this vaccine is used during pregnancy, or if the vaccinee lives in the same household with or has close contact with a pregnant woman, the vaccinee should be apprised of the potential hazard to the fetus. Healthcare providers, state health departments, and other public health staff should report to the National Smallpox Vaccine in Pregnancy Registry all pregnant women who, from 42 days prior to conception onward, received ACAM2000 or had close contact with a person who received ACAM2000 within the previous 28 days.. Civilian women should contact their healthcare provider or state health department for help enrolling in the registry. All civilian and military cases should be reported to the DoD, telephone 619 553-9255, Defense Switched Network (DSN) 553-9255, fax 619 533-7601 or e-mail NHRC-BirthRegistry@med.navy.mil**.**

8.3 Nursing Mothers

ACAM2000 has not been studied in lactating women. It is not known whether vaccine virus or antibodies are secreted in human milk. Live vaccinia virus can be inadvertently transmitted from a lactating mother to her infant. Infants are at high risk of developing serious complications from live vaccinia smallpox vaccination.

8.4 Pediatric Use

The safety and effectiveness of ACAM2000 have not been established in the age groups from birth to age 16. The use of ACAM2000 in all pediatric age groups is supported by evidence from the adequate and well-controlled studies of ACAM2000 in adults and with additional historical data with use of live vaccinia virus smallpox vaccine in pediatrics. Before the eradication of smallpox disease, live vaccinia virus smallpox vaccine was administered routinely in all pediatric age groups, including neonates and infants, and was effective in preventing smallpox disease. During that time, live vaccinia virus was occasionally associated with serious complications in children, the highest risk being in infants younger than 12 months of age. [See Warnings and Precautions (5.6)].

8.5 Geriatric Use

Clinical studies of ACAM2000 did not include sufficient numbers of subjects aged 65 and over to determine whether they respond differently from younger subjects. There are no published data to support the use of this vaccine in geriatric (persons >65 years) populations.

11 DESCRIPTION

ACAM2000, Smallpox (Vaccinia) Vaccine, Live, is a live vaccinia virus derived from plaque purification cloning from Dryvax® (Wyeth Laboratories, Marietta, PA, calf lymph vaccine, New York City Board of Health Strain) and grown in African Green Monkey kidney (Vero) cells and tested to be free of adventitious agents.

ACAM2000 is provided as a lyophilized preparation of purified live virus containing the following non-active excipients: 6-8 mM HEPES (pH 6.5-7.5), 2% human serum albumin USP, 0.5 – 0.7% sodium chloride USP, 5% mannitol USP, and trace amounts of neomycin and polymyxin B.

Diluent for ACAM2000 contains 50% (v/v) Glycerin USP, 0.25% (v/v) Phenol USP in Water for Injection USP, supplied in 3 mL clear glass vials containing 0.6 mL of diluent.

After reconstitution, each vial of ACAM2000 vaccine contains approximately 100 doses (0.0025 mL/dose). The concentration of vaccinia virus is 1.0-5.0 x 10^8 plaque-forming units (PFU)/mL or 2.5-12.5 x 10^5 PFU/dose determined by plaque assay in Vero cells. ACAM2000 is administered by the percutaneous route (scarification) using 15 jabs of a stainless steel bifurcated needle that has been dipped into the vaccine.

12 CLINICAL PHARMACOLOGY

Smallpox vaccine does not contain smallpox virus (variola) and cannot spread or cause smallpox.

12.1 Mechanism of Action

Vaccinia virus is a member of the same taxonomic group (the Orthopox genus) as smallpox (variola) virus, and immunity induced by vaccinia virus cross-protects against variola virus. Vaccinia virus causes a localized virus infection of the epidermis at the site of inoculation, surrounding dermal and subcutaneous tissues, and draining lymph nodes. Virus may be transiently present in blood and infects reticuloendothelial and other tissues. Langerhans cells in the epidermis are specific targets for the early stage of virus replication. The formation of a pustule ('pock' or 'take') at the site of inoculation provides evidence of protective immunity. The virus replicates within cells and viral antigens are presented to the immune system. Neutralizing antibodies and B and T cells provide long-term memory. The level of neutralizing antibody that protects against smallpox is unknown but >95% of persons undergoing primary vaccination develop neutralizing or hemagglutination inhibiting antibodies to vaccinia.

12.2 Pharmacodynamics

12.2.1 Cutaneous Response

The cutaneous responses following smallpox vaccination are dependent on the immune status of the individual, potency of the vaccine, and vaccination technique. Two types of responses have been defined by the WHO Expert Committee on Smallpox, and described by the Advisory Committee on Immunization Practices (ACIP). The responses include: a) major cutaneous reaction, which indicates that virus replication has taken place and vaccination was successful; or b) equivocal reaction. Equivocal reactions may be a consequence of pre-existing immunity adequate to suppress viral multiplication, vaccination technique failure, or use of inactive vaccine or vaccine that has lost potency.

Successful vaccination in persons who are naïve to smallpox vaccination, termed primary vaccination, is represented by a major cutaneous reaction, defined as a vesicular or pustular lesion or an area of definite palpable induration or congestion surrounding a central lesion that might be a crust or an ulcer.

Subjects who have been previously vaccinated and are revaccinated may manifest a reduced cutaneous response compared to vaccinia-naïve subjects, but still exhibit an immune response to the vaccine. [See Dosage and Administration (2.4)]

12.2.2 Neutralizing Antibody and Cellular Immune Responses

Neutralizing antibodies are known to mediate protection against smallpox. Neutralizing antibodies against vaccinia develop in >95% of individuals following primary vaccination, rise rapidly (by day 15-20 after vaccination) and may be boosted on revaccination. Antibody titers are highly variable. Titers may remain high for longer periods following two or more vaccinations than after a primary vaccination. The level of the neutralizing antibody response following primary vaccination is generally in proportion to the intensity of the cutaneous reaction. The level of neutralizing antibody that is required to protect against smallpox has not been clearly established, although some studies indicate that persons with antibody titers > 1:32 are protected. Cellular immune responses are also elicited by vaccination and may contribute to protection and immunological memory.

12.2.3 Virus Shedding

Virus is shed from the vaccination site during the period starting with the development of a papule (day 2-5); shedding ceases when the scab separates and the lesion is re-epithelialized, about 14-21 days after vaccination. Steps should be taken in clinical use to reduce the risk of accidental infection of other sites in the vaccinated patient or of contact spread to other individuals [See Vaccination Instructions (2.3)].

14 CLINICAL STUDIES

Vaccine efficacy was assessed by comparing the immunologic response of ACAM2000 to another US-licensed live vaccinia virus smallpox vaccine, Dryvax®, in two randomized, multi-center active-controlled clinical trials; one study in subjects who previously had not been vaccinated with smallpox vaccine (i.e., vaccinia-naïve subjects) and one study in subjects who had been vaccinated with smallpox vaccine >10 years previously (i.e., previously

vaccinated subjects). In both trials, the co-primary efficacy endpoints were the proportion of subjects with a successful vaccination/revaccination and the geometric mean neutralizing antibody titer (GMT) on Day 30. Successful primary vaccination was defined as a major cutaneous reaction on Day 7 or 10 (Days 6 to 11, with allowable visit window). Successful revaccination was defined as development of any cutaneous lesion on Day 7 (± 1 day) of a measurable size. Successful revaccination was determined by a panel of experts who reviewed digital photographs of the cutaneous lesions.

The statistical method used to compare the proportion of subjects who were successfully vaccinated in the two treatment groups was a test of non-inferiority of ACAM2000 to the active comparator intended to rule out a greater than 5% margin of superiority of the comparator for successful primary vaccination (Study 1) and a 10% margin of superiority of the comparator for successful revaccination (Study 2). Non-inferiority was to be declared if the lower bound of the 1-sided 97.5% confidence interval (CI) for the percent difference between ACAM2000 and the comparator exceeded -5% in naïve subjects and -10% in previously vaccinated subjects.

Analysis of the GMT was performed using a test of non-inferiority of neutralizing antibody titer between ACAM2000 and the comparator, intended to ensure that the ratio of the GMTs of ACAM2000: comparator vaccine was at least 0.5 (equivalent to the difference of the $\log_{10}$ (GMT) being at least -0.301).

In Study 1, a total of 1037 male and female vaccinia-naïve subjects, aged 18 to 30 years inclusive, primarily Caucasian (76%) were randomized in a 3:1 ratio to receive ACAM2000 (780 subjects) or comparator (257 subjects). The ACAM2000 subjects were further stratified to receive one of three lots (Lots A, B and C) at a 1:1:1 ratio (258, 264, and 258 subjects, respectively). All subjects were to be evaluated for their cutaneous response and a random subset was selected for evaluation of neutralizing antibody response.

In Study 2, a total of 1647 male and female previously-vaccinated subjects, aged 31 to 84 years inclusive, primarily Caucasian (81%) were randomized in a 3:1 ratio to receive ACAM2000 (1242 subjects) or the comparator (405 subjects). The ACAM2000 subjects were further stratified to receive one of three lots (Lots A, B and C) at a 1:1:1 ratio (411, 417, and 414 subjects, respectively). All subjects were evaluated for their cutaneous response and a random subset was to be selected for evaluation of neutralizing antibody response.

Tables 4 and 5 present the results of the primary efficacy analyses for both studies.

Table 4 - Cutaneous Response (Vaccination Success) in Subjects Given ACAM2000 vs. Comparator Vaccine, Studies 1 and 2

	Study 1 ACAM 2000	**Study 1 Comparator**	**Study 2 ACAM 2000**	**Study 2 Comparator**
Size of Evaluable Population[a]	776	257	1189	388
Number of Vaccination Successes (%)	747 (96)[b]	255 (99)	998 (84)[d]	381 (98)
97.5% 1-sided CI by normal approx. on percent difference between ACAM2000-Comparator	-4.67% [c]	--	-17% [e]	--
Non-Inferiority to Comparator	Yes	--	No	--

a. Subjects who received study vaccine and were evaluated for a local cutaneous reaction within the protocol-designated timeframe were included in the efficacy evaluable (EE) population.
b. Results for vaccine lots, A, B and C were 95%, 98% and 96%.
c. Since the critical value for the evaluation was declared to be -5%, ACAM2000 is considered to be non-inferior to Comparator for this parameter.
d. Results for vaccine lots, A, B and C were 79%, 87% and 86%.
e. Since the critical value for the evaluation was declared to be -10%, ACAM2000 is not considered to be non-inferior to Comparator for this parameter.

Table 5 - Neutralizing Antibody Response in Subjects Given ACAM2000 vs. Comparator Vaccine, Studies 1 and 2

	Study 1 ACAM 2000	**Study 1 Comparator**	**Study 2 ACAM 2000**	**Study 2 Comparator**
Size of Evaluable Population[a]	565	190	734	376
GMT[b]	166	255	286	445
Log_{10} mean	2.2	2.4	2.5	2.6
97.5% 1-sided CI by ANOVA on difference between ACAM2000-Comparator	-0.307[c]	--	-0.275[d]	--
Meets Non-Inferiority to Comparator	No	--	Yes	--

a. A randomly selected sample of subjects who received study vaccine and had samples collected for neutralizing antibody response at Baseline and at the designated time-point post-treatment were included in the antibody evaluable (AnE) population.
b. GMT – Geometric mean neutralizing antibody titer as measured by Vaccinia 50% plaque reduction neutralization test.
c. Since the critical value for the evaluation was declared to be -0.301, ACAM2000 is not considered to be non-inferior to Comparator for this parameter.
d. Since the critical value for the evaluation was declared to be -0.301, ACAM2000 is considered to be non-inferior to Comparator for this parameter.

The primary determinant for an effective immune response in those naïve to vaccine is a major cutaneous reaction. ACAM2000 was non-inferior to comparator in this population with regard to eliciting a major cutaneous reaction. The measure of the strength of the generated antibody response was similar but did not meet the predefined criterion for non-inferiority. Among subjects who were previously vaccinated, development of a major cutaneous response after revaccination with vaccinia-based smallpox vaccines may not provide an accurate measure of the strength of the immune response because the pre-existing immunity modifies the scope of the cutaneous response. In previously vaccinated subjects, ACAM2000 was non-inferior to the comparator with regard to the strength of the neutralizing antibody immune response. Therefore, ACAM2000 was non-inferior to the comparator in the rate of major cutaneous reaction in those naïve to the vaccine, and the strength of the neutralizing antibody immune response in those previously exposed to vaccinia-based smallpox vaccines.

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16 HOW SUPPLIED / STORAGE AND HANDLING

16.1 How Supplied

ACAM2000, Smallpox (Vaccinia) Vaccine, Live, is supplied in multiple-dose 3 mL clear glass vials containing lyophilized powder (freeze-dried vaccine). After reconstitution with 0.3 mL of diluent, the vial contains approximately 100 nominal doses of 0.0025 mL of vaccinia virus (live), 1.0 - $5.0x10^8$ PFU/mL or 2.5-$12.5x10^5$ PFU/dose.

Diluent for ACAM2000, 50% (v/v) Glycerin USP, 0.25% (v/v) Phenol USP in Water for Injection USP, is supplied in 3 mL clear glass vials containing 0.6 mL of diluent.

Bifurcated needles are supplied in boxes (5 x 5 x 1 in) containing 100 needles.

1 mL tuberculin syringes with 25 gauge x 5/8" needles are supplied for vaccine reconstitution.

Both the vaccine and diluent vial stoppers are not made with natural rubber latex.

16.2 Storage and Handling

ACAM2000 should be stored in a freezer with an average temperature of -15°C to -25°C (+5°F to -13°F).

Prior to reconstitution, ACAM2000 vaccine retains a potency of $1.0x10^8$ PFU or higher per dose for at least 18 months when stored at refrigerated temperatures of +2-8°C (36-46°F).

During shipment, ACAM2000 should be maintained at a temperature of -10°C or colder.

After reconstitution, ACAM2000 vaccine may be administered during a 6 to 8 hour workday at room temperature (20-25°C, 68-77°F). Reconstituted ACAM2000 vaccine may be stored in a refrigerator (2-8°C, 36-46°F) no longer than 30 days, after which it should be discarded [*See Dosage and Administration (2.3)*]. Diluent for Smallpox Vaccine, (Vero Cells) Lyophilized, ACAM2000 should be stored at room temperature (15-30°C, 59-86°F).

ACAM2000 contains live vaccinia virus that is transmissible, and should be handled as an infectious agent once vials are open. See 2.1 [Instructions for Vaccine Preparation] and 2.2 [Preparation / Handling Precautions and Instructions for Disposal] for details on handling and disposal.

17 PATIENT COUNSELING INFORMATION

Please refer patient to the FDA-approved Medication Guide prepared for ACAM2000 Smallpox Vaccine.

17.1 Serious Complications of Vaccination

Patients must be informed of the major serious adverse events associated with vaccination, including myocarditis and/or pericarditis, progressive vaccinia in immunocompromised persons, eczema vaccinatum in persons with skin disorders, auto- and accidental inoculation, generalized vaccinia, urticaria, erythema multiforme major (including Stevens-Johnson syndrome) and fetal vaccinia in pregnant women.

17.2 Protecting Contacts at Highest Risk for Adverse Events

Patients must be informed that they should avoid contact with individuals at high risk of serious adverse effects of vaccinia virus, for instance, those with past or present eczema, immunodeficiency states including HIV infection, pregnancy, or infants less than 12 months of age.

17.3 Self-inoculation and Spread to Close Contacts

Patients must be advised that virus is shed from the cutaneous lesion at the site of inoculation from approximately Day 3 until scabbing occurs, typically between Days 14-21 after primary vaccination. Vaccinia virus may be transmitted by direct physical contact. Accidental infection of skin at sites other than the site of intentional vaccination (self-inoculation) may occur by trauma or scratching. Contact spread may also result in accidental inoculation of household members or other close contacts. The result of accidental infection is a pock lesion(s) at an unwanted site(s) in the vaccinee or contact, and resembles the vaccination site. Self-inoculation occurs most often on the face, eyelid, nose, and mouth, but lesions at any site of traumatic inoculation can occur. Self-inoculation of the eye may result in ocular vaccinia, a potentially serious complication.

17.4 Care of the Vaccination Site and Potentially Contaminated Materials

Patients must be given the following instructions:

- The vaccination site must be completely covered with a semipermeable bandage. Keep site covered until the scab falls off on its own.
- The vaccination site must be kept dry. Normal bathing may continue, but cover the vaccination site with waterproof bandage when bathing. The site should not be scrubbed. Cover the vaccination site with loose gauze bandage after bathing.
- Don’t scratch the vaccination site. Don’t scratch or pick at the scab.
- Do not touch the lesion or soiled bandage and subsequently touch other parts of the body particularly the eyes, anal and genital areas that are susceptible to accidental (auto-) inoculation.
- After changing the bandage or touching the site, wash hands thoroughly with soap and water or >60% alcohol-based hand-rub solutions.
- To prevent transmission to contacts, physical contact of objects that have come into contact with the lesion (e.g. soiled bandages, clothing, fingers) must be avoided.
- Wash separately clothing, towels, bedding or other items that may have come in direct contact with the vaccination site or drainage from the site, using hot water with detergent and/or bleach. Wash hands afterwards.
- Soiled and contaminated bandages must be placed in plastic bags for disposal.
- The vaccinee must wear a shirt with sleeves that covers the vaccination site as an extra precaution to prevent spread of the vaccinia virus. This is particularly important in situations of close physical contact.
- The vaccinee must change the bandage every 1 to 3 days. This will keep skin at the vaccination site intact and minimize softening.
- Don’t put salves or ointments on the vaccination site.
- When the scab fall off, throw it away in a sealed plastic bag and wash hands afterwards.

Manufactured by
Emergent Product Development Gaithersburg Inc.
Gaithersburg, MD USA 20879
Licence No. XXXX

Tetanus / Diphtheria / Pertussis (Tdap)

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use Adacel safely and effectively. See full prescribing information for Adacel.

Adacel (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed), Suspension for Intramuscular Injection
Initial U.S. Approval: 2005

----------RECENT MAJOR CHANGES----------

Warnings and Precautions, deleted latex warning 12/2020

----------INDICATIONS AND USAGE----------

- Adacel is a vaccine indicated for active booster immunization against tetanus, diphtheria and pertussis. Adacel is approved for use in persons 10 through 64 years of age. (1)

----------DOSAGE AND ADMINISTRATION----------

For intramuscular injection only.

- Each dose of Adacel is administered as a 0.5 mL injection. (2.1)
- For routine booster vaccination, a first dose of Adacel is administered 5 years or more after the last dose of Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) series or 5 years or more after vaccination with Tetanus and Diphtheria Toxoids Adsorbed (Td). A second dose of Adacel may be administered 8 years or more after the first dose with Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap).
- Adacel may be administered for tetanus prophylaxis for wound management. For management of a tetanus prone wound, a booster dose of Adacel may be administered if at least 5 years have elapsed since previous receipt of a tetanus toxoid containing vaccine. (2.2)

----------DOSAGE FORMS AND STRENGTHS----------

- Single-dose vials and prefilled syringes containing a 0.5 mL suspension for injection. (3)

----------CONTRAINDICATIONS----------

- Severe allergic reaction (eg, anaphylaxis) to any component of Adacel or any other diphtheria toxoid, tetanus toxoid and pertussis antigen-containing vaccine. (4.1)
- Encephalopathy (eg, coma, decreased level of consciousness, prolonged seizures) within 7 days of administration of a previous pertussis antigen-containing vaccine. (4.2)

----------WARNINGS AND PRECAUTIONS----------

- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following a subsequent dose of Adacel vaccine. (5.3)
- Progressive or unstable neurologic conditions are reasons to defer Adacel vaccination. (5.4)
- Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine should not receive Adacel unless at least 10 years have elapsed since the last dose of a tetanus toxoid-containing vaccine. (5.5)
- Syncope (fainting) can occur in association with administration of injectable vaccines, including Adacel. Procedures should be in place to prevent falling injury and manage syncopal reactions. (5.7)

----------ADVERSE REACTIONS----------

- Following the first vaccination with Adacel, the most common solicited adverse reactions within 0-14 days of vaccination for Adolescents (11-17 years of age)/Adults (18-64 years of age) were:
  injection site pain (77.8%/65.7%), headache (43.7%/33.9%), body ache or muscle weakness (30.4%/21.9%), tiredness (30.2%/24.3%), injection site swelling (20.9%/21.0%), and injection site erythema (20.8%/24.7%). (6.1)
- Following a second vaccination with Adacel, the most common solicited reactions occurring within 0-7 days of vaccination for Adults (18-64 years of age) were:
  injection site pain (87.1%), myalgia (58.1%), headache (41.4%), malaise (33.3%), injection site swelling (6.9%), and injection site erythema (6.4%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Pharmacovigilance Department, Sanofi Pasteur Inc., Discovery Drive, Swiftwater, PA 18370 at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or http://vaers.hhs.gov.

----------DRUG INTERACTIONS----------

- When Adacel was administered concomitantly with trivalent inactivated influenza vaccine (TIV) to adults 19-64 years of age, a lower antibody response was observed for pertactin antigen as compared to Adacel administered alone. (7.1, 14.4)
- Immunosuppressive therapies may reduce the immune response to Adacel. (7.2)
- Do not mix Adacel with any other vaccine in the same syringe or vial.

----------USE IN SPECIFIC POPULATIONS----------

- Pregnancy Exposure Registry: contact Sanofi Pasteur Inc. at 1-800-822-2463 (1-800-VACCINE). (8.1)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 12/2020

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

Adacel® is a vaccine indicated for active booster immunization against tetanus, diphtheria and pertussis. Adacel is approved for use in individuals 10 through 64 years of age.

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Preparation for Administration

Just before use, shake the vial or syringe well until a uniform, white, cloudy suspension results.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exist, the vaccine should not be administered.

Withdraw the 0.5 mL dose of vaccine from the single-dose vial using a sterile needle and syringe.

Adacel should not be combined through reconstitution or mixed with any other vaccine. Discard unused portion in vial.

2.2 Administration, Dose and Schedule

Adacel is administered as a single 0.5 mL intramuscular injection.

Routine Booster Vaccination

A first dose of Adacel is administered 5 years or more after the last dose of the Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) series or 5 years or more after a dose of Tetanus and Diphtheria Toxoids Adsorbed (Td). A second dose of Adacel may be administered 8 years or more after the first dose of Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap).

Wound Management

Adacel may be administered for tetanus prophylaxis for wound management. For management of a tetanus prone wound, a booster dose of Adacel may be administered if at least 5 years have elapsed since previous receipt of a tetanus toxoid containing vaccine.

3 DOSAGE FORMS AND STRENGTHS

Adacel is a suspension for injection available in 0.5 mL single-dose vials and prefilled syringes. [See *HOW SUPPLIED/STORAGE AND HANDLING (16).*]

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A severe allergic reaction (eg, anaphylaxis) after a previous dose of any tetanus toxoid, diphtheria toxoid or pertussis containing vaccine or any other component of this vaccine is a contraindication to administration of Adacel. [See *DESCRIPTION (11).*] Because of uncertainty as to which component of the vaccine may be responsible, none of the components should be

administered. Alternatively, such individuals may be referred to an allergist for evaluation if further immunizations are to be considered.

4.2 Encephalopathy

Encephalopathy (eg, coma, prolonged seizures, or decreased level of consciousness) within 7 days of a previous dose of a pertussis containing vaccine not attributable to another identifiable cause is a contraindication to administration of any pertussis containing vaccine, including Adacel.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

5.2 Guillain-Barré Syndrome and Brachial Neuritis

If Guillain-Barré syndrome occurred within 6 weeks of receipt of prior vaccine containing tetanus toxoid, the risk for Guillain-Barré syndrome may be increased following a dose of Adacel. A review by the Institute of Medicine found evidence for acceptance of a causal relation between tetanus toxoid and brachial neuritis. (1)

5.3 Progressive or Unstable Neurologic Disorders

Progressive or unstable neurologic conditions are reasons to defer Adacel. It is not known whether administration of Adacel to persons with an unstable or progressive neurologic disorder might hasten manifestations of the disorder or affect the prognosis. Administration of Adacel to persons with an unstable or progressive neurologic disorder may result in diagnostic confusion between manifestations of the underlying illness and possible adverse effects of vaccination.

5.4 Arthus-Type Hypersensitivity

Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine should not receive Adacel unless at least 10 years have elapsed since the last dose of a tetanus toxoid containing vaccine.

5.5 Altered Immunocompetence

If Adacel is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained. [See *DRUG INTERACTIONS (7.2).]*

5.6 Syncope

Syncope (fainting) can occur in association with administration of injectable vaccine, including Adacel. Procedures should be in place to prevent falling injury and manage syncopal reactions.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical

trials of another vaccine and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events. As with any vaccine, there is the possibility that broad use of Adacel could reveal adverse reactions not observed in clinical trials.

The safety of a first vaccination with Adacel was evaluated in 5 clinical studies. Three of the studies were conducted in the U.S. and 2 were conducted in Canada. Of the study participants, 86% were Caucasian, 8% Black, 3% Hispanic, 1% Asian and 2% of other ethnic origin. A total of 7,143 individuals 10 through 64 years of age inclusive (4,695 adolescents 10 through 17 years of age and 2,448 adults 18 through 64 years of age) received a single dose of Adacel.

U.S. Adolescent and Adult Study of a First Vaccination with Adacel (Td506)

Clinical study Td506 was a randomized, observer-blind, active-controlled trial that enrolled adolescents 11 through 17 years of age (Adacel N = 1,184; DECAVAC (Tetanus and Diphtheria Toxoids Adsorbed; manufactured by Sanofi Pasteur Inc., Swiftwater, PA) N = 792) and adults 18 through 64 years of age (Adacel N = 1,752; DECAVAC N = 573). Study participants had not received tetanus or diphtheria-containing vaccines within the previous 5 years. Solicited local and systemic reactions and unsolicited adverse events were monitored daily for 14 days post vaccination using a diary card. From days 14 to 28 post vaccination, information on adverse events necessitating a medical contact, such as a telephone call, visit to an emergency room, physician's office or hospitalization, was obtained via telephone interview or at an interim clinic visit. From days 28 to 6 months post vaccination, participants were monitored for unexpected visits to a physician's office or to an emergency room, onset of serious illness, and hospitalizations. Information regarding adverse events that occurred in the 6-month post vaccination time period was obtained from participants via telephone contact. At least 96% of participants completed the 6-month follow-up evaluation.

The frequency of selected solicited adverse reactions (erythema, swelling, pain and fever) occurring during days 0 to 14 following vaccination with Adacel or Td vaccine in adolescents 11 through 17 years of age and adults 18 through 64 years of age are presented in Table 1. Most of these reactions were reported at a similar frequency in recipients of both Adacel and Td vaccine. Pain at the injection site was the most common adverse reaction in 62.9% to 77.8% of all vaccinees. In addition, overall rates of pain were higher in adolescent recipients of Adacel compared to Td vaccine recipients. Rates of moderate and severe pain in adolescents did not significantly differ between the Adacel and Td vaccine groups. Among adults, the rates of pain after receipt of Adacel or Td vaccine did not significantly differ. Fever of 38°C and higher was uncommon, although in the adolescent age group it occurred significantly more frequently in Adacel recipients than Td vaccine recipients.

Table 1: Frequencies of Solicited Injection Site Reactions and Fever for Adolescents and Adults, Days 0-14, Following a First Vaccination with Adacel or Td Vaccine in Study Td506

Adverse Reactions*		Adolescents 11-17 years		Adults 18-64 years	
		Adacel N† = 1,170-1,175 (%)	Td‡ N† = 783-787 (%)	Adacel N† = 1,688-1,698 (%)	Td‡ N† = 551-561 (%)
Injection Site Pain	Any	77.8§	71.0	65.7	62.9
	Moderate¶	18.0	15.6	15.1	10.2
	Severe#	1.5	0.6	1.1	0.9
Injection Site Swelling	Any	20.9	18.3	21.0	17.3
	Moderate¶				
	1.0 to 3.4 cm	6.5	5.7	7.6	5.4
	Severe#				
	≥3.5 cm	6.4	5.5	5.8	5.5
	≥5 cm (2 inches)	2.8	3.6	3.2	2.7
Injection Site Erythema	Any	20.8	19.7	24.7	21.6
	Moderate¶				
	1.0 to 3.4 cm	5.9	4.6	8.0	8.4
	Severe#				
	≥3.5 cm	6.0	5.3	6.2	4.8
	≥5 cm (2 inches)	2.7	2.9	4.0	3.0
Fever	≥38.0°C (≥100.4°F)	5.0§	2.7	1.4	1.1
	≥38.8°C to ≤39.4°C (≥102.0°F to ≤103.0°F)	0.9	0.6	0.4	0.2
	≥39.5°C (≥103.1°F)	0.2	0.1	0.0	0.2

* The study sample size was designed to detect >10% differences between Adacel and Td vaccines for events of 'Any' intensity.

† N = number of participants with available data.

‡ Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur Inc., Swiftwater, PA.

§ Adacel did not meet the non-inferiority criterion for rates of 'Any' Pain in adolescents compared to Td vaccine rates (upper limit of the 95% CI on the difference for Adacel minus Td vaccine was 10.7% whereas the criterion was <10%). For 'Any' Fever the non-inferiority criteria was met, however, 'Any' Fever was statistically higher in adolescents receiving Adacel.

¶ Interfered with activities, but did not necessitate medical care or absenteeism.

Incapacitating, prevented the performance of usual activities, may have/or did necessitate medical care or absenteeism.

The frequency of other solicited adverse reactions (days 0-14) are presented in Table 2. The rates of these reactions following a first vaccination with Adacel were comparable with those observed with Td vaccine. Headache was the most frequent systemic reaction and was usually of mild to moderate intensity.

Table 2: Frequencies of Other Solicited Adverse Reactions for Adolescents and Adults, Days 0-14, Following a First Vaccination with Adacel or Td Vaccine in Study Td506

Adverse Reaction		Adolescents 11-17 years		Adults 18-64 years	
		Adacel N* = 1,174-1,175 (%)	Td† N* = 787 (%)	Adacel N* = 1,697-1,698 (%)	Td† N* = 560-561 (%)
Headache	Any	43.7	40.4	33.9	34.1
	Moderate‡	14.2	11.1	11.4	10.5
	Severe§	2.0	1.5	2.8	2.1
Body Ache or Muscle Weakness	Any	30.4	29.9	21.9	18.8
	Moderate‡	8.5	6.9	6.1	5.7
	Severe§	1.3	0.9	1.2	0.9
Tiredness	Any	30.2	27.3	24.3	20.7
	Moderate‡	9.8	7.5	6.9	6.1
	Severe§	1.2	1.0	1.3	0.5
Chills	Any	15.1	12.6	8.1	6.6
	Moderate‡	3.2	2.5	1.3	1.6
	Severe§	0.5	0.1	0.7	0.5
Sore and Swollen Joints	Any	11.3	11.7	9.1	7.0
	Moderate‡	2.6	2.5	2.5	2.1
	Severe§	0.3	0.1	0.5	0.5
Nausea	Any	13.3	12.3	9.2	7.9
	Moderate‡	3.2	3.2	2.5	1.8
	Severe§	1.0	0.6	0.8	0.5
Lymph Node Swelling	Any	6.6	5.3	6.5	4.1
	Moderate‡	1.0	0.5	1.2	0.5
	Severe§	0.1	0.0	0.1	0.0
Diarrhea	Any	10.3	10.2	10.3	11.3
	Moderate‡	1.9	2.0	2.2	2.7
	Severe§	0.3	0.0	0.5	0.5
Vomiting	Any	4.6	2.8	3.0	1.8
	Moderate‡	1.2	1.1	1.0	0.9
	Severe§	0.5	0.3	0.5	0.2
Rash	Any	2.7	2.0	2.0	2.3

* N = number of participants with available data.
† Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur Inc., Swiftwater, PA.
‡ Interfered with activities, but did not necessitate medical care or absenteeism.
§ Incapacitating, prevented the performance of usual activities, may have/or did necessitate medical care or absenteeism.

Injection site and systemic solicited reactions occurred at similar rates in Adacel and Td vaccine recipients in the 3 day post-vaccination period. Most injection site reactions occurred within the first 3 days after vaccination (with a mean duration of less than 3 days). The rates of unsolicited adverse events reported from days 14-28 post-vaccination were comparable between the two vaccine groups, as were the rates of unsolicited adverse events from day 28 through 6 months. There were no spontaneous reports of extensive limb swelling of the injected limb in study Td506, nor in the other three studies which also contributed to the safety database for Adacel.

Adult Study of a Second Vaccination with Adacel (Td537)

In a randomized, observer-blind, active-controlled, multi-center study (Td537), adults 18 through 64 years of age who had received a first dose of Adacel 8-12 years previously were enrolled and randomized to receive either Adacel (N = 1002) or a US licensed Td vaccine, TENIVAC (Tetanus and Diphtheria Toxoids Adsorbed; manufactured by Sanofi Pasteur, Limited) (N = 328). Subjects were recruited from the primary licensure study Td506 and the Canadian general public and had not received Td or Tdap vaccine since their initial Adacel dose. The demographic characteristics for study participants were similar for both vaccine groups. The mean ages were 28.9 years for the Adacel group and 29.2 years for the Td group. Overall, there were more female participants in both the Adacel group and Td group; 64.5% and 64.6%, respectively. In both vaccine groups, greater than 94% of subjects identified as white and 99% as non-Hispanic or Latino.

Safety data were collected from all participants who received the study vaccine (N = 999 for the Adacel group; N = 328 for the Td group). Solicited local and systemic reactions and unsolicited adverse events were monitored for 7 days post-vaccination using a diary card. Unsolicited adverse events were collected for approximately 28 days post-vaccination. Serious adverse events were collected throughout the study period (up to 6 months post-vaccination).

Solicited adverse reactions reported to occur during days 0-7 following vaccination are presented in Table 3.

Table 3: Frequencies of Solicited Adverse Reactions 0-7 Days Following a Second Vaccination with Adacel Compared to Td Vaccine in Study Td537 - Safety Analysis Set

Adverse Reaction		Adacel (N=999) (%)	Td Adsorbed* (N=328) (%)
Injection site pain	**Any**	87.1	87.4
	Grade 2†	28.5	31.4
	Grade 3‡	3.6	2.8
Injection site erythema	**Any**	6.4	5.5
	Grade 2 (≥51 to ≤100 mm)	2.1	2.8
	Grade 3 (>100 mm)	0.2	0.0
Injection site swelling	**Any**	6.9	8.0
	Grade 2 (≥51 to ≤100 mm)	2.4	2.2
	Grade 3 (>100 mm)	0.3	0.0
Fever	**Any**	0.9	1.8
	Grade 2 (≥38.5°C to ≤38.9°C or ≥101.2°F to ≤102.0°F	0.3	0.6
	Grade 3 (≥102.1°F)	0.2	0.3
Headache	**Any**	41.4	39.1
	Grade 2†	12.4	10.5
	Grade 3‡	2.6	4.0
Malaise	**Any**	33.3	30.8
	Grade 2†	9.3	9.8
	Grade 3‡	3.0	3.7
Myalgia	**Any**	58.1	58.2
	Grade 2†	18.7	16.9
	Grade 3‡	3.0	3.1

N = number of participants with available data

* Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur Limited, Toronto, Ontario, Canada.

† Some interference with activity

‡ Significant; prevents daily activity

Adult Study of a Second Vaccination with Adacel (Td518)

Study Td518 was a descriptive, open-label, post-marketing, multi-center study evaluating the safety of Adacel readministration in adults 5 years following a previous dose of Adacel. The mean age of subjects was 31.7 years, there were more females (52.2%) than males (47.8%) and 89.9% of subjects were Caucasian. Solicited adverse reactions were collected for 14 days following vaccination. SAEs were monitored for 6 months following vaccination. A total of 545 subjects 16-69 years of age were enrolled. All participants in this study received a first dose of Adacel vaccine as part of Sanofi Pasteur studies Td501, Td502, or Td505. Approximately 90% of the participants had at least one solicited injection site reaction. The most frequently reported injection site reactions were pain in 87.6% of subjects, followed by erythema/redness in 28.6%, and swelling in 25.6%. Approximately 77% of the participants had at least one solicited systemic reaction. The most frequently reported solicited systemic adverse reactions in subjects who received a second dose of Adacel were myalgia (61%), followed by headache (53.2%), malaise (38.2%), and fever (6.5%).

Injection Site and Systemic Reactions Following Adacel Given Concomitantly with Hepatitis B Vaccine

In the concomitant vaccination study with Adacel (first vaccination) and Hepatitis B vaccine [Recombivax HB] (Td501) [See *CLINICAL STUDIES (14)*], injection site and systemic adverse events were monitored daily for 14 days post-vaccination using a diary card. Injection site adverse events were only monitored at site/arm of Adacel administration. Unsolicited reactions (including immediate reactions, serious adverse events and events that elicited seeking medical attention) were collected at a clinic visit or via telephone interview for the duration of the trial, ie, up to 6 months post-vaccination.

The rates reported for fever and injection site pain (at the Adacel administration site) were similar when Adacel and Hepatitis B vaccine were given concurrently or separately. However, the rates of injection site erythema (23.4% for concomitant vaccination and 21.4% for separate administration) and swelling (23.9% for concomitant vaccination and 17.9% for separate administration) at the Adacel administration site were increased when coadministered. Swollen and/or sore joints were reported by 22.5% for concomitant vaccination and 17.9% for separate administration. The rates of generalized body aches in the individuals who reported swollen and/or sore joints were 86.7% for concomitant vaccination and 72.2% for separate administration. Most joint complaints were mild in intensity with a mean duration of 1.8 days. The incidence of other solicited and unsolicited adverse events were not different between the 2 study groups.

Injection Site and Systemic Reactions Following Adacel Given Concomitantly with Trivalent Inactivated Influenza Vaccine (TIV)

In the concomitant vaccination study with Adacel (first vaccination) and trivalent inactivated influenza vaccine [Fluzone] (Td502) [See *CLINICAL STUDIES (14)*], injection site and systemic adverse events were monitored for 14 days post-vaccination using a diary card. All unsolicited reactions occurring through day 14 were collected. From day 14 to the end of the trial, ie, up to 84 days, only events that elicited seeking medical attention were collected.

The rates of fever and injection site erythema and swelling were similar for recipients of concurrent and separate administration of Adacel and TIV. However, pain at the Adacel injection site occurred at statistically higher rates following concurrent administration (66.6%) versus separate administration (60.8%). The rates of sore and/or swollen joints were 13% for concurrent administration and 9% for separate administration. Most joint complaints were mild in intensity with a mean duration of 2.0 days. The incidence of other solicited and unsolicited adverse events was similar between the 2 study groups.

Additional Studies

In an additional study (Td505), 1,806 adolescents 11 through 17 years of age received Adacel (first vaccination) as part of the lot consistency study used to support Adacel licensure. This study was a randomized, double-blind, multi-center trial designed to assess lot consistency as measured by the safety and immunogenicity of 3 lots of Adacel when given as a booster dose to adolescents 11 through 17 years of age inclusive. Local and systemic adverse events were monitored for 14 days post-vaccination using a diary card. Unsolicited adverse events and serious adverse events were collected for 28 days post-vaccination. Pain was the most frequently reported local adverse event occurring in approximately 80% of all participants. Headache was

the most frequently reported systemic event occurring in approximately 44% of all participants. Sore and/or swollen joints were reported by approximately 14% of participants. Most joint complaints were mild in intensity with a mean duration of 2.0 days.

An additional 962 adolescents and adults received Adacel in three supportive Canadian studies (TC9704, Td9707 and TD9805) used as the basis for licensure in other countries. Within these clinical trials, the rates of local and systemic reactions following the first vaccination with Adacel were similar to those reported in the four principal trials in the U.S. with the exception of a higher rate (86%) of adults experiencing "any" local injection site pain. The rate of severe pain (0.8%), however, was comparable to the rates reported in four principal trials conducted in the US. There was one spontaneous report of whole-arm swelling of the injected limb among the 277 Td vaccine recipients, and two spontaneous reports among the 962 Adacel recipients in the supportive Canadian studies.

An additional study (Td519) enrolled 1,302 individuals in an open label, two-arm, multicenter trial (651 participants in each group) to evaluate the safety and immunogenicity of a first vaccination with Adacel administered to persons 10 to <11 years of age compared to persons 11 to <12 years of age. Immediate reactions were monitored for 20 minutes post-vaccination. Solicited local and systemic adverse events were monitored for 7 days post-vaccination using a diary card. Unsolicited and serious adverse events were collected for approximately 30 days post-vaccination. Similar rates of immediate, solicited and unsolicited adverse reactions were reported in each of the two age cohorts. One serious adverse event, not related to vaccination, was reported in the younger age group.

Serious Adverse Events

Throughout the 6-month follow-up period following a first vaccination with Adacel in study Td506, SAEs were reported in 1.5% of Adacel recipients and in 1.4% of Td vaccine recipients. Two SAEs in adults were neuropathic events that occurred within 28 days of Adacel administration; one severe migraine with unilateral facial paralysis and one diagnosis of nerve compression in neck and left arm. Similar or lower rates of serious adverse events were reported in the other trials following a first vaccination with Adacel in participants up to 64 years of age and no additional neuropathic events were reported.

In study Td537 when a second vaccination of Adacel was administered 8-12 years following the initial vaccination of Adacel, a total of 8 participants (0.8%) in the Adacel group and 1 participant (0.3%) in the Td group reported SAEs during the 6-month follow-up period. All SAEs were considered by the investigator to be unrelated to the study vaccine.

In study Td518, seven participants experienced an SAE, all of which were considered by the investigator to be unrelated to the study vaccine.

6.2 Postmarketing Experience

The following adverse events of Adacel have been spontaneously reported in the US and other countries. Because these events are reported voluntarily from a population of uncertain size, it may not be possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

The following adverse events were included based on one or more of the following factors: severity, frequency of reporting, or strength of evidence for a causal relationship to Adacel.

- ***Immune system disorders***
  Anaphylactic reaction, hypersensitivity reaction (angioedema, edema, rash, hypotension)
- ***Nervous system disorders***
  Paresthesia, hypoesthesia, Guillain-Barré syndrome, brachial neuritis, facial palsy, convulsion, syncope, myelitis
- ***Cardiac disorders***
  Myocarditis
- ***Skin and subcutaneous tissue disorders***
  Pruritus, urticaria
- ***Musculoskeletal and connective tissue disorders***
  Myositis, muscle spasm
- ***General disorders and administration site conditions***
  Large injection site reactions (>50 mm), extensive limb swelling from the injection site beyond one or both joints
  Injection site bruising, sterile abscess, Arthus hypersensitivity

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

When Adacel is administered concomitantly with other injectable vaccines or Tetanus Immune Globulin, they should be given with separate syringes and at different injection sites. Adacel should not be mixed with any other vaccine in the same syringe or vial.

Trivalent Inactivated Influenza Vaccine (TIV)

In a clinical study Adacel (first vaccination) was administered concomitantly with a US-licensed trivalent inactivated influenza vaccine (TIV). [See *ADVERSE REACTIONS (6.1)* and *CLINICAL STUDIES (14).*]

No interference in tetanus and diphtheria seroprotection rates and responses to influenza vaccine, detoxified pertussis toxin (PT), fimbriae types 2 and 3 (FIM) or filamentous hemagglutinin (FHA) were observed when Adacel vaccine was administered concomitantly with TIV compared to separate administration. A lower pertactin (PRN) GMC was observed when Adacel was administered concomitantly with TIV compared to separate administration.

7.2 Immunosuppressive Treatments

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to vaccines. [See *WARNINGS AND PRECAUTIONS (5.6).*]

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to Adacel during pregnancy. Women who receive Adacel during pregnancy are encouraged to contact directly, or have their healthcare professional contact, Sanofi Pasteur Inc. at 1-800-822-2463 (1-800-VACCINE).

Risk Summary

All pregnancies have a risk of birth defect, loss or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no adequate and well-controlled studies of Adacel administration in pregnant women in the U.S.

Available data suggest the rates of major birth defects and miscarriage in women who receive Adacel within 30 days prior to pregnancy or during pregnancy are consistent with estimated background rates. *(*See *Data)*

Two developmental toxicity studies were performed in female rabbits given 0.5 mL (a single human dose) of Adacel twice prior and during gestation. The studies revealed no evidence of harm to the fetus due to Adacel. *(*See *Data)*

Data

Human Data

An assessment of data from the ongoing pregnancy registry over 12 years (2005-2017) included 1518 reports of exposure to Adacel vaccine from 30 days before or at any time during pregnancy. Of these reports, 543 had known pregnancy outcomes available and were enrolled in the registry prior to the outcomes being known. Among the 543 pregnancies with known outcomes, the timing of Adacel vaccination was not known for 126 of the pregnancies.

Of the prospectively followed pregnancies for whom the timing of Adacel vaccination was known, 374 women received Adacel during the 30 days prior to conception through the second trimester. Outcomes among these prospectively followed pregnancies included 5 infants with major birth defects and 25 cases of miscarriage.

Animal Data

The effect of Adacel on embryo-fetal and pre-weaning development was evaluated in two developmental toxicity studies in female rabbits. Animals were administered 0.5 mL (a single human dose) of Adacel twice prior to gestation, during the period of organogenesis (gestation day 6) and later during pregnancy on gestation day 29. No adverse effects on pregnancy, parturition, lactation, embryo-fetal or pre-weaning development were observed. There were no vaccine related fetal malformations or other evidence of teratogenesis noted in this study.

8.2 Lactation

Risk Summary

It is not known whether Adacel vaccine components are excreted in human milk. Data are not available to assess the effect of administration of Adacel on breast-fed infants or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother’s clinical need for Adacel and any potential adverse effects on the breastfed child from Adacel or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Adacel is not approved for individuals less than 10 years of age. Safety and effectiveness of Adacel in persons less than 10 years of age in the U.S. have not been established.

8.5 Geriatric Use

Adacel is not approved for use in individuals 65 years of age and older.

In a clinical study, individuals 65 years of age and older received a single dose of Adacel. Based on prespecified criteria, persons 65 years of age and older who received a dose of Adacel had lower geometric mean concentrations of antibodies to PT, PRN and FIM when compared to infants who had received a primary series of DAPTACEL®, Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP). [See *CLINICAL STUDIES (14)* for description of DAPTACEL.]

11 DESCRIPTION

Adacel is a sterile isotonic suspension of tetanus and diphtheria toxoids and pertussis antigens adsorbed on aluminum phosphate, for intramuscular injection.

Each 0.5 mL dose contains 5 Lf tetanus toxoid (T), 2 Lf diphtheria toxoid (d), and acellular pertussis antigens [2.5 mcg detoxified pertussis toxin (PT), 5 mcg filamentous hemagglutinin (FHA), 3 mcg pertactin (PRN), 5 mcg fimbriae types 2 and 3 (FIM)]. Other ingredients per 0.5 mL dose include 1.5 mg aluminum phosphate (0.33 mg aluminum) as the adjuvant, ≤5 mcg residual formaldehyde, <50 ng residual glutaraldehyde and 3.3 mg (0.6% v/v) 2-phenoxyethanol (not as a preservative). The antigens are the same as those in DAPTACEL; however, Adacel is formulated with reduced quantities of diphtheria and detoxified PT.

The acellular pertussis vaccine components are produced from *Bordetella pertussis* cultures grown in Stainer-Scholte medium (2) modified by the addition of casamino acids and dimethyl-beta-cyclodextrin. PT, FHA and PRN are isolated separately from the supernatant culture medium. FIM are extracted and copurified from the bacterial cells. The pertussis antigens are purified by sequential filtration, salt-precipitation, ultrafiltration and chromatography. PT is detoxified with glutaraldehyde, FHA is treated with formaldehyde, and the residual aldehydes are removed by ultrafiltration. The individual antigens are adsorbed onto aluminum phosphate.

The tetanus toxin is produced from *Clostridium tetani* grown in modified Mueller-Miller casamino acid medium without beef heart infusion. (3) Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. *Corynebacterium diphtheriae* is grown in modified Mueller's growth medium. (4) After purification by ammonium sulfate fractionation, diphtheria toxin is detoxified with formaldehyde and diafiltered.

The adsorbed diphtheria, tetanus and acellular pertussis components are combined with aluminum phosphate (as adjuvant), 2-phenoxyethanol (not as a preservative) and water for injection. Adacel does not contain a preservative.

In the guinea pig potency test, the tetanus component induces at least 2 neutralizing units/mL of serum and the diphtheria component induces at least 0.5 neutralizing units/mL of serum. The potency of the acellular pertussis vaccine components is evaluated by the antibody response of

immunized mice to detoxified PT, FHA, PRN and FIM as measured by enzyme-linked immunosorbent assay (ELISA).

Diphtheria and tetanus toxoids are individually adsorbed onto aluminum phosphate.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Tetanus

Tetanus is a disease manifested primarily by neuromuscular dysfunction caused by a potent exotoxin released by *C tetani*.

Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assay is considered the minimum protective level. (5) (6)

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection. Antitoxin levels of at least 0.1 IU/mL are generally regarded as protective. (5) Levels of 1.0 IU/mL have been associated with long-term protection. (7)

Pertussis

Pertussis (whooping cough) is a respiratory disease caused by *B pertussis*. This Gram-negative coccobacillus produces a variety of biologically active components, though their role in either the pathogenesis of, or immunity to, pertussis has not been clearly defined.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Adacel has not been evaluated for carcinogenic or mutagenic potential, or impairment of male fertility.

14 CLINICAL STUDIES

The effectiveness of the tetanus toxoid and diphtheria toxoid used in Adacel was based on the immune response to these antigens compared to a US licensed Tetanus and Diphtheria Toxoids Adsorbed For Adult Use (Td) vaccine manufactured by Sanofi Pasteur Inc., Swiftwater, PA. The primary measures for immune response to the diphtheria and tetanus toxoids were the percentage of participants attaining an antibody level of at least 0.1 IU/mL.

The effectiveness of the pertussis antigens used in Adacel was evaluated based on a comparison of pertussis antibody levels achieved in recipients of Adacel with those obtained in infants after three or four doses of DAPTACEL. For the first dose of Adacel, the comparisons were to infants who received three doses of DAPTACEL in the Sweden I Efficacy trial. For the second dose of Adacel, for the evaluation of FHA, PRN, and FIM antibody levels, the comparisons were to infants who received three doses of DAPTACEL in the Sweden I Efficacy trial; for evaluation of PT antibody levels, the comparison was to infants who received four doses of DAPTACEL in a

US safety and immunogenicity study (Study M5A10). In the Sweden I Efficacy Trial, three doses of DAPTACEL vaccine were shown to confer a protective efficacy of 84.9% (95% CI: 80.1%, 88.6%) against WHO defined pertussis (21 days of paroxysmal cough with laboratory-confirmed *B pertussis* infection or epidemiological link to a confirmed case). The protective efficacy against mild pertussis (defined as at least one day of cough with laboratory-confirmed *B pertussis* infection) was 77.9% (95% CI: 72.6%, 82.2%). (8)

In addition, the ability of Adacel to elicit a booster response (defined as rise in antibody concentration after vaccination) to the tetanus, diphtheria and pertussis antigens following vaccination was evaluated.

14.1 Immunological Evaluation in Adolescents and Adults, 11 through 64 Years of Age Following a First Vaccination with Adacel

Study Td506 was a comparative, multi-center, randomized, observer-blind, controlled trial which enrolled 4,480 participants; 2,053 adolescents (11-17 years of age) and 2,427 adults (18-64 years of age). Enrollment was stratified by age to ensure adequate representation across the entire age range. Participants had not received a tetanus or diphtheria toxoid containing vaccine within the previous 5 years. After enrollment participants were randomized to receive one dose of either Adacel or Td vaccine. A total of 4,461 randomized participants were vaccinated. The per-protocol immunogenicity subset included 1,270 Adacel recipients and 1,026 Td vaccine recipients. Sera were obtained before and approximately 35 days after vaccination. [Blinding procedures for safety assessments are described in *ADVERSE REACTIONS (6).*]

Demographic characteristics were similar within age groups and between the vaccine groups. A total of 76% of the adolescents and 1.1% of the adults reported a history of receiving 5 previous doses of diphtheria-tetanus-pertussis containing vaccines. Anti-tetanus and anti-diphtheria seroprotection rates (≥0.1 IU/mL) and booster response rates were comparable between Adacel and Td vaccines. (See Table 4 and Table 5.) Adacel induced pertussis antibody levels that were non-inferior to those of Swedish infants who received three doses of DAPTACEL vaccine (Sweden I Efficacy Study). (See Table 6.) Acceptable booster responses to each of the pertussis antigens were also demonstrated, ie, the percentage of participants with a booster response exceeded the predefined lower limit. (See Table 7.)

Table 4: Pre-vaccination and Post-vaccination Antibody Responses and Booster Response Rates to Tetanus Toxoid Following A First Vaccination with Adacel Vaccine as Compared to Td Vaccine in Adolescents and Adults 11 through 64 Years of Age (Td506)

			Anti-Tetanus toxoid (IU/mL)				
			Pre-vaccination		1 Month Post-vaccination		
Age Group (years)	**Vaccine**	**N***	**% ≥0.10 (95% CI)**	**% ≥1.0 (95% CI)**	**% ≥0.10 (95% CI)**	**% ≥1.0 (95% CI)**	**% Booster† (95% CI)**
11-17	**Adacel**	**527**	99.6 (98.6, 100.0)	44.6 (40.3, 49.0)	100.0‡ (99.3, 100.0)	99.6§ (98.6, 100.0)	91.7‡ (89.0, 93.9)
	Td¶	**516**	99.2 (98.0, 99.8)	43.8 (39.5, 48.2)	100.0 (99.3, 100.0)	99.4 (98.3, 99.9)	91.3 (88.5, 93.6)
18-64	**Adacel**	**742-743**	97.3 (95.9, 98.3)	72.9 (69.6, 76.1)	100.0‡ (99.5, 100.0)	97.8§ (96.5, 98.8)	63.1‡ (59.5, 66.6)
	Td¶	**509**	95.9 (93.8, 97.4)	70.3 (66.2, 74.3)	99.8 (98.9, 100.0)	98.2 (96.7, 99.2)	66.8 (62.5, 70.9)

* N = number of participants in the per-protocol population with available data.

† Booster response is defined as: A 4-fold rise in antibody concentration, if the pre-vaccination concentration was equal to or below the cut-off value and a 2-fold rise in antibody concentration if the pre-vaccination concentration was above the cut-off value. The cut-off value for tetanus was 2.7 IU/mL.

‡ Seroprotection rates at ≥0.10 IU/mL and booster response rates to Adacel were non-inferior to Td vaccine (upper limit of the 95% CI on the difference for Td vaccine minus Adacel <10%).

§ Seroprotection rates at ≥1.0 IU/mL were not prospectively defined as a primary endpoint.

¶ Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur Inc., Swiftwater, PA.

Table 5: Pre-vaccination and Post-vaccination Antibody Responses and Booster Response Rates to Diphtheria Toxoid Following A First Vaccination with Adacel as Compared to Td Vaccine in Adolescents and Adults 11 through 64 Years of Age (Td506)

			Anti-Diphtheria toxin (IU/mL)				
			Pre-vaccination		1 Month Post-vaccination		
Age Group (years)	**Vaccine**	**N***	**% ≥0.10 (95% CI)**	**% ≥1.0 (95% CI)**	**% ≥0.10 (95% CI)**	**% ≥1.0 (95% CI)**	**% Booster† (95% CI)**
11-17	**Adacel**	**527**	72.5 (68.5, 76.3)	15.7 (12.7, 19.1)	99.8‡ (98.9, 100.0)	98.7§ (97.3, 99.5)	95.1‡ (92.9, 96.8)
	Td¶	**515-516**	70.7 (66.5, 74.6)	17.3 (14.1, 20.8)	99.8 (98.9, 100.0)	98.4 (97.0, 99.3)	95.0 (92.7, 96.7)
18-64	**Adacel**	**739-741**	62.6 (59.0, 66.1)	14.3 (11.9, 17.0)	94.1‡ (92.1, 95.7)	78.0§ (74.8, 80.9)	87.4‡ (84.8, 89.7)
	Td¶	**506-507**	63.3 (59.0, 67.5)	16.0 (12.9, 19.5)	95.1 (92.8, 96.8)	79.9 (76.1, 83.3)	83.4 (79.9, 86.5)

* N = number of participants in the per-protocol population with available data.

† Booster response is defined as: A 4-fold rise in antibody concentration, if the pre-vaccination concentration was equal to or below the cut-off value and a 2-fold rise in antibody concentration if the pre-vaccination concentration was above the cut-off value. The cut-off value for diphtheria was 2.56 IU/mL.

‡ Seroprotection rates at ≥0.10 IU/mL and booster response rates to Adacel were non-inferior to Td vaccine (upper limit of the 95% CI on the difference for Td vaccine minus Adacel <10%).

§ Seroprotection rates at ≥1.0 IU/mL were not prospectively defined as a primary endpoint.

¶ Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur Inc., Swiftwater, PA.

Table 6: Ratio of Pertussis Antibody Geometric Mean Concentrations (GMCs)* Observed One Month Following A First Vaccination with Adacel in Adolescents and Adults 11 through 64 Years of Age Compared with Those Observed in Infants One Month following Vaccination at 2,4 and 6 Months of Age in the Efficacy Trial with DAPTACEL (Sweden I Efficacy Study)

	Adolescents 11-17 Years of Age	Adults 18-64 Years of Age
	Adacel[†]/DAPTACEL[‡] GMC Ratio (95% CIs)	Adacel[§]/DAPTACEL[‡] GMC Ratio (95% CIs)
Anti-PT	3.6 (2.8, 4.5)[¶]	2.1 (1.6, 2.7)[¶]
Anti-FHA	5.4 (4.5, 6.5)[¶]	4.8 (3.9, 5.9)[¶]
Anti-PRN	3.2 (2.5, 4.1)[¶]	3.2 (2.3, 4.4)[¶]
Anti-FIM	5.3 (3.9, 7.1)[¶]	2.5 (1.8, 3.5)[¶]

* Antibody GMCs, measured in arbitrary ELISA units were calculated separately for infants, adolescents and adults.

† N = 524 to 526, number of adolescents in the per-protocol population with available data for Adacel.

‡ N = 80, number of infants who received DAPTACEL with available data post dose 3 (Sweden Efficacy I).

§ N = 741, number of adults in the per-protocol population with available data for Adacel.

¶ GMC following Adacel was non-inferior to GMC following DAPTACEL (lower limit of 95% CI on the ratio of GMC for Adacel divided by DAPTACEL >0.67).

Table 7: Booster Response Rates to the Pertussis Antigens Observed One Month Following a First Vaccination with Adacel in Adolescents and Adults 11 through 64 Years of Age

	Adolescents 11-17 Years of Age		Adults 18-64 Years of Age		Predefined Acceptable Rates* %[†]
	N[‡]	% (95% CI)	N[‡]	% (95% CI)	
Anti-PT	524	92.0 (89.3, 94.2)	739	84.4 (81.6, 87.0)	81.2
Anti-FHA	526	85.6 (82.3, 88.4)	739	82.7 (79.8, 85.3)	77.6
Anti-PRN	525	94.5 (92.2, 96.3)	739	93.8 (91.8, 95.4)	86.4
Anti-FIM	526	94.9 (92.6, 96.6)	739	85.9 (83.2, 88.4)	82.4

* The acceptable response rate for each antigen was defined as the lower limit of the 95% CI for the rate being no more than 10% lower than the response rate observed in previous clinical trials.

† A booster response for each antigen was defined as a 4-fold rise in antibody concentration if the pre-vaccination concentration was equal to or below the cut-off value and a 2-fold rise in antibody concentration if the pre-vaccination concentration was above the cut-off value. The cut-off values for pertussis antigens were established based on antibody data from both adolescents and adults in previous clinical trials. The cut-off values were 85 EU/mL for PT, 170 EU/mL for FHA, 115 EU/mL for PRN and 285 EU/mL for FIM.

‡ N = number of participants in the per-protocol population with available data.

Study Td519 assessed the comparative immunogenicity of a first vaccination with Adacel administered to adolescents (10 to <11 years of age and 11 to <12 years of age) [See *ADVERSE REACTIONS (6.1)*.] In this study non-inferiority was demonstrated for booster responses to tetanus and diphtheria toxoids, GMCs to the pertussis antigens (PT, FHA, PRN and FIM) and booster responses to the pertussis antigens PT, FHA and PRN. For FIM, non-inferiority was not demonstrated as the lower bound of the 95% CI of the difference in booster response rates (-5.96%) did not meet the predefined criterion (>-5% when the booster response in the older age group was >95%).

14.2 Immunological Evaluation in Adults, 18 through 64 Years of Age Following a Second Vaccination with Adacel

In study Td537 [See *ADVERSE REACTIONS (6.1)*.], subjects 18 to 64 years of age who had received a dose of Adacel 8-12 years previously, were randomized to receive a second dose of Adacel or Td vaccine (Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur, Limited). Blood samples for immunogenicity analyses were obtained from participants pre-vaccination and approximately 28 days post-vaccination. The per-protocol analysis set was used for all immunogenicity analyses, and included 948 participants in the Adacel group and 317 participants in the Td control vaccine group. Of the study participants, 35% were male. Of subjects who reported a racial/ethnic demographic, 95% were Caucasian, 2% Black, 0.5% American Indian or Alaska native, 1% Asian and 1.5% were of mixed or other origin.

A tetanus antitoxoid level of ≥0.1 IU/mL, measured by the ELISA used in this study was considered protective. An anti-diphtheria anti-toxin level of ≥0.1 IU/mL was considered protective. Pre-vaccination and post-vaccination seroprotection rates and booster response rates are presented in Table 8.

Table 8: Pre-vaccination and Post-vaccination Seroprotection Rates and Booster Response Rates to Tetanus Toxoid and Diphtheria Toxoid Following a Second Vaccination with Adacel Compared to Td Vaccine in Persons 18 through 64 Years of Age, Per Protocol Analysis Set

	Vaccine	N*	Pre-vaccination		1 month post-vaccination		
			≥0.1 IU/mL (95% CI)	≥1.0 IU/mL (95% CI)	≥0.1 IU/mL (95% CI)[†]	≥1.0 IU/mL (95% CI)[‡]	%Booster[§] (95% CI)
Anti-Tetanus Toxoid (ELISA - IU/mL)	Adacel	944-948	97.2 (96.0; 98.2)	62.3 (59.1; 65.4)	100.0 (99.6; 100.0)	99.9 (99.4; 100.0)	74.5[¶ #] (71.6; 77.2)
	Td[þ] Adsorbed	315-317	96.5 (93.8; 98.2)	63.8 (58.2; 69.1)	100.0 (98.8; 100.0)	100.0 (98.8; 100.0)	81.6[¶ #] (76.9; 85.7)
Anti-Diphtheria Toxin (ELISA - IU/mL)	Adacel	945-948	84.7 (82.2; 86.9)	29.1 (26.2; 32.1)	99.8 (99.2; 100.0)	94.9 (93.3; 96.2)	83.2[¶] (80.6; 85.5)
	Td[þ] Adsorbed	315-317	83.8 (79.3; 87.7)	29.8 (24.8; 35.2)	99.4 (97.7; 99.9)	94.0 (90.8; 96.4)	84.1[¶] (79.6; 88.0)

* N = number of participants in the per-protocol population with available data.

† Seroprotection rates at ≥0.10 IU/mL for Adacel were non-inferior to Td for diphtheria toxin and tetanus toxoid (upper limit of the 95% CI on the difference for Td vaccine minus Adacel <10%).

‡ Seroprotection rates at ≥1.0 IU/mL were not prospectively defined as a primary or secondary endpoint.

§ Booster response is defined as a minimum rise in antibody concentration from pre to post-vaccination. The minimum rise is at least 2 times if the pre-vaccination concentration is above the cutoff value, or at least 4 times if it is at or below the cutoff value. The cutoff values for to tetanus and diphtheria are 2.7 IU/mL and 2.56 IU/mL, respectively.

¶ n/M: defines the number n of participants with booster response / the number M of subjects with available data to evaluate booster response. There were (n/M) 703/944, 257/315, 786/945 and 265/315 for Adacel/Tetanus, Td Adsorbed/Tetanus, Adacel/Diphtheria, and Td Adsorbed/Diphtheria, respectively.

Booster response rates for tetanus toxoid in Adacel did not meet the pre-specified non-inferiority criteria.

þ Tetanus and Diphtheria Toxoids Adsorbed manufactured by Sanofi Pasteur Limited, Toronto, Ontario, Canada.

For all pertussis antigens (PT, FHA, PRN and FIM), post-vaccination anti-pertussis GMCs in the Adacel group were non-inferior to GMCs induced by 3 or 4 doses of DAPTACEL in historical studies as are presented in Table 9.

Table 9: Ratio of Pertussis Antibody Geometric Mean Concentrations (GMCs) Observed One Month Following a Second Vaccination with Adacel in Adults Compared with Those Observed in Infants One Month following Vaccination with 3 or 4 Doses of DAPTACEL (Per-Protocol Analysis Set)

Antigen	Adacel			DAPTACEL*			Adacel/DAPTACEL*	
	N	GMC (EU/mL)	(95% CI)	N	GMC (EU/mL)	(95% CI)	GMC Ratio	(95% CI)†
PT	935	102	(94.9; 110)	366	98.1	(90.9; 106)	1.04	(0.92; 1.18)
FHA	948	209	(200; 217)	80	39.9	(34.6; 46.1)	5.22	(4.51; 6.05)
PRN	948	318	(302; 334)	80	108	(91.4; 128)	2.94	(2.46; 3.51)
FIM	948	745	(711; 781)	80	341	(270; 431)	2.18	(1.84; 2.60)

* DAPTACEL: Historical controls who received DAPTACEL in Sanofi Pasteur studies. PT antibody GMC were compared to GMC following 4 doses of DAPTACEL in M5A10. FHA, PRN and FIM antibody GMCs were compared to GMCs following 3 doses of Daptacel in the Sweden I Efficacy trial.

† For each pertussis antigen, non-inferiority was demonstrated if the lower limit of the 2-sided 95% CI of the GMC ratio (Adacel divided by the historical control) was > 0.66.

Booster response rates for PT and FHA were non-inferior in Adacel participants compared to pre-specified criteria for booster response rates, but non-inferiority was not achieved for PRN and FIM booster response rates (See Table 10).

Table 1: Comparison of Booster Response* Rates for Pertussis Antigens Following a Second Vaccination with Adacel (Per-Protocol Analysis Set)

	Adacel (N=948)		Pre-specified criteria for Booster Response Rates†	Adacel minus Pre-specified Booster Response Rates†	
Antigen	**n/M**	**% (95% CI)**	**%**	**Difference (%)**	**(95% CI)‡**
PT	693/894	77.5 (74.6; 80.2)	61.4	16.12	(13.27; 18.73)
FHA	651/945	68.9 (65.8; 71.8)	73.1	-4.21	(-7.23; -1.34)
PRN	617/945	65.3 (62.2; 68.3)	83.9	-18.61	(-21.7; -15.6)
FIM	537/945	56.8 (53.6; 60.0)	75.9	-19.07	(-22.3; -16.0)

N = number of subjects analyzed according to Per-Protocol Analysis Set

M = number of subjects with available data for the considered endpoint

n = number of subjects fulfilling the item listed in the first column

* Booster response is defined as a minimum rise in antibody concentration from pre to post-vaccination. The minimum rise is at least 2-fold if the pre-vaccination concentration is above the cutoff value, or at least 4-fold if it is at or below the cutoff value. The cutoff values for Study Td537 for the pertussis antigens are: 93 EU/mL for PT, 170 EU/mL for FHA, 115 EU/mL for PRN, and 285 EU/mL for FIM.

† Pre-specified criteria for booster response rates were derived from participants 21 to <65 years of age who received Adacel in Study Td506.

‡ Non-inferiority in booster response rate for each pertussis antigen was demonstrated if the lower limit of the 2-sided 95% CI of the difference of booster response rates between participants receiving Adacel in Study Td537

and expected booster response rates based on Study Td506 was >-10%.

14.3 Concomitant Hepatitis B Vaccine Administration

The concomitant use of Adacel (first vaccination) and hepatitis B (Hep B) vaccine (Recombivax HB®, 10 mcg per dose using a two-dose regimen, manufactured by Merck and Co., Inc.) was evaluated in a multi-center, open-labeled, randomized, controlled study that enrolled 410 adolescents, 11 through 14 years of age inclusive. One group received Adacel and Hep B vaccines concurrently (N = 206). The other group (N = 204) received Adacel at the first visit, then 4-6 weeks later received Hep B vaccine. The second dose of Hep B vaccine was given 4-6 weeks after the first dose. Serum samples were obtained prior to and 4-6 weeks after Adacel administration, as well as 4-6 weeks after the 2nd dose of Hep B for all participants. No interference was observed in the immune responses to any of the vaccine antigens when Adacel and Hep B vaccines were given concurrently or separately. [See *ADVERSE REACTIONS (6.1)*.]

14.4 Concomitant Influenza Vaccine Administration

The concomitant use of Adacel (first vaccination) and trivalent inactivated influenza vaccine (TIV, Fluzone®, manufactured by Sanofi Pasteur Inc., Swiftwater, PA) was evaluated in a multi-center, open-labeled, randomized, controlled study conducted in 720 adults, 19-64 years of age inclusive. In one group, participants received Adacel and TIV vaccines concurrently (N = 359). The other group received TIV at the first visit, then 4-6 weeks later received Adacel (N = 361). Sera were obtained prior to and 4-6 weeks after Adacel, as well as 4-6 weeks after the TIV. The immune responses were comparable for concurrent and separate administration of Adacel and TIV vaccines for diphtheria (percent of participants with seroprotective concentration ≥0.10 IU/mL and booster responses), tetanus (percent of participants with seroprotective concentration ≥0.10 IU/mL), pertussis antigens (booster responses and GMCs except lower PRN GMC in the concomitant group, lower bound of the 90% CI was 0.61 and the prespecified criterion was ≥0.67) and influenza antigens (percent of participants with hemagglutination-inhibition [HI] antibody titer ≥1:40 IU/mL and ≥4-fold rise in HI titer). Although tetanus booster response rates were significantly lower in the group receiving the vaccines concurrently versus separately, greater than 98% of participants in both groups achieved seroprotective levels of ≥0.1 IU/mL. [See *ADVERSE REACTIONS (6.1)*.]

15 REFERENCES

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16 HOW SUPPLIED/STORAGE AND HANDLING

Syringe, without needle, single-dose – NDC 49281-400-89 (not made with natural rubber latex); in package of 5 syringes, NDC 49281-400-20.

Vial, single-dose – NDC 49281-400-58; in package of 5 vials; NDC 49281-400-05. The vial stopper is not made with natural rubber latex. Discard unused portion in vial.

Vial, single-dose – NDC 49281-400-58; in package of 10 vials; NDC 49281-400-10. The vial stopper is not made with natural rubber latex. Discard unused portion in vial.

Not all pack sizes may be marketed.

Adacel should be stored at 2°C to 8°C (35°F to 46°F). DO NOT FREEZE. Product which has been exposed to freezing should not be used. Do not use after expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

Before administration of Adacel, healthcare providers should inform the patient, parent or guardian of the benefits and risks of the vaccine and the importance of receiving recommended booster dose unless a contraindication to further immunization exists.

The healthcare provider should inform the patient, parent or guardian about the potential for adverse reactions that have been temporally associated with Adacel or other vaccines containing similar components. The healthcare provider should provide the Vaccine Information Statements (VISs) that are required by the National Childhood Vaccine Injury Act of 1986 to be given with each immunization. The patient, parent or guardian should be instructed to report any serious adverse reactions to their healthcare provider.

Pregnancy Exposure Registry

[See *USE IN SPECIFIC POPULATIONS (8.1)*.]

Manufactured by:
Sanofi Pasteur Limited
Toronto Ontario Canada

Distributed by:
Sanofi Pasteur Inc.
Swiftwater PA 18370 USA

Adacel® is a registered trademark of Sanofi, its affiliates, and its subsidiaries.

R13-1220 USA

SANOFI PASTEUR

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use BOOSTRIX safely and effectively. See full prescribing information for BOOSTRIX.

BOOSTRIX (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed) injectable suspension, for intramuscular use

Initial U.S. Approval: 2005

---------- RECENT MAJOR CHANGES----------

Indications and Usage (1)	10/2022
Dosage and Administration (2.2)	10/2022

----------INDICATIONS AND USAGE ----------

BOOSTRIX is a vaccine indicated for:

- active booster immunization against tetanus, diphtheria, and pertussis in individuals aged 10 years and older, (1)
- immunization during the third trimester of pregnancy to prevent pertussis in infants younger than 2 months of age. (1)

----------DOSAGE AND ADMINISTRATION----------

For intramuscular use only.

- Each dose of BOOSTRIX is administered as a 0.5-mL injection. (2.2)
- An initial dose of BOOSTRIX is administered 5 years or more after the last dose of the Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) series or 5 years or more after a dose of Tetanus and Diphtheria Toxoids Adsorbed (Td). BOOSTRIX may be administered as an additional dose 9 years or more after the initial dose of Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap). (2.2)
- BOOSTRIX may be administered for tetanus prophylaxis for wound management. For management of a tetanus-prone wound, a dose of BOOSTRIX may be administered if at least 5 years have elapsed since previous receipt of a tetanus toxoid-containing vaccine. (2.2)
- To provide protection against pertussis in infants younger than 2 months of age, administer BOOSTRIX during the third trimester of pregnancy. (2.2)

---------- DOSAGE FORMS AND STRENGTHS----------

Single-dose vials and single-dose prefilled syringes containing a 0.5-mL suspension for injection. (3)

----------CONTRAINDICATIONS ----------

- Severe allergic reaction (e.g., anaphylaxis) after a previous dose of any tetanus toxoid-, diphtheria toxoid-, or pertussis antigen-containing vaccine or to any component of BOOSTRIX. (4.1)
- Encephalopathy within 7 days of administration of a previous pertussis antigen-containing vaccine. (4.2)

----------WARNINGS AND PRECAUTIONS----------

- The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions. (5.2)
- If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk of Guillain-Barré syndrome may be increased following a subsequent dose of tetanus toxoid-containing vaccine, including BOOSTRIX. (5.3)
- Progressive or unstable neurologic conditions are reasons to defer vaccination with a pertussis-containing vaccine, including BOOSTRIX. (5.4)
- Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine should not receive BOOSTRIX unless at least 10 years have elapsed since the last dose of a tetanus toxoid-containing vaccine. (5.5)

----------ADVERSE REACTIONS----------

- Common solicited adverse reactions (≥15%) in adolescents (aged 10 to 18 years) were pain, redness, and swelling at the injection site; increase in arm circumference of the injected arm; headache; fatigue; and gastrointestinal symptoms. (6.1)
- Common solicited adverse reactions (≥15%) in adults (aged 19 to 64 years) were pain, redness, and swelling at the injection site; headache; fatigue; and gastrointestinal symptoms. (6.1)
- The most common solicited adverse reaction (≥15%) in the elderly (aged 65 years and older) was pain at the injection site. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

BOOSTRIX is indicated for:

- active booster immunization against tetanus, diphtheria, and pertussis in individuals aged 10 years and older,
- immunization during the third trimester of pregnancy to prevent pertussis in infants younger than 2 months of age.

2 DOSAGE AND ADMINISTRATION

For intramuscular use only.

2.1 Preparation for Administration

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use the vaccine if either of these conditions exist. Shake vigorously to obtain a homogeneous, turbid, white suspension before administration. Do not use if resuspension does not occur with vigorous shaking.

For the prefilled syringes, attach a sterile needle and administer intramuscularly.

For the vials, use a sterile needle and sterile syringe to withdraw the 0.5-mL dose and administer intramuscularly. Changing needles between drawing vaccine from a vial and injecting it into a recipient is not necessary unless the needle has been damaged or contaminated.

2.2 Administration, Dose, and Schedule

BOOSTRIX is administered as a 0.5-mL intramuscular injection into the deltoid muscle of the upper arm.

Active Booster Immunization

An initial dose of BOOSTRIX is administered 5 years or more after the last dose of the Diphtheria and Tetanus Toxoids and Acellular Pertussis (DTaP) series or 5 years or more after a dose of Tetanus and Diphtheria Toxoids Adsorbed (Td).

BOOSTRIX may be administered as an additional dose 9 years or more after the initial dose of Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed (Tdap).

BOOSTRIX may be administered for tetanus prophylaxis for wound management. For management of a tetanus-prone wound, a dose of BOOSTRIX may be administered if at least 5 years have elapsed since previous receipt of a tetanus toxoid-containing vaccine.

Immunization During the Third Trimester of Pregnancy

To prevent pertussis in infants younger than 2 months of age, administer BOOSTRIX to

pregnant individuals during the third trimester of pregnancy.

2.3 Additional Dosing Information

The use of BOOSTRIX as a primary series or to complete the primary series for diphtheria, tetanus, or pertussis has not been established.

3 DOSAGE FORMS AND STRENGTHS

BOOSTRIX is a suspension for injection available in 0.5-mL single-dose vials and prefilled TIP-LOK syringes.

4 CONTRAINDICATIONS

4.1 Severe Allergic Reaction

Do not administer BOOSTRIX to individuals with a known history of severe allergic reaction (e.g., anaphylaxis) to any component of BOOSTRIX or after a previous dose of any tetanus toxoid-, diphtheria toxoid-, or pertussis antigen-containing vaccine *[see Description (11)]*.

4.2 Encephalopathy

Encephalopathy within 7 days of administration of a previous dose of a pertussis antigen-containing vaccine that is not attributable to another identifiable cause is a contraindication to administration of any pertussis antigen-containing vaccine, including BOOSTRIX.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Appropriate medical treatment to manage allergic reactions must be immediately available in the event an acute allergic reaction occurs following administration of BOOSTRIX.

5.2 Latex

The tip caps of the prefilled syringes contain natural rubber latex which may cause allergic reactions.

5.3 Guillain-Barré Syndrome and Brachial Neuritis

If Guillain-Barré syndrome occurred within 6 weeks of receipt of a prior vaccine containing tetanus toxoid, the risk of Guillain-Barré syndrome may be increased following a subsequent dose of tetanus toxoid-containing vaccine, including BOOSTRIX. A review by the Institute of Medicine (IOM) found evidence for a causal relationship between receipt of tetanus toxoid and both Guillain-Barré syndrome and brachial neuritis.[1]

5.4 Progressive or Unstable Neurologic Disorders

Progressive or unstable neurologic conditions (e.g., cerebrovascular events, acute encephalopathic conditions) are reasons to defer vaccination with a pertussis-containing vaccine,

including BOOSTRIX. It is not known whether administration of BOOSTRIX to persons with an unstable or progressive neurologic disorder might hasten manifestations of the disorder or affect the prognosis. Administration of BOOSTRIX to persons with an unstable or progressive neurologic disorder may result in diagnostic confusion between manifestations of the underlying illness and possible adverse effects of vaccination.

5.5 Arthus-Type Hypersensitivity

Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine usually have a high serum tetanus antitoxin level and should not receive BOOSTRIX or other tetanus toxoid-containing vaccines unless at least 10 years have elapsed since the last dose of tetanus toxoid-containing vaccine.

5.6 Altered Immunocompetence

As with any vaccine, if administered to immunosuppressed persons, including individuals receiving immunosuppressive therapy, the expected immune response may not be obtained.

5.7 Syncope

Syncope (fainting) may occur in association with administration of injectable vaccines, including BOOSTRIX. Procedures should be in place to avoid injury from fainting.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice.

Table 1 provides an overview of the studies that evaluated the safety of BOOSTRIX in various populations.

Table 1. Studies Conducted with BOOSTRIX and the Non-U.S. Formulation of BOOSTRIX

Clinical Studies	Age	Trial Arms (Number of Subjects Vaccinated)
Initial-Dose Studies		
U.S. Adolescent Study (NCT00109330)	10 to 18 Years	BOOSTRIX (3,080) Td (1,034)
German Adolescent Study (NCT00263679)	10 to 12 Years	BOOSTRIX (319)
U.S. Adult Study (NCT00346073)	19 to 64 Years	BOOSTRIX (1,522) Tdap (762)
U.S Elderly Study (NCT00835237)	≥65 Years	BOOSTRIX (887) Td (445)
Revaccination Studies (all subjects were vaccinated with BOOSTRIX and trial arms were defined based on initial-dose vaccination; subjects in the Control trial arm received a first dose of BOOSTRIX)		
U.S. Revaccination Study – 10 Years after Initial Td or BOOSTRIX Dose (NCT01738477)	20 to 29 Years	BOOSTRIX (128) Td (37)
U.S. Revaccination Study – 9 Years after Initial BOOSTRIX or Tdap Dose (NCT00489970)	28 to 73 Years	BOOSTRIX (309) Tdap (138) Control (362)
Concomitant Vaccine Studies		
Concomitant Vaccination with Meningococcal Conjugate Vaccine (MCV4) (NCT00282295)	11 to 18 Years	BOOSTRIX+MCV4 (446) BOOSTRIX→MCV4 (446) MCV4→BOOSTRIX (449)
Concomitant Vaccination with Inactivated Trivalent Influenza Vaccine (TIV) (NCT00385255)	19 to 64 Years	BOOSTRIX+TIV (748) TIV→BOOSTRIX (749)
Study in Pregnant Women		
Vaccination During the Third Trimester of Pregnancy (NCT02377349)	18 to 45 Years	Non-U.S. formulation of BOOSTRIX/Tdap[a] (341) Placebo (346)

[a] Non-U.S. formulation of BOOSTRIX contains the same antigens and in the same quantities as BOOSTRIX. The non-U.S. formulation is manufactured to contain 0.5 mg aluminum per dose. The U.S. formulation is manufactured to contain 0.3 mg aluminum per dose.

In these studies, subjects were monitored for solicited adverse events using standardized diary cards during the 4 days (Days 0 to 3), 8 days (Days 0 to 7), or 15 days (Days 0 to 14) following vaccination. Unsolicited adverse events were monitored for the 31-day period following vaccination (Days 0 to 30).

Serious adverse events were monitored for 6 months post-vaccination in the initial-dose studies (NCT00109330, NCT00263679, NCT00346073, NCT00835237), for the 31-day (Days 0 to 30) period post-vaccination in the revaccination (NCT01738477, NCT00489970) and the concomitant-vaccine administration studies (NCT00282295, NCT00385255), and from vaccination through 2 months after delivery in the study of pregnant individuals (NCT02377349).

Initial-Dose Studies

In clinical studies, 4,949 adolescents (aged 10 to 18 years) and 4,076 adults (aged 19 years and older) were vaccinated with a single dose of BOOSTRIX. Of these adolescents, 1,341 were vaccinated with BOOSTRIX in a coadministration study with meningococcal conjugate vaccine *[see Drug Interactions (7.1), Clinical Studies (14.5)]*. Of these adults, 1,104 were aged 65 years and older *[see Clinical Studies (14.2)]*. A total of 860 adults aged 19 years and older received concomitant vaccination with BOOSTRIX and influenza vaccines in a coadministration study *[see Drug Interactions (7.1), Clinical Studies (14.6)]*.

Solicited Adverse Events in the U.S. Adolescent Study: Table 2 presents the solicited local adverse reactions and general adverse events within 15 days of vaccination with BOOSTRIX or Td vaccine for the total vaccinated cohort.

The primary safety endpoint was the incidence of Grade 3 pain (spontaneously painful and/or prevented normal activity) at the injection site within 15 days of vaccination. Grade 3 pain was reported in 4.6% of those who received BOOSTRIX compared with 4.0% of those who received the Td vaccine. The difference in rate of Grade 3 pain was within the pre-defined clinical limit for non-inferiority (upper limit of the 95% CI for the difference [BOOSTRIX minus Td] ≤4%).

Table 2. Rates of Solicited Local Adverse Reactions or General Adverse Events within the 15-Day[a] Post-Vaccination Period in Adolescents Aged 10 to 18 Years (Total Vaccinated Cohort)

Adverse Reactions/Adverse Events	BOOSTRIX (n = 3,032) %	Td (n = 1,013) %
Local		
Pain, any[b]	75	72
Pain, Grade 2 or 3[b]	51	43
Pain, Grade 3[c]	5	4
Redness, any	23	20
Redness, >20 mm	4	4
Redness, ≥50 mm	2	2
Swelling, any	21	20
Swelling, >20 mm	5	5
Swelling, ≥50 mm	3	3

Arm circumference increase, >5 mm[d]	28	30
Arm circumference increase, >20 mm[d]	2	2
Arm circumference increase, >40 mm[d]	1	0.3
General		
Headache, any	43	42
Headache, Grade 2 or 3[b]	16	13
Headache, Grade 3	4	3
Fatigue, any	37	37
Fatigue, Grade 2 or 3	14	13
Fatigue, Grade 3	4	3
Gastrointestinal symptoms, any[e]	26	26
Gastrointestinal symptoms, Grade 2 or 3[e]	10	10
Gastrointestinal symptoms, Grade 3[e]	3	3
Fever, ≥99.5°F (37.5°C)[f]	14	13
Fever, >100.4°F (38.0°C)[f]	5	5
Fever, >102.2°F (39.0°C)[f]	1	1

Td = Tetanus and Diphtheria Toxoids Adsorbed manufactured by MassBiologics.
n = Number of subjects in the total vaccinated cohort with local/general symptoms sheets completed.
Grade 2 = Local: painful when limb moved; General: interfered with normal activity.
Grade 3 = Local: spontaneously painful and/or prevented normal activity; General: prevented normal activity.

[a] Day of vaccination and the next 14 days.

[b] Statistically significantly higher (P <0.05) following BOOSTRIX as compared with Td vaccine.

[c] Grade 3 injection site pain following BOOSTRIX was not inferior to Td vaccine (upper limit of 2-sided 95% CI for the difference [BOOSTRIX minus Td] in the percentage of subjects ≤4%).

[d] Mid-upper region of the vaccinated arm.

[e] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.

[f] Oral temperatures or axillary temperatures.

Unsolicited Adverse Events in the U.S. Adolescent Study: The incidence of unsolicited adverse events reported in the 31 days after vaccination was comparable between the 2 groups (25.4% and 24.5% for BOOSTRIX and Td vaccine, respectively).

Solicited Adverse Events in the German Adolescent Study: BOOSTRIX was administered to 319 children aged 10 to 12 years previously vaccinated with 5 doses of acellular pertussis antigen-containing vaccines; 193 of these subjects received 5 doses of INFANRIX (Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed). Table 3 presents the rates of solicited local adverse reactions and fever within 15 days of vaccination for those subjects who had previously been vaccinated with 5 doses of INFANRIX. No cases of whole arm swelling

were reported. Two individuals (2/193) reported large injection site swelling (range: 110 to 200 mm diameter), in 1 case associated with Grade 3 pain. Neither individual sought medical attention. These episodes were reported to resolve without sequelae within 5 days.

Table 3. Rates of Solicited Local Adverse Reactions and Fever Reported within the 15-Day[a] Post-Vaccination Period following Administration of BOOSTRIX in Adolescents Aged 10 to 12 Years Who Had Previously Received 5 Doses of INFANRIX

Adverse Reactions and Fever	BOOSTRIX (n = 193) %
Pain, any	62
Pain, Grade 2 or 3	33
Pain, Grade 3	6
Redness, any	48
Redness, >20 mm	15
Redness, ≥50 mm	11
Swelling, any	39
Swelling, >20 mm	18
Swelling, ≥50 mm	14
Fever, ≥99.5°F (37.5°C)[b]	9
Fever, >100.4°F (38.0°C)[b]	4
Fever, >102.2°F (39.0°C)[b]	1

INFANRIX = Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed manufactured by GlaxoSmithKline Biologics.
n = Number of subjects with local/general symptoms sheets completed.
Grade 2 = Painful when limb moved.
Grade 3 = Spontaneously painful and/or prevented normal activity.
[a] Day of vaccination and the next 14 days.
[b] Oral temperatures or axillary temperatures.

Solicited Adverse Events in the U.S. Adult (Aged 19 to 64 Years) Study: Table 4 presents solicited local adverse reactions and general adverse events within 15 days of vaccination with BOOSTRIX or the comparator Tdap vaccine for the total vaccinated cohort.

Table 4. Rates of Solicited Local Adverse Reactions or General Adverse Events within the 15-Day[a] Post-Vaccination Period in Adults Aged 19 to 64 Years (Total Vaccinated Cohort)

Adverse Reactions/Adverse Events	BOOSTRIX (n = 1,480) %	Tdap (n = 741) %
Local		
Pain, any	61	69
Pain, Grade 2 or 3	35	44
Pain, Grade 3	2	2
Redness, any	21	27
Redness, >20 mm	4	6
Redness, ≥50 mm	2	2
Swelling, any	18	26
Swelling, >20 mm	4	6
Swelling, ≥50 mm	1	3
General		
Headache, any	30	31
Headache, Grade 2 or 3	11	11
Headache, Grade 3	2	2
Fatigue, any	28	29
Fatigue, Grade 2 or 3	9	9
Fatigue, Grade 3	3	1
Gastrointestinal symptoms, any[b]	16	18
Gastrointestinal symptoms, Grade 2 or 3[b]	4	6
Gastrointestinal symptoms, Grade 3[b]	1	1
Fever, ≥99.5°F (37.5°C)[c]	6	8
Fever, >100.4°F (38.0°C)[c]	1	2
Fever, >102.2°F (39.0°C)[c]	0.1	0.4

Tdap = ADACEL (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed, a Tdap vaccine manufactured by Sanofi Pasteur).
n = Number of subjects in the total vaccinated cohort with local/general symptoms sheets completed.
Grade 2 = Local: painful when limb moved; General: interfered with normal activity.
Grade 3 = Local/General: prevented normal activity.
[a] Day of vaccination and the next 14 days.
[b] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.
[c] Oral temperatures.

Unsolicited Adverse Events in the U.S. Adult (Aged 19 to 64 Years) Study: The incidence of unsolicited adverse events reported in the 31 days after vaccination was comparable between the

2 groups (17.8% and 22.2% for BOOSTRIX and Tdap vaccine, respectively).

Solicited Adverse Events in the U.S. Elderly (Aged 65 Years and Older) Study: Table 5 presents solicited local adverse reactions and general adverse events within 4 days of vaccination with BOOSTRIX or the comparator Td vaccine for the total vaccinated cohort.

Table 5. Rates of Solicited Local Adverse Reactions or General Adverse Events within 4 Days[a] of Vaccination in the Elderly Aged 65 Years and Older (Total Vaccinated Cohort)

Adverse Reactions/Adverse Events	BOOSTRIX %	Td %
Local	**(n= 882)**	**(n = 444)**
Pain, any	22	28
Pain, Grade 2 or 3	8	10
Pain, Grade 3	0.2	1
Redness, any	11	13
Redness, >20 mm	1	3
Redness, ≥50 mm	1	1
Swelling, any	8	12
Swelling, >20 mm	2	3
Swelling, ≥50 mm	1	1
General	**(n = 882)**	**(n = 445)**
Fatigue, any	13	15
Fatigue, Grade 2 or 3	3	3
Fatigue, Grade 3	1	1
Headache, any	12	12
Headache, Grade 2 or 3	2	2
Headache, Grade 3	1	0
Gastrointestinal symptoms, any[b]	8	9
Gastrointestinal symptoms, Grade 2 or 3[b]	2	2
Gastrointestinal symptoms, Grade 3[b]	0.3	0.4
Fever, ≥99.5°F (37.5°C)[c]	2	3
Fever, >100.4°F (38.0°C)[c]	0.2	0.2
Fever, >102.2°F (39.0°C)[c]	0	0

Td = DECAVAC (Tetanus and Diphtheria Toxoids Adsorbed, a U.S.-licensed Td vaccine, manufactured by Sanofi Pasteur).
n = Number of subjects with a documented dose.
Grade 2 = Local: painful when limb moved; General: interfered with normal activity.
Grade 3 = Local/General: prevented normal activity.
[a] Day of vaccination and the next 3 days.
[b] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.
[c] Oral temperatures.

Unsolicited Adverse Events in the U.S. Elderly (Aged 65 Years and Older) Study: The incidence of unsolicited adverse events reported in the 31 days after vaccination was comparable between the 2 groups (17.1% and 14.4% for BOOSTRIX and Td vaccine, respectively).

Serious Adverse Events (SAEs): In the U.S. and German adolescent safety studies, no serious adverse events were reported to occur within 31 days of vaccination. During the 6-month extended safety evaluation period, no serious adverse events that were of potential autoimmune origin or new onset and chronic in nature were reported to occur. In non-U.S. adolescent studies in which serious adverse events were monitored for up to 37 days, 1 subject was diagnosed with insulin-dependent diabetes 20 days following administration of BOOSTRIX. No other serious adverse events of potential autoimmune origin or that were new onset and chronic in nature were reported to occur in these studies. In the U.S. adult (aged 19 to 64 years) study, serious adverse events were reported to occur during the entire study period (0-6 months) by 1.4% and 1.7% of subjects who received BOOSTRIX and the comparator Tdap vaccine, respectively. During the 6-month extended safety evaluation period, no serious adverse events of a neuroinflammatory nature or with information suggesting an autoimmune etiology were reported in subjects who received BOOSTRIX. In the U.S. elderly (aged 65 years and older) study, serious adverse events were reported to occur by 0.7% and 0.9% of subjects who received BOOSTRIX and the comparator Td vaccine, respectively, during the 31-day period after vaccination. Serious adverse events were reported to occur by 4.2% and 2.2% of subjects who received BOOSTRIX and the comparator Td vaccine, respectively, during the 6-month period after vaccination.

Revaccination Studies

U.S. Revaccination Studies in Adults: In 2 clinical studies, 974 adults (aged 20 years and older) were vaccinated with a dose of BOOSTRIX *[see Clinical Studies (14.4)]*.

Solicited Adverse Events in the U.S. Revaccination Studies: Table 6 presents solicited local adverse reactions and general adverse events within 4 days of vaccination with BOOSTRIX for the total vaccinated cohort in both studies.

Table 6. Rates of Solicited Local Adverse Reactions or General Adverse Events within 4 Days[a] of Vaccination in Adults Aged 20 to 73 Years (Total Vaccinated Cohort)

Adverse Reactions/ Adverse Events	Adults Aged 20 to 29 Years		Adults Aged 28 to 73 Years		
	BOOSTRIX[b] (n = 125) %	Td[c] (n = 36) %	BOOSTRIX[d] (n = 306) %	Tdap[e] (n = 137) %	Control[f] (n = 358) %
Local					
Pain, any	78	58	59	61	37
Pain, Grade 2 or 3	33	19	17	15	9
Pain, Grade 3	5	6	1	1	1
Redness, any	38	42	24	23	15
Redness, >20 mm	4	0	6	4	1
Redness, ≥50 mm[g]	1	0	2	2	0
Swelling, any	24	19	19	19	12
Swelling, >20 mm	2	3	3	3	3
Swelling, ≥50 mm[h]	0	0	1	2	1
General					
Headache, any	32	22	17	18	15
Headache, Grade 2 or 3	10	3	4	4	2
Headache, Grade 3	2	0	0	1	0.3
Fatigue, any	30	22	23	17	14
Fatigue, Grade 2 or 3	14	3	8	7	3
Fatigue, Grade 3	2	0	1	1	0
Gastrointestinal symptoms, any[i]	9	3	9	3	8
Gastrointestinal symptoms, Grade 2 or 3[i]	2	0	2	0	3
Gastrointestinal symptoms, Grade 3[i]	2	0	0	0	0
Fever, ≥100.4°F (38.0°C)[j]	1	0	1	0	1
Fever, >102.2°F (39.0°C)[j]	0	0	0.3	0	0

Td = Tetanus and Diphtheria Toxoids Adsorbed for Adult Use manufactured by MassBiologics.
Tdap = ADACEL (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine Adsorbed, a Tdap vaccine manufactured by Sanofi Pasteur).
n = Number of subjects with a documented dose.
Grade 2 = Local: painful when limb moved and interfered with normal activities; General: interfered with normal activity.
Grade 3 = Local: significant pain at rest and/or prevented normal activity; General: prevented normal activity.

[a] Day of vaccination and the next 3 days.
[b] Subjects who were revaccinated with BOOSTRIX 10 years after initial vaccination with BOOSTRIX.
[c] Subjects who received a dose of BOOSTRIX 10 years after initial vaccination with Td vaccine.
[d] Subjects who were revaccinated with BOOSTRIX 9 years after initial vaccination with BOOSTRIX.
[e] Subjects who received a dose of BOOSTRIX 9 years after initial vaccination with Tdap vaccine.
[f] Control Group = Newly enrolled subjects who received an initial dose of BOOSTRIX.
[g] In the study of adults aged 20 to 29 years, redness >50 mm was recorded.
[h] In the study of adults aged 20 to 29 years, swelling >50 mm was recorded.
[i] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.
[j] Oral temperatures.

Concomitant Vaccination with Meningococcal Conjugate Vaccine in Adolescents

Table 7 presents the percentages of subjects experiencing local reactions at the injection site for BOOSTRIX and solicited general events following BOOSTRIX. The incidence of unsolicited adverse events reported in the 31 days after any vaccination was similar following each dose of BOOSTRIX in all cohorts.

Table 7. Rates of Solicited Local Adverse Reactions or General Adverse Events Reported within the 4-Day Post-Vaccination Period following Administration of BOOSTRIX in Individuals Aged 11 to 18 Years (Total Vaccinated Cohort)

Adverse Reactions/Adverse Events	BOOSTRIX+MCV4[a] (n = 441) %	BOOSTRIX→MCV4[b] (n = 432-433) %	MCV4→BOOSTRIX[c] (n = 441) %
Local (at injection site for BOOSTRIX)			
Pain, any	70	70	48
Redness, any	23	26	18
Swelling, any	18	18	12
General (following administration of BOOSTRIX)			
Fatigue	34	32	20
Headache	34	31	17
Gastrointestinal symptoms[d]	15	15	8
Fever, ≥99.5°F (37.5°C)[e]	5	4	2

MCV4 = MENACTRA (Meningococcal [Groups A, C, Y, and W-135] Polysaccharide Diphtheria Toxoid Conjugate Vaccine), Sanofi Pasteur.
n = Number of subjects in the total vaccinated cohort with local/general symptoms sheets

completed.
[a] BOOSTRIX+MCV4 = Concomitant vaccination with BOOSTRIX and MENACTRA.
[b] BOOSTRIX→MCV4 = BOOSTRIX followed by MCV4 1 month later.
[c] MCV4→BOOSTRIX = MCV4 followed by BOOSTRIX 1 month later.
[d] Gastrointestinal symptoms included nausea, vomiting, diarrhea, and/or abdominal pain.
[e] Oral temperatures.

Vaccination During Pregnancy

Safety of non-U.S. formulation BOOSTRIX (0.5 mg aluminum/per dose) during the third trimester of pregnancy was evaluated in study NCT02377349. The safety data with the non-U.S. formulation are relevant because the non-U.S. formulation of BOOSTRIX contains the same antigens and in the same quantities as BOOSTRIX. However, the non-U.S. formulation contains more aluminum per dose (see Table 1).

In the randomized, controlled study NCT02377349, 687 pregnant individuals received the non-U.S. formulation of BOOSTRIX or placebo during the third trimester (341 non-U.S. formulation of BOOSTRIX, 346 placebo [saline]). The placebo recipients received the non-U.S. formulation of BOOSTRIX postpartum. The rates of reported solicited adverse reactions following receipt of the non-U.S. formulation of BOOSTRIX administered during pregnancy were consistent with the rates following receipt of the non-U.S. formulation of BOOSTRIX administered to study participants postpartum. For further information about pregnancy outcomes *[see Use in Specific Populations (8.1)]*.

6.2 Postmarketing Experience

In addition to reports in clinical trials for BOOSTRIX, the following adverse events have been identified in persons aged 10 years and older during postapproval use of BOOSTRIX worldwide. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

Blood and Lymphatic System Disorders

Lymphadenitis, lymphadenopathy.

Immune System Disorders

Allergic reactions, including anaphylactic and anaphylactoid reactions.

Cardiac Disorders

Myocarditis.

General Disorders and Administration Site Conditions

Extensive swelling of the injected limb, injection site induration, injection site inflammation, injection site mass, injection site pruritus, injection site nodule, injection site warmth, injection site reaction.

Musculoskeletal and Connective Tissue Disorders

Arthralgia, back pain, myalgia.

Nervous System Disorders

Convulsions (with and without fever), encephalitis, facial palsy, loss of consciousness, paresthesia, syncope.

Skin and Subcutaneous Tissue Disorders

Angioedema, exanthem, Henoch-Schönlein purpura, rash, urticaria.

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

In a clinical study of participants 11 to 18 years of age, BOOSTRIX was administered concomitantly with MENACTRA *[see Clinical Studies (14.5)]*. Post-vaccination geometric mean antibody concentrations (GMCs) to pertactin (PRN) were lower following BOOSTRIX administered concomitantly with meningococcal conjugate vaccine compared with BOOSTRIX administered first. It is not known if the efficacy of BOOSTRIX is affected by the reduced response to PRN.

In a clinical study of adults 19 to 64 years of age, BOOSTRIX was administered concomitantly with FLUARIX (Influenza Virus Vaccine) *[see Clinical Studies (14.6)]*. Lower GMCs for antibodies to the pertussis antigens filamentous hemagglutinin (FHA) and PRN were observed when BOOSTRIX was administered concomitantly with FLUARIX as compared with BOOSTRIX alone. It is not known if the efficacy of BOOSTRIX is affected by the reduced response to FHA and PRN.

7.2 Immunosuppressive Therapies

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs, and corticosteroids (used in greater than physiologic doses), may reduce the immune response to BOOSTRIX.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Pregnancy Exposure Registry

There is a pregnancy exposure registry that monitors pregnancy outcomes in women exposed to BOOSTRIX during pregnancy. Healthcare providers are encouraged to register women by calling 1-888-452-9622 or visiting http://pregnancyregistry.gsk.com/boostrix.html.

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general

population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

In a randomized, controlled clinical study (NCT02377349), in which the non-U.S. formulation of BOOSTRIX was administered during the third trimester of pregnancy, there were no identified vaccine-related adverse effects on pregnancy or on the fetus/newborn child *(see Data)*.

Available data from the pregnancy registry and from spontaneous and postmarketing reports suggest that the rates of major birth defects and miscarriage in women who received BOOSTRIX within 28 days prior to conception or during pregnancy are consistent with estimated background rates *(see Data)*.

A developmental toxicity study was performed in female rats administered INFANRIX prior to mating and BOOSTRIX during gestation, 0.1 mL at each occasion (a single human dose is 0.5 mL). In a second study, female rats were administered 0.2 mL of BOOSTRIX prior to mating and during the gestation and lactation period. In a third study, female New Zealand White rabbits were given 0.5 mL (full human dose) of BOOSTRIX (non-U.S. formulation) prior to mating and during gestation. These studies revealed no evidence of harm to the fetus due to BOOSTRIX *(see Data)*.

Data

Human Data: Safety data from a randomized (1:1), controlled clinical study (NCT02377349) (341 non-U.S. formulation of BOOSTRIX, 346 placebo pregnancy outcomes) in which the non-U.S. formulation of BOOSTRIX was administered to pregnant women during the third trimester did not reveal any vaccine-related adverse effects on pregnancy or on the fetus/newborn child. Safety data from prospective clinical studies on the use of BOOSTRIX during the first and second trimester of pregnancy are not available.

An assessment of data from the U.S. pregnancy exposure registry over approximately 17 years (2005-2022) included 1,523 prospective reports of exposure to BOOSTRIX within 28 days prior to conception or during pregnancy. Among the 256 reports with known pregnancy outcomes, 19 women were exposed to BOOSTRIX in the first trimester with no major birth defects reported and 3 spontaneous abortions with no apparent birth defect; 28 women were exposed to BOOSTRIX in the second trimester, and 199 women were exposed to BOOSTRIX in the third trimester with no major birth defects reported; 10 women were exposed to BOOSTRIX at an unknown timing in pregnancy with no major birth defects reported.

An assessment of U.S. spontaneous reports and postmarketing data included 810 prospective reports of exposure to BOOSTRIX during pregnancy since May 2005 through 31 August 2022. Among the 138 reports with known pregnancy outcomes, 17 women were exposed to BOOSTRIX in the first trimester with no major birth defects reported and 2 spontaneous abortions with no apparent birth defect; 26 women were exposed to BOOSTRIX in the second trimester, and 92 women were exposed to BOOSTRIX in the third trimester with no major birth

defects reported; 3 women were exposed to BOOSTRIX at an unknown timing in pregnancy with no major birth defects reported.

Animal Data: Developmental toxicity studies were performed in female rats and New Zealand White rabbits. In one study, female rats were administered 0.1 mL of INFANRIX (a single human dose is 0.5 mL) by intramuscular injection 30 days prior to mating and 0.1 mL of BOOSTRIX (a single human dose is 0.5 mL) by intramuscular injection on Gestation Days 6, 8, 11, and 15. The antigens in INFANRIX are the same as those in BOOSTRIX, but INFANRIX is formulated with higher quantities of these antigens. In a second study, female rats were administered 0.2 mL of BOOSTRIX by intramuscular injection 28 days and 14 days prior to mating, on Gestation Days 3, 8, 11, and 15, and on Lactation Day 7. In these studies, no adverse effects on embryo-fetal or pre-weaning development up to Postnatal Day 25 were observed; there were no fetal malformations or variations observed. In a third study, female New Zealand White rabbits were administered 0.5 mL (full human dose) of BOOSTRIX (non-U.S. formulation) by intramuscular injection on Premating Days -28 and -14 and on Gestation Days 3, 8, 11, 15, and 24. In this study, no adverse effects on embryo-fetal development related to BOOSTRIX were observed; postnatal development was not evaluated.

8.2 Lactation

Risk Summary

It is not known whether the vaccine components of BOOSTRIX are excreted in human milk. Data are not available to assess the effect of administration of BOOSTRIX on breastfed infants or on milk production/excretion. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for BOOSTRIX and any potential adverse effects on the breastfed child from BOOSTRIX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

BOOSTRIX is not indicated for use in children aged younger than 10 years. Safety and effectiveness of BOOSTRIX in this age group have not been established.

8.5 Geriatric Use

In the initial-dose clinical trials, 1,104 subjects aged 65 years and older received BOOSTRIX; of these subjects, 299 were aged 75 years and older. Adverse events following BOOSTRIX were similar in frequency to those reported with the comparator Td vaccine *[see Adverse Reactions (6.1)]*.

A revaccination study of BOOSTRIX in adults aged 28 to 73 years *[see Clinical Studies (14.4)]* did not include sufficient numbers of subjects aged 65 and older to determine whether they respond differently from younger subjects.

11 DESCRIPTION

BOOSTRIX (Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed) is a noninfectious, sterile, vaccine for intramuscular administration. It contains tetanus toxoid, diphtheria toxoid, and pertussis antigens (inactivated pertussis toxin [PT] and formaldehyde-treated FHA and PRN). The antigens are the same as those in INFANRIX, but BOOSTRIX is formulated with reduced quantities of these antigens.

Tetanus toxin is produced by growing *Clostridium tetani (C. tetani)* in a modified Latham medium derived from bovine casein. The diphtheria toxin is produced by growing *Corynebacterium diphtheriae (C. diphtheriae)* in Fenton medium containing a bovine extract. The bovine materials used in these extracts are sourced from countries which the United States Department of Agriculture (USDA) has determined neither have nor are at risk of bovine spongiform encephalopathy (BSE). Both toxins are detoxified with formaldehyde, concentrated by ultrafiltration, and purified by precipitation, dialysis, and sterile filtration.

The acellular pertussis antigens (PT, FHA, and PRN) are isolated from *Bordetella pertussis* (*B. pertussis)* culture grown in modified Stainer-Scholte liquid medium. PT and FHA are isolated from the fermentation broth; PRN is extracted from the cells by heat treatment and flocculation. The antigens are purified in successive chromatographic and precipitation steps. PT is detoxified using glutaraldehyde and formaldehyde. FHA and PRN are treated with formaldehyde.

Each antigen is individually adsorbed onto aluminum hydroxide. Each 0.5-mL dose is formulated to contain 5 Lf of tetanus toxoid, 2.5 Lf of diphtheria toxoid, 8 mcg of inactivated PT, 8 mcg of FHA, and 2.5 mcg of PRN (69 kiloDalton outer membrane protein).

Tetanus and diphtheria toxoid potency is determined by measuring the amount of neutralizing antitoxin in previously immunized guinea pigs. The potency of the acellular pertussis components (inactivated PT and formaldehyde-treated FHA and PRN) is determined by enzyme-linked immunosorbent assay (ELISA) on sera from previously immunized mice.

Each 0.5-mL dose contains aluminum hydroxide as adjuvant (formulated to contain 0.3 mg aluminum) and 4.4 mg of sodium chloride. The aluminum content is measured by assay. Each dose also contains ≤100 mcg of residual formaldehyde and ≤100 mcg of polysorbate 80 (Tween 80).

BOOSTRIX is available in vials and prefilled syringes. The tip caps of the prefilled syringes contain natural rubber latex; the plungers are not made with natural rubber latex. The vial stoppers are not made with natural rubber latex.

BOOSTRIX is formulated without preservatives.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Active Immunization

Tetanus is a condition manifested primarily by neuromuscular dysfunction caused by a potent exotoxin released by *C. tetani*. Protection against disease is due to the development of neutralizing antibodies to the tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assays, is considered the minimum protective level.[2] A level ≥0.1 IU/mL by ELISA has been considered as protective.

Diphtheria is an acute toxin-mediated infectious disease caused by toxigenic strains of *C. diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to the diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL, measured by neutralization assays, is the lowest level giving some degree of protection; a level of 0.1 IU/mL by ELISA is regarded as protective. Diphtheria antitoxin levels ≥1.0 IU/mL by ELISA have been associated with long-term protection.[3]

Pertussis (whooping cough) is a disease of the respiratory tract caused by *B. pertussis*. The role of the different components produced by *B. pertussis* in either the pathogenesis of, or the immunity to, pertussis is not well understood.

Passive Immunization to Prevent Pertussis in Infants

Antibodies to pertussis antigens from individuals vaccinated during the third trimester of pregnancy are transferred transplacentally to prevent pertussis in infants younger than 2 months of age.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

BOOSTRIX has not been evaluated for carcinogenic or mutagenic potential, or for impairment of male fertility in animals. Vaccination of female rabbits and rats with BOOSTRIX had no effect on fertility. *[See Use in Specific Populations (8.1).]*

14 CLINICAL STUDIES

14.1 Effectiveness of BOOSTRIX, INFANRIX, and PEDIARIX

Effectiveness of BOOSTRIX

The effectiveness of the tetanus and diphtheria toxoid components of BOOSTRIX is based on the immunogenicity of the individual antigens compared with U.S.-licensed vaccines using established serologic correlates of protection. The effectiveness of the pertussis components of BOOSTRIX was evaluated by comparison of the immune response of adolescents and adults following an initial dose of BOOSTRIX to the immune response of infants following a 3-dose

primary series of INFANRIX or by comparison of the immune response of adults following an additional dose of BOOSTRIX to the immune response of infants following a 3-dose primary series of PEDIARIX. In addition, the ability of BOOSTRIX to induce a booster response to each of the antigens was evaluated.

Efficacy of INFANRIX Against Pertussis

The efficacy of a 3-dose primary series of INFANRIX in infants has been assessed in 2 clinical studies: A prospective efficacy trial conducted in Germany employing a household contact study design and a double-blind, randomized, active Diphtheria and Tetanus Toxoids (DT)-controlled trial conducted in Italy sponsored by the National Institutes of Health (NIH) (for details see INFANRIX prescribing information). In the household contact study, the protective efficacy of INFANRIX in infants against WHO-defined pertussis (21 days or more of paroxysmal cough with infection confirmed by culture and/or serologic testing) was calculated to be 89% (95% CI: 77%, 95%). When the definition of pertussis was expanded to include clinically milder disease, with infection confirmed by culture and/or serologic testing, the efficacy of INFANRIX against ≥7 days of any cough was 67% (95% CI: 52%, 78%) and against ≥7 days of paroxysmal cough was 81% (95% CI: 68%, 89%) (for details see INFANRIX prescribing information).

Immune Responses to Pertussis Antigens of PEDIARIX Compared with INFANRIX

The diphtheria, tetanus, and pertussis components in PEDIARIX are the same as those in INFANRIX. The effectiveness of the pertussis component of PEDIARIX was determined in clinical trials by comparison to antibody responses to INFANRIX (for details see PEDIARIX prescribing information).

Immune Responses to Pertussis Antigens of BOOSTRIX Compared with INFANRIX or PEDIARIX

Although a serologic correlate of protection for pertussis has not been established, serological data from a subset of infants immunized with a 3-dose primary series of INFANRIX in the German household contact study were compared with the sera of adolescents and adults immunized with an initial dose of BOOSTRIX *[see Clinical Studies (14.2)]*. Serological data from infants immunized with a 3-dose primary series of PEDIARIX in an additional pediatric study were compared with the sera of adults immunized with an additional dose of BOOSTRIX *[see Clinical Studies (14.4)]*. The GMCs to each of the pertussis antigens 1 month following a dose of BOOSTRIX were compared with the GMCs of infants following INFANRIX administered at 3, 4, and 5 months of age or were compared with the GMCs of infants following PEDIARIX administered at 2, 4, and 6 months of age. The majority of subjects in the study of INFANRIX had only anti-PT serology data.

14.2 Immunological Evaluation following an Initial Dose of BOOSTRIX

Adolescents (Aged 10 to 18 years)

In a multicenter, randomized, observer-blinded, controlled study conducted in the United States

(NCT00109330), the immune responses to each of the antigens contained in BOOSTRIX were evaluated in sera obtained approximately 1 month after administration of a single dose of vaccine to adolescent subjects (aged 10 to 18 years). Of the subjects enrolled in this study, approximately 76% were aged 10 to 14 years and 24% were aged 15 to 18 years. Approximately 98% of participants in this study had received the recommended series of 4 or 5 doses of either DTwP or a combination of DTwP and DTaP in childhood. The racial/ethnic demographics were as follows: White 85.8%, Black 5.7%, Hispanic 5.6%, Oriental 0.8%, and other 2.1%.

Response to Tetanus and Diphtheria Toxoids: The antibody responses to the tetanus and diphtheria toxoids of BOOSTRIX compared with Td vaccine are shown in Table 8. One month after a single dose, anti-tetanus and anti-diphtheria seroprotective rates (≥0.1 IU/mL by ELISA) and booster response rates were comparable between BOOSTRIX and the comparator Td vaccine.

Table 8. Antibody Responses to Tetanus and Diphtheria Toxoids following BOOSTRIX Compared with Td Vaccine in Adolescents Aged 10 to 18 Years (ATP Cohort for Immunogenicity)

Antibodies	n	% ≥0.1 IU/mL[a] (95% CI)	% ≥1.0 IU/mL[a] (95% CI)	% Booster Response[b] (95% CI)
Anti-tetanus				
BOOSTRIX	2,469-2,516			
Pre-vaccination		97.7 (97.1, 98.3)	36.8 (34.9, 38.7)	–
Post-vaccination		100 (99.8, 100)[c]	99.5 (99.1, 99.7)[d]	89.7 (88.4, 90.8)[c]
Td	817-834			
Pre-vaccination		96.8 (95.4, 97.9)	39.9 (36.5, 43.4)	–
Post-vaccination		100 (99.6, 100)	99.8 (99.1, 100)	92.5 (90.5, 94.2)
Anti-diphtheria				
BOOSTRIX	2,463-2,515			
Pre-vaccination		85.8 (84.3, 87.1)	17.1 (15.6, 18.6)	–
Post-vaccination		99.9 (99.7, 100)[c]	97.3 (96.6, 97.9)[d]	90.6 (89.4, 91.7)[c]
Td	814-834			
Pre-vaccination		84.8 (82.1, 87.2)	19.5 (16.9, 22.4)	–
Post-vaccination		99.9 (99.3, 100)	99.3 (98.4, 99.7)	95.9 (94.4, 97.2)

Td = Tetanus and Diphtheria Toxoids, Adsorbed manufactured by MassBiologics.

ATP = According-to-protocol; CI = Confidence Interval.

[a] Measured by ELISA.

[b] Booster response: In subjects with pre-vaccination <0.1 IU/mL, post-vaccination concentration ≥0.4 IU/mL. In subjects with pre-vaccination concentration ≥0.1 IU/mL, an increase of at least 4 times the pre-vaccination concentration.

[c] Seroprotection rate or booster response rate to BOOSTRIX was non-inferior to Td (upper limit of 2-sided 95% CI on the difference for Td minus BOOSTRIX ≤10%).

[d] Non-inferiority criteria not prospectively defined for this endpoint.

Response to Pertussis Antigens: The booster response rates of adolescents to the pertussis antigens are shown in Table 9. For each of the pertussis antigens the lower limit of the 2-sided 95% CI for the percentage of subjects with a booster response exceeded the pre-defined lower limit of 80% for demonstration of an acceptable booster response.

Table 9. Booster Responses to the Pertussis Antigens following BOOSTRIX in Adolescents Aged 10 to 18 Years (ATP Cohort for Immunogenicity)

Pertussis Antibodies	n	BOOSTRIX % Booster Response[a] (95% CI)
Anti-PT	2,677	84.5 (83.0, 85.9)
Anti-FHA	2,744	95.1 (94.2, 95.9)
Anti-PRN	2,752	95.4 (94.5, 96.1)

ATP = According-to-protocol; CI = Confidence Interval; PT = Pertussis toxin; FHA = Filamentous hemagglutinin; PRN = Pertactin.

[a] Booster response: In initially seronegative subjects (<5 EL.U./mL), post-vaccination antibody concentrations ≥20 EL.U./mL. In initially seropositive subjects with pre-vaccination antibody concentrations ≥5 EL.U./mL and <20 EL.U./mL, an increase of at least 4 times the pre-vaccination antibody concentration. In initially seropositive subjects with pre-vaccination antibody concentrations ≥20 EL.U./mL, an increase of at least 2 times the pre-vaccination antibody concentration.

The GMCs to each of the pertussis antigens 1 month following a single dose of BOOSTRIX were compared with the GMCs of a subset of infants following a 3-dose primary series of INFANRIX in the German household contact study *[see Clinical Studies (14.1)]*. Table 10 presents the results for the total immunogenicity cohort in both studies (vaccinated subjects with serology data available for at least 1 pertussis antigen). Anti-PT, anti-FHA, and anti-PRN antibody concentrations observed in adolescents 1 month after a single dose of BOOSTRIX were non-inferior to those infants following a primary vaccination series with INFANRIX.

Table 10. Ratio of GMCs to Pertussis Antigens following 1 Dose of BOOSTRIX in Adolescents Aged 10 to 18 Years Compared with 3 Doses of INFANRIX in Infants (Total Immunogenicity Cohort)

Pertussis Antibodies	BOOSTRIX (n)	INFANRIX (n)	GMC Ratio: BOOSTRIX/INFANRIX (95% CI)
Anti-PT	2,941	2,884	1.90 (1.82, 1.99)[a]
Anti-FHA	2,979	685	7.35 (6.85, 7.89)[a]
Anti-PRN	2,978	631	4.19 (3.73, 4.71)[a]

GMC = Geometric mean antibody concentration, measured in ELISA units; CI = Confidence Interval; PT = Pertussis toxin; FHA = Filamentous hemagglutinin; PRN = Pertactin.
n = Number of subjects for GMC evaluation.

[a] GMC following BOOSTRIX was non-inferior to GMC following INFANRIX (lower limit of 95% CI for the GMC ratio of BOOSTRIX/INFANRIX >0.67).

Adults (Aged 19 to 64 Years)

A multicenter, randomized, observer-blinded study, conducted in the United States (NCT00346073), evaluated the immunogenicity of BOOSTRIX compared with the licensed comparator Tdap vaccine (Sanofi Pasteur). Vaccines were administered as a single dose to subjects (N = 2,284) who had not received a tetanus-diphtheria booster within 5 years. The immune responses to each of the antigens contained in BOOSTRIX were evaluated in sera obtained approximately 1 month after administration. Approximately 33% of subjects were aged 19 to 29 years, 33% were aged 30 to 49 years, and 34% were aged 50 to 64 years. Among subjects in the combined vaccine groups, 62% were female; 84% of subjects were White, 8% Black, 1% Asian, and 7% were of other racial/ethnic groups.

Response to Tetanus and Diphtheria Toxoids: The antibody responses to the tetanus and diphtheria toxoids of BOOSTRIX compared with the comparator Tdap vaccine are shown in Table 11. One month after a single dose, anti-tetanus and anti-diphtheria seroprotective rates (≥0.1 IU/mL by ELISA) were comparable between BOOSTRIX and the comparator Tdap vaccine.

Table 11. Antibody Responses to Tetanus and Diphtheria Toxoids following 1 Dose of BOOSTRIX Compared with the Comparator Tdap Vaccine in Adults Aged 19 to 64 Years (ATP Cohort for Immunogenicity)

Antibodies	n	% ≥0.1 IU/mL[a] (95% CI)	% ≥1.0 IU/mL[a] (95% CI)
Anti-tetanus			
BOOSTRIX	1,445-1,447		
Pre-vaccination		95.9 (94.8, 96.9)	71.9 (69.5, 74.2)
Post-vaccination		99.6 (99.1, 99.8)[b]	98.3 (97.5, 98.9)[b]
Tdap	727-728		
Pre-vaccination		97.2 (95.8, 98.3)	74.7 (71.4, 77.8)
Post-vaccination		100 (95.5, 100)	99.3 (98.4, 99.8)
Anti-diphtheria			
BOOSTRIX	1,440-1,444		
Pre-vaccination		85.2 (83.3, 87.0)	23.7 (21.5, 26.0)
Post-vaccination		98.2 (97.4, 98.8)[b]	87.9 (86.1, 89.5)[c]
Tdap	720-727		
Pre-vaccination		89.2 (86.7, 91.3)	26.5 (23.3, 29.9)
Post-vaccination		98.6 (97.5, 99.3)	92.0 (89.8, 93.9)

Tdap = Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed

manufactured by Sanofi Pasteur.
ATP = According-to-protocol; CI = Confidence Interval.
[a] Measured by ELISA.
[b] Seroprotection rates for BOOSTRIX were non-inferior to the comparator Tdap vaccine (lower limit of 95% CI on the difference of BOOSTRIX minus Tdap ≥-10%).
[c] Non-inferiority criteria not prospectively defined for this endpoint.

Response to Pertussis Antigens: Booster response rates to the pertussis antigens are shown in Table 12. For the FHA and PRN antigens, the lower limit of the 95% CI for the booster responses exceeded the pre-defined limit of 80% demonstrating an acceptable booster response following BOOSTRIX. The PT antigen booster response lower limit of the 95% CI (74.9%) did not exceed the pre-defined limit of 80%.

Table 12. Booster Responses to the Pertussis Antigens following 1 Dose of BOOSTRIX in Adults Aged 19 to 64 Years (ATP Cohort for Immunogenicity)

Pertussis Antibodies	n	BOOSTRIX % Booster Response[a] (95% CI)
Anti-PT	1,419	77.2 (74.9, 79.3)[b]
Anti-FHA	1,433	96.9 (95.8, 97.7)[c]
Anti-PRN	1,441	93.2 (91.8, 94.4)[c]

ATP = According-to-protocol; CI = Confidence Interval; PT = Pertussis toxin;
FHA = Filamentous hemagglutinin; PRN = Pertactin.
[a] Booster response: In initially seronegative subjects (<5 EL.U./mL), post-vaccination antibody concentrations ≥20 EL.U./mL. In initially seropositive subjects with pre-vaccination antibody concentrations ≥5 EL.U./mL and <20 EL.U./mL, an increase of at least 4 times the pre-vaccination antibody concentration. In initially seropositive subjects with pre-vaccination antibody concentrations ≥20 EL.U./mL, an increase of at least 2 times the pre-vaccination antibody concentration.
[b] The PT antigen booster response lower limit of the 95% CI did not exceed the pre-defined limit of 80%.
[c] The FHA and PRN antigens booster response lower limit of the 95% CI exceeded the pre-defined limit of 80%.

The GMCs to each of the pertussis antigens 1 month following a single dose of BOOSTRIX were compared with the GMCs of a subset of infants following a 3-dose primary series of INFANRIX in the German household contact study *[see Clinical Studies (14.1)]*. Table 13 presents the results for the total immunogenicity cohort in both studies (vaccinated subjects with serology data available for at least 1 pertussis antigen). Anti-PT, anti-FHA, and anti-PRN antibody concentrations observed in adults 1 month after a single dose of BOOSTRIX were non-inferior to those infants following a primary vaccination series with INFANRIX.

Table 13. Ratio of GMCs to Pertussis Antigens following 1 Dose of BOOSTRIX in Adults Aged 19 to 64 Years Compared with 3 Doses of INFANRIX in Infants (Total Immunogenicity Cohort)

Pertussis Antibodies	BOOSTRIX (n)	INFANRIX (n)	GMC Ratio: BOOSTRIX/INFANRIX (95% CI)
Anti-PT	1,460	2,884	1.39 (1.32, 1.47)[a]
Anti-FHA	1,472	685	7.46 (6.86, 8.12)[a]
Anti-PRN	1,473	631	3.56 (3.10, 4.08)[a]

GMC = Geometric mean antibody concentration; CI = Confidence Interval; PT = Pertussis toxin; FHA = Filamentous hemagglutinin; PRN = Pertactin.
n = Number of subjects for GMC evaluation.
[a] BOOSTRIX was non-inferior to INFANRIX (lower limit of 95% CI for the GMC ratio of BOOSTRIX/INFANRIX ≥0.67).

Elderly (Aged 65 Years and Older)

The U.S. elderly (aged 65 years and older) study, a randomized, observer-blinded study (NCT00835237), evaluated the immunogenicity of BOOSTRIX (n = 887) compared with a U.S.-licensed comparator Td vaccine (n = 445) (Sanofi Pasteur). Vaccines were administered as a single dose to subjects who had not received a tetanus-diphtheria booster within 5 years. Among all vaccine recipients, the mean age was approximately 72 years; 54% were female and 95% were White. The immune responses to each of the antigens contained in BOOSTRIX were evaluated in sera obtained approximately 1 month after administration.

Response to Tetanus and Diphtheria Toxoids: Immune responses to tetanus and diphtheria toxoids were measured 1 month after administration of a single dose of BOOSTRIX or a comparator Td vaccine. Anti-tetanus and anti-diphtheria seroprotective rates (≥0.1 IU/mL) were comparable between BOOSTRIX and the comparator Td vaccine (Table 14).

Table 14. Immune Responses to Tetanus and Diphtheria Toxoids following BOOSTRIX or Comparator Td Vaccine in the Elderly Aged 65 Years and Older (ATP Cohort for Immunogenicity)

Anti-Tetanus and Anti-Diphtheria Titers	BOOSTRIX (n = 844-864)	Td (n = 430-439)
Anti-tetanus		
% ≥0.1 IU/mL (95% CI)	96.8 (95.4, 97.8)[a]	97.5 (95.6, 98.7)
% ≥1.0 IU/mL (95% CI)	88.8 (86.5, 90.8)[a]	90.0 (86.8, 92.6)
Anti-diphtheria		
% ≥0.1 IU/mL (95% CI)	84.9 (82.3, 87.2)[a]	86.6 (83.0, 89.6)
% ≥1.0 IU/mL (95% CI)	52.0 (48.6, 55.4)[b]	51.2 (46.3, 56.0)

Td = Tetanus and Diphtheria Toxoids Adsorbed, a U.S.-licensed Td vaccine, manufactured by Sanofi Pasteur.

ATP = According-to-protocol; CI = Confidence Interval.

[a] Seroprotection rates for BOOSTRIX were non-inferior to the comparator Td vaccine (lower limit of 95% CI on the difference of BOOSTRIX minus Td ≥-10%).

[b] Non-inferiority criteria not prospectively defined for this endpoint.

Response to Pertussis Antigens: The GMCs to each of the pertussis antigens 1 month following a single dose of BOOSTRIX were compared with the GMCs of a subset of infants following a 3-dose primary series of INFANRIX in the German household contact study *[see Clinical Studies (14.1)]*. Table 15 presents the results for the total immunogenicity cohort in both studies (vaccinated subjects with serology data available for at least 1 pertussis antigen). Anti-PT, anti-FHA, and anti-PRN antibody concentrations in the elderly 1 month after a single dose of BOOSTRIX were non-inferior to those of infants following a primary vaccination series with INFANRIX.

Table 15. Ratio of GMCs to Pertussis Antigens following 1 Dose of BOOSTRIX in the Elderly Aged 65 Years and Older Compared with 3 Doses of INFANRIX in Infants (Total Immunogenicity Cohort)

Pertussis Antibodies	BOOSTRIX (n)	INFANRIX (n)	GMC Ratio: BOOSTRIX/INFANRIX (95% CI)
Anti-PT	865	2,884	1.07 (1.00, 1.15)[a]
Anti-FHA	847	685	8.24 (7.45, 9.12)[a]
Anti-PRN	878	631	0.93 (0.79, 1.10)[a]

GMC = Geometric mean antibody concentration; CI = Confidence Interval; PT = Pertussis toxin; FHA = Filamentous hemagglutinin; PRN = Pertactin.

n = Number of subjects for GMC evaluation.

[a] BOOSTRIX was non-inferior to INFANRIX (lower limit of 95% CI for the GMC ratio of

BOOSTRIX/INFANRIX ≥0.67).

14.3 Study in Pregnant Women

The effectiveness of BOOSTRIX immunization during the third trimester of pregnancy to prevent pertussis among infants younger than 2 months of age was based on a re-analysis within a Bayesian meta-analysis framework of the BOOSTRIX-relevant data from an observational case-control study of Tdap vaccine effectiveness.[4] In this re-analysis, a conditional logistic regression model controlling for age, maternal education, and family size was fit to data from 108 cases (including 4 cases whose mothers received BOOSTRIX during the third trimester) and 183 controls (including 18 whose mothers received BOOSTRIX during the third trimester) matched by age group (<2 weeks old, ≥2 weeks old) and birth hospital. This yielded a preliminary vaccine effectiveness estimate of 78.0% (95% CI: -38.0, 96.5) for vaccination during the third trimester of pregnancy. This preliminary effectiveness estimate was updated using a Bayesian meta-analysis with an informative prior constructed from four observational studies that provided estimates of the vaccine effectiveness of the non-U.S. formulation of BOOSTRIX against pertussis in infants whose mothers were immunized during pregnancy.[5, 6, 7, 8] To account for potential publication bias, this informative prior was downweighted by combining it with an uninformative prior. When the informative prior has 20% weight, the Bayesian update resulted in estimates of effectiveness of vaccination during the third trimester of pregnancy of 81.5% (95% credible interval:12.9, 94.5). When the informative prior has 90% weight, the Bayesian update resulted in estimates of effectiveness of vaccination during the third trimester of pregnancy of 83.4% (95% credible interval: 55.7, 92.5). The vaccine effectiveness point estimates were consistent, regardless of the weight applied to the informative prior.

14.4 Immune Responses to Vaccination in Infants Born to Mothers Who Received BOOSTRIX During Pregnancy

Data are not available on immune responses to US licensed vaccines administered on the US schedule among infants born to mothers who received BOOSTRIX during pregnancy.

In infants whose mothers received BOOSTRIX (non-US formulation) during the third trimester of pregnancy, antibody responses to a non-US licensed DTaP-containing vaccine were diminished for anti-PT, anti-FHA and anti-PRN following the primary series (NCT 02422264), and for anti-PT and anti-FHA following a booster dose (NCT 02853929) compared to infants who received the same vaccine but whose mothers received placebo during pregnancy. Whether the diminished immune responses observed in vaccinated infants whose mothers received BOOSTRIX (non-US formulation) during pregnancy result in diminished effectiveness of pertussis vaccination in infants is unknown.

14.5 Immunological Evaluation following Revaccination with BOOSTRIX

Adults (Aged 20 to 29 Years)

A multicenter, open-label, controlled study conducted in the United States evaluated the immunogenicity of BOOSTRIX in adults aged 20 to 29 years who received an initial dose of BOOSTRIX (n = 128) or the comparator Td vaccine (MassBiologics) (n = 37) in the U.S. adolescent (aged 10 to 18 years) study (NCT01738477). BOOSTRIX was administered to all subjects 10 years after initial vaccination. The immune responses to each of the antigens contained in BOOSTRIX were evaluated in sera obtained approximately 1 month after vaccine administration. Among all vaccine recipients, the mean age was 23.5 years; 45.5% were female, and 87.9% were White.

Response to Tetanus and Diphtheria Toxoids: The antibody responses to the tetanus and diphtheria toxoids of BOOSTRIX are shown in Table 16. One month after vaccination, anti-tetanus and anti-diphtheria seroprotective rates (≥0.1 IU/mL by ELISA) were comparable between groups.

Table 16. Antibody Responses to Tetanus and Diphtheria Toxoids following BOOSTRIX in Adults Aged 20 to 29 Years (ATP Cohort for Immunogenicity)

Antibodies	n	% ≥0.1 IU/mL[a] (95% CI)	% ≥1.0 IU/mL[a] (95% CI)
Anti-tetanus			
BOOSTRIX[b]	115		
Pre-vaccination		100 (96.8, 100)	74.8 (65.8, 82.4)
Post-vaccination		100 (96.8, 100)[c]	100 (96.8, 100)[d]
Td[e]	35		
Pre-vaccination		100 (90, 100)	77.1 (59.9, 89.6)
Post-vaccination		100 (90, 100)	100 (90, 100)
Anti-diphtheria			
BOOSTRIX[b]	115		
Pre-vaccination		100 (96.8, 100)	60.9 (51.3, 69.8)
Post-vaccination		100 (96.8, 100)[c]	100 (96.8, 100)[d]
Td[e]	35		
Pre-vaccination		100 (90, 100)	65.7 (47.8, 80.9)
Post-vaccination		100 (90,100)	97.1 (85.1, 99.9)

Td manufactured by MassBiologics.

ATP = According-to-protocol; CI = Confidence Interval.

n = Number of subjects with available results.

[a] Measured by ELISA.

[b] Subjects who were revaccinated with BOOSTRIX 10 years after initial vaccination with BOOSTRIX.

[c] Seroprotection rates following revaccination with BOOSTRIX were non-inferior to an initial dose of BOOSTRIX (Td group) (lower limit of 2-sided 95% CI on the difference for second dose of BOOSTRIX minus first dose of BOOSTRIX ≥-10%).

[d] Non-inferiority criteria not prospectively defined for this endpoint.

[e] Subjects who received a dose of BOOSTRIX 10 years after initial vaccination with Td vaccine.

Response to Pertussis Antigens: The GMCs to each of the pertussis antigens 1 month following revaccination with BOOSTRIX in subjects who had received an initial dose of BOOSTRIX 10 years earlier were compared with the GMCs of infants following a 3-dose primary series of PEDIARIX *[see Clinical Studies (14.1)]*. Table 17 presents the results for the ATP cohort for immunogenicity in both studies. Anti-PT, anti-FHA, and anti-PRN antibody concentrations observed in adults 1 month after revaccination with BOOSTRIX were non-inferior to those of infants following a primary vaccination series with PEDIARIX.

Table 17. Ratio of GMCs to Pertussis Antigens following BOOSTRIX in Adults Aged 20 to 29 Years Compared with 3 Doses of PEDIARIX in Infants (ATP Cohort for Immunogenicity)

Pertussis Antibodies	BOOSTRIX[a] (n)	PEDIARIX (n)	GMC Ratio: BOOSTRIX/PEDIARIX (95% CI)
Anti-PT	115	149	1.81 (1.48, 2.21)[b]
Anti-FHA	115	149	2.37 (1.98, 2.83)[b]
Anti-PRN	115	149	9.87 (7.80, 12.49)[b]

GMC = Geometric mean antibody concentration; CI = Confidence Interval; PT = Pertussis toxin; FHA = Filamentous hemagglutinin; PRN = Pertactin.

n = Number of subjects for GMC evaluation.

[a] Subjects who were revaccinated with BOOSTRIX 10 years after initial vaccination with BOOSTRIX.

[b] BOOSTRIX was non-inferior to PEDIARIX (lower limit of 95% CI for the GMC ratio of BOOSTRIX/PEDIARIX ≥0.67).

Adults (Aged 28 to 73 Years)

A multicenter, open-label, controlled study conducted in the United States evaluated the immunogenicity of BOOSTRIX in adults aged 28 to 73 years who received an initial dose of BOOSTRIX (n = 309) or the licensed comparator Tdap vaccine (Sanofi Pasteur) (n = 138) in the U.S. adult (aged 19 to 64 years) study (NCT00489970). BOOSTRIX was administered to all subjects 9 years after initial vaccination. A control group of newly enrolled adult subjects received an initial dose of BOOSTRIX (n = 362). The immune responses to each of the antigens contained in BOOSTRIX were evaluated in sera obtained approximately 1 month after vaccine administration. Of the subjects enrolled in this study, the mean age was 52.1 years; 62.6% were female, and 86.6% were White.

Response to Tetanus and Diphtheria Toxoids: The antibody responses to the tetanus and diphtheria toxoids of BOOSTRIX are shown in Table 18. One month after vaccination, anti-tetanus and anti-diphtheria seroprotective rates (≥0.1 IU/mL by ELISA) were comparable between groups.

Table 18. Antibody Responses to Tetanus and Diphtheria Toxoids following BOOSTRIX in Adults Aged 28 to 73 Years (ATP Cohort for Immunogenicity)

Antibodies	n	% ≥0.1 IU/mL[a] (95% CI)	% ≥1.0 IU/mL[a] (95% CI)	% Booster Response[b] (95% CI)
Anti-tetanus				
BOOSTRIX[c]	268-271			
Pre-vaccination		98.1 (95.7, 99.4)	78.7 (73.3, 83.5)	
Post-vaccination		100 (98.6, 100)[d]	99.3 (97.4, 99.9)[e]	47.0 (40.9, 53.2)[f]
Tdap[g]	120-121			
Pre-vaccination		100 (97.0, 100)	84.2 (76.4, 90.2)	
Post-vaccination		100 (97.0, 100)[d]	100 (97.0, 100)[e]	36.7 (28.1, 45.9)[f]
Control[h]	324-327			
Pre-vaccination		93.8 (90.6, 96.2)	71.3 (66.0, 76.2)	
Post-vaccination		99.7 (98.3, 100)	97.6 (95.2, 98.9)	48.5 (42.9, 54.0)
Anti-diphtheria				
BOOSTRIX[c]	269-271			
Pre-vaccination		91.1 (87.0, 94.2)	42.4 (36.4, 48.5)	
Post-vaccination		99.3 (97.4, 99.9)[d]	91.9 (88.0, 94.8)[e]	62.8 (56.7, 68.6)[f]
Tdap[g]	118-121			
Pre-vaccination		95.8 (90.4, 98.6)	45.8 (36.6, 55.2)	
Post-vaccination		99.2 (95.5, 100)[d]	93.4 (87.4, 97.1)[e]	60.2 (50.7, 69.1)[f]
Control[h]	324-326			
Pre-vaccination		81.8 (77.1, 85.8)	28.4 (23.5, 33.6)	
Post-vaccination		97.9 (95.6, 99.1)	86.5 (82.3, 90.0)	68.7 (63.4, 73.7)

Tdap = Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed manufactured by Sanofi Pasteur.

ATP = According-to-protocol; CI = Confidence Interval.

n = Number of subjects with available results.

[a] Measured by ELISA.

[b] Booster response: In subjects with pre-vaccination <0.1 IU/mL, post-vaccination concentration ≥0.4 IU/mL. In subjects with pre-vaccination concentration ≥0.1 IU/mL, an increase of at least 4 times the pre-vaccination concentration.

[c] Subjects who were revaccinated with BOOSTRIX 9 years after initial vaccination with BOOSTRIX.

[d] Seroprotection rates following a dose of BOOSTRIX in subjects who had received an initial dose of BOOSTRIX or the licensed comparator Tdap vaccine were non-inferior to an initial

dose of BOOSTRIX (Control Group) (lower limit of 97.5% CI on the difference of BOOSTRIX minus Control Group ≥-10%).

[e] Non-inferiority criteria not prospectively defined for this endpoint.

[f] The booster response rates following a dose of BOOSTRIX in subjects who had received an initial dose of BOOSTRIX or the licensed comparator Tdap vaccine did not meet the pre-defined non-inferiority criteria (lower limit of the 97.5% CIs ≥-10% [BOOSTRIX minus Control Group] and [Tdap minus Control Group]).

[g] Subjects who received a dose of BOOSTRIX 9 years after initial vaccination with Tdap vaccine.

[h] Control Group = Newly enrolled subjects who received an initial dose of BOOSTRIX.

Response to Pertussis Antigens: The GMCs to each of the pertussis antigens 1 month following a dose of BOOSTRIX in subjects who had received an initial dose of BOOSTRIX or the licensed comparator Tdap vaccine (Sanofi Pasteur) 9 years earlier were compared with the GMCs of infants following a 3-dose primary series of PEDIARIX *[see Clinical Studies (14.1)]*. Table 19 presents the results for the ATP cohort for immunogenicity in both studies. Anti-PT, anti-FHA, and anti-PRN antibody concentrations observed in adults 1 month after a dose of BOOSTRIX were non-inferior to those of infants following a primary vaccination series with PEDIARIX.

Table 19. Ratio of GMCs to Pertussis Antigens following BOOSTRIX in Adults Aged 28 to 73 Years Compared with 3 Doses of PEDIARIX in Infants (ATP Cohort for Immunogenicity)

	Vaccinated with BOOSTRIX 9 Years after Initial Vaccination with:		**PEDIARIX (n)**	**GMC Ratio:**	
Pertussis Antibodies	**BOOSTRIX (n)**	**Tdap (n)**		**BOOSTRIX/ PEDIARIX (97.5% CI)**	**Tdap/ PEDIARIX (97.5% CI)**
Anti-PT	271	121	149	1.33 (1.09, 1.61)[a]	1.46 (1.14, 1.87)[a]
Anti-FHA	271	121	149	2.02 (1.72, 2.37)[a]	2.07 (1.68, 2.57)[a]
Anti-PRN	271	121	149	8.64 (6.85, 10.89)[a]	10.90 (8.27, 14.38)[a]

Tdap = Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed manufactured by Sanofi Pasteur.

GMC = Geometric mean antibody concentration; CI = Confidence Interval; PT = Pertussis toxin;

FHA = Filamentous hemagglutinin; PRN = Pertactin.
n = Number of subjects for GMC evaluation.
[a] BOOSTRIX was non-inferior to PEDIARIX (lower limit of 97.5% CI for the GMC ratio of BOOSTRIX/PEDIARIX ≥0.67).

Compared with the Control Group, non-inferiority of booster response rates to the pertussis antigens following a dose of BOOSTRIX in subjects who had received an initial dose of BOOSTRIX or another licensed Tdap vaccine (Sanofi Pasteur) was achieved for the PT antigen [BOOSTRIX minus Control Group] and the FHA antigen [Tdap minus Control Group], respectively (Table 20). Non-inferiority was not achieved for FHA and PRN booster response rates [BOOSTRIX minus Control Group] or for PT and PRN booster response rates [Tdap minus Control Group].

Table 20. Booster Responses to the Pertussis Antigens following BOOSTRIX in Adults Aged 28 to 73 Years (ATP Cohort for Immunogenicity)

Pertussis Antibodies	n	% Booster Response[a] (95% CI)	Difference in Booster Response Rates	
			BOOSTRIX minus Control Group (97.5 % CI)	Tdap minus Control Group (97.5% CI)
Anti-PT				
BOOSTRIX[b]	271	86.7 (82.1, 90.5)	-2.85 (-9.09, 3.08)[c]	
Tdap[d]	120	88.3 (81.2, 93.5)		-1.24 (-10.03, 5.57)
Control[e]	326	89.6 (85.7, 92.7)		
Anti-FHA				
BOOSTRIX[b]	271	85.6 (80.9, 89.6)	-7.05 (-13.16, -1.40)	
Tdap[d]	120	96.7 (91.7, 99.1)		4.01 (-2.38, 8.66)[c]
Control[e]	327	92.7 (89.3, 95.2)		
Anti-PRN				
BOOSTRIX[b]	271	77.5 (72.0, 82.3)	-10.32 (-17.50, -3.38)	
Tdap[d]	118	83.1 (75.0, 89.3)		-4.76 (-14.53, 3.18)
Control[e]	320	87.8 (83.7, 91.2)		

Tdap = Tetanus Toxoid, Reduced Diphtheria Toxoid and Acellular Pertussis Vaccine, Adsorbed manufactured by Sanofi Pasteur.
ATP = According-to-protocol; CI = Confidence Interval; PT = Pertussis toxin;
FHA = Filamentous hemagglutinin; PRN = Pertactin.
n = Number of subjects with available results.
[a] Booster response: In initially seronegative subjects (pre-vaccination antibody concentration below the assay cut-off), post-vaccination antibody concentrations ≥4 times the assay cut-off. In initially seropositive subjects with pre-vaccination antibody concentrations <4 times the assay cut-off, an increase of at least 4 times the pre-vaccination antibody concentration. In

initially seropositive subjects with pre-vaccination antibody concentrations ≥4 times the assay cut-off, an increase of at least 2 times the pre-vaccination antibody concentration. Assay cut-offs: anti-PT = 2.693 IU/mL; anti-FHA = 2.046 IU/mL; anti-PRN = 2.187 IU/mL.

[b] Subjects who were revaccinated with BOOSTRIX 9 years after initial vaccination with BOOSTRIX.

[c] Non-inferiority of the booster response rate for each pertussis antigen was demonstrated if the lower limit of the 97.5% CI [BOOSTRIX minus Control Group] or [Tdap minus Control Group] was above the pre-defined limit of -10%.

[d] Subjects who received a dose of BOOSTRIX 9 years after initial vaccination with Tdap vaccine.

[e] Control Group = Newly enrolled subjects who received an initial dose of BOOSTRIX.

14.6 Concomitant Administration with Meningococcal Conjugate Vaccine

The concomitant use of BOOSTRIX and a tetravalent meningococcal (groups A, C, Y, and W-135) conjugate vaccine (Sanofi Pasteur) was evaluated in a randomized study in healthy adolescents aged 11 to 18 years (NCT00282295). A total of 1,341 adolescents were vaccinated with BOOSTRIX. Of these, 446 subjects received BOOSTRIX administered concomitantly with meningococcal conjugate vaccine at different injection sites, 446 subjects received BOOSTRIX followed by meningococcal conjugate vaccine 1 month later, and 449 subjects received meningococcal conjugate vaccine followed by BOOSTRIX 1 month later.

Immune responses to diphtheria and tetanus toxoids (% of subjects with anti-tetanus and anti-diphtheria antibodies ≥1.0 IU/mL by ELISA), pertussis antigens (booster responses and GMCs), and meningococcal antigens (vaccine responses) were measured 1 month (range: 30 to 48 days) after concomitant or separate administration of BOOSTRIX and meningococcal conjugate vaccine. For BOOSTRIX given concomitantly with meningococcal conjugate vaccine compared with BOOSTRIX administered first, non-inferiority was demonstrated for all antigens, with the exception of the anti-PRN GMC. The lower limit of the 95% CI for the GMC ratio was 0.54 for anti-PRN (pre-specified limit ≥0.67). For the anti-PRN booster response, non-inferiority was demonstrated. It is not known if the efficacy of BOOSTRIX is affected by the reduced response to PRN.

There was no evidence that BOOSTRIX interfered with the antibody responses to the meningococcal antigens when measured by rabbit serum bactericidal assays (rSBA) when given concomitantly or sequentially (meningococcal conjugate vaccine followed by BOOSTRIX or BOOSTRIX followed by meningococcal conjugate vaccine).

14.7 Concomitant Administration with FLUARIX (Inactivated Influenza Vaccine)

The concomitant use of BOOSTRIX and FLUARIX was evaluated in a multicenter, open-label, randomized, controlled study (NCT00385255) of 1,497 adults aged 19 to 64 years. In one group, subjects received BOOSTRIX and FLUARIX concurrently (n = 748). The other group received

FLUARIX at the first visit, then 1 month later received BOOSTRIX (n = 749). Sera was obtained prior to and 1 month following concomitant or separate administration of BOOSTRIX and/or FLUARIX, as well as 1 month after the separate administration of FLUARIX.

Immune responses following concurrent administration of BOOSTRIX and FLUARIX were non-inferior to separate administration for diphtheria (seroprotection defined as ≥0.1 IU/mL), tetanus (seroprotection defined as ≥0.1 IU/mL and based on concentrations ≥1.0 IU/mL), PT antigen (anti-PT GMC), and influenza antigens (percent of subjects with hemagglutination-inhibition [HI] antibody titer ≥1:40 and ≥4-fold rise in HI titer). Non-inferiority criteria were not met for the anti-pertussis antigens FHA and PRN. The lower limit of the 95% CI of the GMC ratio was 0.64 for anti-FHA and 0.60 for anti-PRN and the pre-specified limit was ≥0.67. It is not known if the efficacy of BOOSTRIX is affected by the reduced response to FHA and PRN.

15 REFERENCES

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8. Andrews A, Campbell H, Riberio S, Fry N, Amirthalingham G. Boostrix-IPV Report: Effectiveness of Maternal Pertussis Vaccination in Prevention of Confirmed Pertussis in Children in England Using the Screening Method Report to 30 September 2018. Public

Health England. Unpublished. 2020.

16 HOW SUPPLIED/STORAGE AND HANDLING

BOOSTRIX is available in 0.5-mL single-dose vials and single-dose, disposable, prefilled TIP-LOK syringes (packaged without needles):

NDC 58160-842-01 Vial in Package of 10: NDC 58160-842-11

NDC 58160-842-43 Syringe in Package of 10: NDC 58160-842-52

Store refrigerated between 2º and 8ºC (36º and 46ºF). Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

Provide the following information to the vaccine recipient, parent, or guardian:

- Inform of the potential benefits and risks of immunization with BOOSTRIX.
- Inform about the potential for adverse reactions that have been temporally associated with administration of BOOSTRIX or other vaccines containing similar components.
- Instruct vaccine recipient to report any adverse events to their healthcare provider.
- Advise women who receive BOOSTRIX during pregnancy to enroll in the pregnancy registry *[see Use in Specific Populations (8.1)]*.
- Give the Vaccine Information Statements, which are required by the National Childhood Vaccine Injury Act of 1986 prior to immunization. These materials are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

Manufactured by **GlaxoSmithKline Biologicals**

Rixensart, Belgium, U.S. License 1617, and

GSK Vaccines GmbH

Marburg, Germany, U.S. License 1617

Distributed by **GlaxoSmithKline**

Durham, NC 27701

BTX:35PI

HIGHLIGHTS OF PRESCRIBING INFORMATION

These highlights do not include all the information needed to use TENIVAC® safely and effectively. See full prescribing information for TENIVAC.

TENIVAC (Tetanus and Diphtheria Toxoids Adsorbed)
Suspension for Intramuscular Injection
Initial U.S. Approval: 2003

----------INDICATIONS AND USAGE----------

TENIVAC is a vaccine indicated for active immunization for the prevention of tetanus and diphtheria in persons 7 years of age and older. (1)

----------DOSAGE AND ADMINISTRATION----------

- Each 0.5 mL dose should be administered intramuscularly. (2.5)
- Primary immunization with TENIVAC consists of 3 doses. The first 2 doses are administered 2 months apart and the third dose is administered 6-8 months after the second dose. (2.1)
- TENIVAC may be used for booster immunization against tetanus and diphtheria. Routine booster immunization against tetanus and diphtheria is recommended at 11-12 years of age and every 10 years thereafter. (2.2)
- For post-exposure diphtheria prophylaxis and for management of a tetanus prone wound, a booster dose of TENIVAC may be administered if at least 5 years have elapsed since previous receipt of a diphtheria toxoid and tetanus toxoid containing vaccine. (2.3) (2.4)

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection supplied in 0.5 mL single-dose vials or syringes. (3)

----------CONTRAINDICATIONS----------

Severe allergic reaction (e.g., anaphylaxis) to a previous dose of TENIVAC, or any other tetanus or diphtheria toxoid-containing vaccine, or any component of this vaccine. (4.1)

----------WARNINGS AND PRECAUTIONS----------

- The tip caps of the prefilled syringes may contain natural rubber latex which may cause allergic reactions in latex sensitive individuals. (5.2)
- More frequent administration of TENIVAC than described in Dosage and Administration (2.1, 2.2, 2.3, 2.4) may be associated with increased incidence and severity of adverse reactions. (5.3)
- Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine should not receive TENIVAC more frequently than every 10 years, even for tetanus prophylaxis as part of wound management. (5.4)
- Carefully consider benefits and risks before administering TENIVAC to persons with a history of Guillain-Barré syndrome within 6 weeks of a previous tetanus toxoid-containing vaccine. (5.5)

----------ADVERSE REACTIONS----------

- The most frequent solicited injection site reaction within 0-3 days following TENIVAC was pain, reported in 78.3% of study participants 11-59 years of age and 35.3% of participants ≥60 years of age. (6.1)
- The most frequent solicited systemic reaction within 0-3 days following TENIVAC was headache, reported in 17.9% of participants, overall. (6.1)
- Other common (≥10%) solicited adverse reactions within 0-3 days following TENIVAC were injection site redness, injection site swelling, malaise, muscle weakness and pain in joints. (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Sanofi Pasteur Inc. at 1-800-822-2463 (1-800-VACCINE) or VAERS at 1-800-822-7967 or http://vaers.hhs.gov

----------DRUG INTERACTIONS----------

- No safety and immunogenicity data are available on the concomitant administration of TENIVAC with other US licensed vaccines. (7.1)
- If passive protection against tetanus is required, Tetanus Immune Globulin (TIG) (Human) may be administered concomitantly at a separate site with a separate needle and syringe. (7.2)
- Immunosuppressive therapies may reduce the immune response to TENIVAC. (7.3)

----------USE IN SPECIFIC POPULATIONS----------

Pre- and post-vaccination tetanus and diphtheria seroprotection rates were lower in study participants ≥65 years of age compared to younger participants. In general, rates of solicited adverse reactions were not higher in participants ≥65 years of age compared to younger participants. (8.5)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 11/2019

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

TENIVAC® is a vaccine indicated for active immunization for the prevention of tetanus and diphtheria in persons 7 years of age and older.

2 DOSAGE AND ADMINISTRATION

2.1 Primary Immunization

In persons who have not been immunized previously against tetanus and diphtheria, primary immunization with TENIVAC consists of three 0.5 mL doses. The first 2 doses are administered 2 months apart and the third dose is administered 6-8 months after the second dose.

TENIVAC may be used to complete the primary immunization series for tetanus and diphtheria, following one or two doses of Diphtheria and Tetanus Toxoids and Pertussis Vaccine Adsorbed (whole-cell DTP), Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP), and/or Diphtheria and Tetanus Toxoids Adsorbed (DT). However, the safety and efficacy of TENIVAC in such regimens have not been evaluated.

2.2 Routine Booster Immunization

TENIVAC may be used for routine booster immunization against tetanus and diphtheria in persons 7 years of age and older. Routine booster immunization against tetanus and diphtheria is recommended in children 11-12 years of age and every 10 years thereafter.

2.3 Diphtheria Prophylaxis for Case Contacts

TENIVAC may be used for post-exposure diphtheria prophylaxis in persons 7 years of age and older who have not completed primary vaccination, whose vaccination status is unknown, or who have not been vaccinated with diphtheria toxoid within the previous 5 years. Consult recommendations of the Advisory Committee on Immunization Practices for additional interventions for diphtheria prophylaxis in close contacts of diphtheria patients. (1)

2.4 Tetanus Prophylaxis in Wound Management

For active tetanus immunization in wound management of patients 7 years of age and older, a preparation containing tetanus and diphtheria toxoids is preferred instead of single-antigen tetanus toxoid to enhance diphtheria protection. (1) TENIVAC is approved for wound management of patients 7 years of age and older.

The need for active immunization with a tetanus toxoid-containing preparation, with or without passive immunization with Tetanus Immune Globulin (TIG) (Human) depends on both the condition of the wound and the patient's vaccination history. (See Table 1.)

When indicated, TIG (Human) should be administered at a separate site, with a separate needle and syringe, according to the manufacturer's package insert. If a contraindication to using tetanus toxoid-containing preparations exists in a person who has not completed a primary immunizing course of tetanus toxoid and other than a clean, minor wound is sustained, only passive immunization with TIG (Human) should be given. (1)

Table 1: Guide for use of Tetanus and Diphtheria Toxoids Adsorbed (Td) for Tetanus Prophylaxis in Routine Wound Management in Persons 7 Years of Age and Older

History of Adsorbed Tetanus Toxoid (Doses)	Clean, Minor Wounds		All Other Wounds*	
	Td	TIG	Td	TIG
Unknown or < three	Yes	No	Yes	Yes
≥ Three†	No‡	No	No§	No

* Such as, but not limited to, wounds contaminated with dirt, puncture wounds and traumatic wounds.
† If only three doses of fluid tetanus toxoid have been received, then a fourth dose of toxoid, preferably an adsorbed toxoid should be given.
‡ Yes, if >10 years since last dose.
§ Yes, if >5 years since last dose. (More frequent boosters are not needed and can accentuate side effects.)

2.5 Administration

Just before use, shake the single-dose vial or syringe well until a uniform, white, cloudy suspension results. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If these conditions exist, the product should not be administered.

Administer the 0.5 mL dose of TENIVAC intramuscularly. Discard unused portion.

The preferred site is the deltoid muscle. The vaccine should not be injected into the gluteal area or areas where there may be a major nerve trunk.

Do not administer this product intravenously or subcutaneously.

TENIVAC should not be combined through reconstitution or mixed with any other vaccine.

3 DOSAGE FORMS AND STRENGTHS

TENIVAC is a suspension for injection available in 0.5 mL single-dose vials or syringes. [See *Description (11).*]

4 CONTRAINDICATIONS

4.1 Hypersensitivity

A severe allergic reaction (e.g., anaphylaxis) after a previous dose of TENIVAC or any other tetanus toxoid or diphtheria toxoid-containing vaccine or any other component of this vaccine is a contraindication to administration of TENIVAC. [See *Description (11).*] Because of uncertainty as to which component of the vaccine may be responsible, none of the components should be administered. Alternatively, such individuals may be referred to an allergist for evaluation if further immunizations are to be considered.

5 WARNINGS AND PRECAUTIONS

5.1 Management of Acute Allergic Reactions

Epinephrine hydrochloride solution (1:1,000) and other appropriate agents and equipment must be available for immediate use in case an anaphylactic or acute hypersensitivity reaction occurs.

5.2 Latex

The tip caps of the TENIVAC prefilled syringes may contain natural rubber latex, which may cause allergic reactions in latex sensitive individuals.

5.3 Frequency of Administration

More frequent doses of TENIVAC than described in Section 2, Dosage and Administration, may be associated with increased incidence and severity of adverse reactions. [See *Dosage and Administration (2.1, 2.2, 2.3, 2.4).*]

5.4 Arthus Reactions

Persons who experienced an Arthus-type hypersensitivity reaction following a prior dose of a tetanus toxoid-containing vaccine usually have high serum tetanus antitoxin levels and should not receive TENIVAC more frequently than every 10 years, even for tetanus prophylaxis as part of wound management.

5.5 Guillain-Barré Syndrome and Brachial Neuritis

A review by the Institute of Medicine found evidence for a causal relation between tetanus toxoid and both brachial neuritis and Guillian-Barré syndrome. (2) If Guillain-Barré syndrome occurred within 6 weeks of receipt of prior vaccine containing tetanus toxoid, the decision to give TENIVAC or any vaccine containing tetanus toxoid should be based on careful consideration of the potential benefits and possible risks.

5.6 Limitations of Vaccine Effectiveness

Vaccination with TENIVAC may not protect all individuals.

5.7 Altered Immunocompetence

If TENIVAC is administered to immunocompromised persons, including persons receiving immunosuppressive therapy, the expected immune response may not be obtained. [See *Drug Interactions (7.3).*]

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to vaccine use and for approximating rates of those events.

In a primary immunization study conducted in Canada, 18 participants, 8 of whom were 6 to 9 years of age and 10 of whom were 17 to 56 years of age, received three doses of TENIVAC. In four booster immunization studies conducted in either the US or Canada, TENIVAC was administered to 3,723 participants overall, ranging in age from 11 to 93 years.

In one of these studies, a US multi-center booster immunization study (TDC01), 2,250 adolescents and adults ages 11-59 years of age received TENIVAC in an open-label design and adults 60 years of age and over were randomized to receive either TENIVAC (N = 700) or DECAVAC (Td manufactured by Sanofi Pasteur Inc.) (N = 701). Vaccine assignment for

participants ≥60 years of age was unblinded to pharmacists and vaccination nurses, but was blinded to other study personnel and participants. Among participants who received TENIVAC, overall, 80.4% were Caucasian, 3.3% Black, 5.1% Hispanic, 4.5% Asian and 6.6% other races. Among participants ≥60 years of age, the racial distribution was similar for the TENIVAC and DECAVAC groups. Among participants who received TENIVAC, the proportion of participants who were female varied by age group (44.4% of participants 11-18 years of age, 70.1% of participants 19-59 years of age and 62.4% of participants ≥60 years of age). Among participants ≥60 years of age who received DECAVAC, 57.6% were female. Nearly all (99.8%) enrolled participants and all participants in the per-protocol immunogenicity population had a reported or documented history of previous immunization against tetanus and diphtheria and, by report, had not received a vaccine containing tetanus or diphtheria toxoid within 5 years prior to enrollment.

In the US multi-center booster immunization study, solicited injection site reactions and systemic adverse events were monitored on diary cards for a subset of participants 11-59 years of age and for all participants ≥60 years of age. The incidence and severity of solicited injection site reactions and selected solicited systemic adverse events that occurred within 3 days following vaccination are shown in Table 2.

Table 2: Frequency and Severity of Selected Solicited Adverse Events Within 0-3 Days Following TENIVAC or DECAVAC in a US Study

	TENIVAC			**DECAVAC**
	Adolescents 11 to 18 years N = 491-492 %	**Adults 19 to 59 years N = 247 %**	**Adults ≥60 years N = 688-695 %**	**Adults ≥60 years N = 686-693 %**
Injection Site Adverse Reactions				
Pain				
Any	80.1	74.9	35.3	29.4
Moderate*	15.0	18.2	2.9	2.3
Severe†	0.2	0.4	0.6	0.7
Redness				
Any	25.6	15.8	18.1	18.0
≥35 mm to <50 mm	1.2	2.4	0.7	1.3
≥50 mm	0.4	0.4	2.3	1.9
Swelling				
Any	15.0	17.0	12.1	13.0
≥35 mm to <50 mm	1.2	2.8	1.0	1.3
≥50 mm	1.8	2.8	1.7	1.3
Systemic Adverse Events				
Fever				
≥37.5°C	4.3	5.7	2.5	3.8
≥38.0°C to <39°C	0.8	1.6	0.6	0.9
≥39°C	0.0	0.0	0.1	0.1
Headache				
Any	23.0	25.1	11.7	10.8
Moderate*	4.3	7.3	1.6	1.4
Severe†	0.6	0.8	0.0	0.3
Muscle Weakness				

	TENIVAC			DECAVAC
	Adolescents 11 to 18 years N = 491-492 %	**Adults 19 to 59 years N = 247 %**	**Adults ≥60 years N = 688-695 %**	**Adults ≥60 years N = 686-693 %**
Any	32.3	17.4	4.9	5.9
Moderate*	7.3	3.2	1.3	1.0
Severe†	0.6	0.4	0.1	0.1
Malaise				
Any	14.5	17.0	8.9	8.8
Moderate*	3.5	3.2	2.4	1.2
Severe†	0.8	0.4	0.1	0.4
Pain in Joints				
Any	15.7	10.9	8.5	7.4
Moderate*	2.8	1.6	2.2	1.4
Severe†	0.6	0.4	0.1	0.0

* Moderate: interfered with activities, but did not require medical care or absenteeism.
† Severe: incapacitating, unable to perform usual activities, may have/or required medical care or absenteeism.

In the US booster immunization study, among participants ≥60 years of age, 7 (1.0%) participants in the TENIVAC group and 10 (1.4%) participants in the DECAVAC group experienced a serious adverse event within 30 days following vaccination. During this period, 2 (0.3%) participants 19-59 years of age and no participants 11-18 years of age experienced a serious adverse event following TENIVAC. Serious adverse events within 30 days following TENIVAC included localized infection, asthma, colonic polyp, cellulitis, angina pectoris, hip and wrist fracture, cholecystitis, chest pain and cerebrovascular accident.

There were five deaths reported during the study. All of the reported deaths were in participants ≥60 years of age and occurred >30 days post-vaccination: three in the TENIVAC group (cardiopulmonary arrest; myocardial infarction and septic shock; and unknown cause) and two in the DECAVAC group (myocardial infarction and congestive heart failure; and liver cancer).

In the primary immunization study (N = 18) in which serious adverse events were monitored for 3 days following each vaccination and in three other booster immunization studies in which serious adverse events were monitored for either four days (N = 347) or one month (N = 426) following vaccination, no serious adverse events were reported.

6.2 Postmarketing Experience

The following adverse events have been spontaneously reported during the postmarketing use of TENIVAC. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

The following adverse events were included based on severity, frequency of reporting or the strength of causal association to TENIVAC:

- **Blood and lymphatic system disorders**

  Lymphadenopathy

- **Immune system disorders**

Allergic reactions (such as erythematous rash, maculopapular rash, urticaria and pruritus); anaphylactic reaction (bronchospasm and angioedema).

- **Nervous system disorders**

  Paresthesia, dizziness, syncope

  Guillain-Barré syndrome

- **Gastrointestinal disorders**

  Vomiting

- **Musculoskeletal, connective tissue and bone disorders**

  Myalgia, pain in extremities

- **General disorders and administration site conditions**

  Injection site reactions (including inflammation, mass, edema, induration, warmth, pruritus, cellulitis, discomfort)

  Fatigue, edema peripheral

7 DRUG INTERACTIONS

7.1 Concomitant Vaccine Administration

No safety and immunogenicity data are available on the concomitant administration of TENIVAC with other US licensed vaccines.

7.2 Tetanus Immune Globulin (Human)

If passive protection against tetanus is required, TIG (Human) may be administered according to its prescribing information, concomitantly with TENIVAC at a separate site with a separate needle and syringe. [See *Dosage and Administration (2.4).*]

7.3 Immunosuppressive Treatments

Immunosuppressive therapies, including irradiation, antimetabolites, alkylating agents, cytotoxic drugs and corticosteroids (used in greater than physiologic doses), may reduce the immune response to TENIVAC. [See *Warnings and Precautions (5.7).*]

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. There are no adequate and well-controlled studies of TENIVAC administration in pregnant women in the U.S. There are insufficient human data from TENIVAC administered during pregnancy to establish the presence or absence of a vaccine-associated risk.

A developmental toxicity study has been performed in female rabbits administered a single human dose of TENIVAC prior to mating and during gestation. This study revealed no evidence of harm to the fetus due to TENIVAC. (See *Animal data*)

Data

Animal data

In a developmental toxicity study, female rabbits received a single human dose (0.5 mL) of TENIVAC by intramuscular injection 17 and 10 days prior to mating, and on gestation days 6 and 29. No adverse effects on pre-weaning development up to post-natal day 35 were observed. There were no vaccine-related fetal malformations or variations observed.

8.2 Lactation

It is not known whether TENIVAC components are excreted in human milk. Data are not available to assess the effect of administration of TENIVAC on breastfed infants or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for TENIVAC and any potential adverse effects on the breastfed child from TENIVAC or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

TENIVAC is not approved for use in infants and children younger than 7 years of age. Safety and effectiveness of TENIVAC in this age group have not been established.

8.5 Geriatric Use

In one clinical study, (TDC01) 449 participants 65 years of age and over, including 192 participants who were 75 years of age and over received a dose of TENIVAC. A lower proportion of participants 65 years of age and over had a pre-vaccination seroprotective level of antibody to tetanus toxoid and diphtheria toxin compared to adolescents and adults less than 65 years of age. The proportion of participants 65 years of age and over with a seroprotective level of antibody following TENIVAC was marginally lower for tetanus and lower for diphtheria compared to younger participants. In general, rates of solicited adverse events were not higher in participants 65 years of age and over compared to younger participants. [See *Adverse Reactions (6), Clinical Pharmacology (12.1),* and *Clinical Studies (14.2).*]

11 DESCRIPTION

TENIVAC, Tetanus and Diphtheria Toxoids Adsorbed, is a sterile isotonic suspension of tetanus and diphtheria toxoids adsorbed on aluminum phosphate.

Each 0.5 mL dose of TENIVAC contains the following active ingredients:

Tetanus Toxoid	5 Lf
Diphtheria Toxoid	2 Lf

Other ingredients per 0.5 mL dose include 1.5 mg of aluminum phosphate (0.33 mg of aluminum) as the adjuvant and ≤5.0 mcg of residual formaldehyde.

Clostridium tetani is grown in modified Mueller-Miller casamino acid medium without beef heart infusion. (3) Tetanus toxin is detoxified with formaldehyde and purified by ammonium sulfate fractionation and diafiltration. *Corynebacterium diphtheriae* is grown in modified Mueller's growth medium. (4) After purification by ammonium sulfate fractionation, diphtheria toxin is detoxified with formaldehyde and diafiltered. Tetanus and diphtheria toxoids are individually adsorbed onto aluminum phosphate.

The adsorbed tetanus and diphtheria toxoids are combined with aluminum phosphate (as adjuvant), sodium chloride and water for injection. This product contains no preservative.

In the guinea pig potency test, the tetanus toxoid component induces at least 2 neutralizing units/mL of serum and the diphtheria toxoid component induces at least 0.5 neutralizing units/mL of serum.

The tip caps of the prefilled syringes may contain natural rubber latex. The vial stoppers do not contain latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Tetanus

Tetanus is an acute disease caused by an extremely potent neurotoxin produced by *C tetani*. Protection against disease is due to the development of neutralizing antibodies to tetanus toxin. A serum tetanus antitoxin level of at least 0.01 IU/mL, measured by neutralization assay is considered the minimum protective level. (5) (6) A tetanus antitoxoid level of ≥0.1 IU/mL as measured by the ELISA used in some clinical studies of TENIVAC is considered protective.

Diphtheria

Diphtheria is an acute toxin-mediated disease caused by toxigenic strains of *C diphtheriae*. Protection against disease is due to the development of neutralizing antibodies to diphtheria toxin. A serum diphtheria antitoxin level of 0.01 IU/mL is the lowest level giving some degree of protection. Antitoxin levels of at least 0.1 IU/mL are generally regarded as protective. (5) A level of at least of 1.0 IU/mL has been associated with long-term protection. (7)

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

TENIVAC has not been evaluated for carcinogenic or mutagenic potential or impairment of male fertility in animals. Vaccination of female rabbits with TENIVAC had no effects on fertility. [See *Use in Specific Populations (8.1)*].

14 CLINICAL STUDIES

14.1 Primary Immunization

A three-dose primary immunization series with TENIVAC was evaluated in 17 participants ages 6 to 56 years in a study conducted in Canada. [See *Adverse Reactions (6.1)*.] The first two doses were administered two months apart, followed by a third dose six to eight months after the second dose. Serum tetanus antitoxin levels were measured by an *in vivo* neutralizing assay and

serum diphtheria antitoxin levels were measured by an *in vitro* neutralizing assay. [See *Clinical Pharmacology (12.1)*.] All 17 participants had serum tetanus and diphtheria antitoxin levels pre-vaccination and 7 days post-vaccination <0.01 IU/mL, consistent with no previous immunization. Four weeks following the second dose, all 17 participants had a serum tetanus antitoxin level >0.1 IU/mL and a serum diphtheria antitoxin level ≥0.01 IU/mL. Four weeks following the third dose, all 17 participants had a serum diphtheria antitoxin level >0.1 IU/mL.

14.2 Booster Immunization

In the US multicenter booster immunization study (TDC01) [see *Adverse Reactions (6.1)*], the immune response to a dose of TENIVAC was evaluated in an open-label manner in a subset of participants 11 to 59 years of age, and in comparison to DECAVAC in participants ≥60 years of age who were randomized to receive a dose of either TENIVAC or DECAVAC . Tetanus immune responses, measured by ELISA [see *Clinical Pharmacology (12.1)*] are presented in Table 3. Diphtheria immune responses, measured by a microneutralization assay [see *Clinical Pharmacology (12.1)*], are presented in Table 4.

Among adults 65 years of age and over who received TENIVAC (N = 419), 94.5% (95% confidence interval 91.9, 96.5) had a post-vaccination tetanus antitoxoid level ≥0.1 IU/mL and 61.1% (95% confidence interval 56.2, 65.8) had a post-vaccination diphtheria antitoxoid level ≥0.1 IU/mL.

Table 3: Tetanus Antitoxoid Levels and Booster Response Rates Following a Dose of TENIVAC, by Age Group, and for Adults ≥60 Years of Age, Compared to DECAVAC, per Protocol Immunogenicity Population

Treatment Group	Age Group	Timing	Percent of Participants With Specified Level of Tetanus Antitoxoid and Booster Response		
			≥0.1 IU/mL % (95% CI)	≥1.0 IU/mL % (95% CI)	Booster Response* % (95% CI)
TENIVAC	Adolescents 11 to 18 years (N = 470)	Pre-	97.9 (96.1, 99.0)	48.7 (44.1, 53.3)	-
		Post-	100.0 (99.2, 100)	99.8 (98.8, 100)	92.8 (90.0, 94.9)
	Adults 19 to 59 years (N = 237)	Pre-	97.5 (94.6, 99.1)	77.6 (71.8, 82.8)	-
		Post-	100.0 (98.5, 100)	99.6 (97.7, 100)	84.0 (78.7, 88.4)
	Adults ≥60 years (N = 661)	Pre-	76.2 (72.8, 79.4)	43.7 (39.9, 47.6)	-
		Post-	96.1† (94.3, 97.4)	90.6‡ (88.1, 92.7)	82.3§ (79.2, 85.1)
DECAVAC	Adults ≥60 years (N = 658)	Pre-	75.2 (71.7, 78.5)	45.7 (41.9, 49.6)	-
		Post-	97.3 (95.7, 98.4)	91.9 (89.6, 93.9)	83.7 (80.7, 86.5)

* Booster response: If pre-vaccination level ≤0.10 IU/mL, 4-fold increase and post-vaccination level ≥0.10 IU/mL. If pre-vaccination level >0.10 IU/mL and ≤2.7 IU/mL, 4-fold increase. If pre-vaccination level >2.7 IU/mL, 2-fold increase.

† TENIVAC non-inferior to DECAVAC [upper limit of 95% CI for difference (DECAVAC minus TENIVAC) <5%].
‡ Non-inferiority criteria not prospectively specified for this endpoint.
§ TENIVAC non-inferior to DECAVAC [upper limit of 95% CI for difference (DECAVAC minus TENIVAC) <10%].
Pre- indicates pre-vaccination bleed.
Post- indicates 26-42 days post-vaccination bleed.

Table 4: Diphtheria Antitoxin Levels and Booster Response Rates Following a Dose of TENIVAC, by Age Group, and for Adults ≥60 Years of Age, Compared to DECAVAC, per Protocol Immunogenicity Population

Treatment Group	Age Group	Timing	Percent of Participants With Specified Level of Diphtheria Antitoxin and Booster Response			
			≥0.01 IU/mL % (95% CI)	≥0.1 IU/mL % (95% CI)	≥1.0 IU/mL % (95% CI)	Booster Response* % (95% CI)
TENIVAC	Adolescents 11 to 18 years (N = 470)	Pre-	99.1 (97.8, 99.8)	78.7 (74.7, 82.3)	18.5 (15.1, 22.3)	-
		Post-	100.0 (99.2, 100)	99.8 (98.8, 100)	98.9 (97.5, 99.7)	95.7 (93.5, 97.4)
	Adults 19 to 59 years (N = 237)	Pre-	96.6 (93.5, 98.5)	73.0 (66.9, 78.5)	18.6 (13.8, 24.1)	-
		Post-	99.2 (97.0, 99.9)	97.5 (94.6, 99.1)	91.1 (86.8, 94.4)	89.9 (85.3, 93.4)
	Adults ≥60 years (N = 661)	Pre-	61.9 (58.1, 65.6)	29.0 (25.6, 32.7)	8.5 (6.5, 10.9)	-
		Post-	88.0† (85.3, 90.4)	71.1‡ (67.5, 74.5)	47.5† (43.6, 51.4)	65.5‡ (61.7, 69.1)
DECAVAC	Adults ≥60 years (N = 658)	Pre-	61.7 (57.9, 65.4)	32.2 (28.7, 35.9)	10.5 (8.3, 13.1)	-
		Post-	87.4 (84.6, 89.8)	70.7 (67.0, 74.1)	45.7 (41.9, 49.6)	62.9 (59.1, 66.6)

* Booster response: If pre-vaccination level ≤0.10 IU/mL, 4-fold increase and post-vaccination level ≥0.10 IU/mL. If pre-vaccination level >0.10 IU/mL and ≤2.56 IU/mL, 4-fold increase. If pre-vaccination level >2.56 IU/mL, 2-fold increase.
† Non-inferiority criteria not prospectively specified for this endpoint.
‡ TENIVAC non-inferior to DECAVAC [upper limit of 95% CI for difference (DECAVAC minus TENIVAC) <10%].
Pre- indicates pre-vaccination bleed.
Post- indicates 26-42 days post-vaccination bleed.

15 REFERENCES

1 CDC. Diphtheria, tetanus and pertussis: recommendations for vaccine use and other preventive measures. Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR 1991;40(RR-10):1-28.

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16 HOW SUPPLIED/STORAGE AND HANDLING

Single-dose Vial, NDC No. 49281-215-58; in package of 10 vials, NDC No. 49281-215-10. Contains no latex.

Single-dose Syringe, NDC No. 49281-215-88; in package of 10 syringes, NDC No. 49281-215-15. The tip caps of the prefilled syringes may contain natural rubber latex. No other components contain latex.

TENIVAC should be stored at 2° to 8°C (35° to 46°F). DO NOT FREEZE. Product which has been exposed to freezing should not be used. Do not use after expiration date shown on the label.

17 PATIENT COUNSELING INFORMATION

Before administration of TENIVAC health-care providers should inform the patient, parent or guardian of the benefits and risks of the vaccine and the importance of completing the primary immunization series or receiving recommended booster doses, as appropriate, unless a contraindication to further immunization exists.

The health-care provider should inform the patient, parent or guardian about the potential for adverse reactions that have been temporally associated with TENIVAC or other vaccines containing similar components. The health-care provider should provide the Vaccine Information Statements (VISs) which are required by the National Childhood Vaccine Injury Act of 1986 to be given with each immunization. Patients, parents, or guardians should be instructed to report adverse reactions to their health-care provider.

Manufactured by:
Sanofi Pasteur Limited
Toronto Ontario Canada

Distributed by:
Sanofi Pasteur Inc.
Swiftwater PA 18370 USA

TENIVAC® is a registered trademark of the Sanofi Pasteur group and its subsidiaries.

R6-1119 USA

Varicella (Chickenpox)

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use VARIVAX safely and effectively. See full prescribing information for VARIVAX.

VARIVAX®
Varicella Virus Vaccine Live
Suspension for subcutaneous injection
Initial U.S. Approval: 1995

----------INDICATIONS AND USAGE----------
VARIVAX is a vaccine indicated for active immunization for the prevention of varicella in individuals 12 months of age and older. (1)

---------- DOSAGE AND ADMINISTRATION ----------
Administer a 0.5-mL dose of VARIVAX subcutaneously. (2.1)
Children (12 months to 12 years of age)
- The first dose is administered at 12 to 15 months of age. (2.1)
- The second dose is administered at 4 to 6 years of age. (2.1)
- There should be a minimum interval of 3 months between doses. (2.1)

Adolescents (≥13 years of age) and Adults
- Two doses are administered at a minimum interval of 4 weeks. (2.1)

---------- DOSAGE FORMS AND STRENGTHS ----------
Suspension for injection (0.5-mL dose) supplied as a lyophilized vaccine to be reconstituted using the accompanying sterile diluent. (2.2, 3, 16)

----------CONTRAINDICATIONS----------
- History of severe allergic reaction to any component of the vaccine (including neomycin and gelatin) or to a previous dose of varicella vaccine. (4.1)
- Immunosuppression. (4.2)
- Moderate or severe febrile illness. (4.3)
- Active untreated tuberculosis. (4.4)
- Pregnancy. (4.5, 8.1, 17)

---------- WARNINGS AND PRECAUTIONS----------
- Evaluate individuals for immune competence prior to administration of VARIVAX if there is a family history of congenital or hereditary immunodeficiency. (5.1)
- Avoid close contact with high-risk individuals susceptible to varicella because of possible transmission of varicella vaccine virus. (5.3)
- Immune Globulins (IG) and other blood products should not be given concomitantly with VARIVAX. (5.4, 7.2)
- Avoid use of salicylates for 6 weeks following administration of VARIVAX to children and adolescents. (5.5, 7.1)

---------- ADVERSE REACTIONS ----------
- Frequently reported (≥10%) adverse reactions in children ages 1 to 12 years include:
  - fever ≥102.0°F (38.9°C) oral: 14.7%
  - injection-site complaints: 19.3% (6.1)
- Frequently reported (≥10%) adverse reactions in adolescents and adults ages 13 years and older include:
  - fever ≥100.0°F (37.8°C) oral: 10.2%
  - injection-site complaints: 24.4% (6.1)
- Other reported adverse reactions in all age groups include:
  - varicella-like rash (injection site)
  - varicella-like rash (generalized) (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact Merck Sharp & Dohme Corp., a subsidiary of Merck & Co., Inc., at 1-877-888-4231 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov .

----------DRUG INTERACTIONS----------
- Reye syndrome has been reported in children and adolescents following the use of salicylates during wild-type varicella infection. (5.5, 7.1)
- Administration of immune globulins and other blood products concurrently with VARIVAX vaccine may interfere with the expected immune response. (5.4, 7.2)
- VARIVAX vaccination may result in a temporary depression of purified protein derivative (PPD) tuberculin skin sensitivity. (7.3)

---------- USE IN SPECIFIC POPULATIONS ----------
Pregnancy: Do not administer VARIVAX to females who are pregnant. Pregnancy should be avoided for 3 months following vaccination with VARIVAX. (4.5, 8.1, 17)

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.

Revised: 11/2022

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

VARIVAX® is a vaccine indicated for active immunization for the prevention of varicella in individuals 12 months of age and older.

2 DOSAGE AND ADMINISTRATION

Subcutaneous administration only

2.1 Recommended Dose and Schedule

Each 0.5 mL dose of VARIVAX is administered subcutaneously.

Children (12 months to 12 years of age)

The first dose is administered at 12 to 15 months of age but may be given anytime through 12 years of age.

The second dose is administered at 4 to 6 years of age. At least 3 months should elapse between a dose of varicella-containing vaccine and VARIVAX.

At least 1 month should elapse between a dose of measles-containing vaccine and a dose of VARIVAX if the vaccines are not given concurrently *[see Clinical Studies (14.1)].*

Adolescents (≥13 years of age) and Adults

Two doses of VARIVAX are administered at a minimum interval of 4 weeks *[see Clinical Studies (14.1)]*.

2.2 Reconstitution Instructions

Use a sterile syringe free of preservatives, antiseptics, and detergents for each reconstitution and injection of VARIVAX because these substances may inactivate the vaccine virus. When reconstituting the vaccine, use only the sterile diluent supplied with VARIVAX. The sterile diluent does not contain preservatives or other anti-viral substances which might inactivate the vaccine virus.

To reconstitute the vaccine, withdraw the total volume of supplied sterile diluent and inject into the lyophilized vaccine vial. Agitate to dissolve completely. Discard if the lyophilized vaccine cannot be dissolved.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Do not use the product if particulates are present or if it appears discolored. Visually inspect the vaccine before and after reconstitution prior to administration. Before reconstitution, the lyophilized vaccine is a white compact crystalline plug. VARIVAX, when reconstituted, is a clear, colorless to pale yellow liquid.

Withdraw the entire amount of reconstituted vaccine, inject the total volume and discard vial.

To minimize loss of potency, administer VARIVAX immediately after reconstitution. Discard if reconstituted vaccine is not used within 30 minutes.

Do not freeze reconstituted vaccine.

Do not combine VARIVAX with any other vaccine through reconstitution or mixing.

2.3 Method of Administration

Inject the vaccine subcutaneously into the outer aspect of the deltoid region of the upper arm or into the higher anterolateral area of the thigh.

3 DOSAGE FORMS AND STRENGTHS

VARIVAX is a suspension for injection supplied as a single-dose vial of lyophilized vaccine to be reconstituted using the accompanying sterile diluent *[see Dosage and Administration (2.2) and How Supplied/Storage and Handling (16)]*. A single dose after reconstitution is 0.5 mL.

4 CONTRAINDICATIONS

4.1 Severe Allergic Reaction

Do not administer VARIVAX to individuals with a history of anaphylactic or severe allergic reaction to any component of the vaccine (including neomycin and gelatin) or to a previous dose of a varicella-containing vaccine.

4.2 Immunosuppression

Do not administer VARIVAX to individuals who are immunodeficient or immunosuppressed due to disease or medical therapy.

Disseminated varicella disease and extensive vaccine-associated rash have been reported in individuals who are immunosuppressed or immunodeficient who were inadvertently vaccinated with a varicella-containing vaccine.

4.3 Moderate or Severe Febrile Illness

Do not administer VARIVAX to individuals with an active febrile illness with fever >101.3°F (>38.5°C).

4.4 Active Untreated Tuberculosis

Do not administer VARIVAX to individuals with active, untreated tuberculosis (TB).

4.5 Pregnancy

Do not administer VARIVAX to individuals who are pregnant or planning on becoming pregnant in the next 3 months. Wild-type varicella is known to cause fetal harm *[see Use in Specific Populations (8.1) and Patient Counseling Information (17)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Family History of Immunodeficiency

Vaccination should be deferred in individuals with a family history of congenital or hereditary immunodeficiency until the individual's immune status has been evaluated and the individual has been found to be immunocompetent.

5.2 Use in HIV-Infected Individuals

The Advisory Committee on Immunization Practices (ACIP) has recommendations on the use of varicella vaccine in HIV-infected individuals.

5.3 Risk of Vaccine Virus Transmission

Post-marketing experience suggests that transmission of varicella vaccine virus (Oka/Merck) resulting in varicella infection including disseminated disease may occur between vaccine recipients (who develop or do not develop a varicella-like rash) and contacts susceptible to varicella including healthy as well as high-risk individuals.

Due to the concern for transmission of vaccine virus, vaccine recipients should attempt to avoid whenever possible close association with susceptible high-risk individuals for up to six weeks following vaccination with VARIVAX. Susceptible high-risk individuals include:

- Immunocompromised individuals;
- Pregnant women without documented history of varicella or laboratory evidence of prior infection;
- Newborn infants of mothers without documented history of varicella or laboratory evidence of prior infection and all newborn infants born at <28 weeks gestation regardless of maternal varicella immunity.

5.4 Immune Globulins and Transfusions

Immune Globulins (IG) and other blood products should not be given concomitantly with VARIVAX *[see Drug Interactions (7.2)]*. These products may contain antibodies that interfere with vaccine virus replication and decrease the expected immune response.

The ACIP has specific recommendations for intervals between administration of antibody-containing products and live virus vaccines.

5.5 Salicylate Therapy

Avoid use of salicylates (aspirin) or salicylate-containing products in children and adolescents 12 months through 17 years of age for six weeks following vaccination with VARIVAX because of the association of Reye syndrome with salicylate therapy and wild-type varicella infection *[see Drug Interactions (7.1)].*

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared to rates in the clinical trials of another vaccine and may not reflect the rates observed in clinical practice. Vaccine-related adverse reactions reported during clinical trials were assessed by the study investigators to be possibly, probably, or definitely vaccine-related and are summarized below.

*"The placebo (Lot 909/C-H663) was identical in appearance to the vaccine in both lyophilized and reconstituted forms but contained no viral material. The placebo consisted of lyophilized stabilizer containing approximately 45 mg neomycin per milliiter." Reference: https://pubmed.ncbi.nlm.nih.gov/6325909/

In clinical trials {1-8}, VARIVAX was administered to over 11,000 healthy children, adolescents, and adults.

In a double-blind, placebo-controlled study among 914 healthy children and adolescents who were serologically confirmed to be susceptible to varicella, the only adverse reactions that occurred at a significantly ($p<0.05$) greater rate in vaccine recipients than in placebo recipients were pain and redness at the injection site {1}.

Children 1 to 12 Years of Age

One-Dose Regimen in Children

In clinical trials involving healthy children monitored for up to 42 days after a single dose of VARIVAX, the frequency of fever, injection-site complaints, or rashes were reported as shown in Table 1:

Table 1: Fever, Local Reactions, and Rashes (%) in Children 1 to 12 Years of Age 0 to 42 Days After Receipt of a Single Dose of VARIVAX

Reaction	N	% Experiencing Reaction	Peak Occurrence During Postvaccination Days
Fever ≥102.0°F (38.9°C) Oral	8827	14.7%	0 to 42
Injection-site complaints (pain/soreness, swelling and/or erythema, rash, pruritus, hematoma, induration, stiffness)	8916	19.3%	0 to 2
Varicella-like rash (injection site) Median number of lesions	8916	3.4% 2	8 to 19
Varicella-like rash (generalized) Median number of lesions	8916	3.8% 5	5 to 26

In addition, adverse events occurring at a rate of ≥1% are listed in decreasing order of frequency: upper respiratory illness, cough, irritability, fatigue, disturbed sleep, diarrhea, loss of appetite, vomiting, otitis, headache, malaise, abdominal pain, other rash, nausea, chills, lymphadenopathy, myalgia, lower respiratory illness, allergic reactions (including allergic rash, hives), stiff neck, arthralgia, itching.

Pneumonitis has been reported rarely (<1%) in children vaccinated with VARIVAX.

Febrile seizures have occurred at a rate of <0.1% in children vaccinated with VARIVAX.

Two-Dose Regimen in Children

Nine hundred eighty-one (981) subjects in a clinical trial received 2 doses of VARIVAX 3 months apart and were actively followed for 42 days after each dose. The 2-dose regimen of varicella vaccine had a safety profile comparable to that of the 1-dose regimen. The overall incidence of injection-site clinical complaints (primarily erythema and swelling) observed in the first 4 days following vaccination was 25.4% Postdose 2 and 21.7% Postdose 1, whereas the overall incidence of systemic clinical complaints in the 42-day follow-up period was lower Postdose 2 (66.3%) than Postdose 1 (85.8%).

Adolescents (13 Years of Age and Older) and Adults

In clinical trials involving healthy adolescents and adults, the majority of whom received two doses of VARIVAX and were monitored for up to 42 days after any dose, the frequencies of fever, injection-site complaints, or rashes are shown in Table 2.

Table 2: Fever, Local Reactions, and Rashes (%) in Adolescents and Adults 0 to 42 Days After Receipt of VARIVAX

Reaction	N	% Post Dose 1	Peak Occurrence in Postvaccination Days	N	% Post Dose 2	Peak Occurrence in Postvaccination Days
Fever ≥100.0°F (37.8°C) Oral	1584	10.2%	14 to 27	956	9.5%	0 to 42
Injection-site complaints (soreness, erythema, swelling, rash, pruritus, pyrexia, hematoma, induration, numbness)	1606	24.4%	0 to 2	955	32.5%	0 to 2
Varicella-like rash (injection site)	1606	3%	6 to 20	955	1%	0 to 6
Median number of lesions		2			2	
Varicella-like rash (generalized)	1606	5.5%	7 to 21	955	0.9%	0 to 23
Median number of lesions		5			5.5	

In addition, adverse events reported at a rate of ≥1% are listed in decreasing order of frequency: upper respiratory illness, headache, fatigue, cough, myalgia, disturbed sleep, nausea, malaise, diarrhea, stiff neck, irritability, lymphadenopathy, chills, abdominal pain, loss of appetite, arthralgia, otitis, itching, vomiting, other rashes, lower respiratory illness, allergic reactions (including allergic rash, hives).

6.2 Post-Marketing Experience

The following adverse events have been identified during post approval use of VARIVAX. Because the events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to vaccine exposure.

Body as a Whole

Anaphylaxis (including anaphylactic shock) and related phenomena such as angioneurotic edema, facial edema, and peripheral edema.

Eye Disorders

Necrotizing retinitis (in immunocompromised individuals).

Hemic and Lymphatic System

Aplastic anemia; thrombocytopenia (including idiopathic thrombocytopenic purpura (ITP)).

Infections and Infestations

Varicella (vaccine strain).

Nervous/Psychiatric

Encephalitis; cerebrovascular accident; transverse myelitis; Guillain-Barré syndrome; Bell's palsy; ataxia; non-febrile seizures; aseptic meningitis; meningitis; dizziness; paresthesia.

Cases of encephalitis or meningitis caused by vaccine strain varicella virus have been reported in immunocompetent individuals previously vaccinated with VARIVAX months to years after vaccination. Reported cases were commonly associated with preceding or concurrent herpes zoster rash *[see Clinical Pharmacology (12.2)]*.

Respiratory

Pharyngitis; pneumonia/pneumonitis.

Skin

Stevens-Johnson syndrome; erythema multiforme; Henoch-Schönlein purpura; secondary bacterial infections of skin and soft tissue, including impetigo and cellulitis; herpes zoster.

The vaccine virus (Oka/Merck strain) contained in VARIVAX may establish latency of varicella zoster virus in immunocompetent individuals, with the potential for later development of herpes zoster *[see Clinical Pharmacology (12.2)].*

7 DRUG INTERACTIONS

7.1 Salicylates

No cases of Reye syndrome have been observed following vaccination with VARIVAX. Vaccine recipients should avoid use of salicylates for 6 weeks after vaccination with VARIVAX, as Reye syndrome has been reported following the use of salicylates during wild-type varicella infection *[see Warnings and Precautions (5.5)]*.

7.2 Immune Globulins and Transfusions

Administration of immune globulins and other blood products concurrently with VARIVAX may interfere with the expected immune response *[see Warnings and Precautions (5.4)]* {9}. The ACIP has specific recommendations for intervals between administration of antibody-containing products and live virus vaccines.

7.3 Tuberculin Skin Testing

Tuberculin skin testing, with tuberculin purified protein derivative (PPD), may be performed before VARIVAX is administered or on the same day, or at least 4 weeks following vaccination with VARIVAX, as other live virus vaccines may cause a temporary depression of tuberculin skin test sensitivity leading to false negative results.

7.4 Use with Other Vaccines

VARIVAX can be administered concurrently with other live viral vaccines. If not given concurrently, at least 1 month should elapse between a dose of a live attenuated measles virus-containing vaccine and a dose of VARIVAX. In children through the age of 12 years at least 3 months should elapse between administration of 2 doses of a live attenuated varicella virus-containing vaccine. For adolescents and adults, 2 doses of VARIVAX may be separated by 1 month *[see Dosage and Administration (2.1)].*

VARIVAX may be administered concomitantly with M-M-R II® (Measles, Mumps, and Rubella Virus Vaccine Live), *Haemophilus influenzae* type b conjugate (meningococcal protein conjugate) and hepatitis B (recombinant). Additionally, VARIVAX may be administered concomitantly with inactivated diphtheria-tetanus and acellular pertussis vaccines *[see Clinical Studies (14.4)].*

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

VARIVAX is contraindicated for use in pregnant women because the vaccine contains live, attenuated varicella virus, and it is known that wild-type varicella virus, if acquired during pregnancy, can cause congenital varicella syndrome *[see Contraindications (4.5) and Patient Counseling Information (17)]*. No increased risk for miscarriage, major birth defect or congenital varicella syndrome was observed in a pregnancy exposure registry that monitored outcomes after inadvertent use. There are no relevant animal data.

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the US general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4%, and 15% to 20%, respectively.

Human Data

A pregnancy exposure registry was maintained from 1995 to 2013 to monitor pregnancy and fetal outcomes following inadvertent administration of VARIVAX. The registry prospectively enrolled 1522 women who received a dose of VARIVAX during pregnancy or within three months prior to conception. After excluding elective terminations (n=60), ectopic pregnancies (n=1) and those lost to follow-up (n=556), there were 905 pregnancies with known outcomes. Of these 905 pregnancies, 271 (30%) were in women who were vaccinated within the three months prior to conception. Miscarriage was reported for 10% of pregnancies (95/905), and major birth defects were reported for 2.6% of live born infants (21/819). These rates of assessed outcomes were consistent with estimated background rates. None of the women who received VARIVAX vaccine delivered infants with abnormalities consistent with congenital varicella syndrome.

8.2 Lactation

Risk Summary

It is not known whether varicella vaccine virus is excreted in human milk. The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for VARIVAX, and any potential adverse effects on the breastfed child from VARIVAX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

No clinical data are available on safety or efficacy of VARIVAX in children less than 12 months of age.

8.5 Geriatric Use

Clinical studies of VARIVAX did not include sufficient numbers of seronegative subjects aged 65 and over to determine whether they respond differently from younger subjects.

11 DESCRIPTION

VARIVAX [Varicella Virus Vaccine Live] is a preparation of the Oka/Merck strain of live, attenuated varicella virus. The virus was initially obtained from a child with wild-type varicella, then introduced into human embryonic lung cell cultures, adapted to and propagated in embryonic guinea pig cell cultures and finally propagated in human diploid cell cultures (WI-38). Further passage of the virus for varicella vaccine was performed at Merck Research Laboratories (MRL) in human diploid cell cultures (MRC-5) that were free of adventitious agents. This live, attenuated varicella vaccine is a lyophilized preparation containing sucrose, phosphate, glutamate, and processed gelatin as stabilizers.

VARIVAX, when reconstituted as directed, is a sterile preparation for subcutaneous injection. Each approximately 0.5-mL dose contains a minimum of 1350 plaque-forming units (PFU) of Oka/Merck varicella virus when reconstituted and stored at room temperature for a maximum of 30 minutes. Each 0.5-mL dose also contains approximately 24 mg of sucrose, 12.0 mg hydrolyzed gelatin, 3.1 mg of sodium chloride, 0.5 mg of monosodium L-glutamate, 0.44 mg of sodium phosphate dibasic, 0.08 mg of potassium phosphate monobasic, and 0.08 mg of potassium chloride. The product also contains residual components of MRC-5 cells including DNA and protein and trace quantities of sodium phosphate monobasic, EDTA, neomycin and fetal bovine serum. The product contains no preservative.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

VARIVAX induces both cell-mediated and humoral immune responses to varicella-zoster virus. The relative contributions of humoral immunity and cell-mediated immunity to protection from varicella are unknown.

12.2 Pharmacodynamics

Transmission

In the placebo-controlled efficacy trial, transmission of vaccine virus was assessed in household settings (during the 8-week postvaccination period) in 416 susceptible placebo recipients who were household contacts of 445 vaccine recipients. Of the 416 placebo recipients, three developed varicella and seroconverted, nine reported a varicella-like rash and did not seroconvert, and six had no rash but seroconverted. If vaccine virus transmission occurred, it did so at a very low rate and possibly without recognizable clinical disease in contacts. These cases may represent either wild-type varicella from community contacts or a low incidence of transmission of vaccine virus from vaccinated contacts. Post-marketing experience suggests that transmission of varicella vaccine virus (Oka/Merck) resulting in varicella infection including disseminated disease may occur rarely between vaccine recipients (who develop or do not develop a varicella-like rash) and contacts susceptible to varicella including healthy as well as high risk individuals *[see Warnings and Precautions (5.3)]* {1,10}.

Herpes Zoster

Overall, 9454 healthy children (12 months to 12 years of age) and 1648 adolescents and adults (13 years of age and older) have been vaccinated with VARIVAX in clinical trials. Eight cases of herpes zoster have been reported in children during 42,556 person-years of follow-up in clinical trials, resulting in a calculated incidence of at least 18.8 cases per 100,000 person-years. The completeness of this reporting has not been determined. One case of herpes zoster has been reported in the adolescent and adult age group during 5410 person-years of follow-up in clinical trials, resulting in a calculated incidence of 18.5 cases per 100,000 person-years. All 9 cases were mild and without sequelae. Two cultures (one child and one adult) obtained from vesicles were positive for wild-type VZV as confirmed by restriction endonuclease analysis {11}. The long-term effect of VARIVAX on the incidence of herpes zoster, particularly in those vaccinees exposed to wild-type varicella, is unknown at present.

In children, the reported rate of herpes zoster in vaccine recipients appears not to exceed that previously determined in a population-based study of healthy children who had experienced wild-type varicella {12}. The incidence of herpes zoster in adults who have had wild-type varicella infection is higher than that in children.

12.6 Duration of Protection

The duration of protection of VARIVAX is unknown; however, long-term efficacy studies have demonstrated continued protection up to 10 years after vaccination *[see Clinical Studies (14.1)]* {13}. A boost in antibody levels has been observed in vaccinees following exposure to wild-type varicella which could account for the apparent long-term protection after vaccination in these studies.

14 CLINICAL STUDIES

14.1 Clinical Efficacy

The protective efficacy of VARIVAX was established by: (1) a placebo-controlled, double-blind clinical trial, (2) comparing varicella rates in vaccinees versus historical controls, and (3) assessing protection from disease following household exposure.

Clinical Data in Children

One-Dose Regimen in Children

Although no placebo-controlled trial was carried out with VARIVAX using the current vaccine, a placebo-controlled trial was conducted using a formulation containing 17,000 PFU per dose {1,14}. In this trial, a single dose of VARIVAX protected 96 to 100% of children against varicella over a two-year period. The study enrolled healthy individuals 1 to 14 years of age (n=491 vaccine, n=465 placebo). In the first year, 8.5% of placebo recipients contracted varicella, while no vaccine recipient did, for a calculated protection rate of 100% during the first varicella season. In the second year, when only a subset of individuals agreed to remain in the blinded study (n=163 vaccine, n=161 placebo), 96% protective efficacy was calculated for the vaccine group as compared to placebo.

In early clinical trials, a total of 4240 children 1 to 12 years of age received 1000 to 1625 PFU of attenuated virus per dose of VARIVAX and have been followed for up to nine years post single-dose vaccination. In this group there was considerable variation in varicella rates among studies and study sites, and much of the reported data were acquired by passive follow-up. It was observed that 0.3 to 3.8% of vaccinees per year reported varicella (called breakthrough cases). This represents an approximate 83% (95% confidence interval [CI], 82%, 84%) decrease from the age-adjusted expected incidence rates in susceptible subjects over this same period {12}. In those who developed breakthrough varicella postvaccination, the majority experienced mild disease (median of the maximum number of lesions <50). In one study, a total of 47% (27/58) of breakthrough cases had <50 lesions compared with 8% (7/92) in unvaccinated individuals, and 7% (4/58) of breakthrough cases had >300 lesions compared with 50% (46/92) in unvaccinated individuals {15}.

Among a subset of vaccinees who were actively followed in these early trials for up to nine years postvaccination, 179 individuals had household exposure to varicella. There were no reports of breakthrough varicella in 84% (150/179) of exposed children, while 16% (29/179) reported a mild form of varicella (38% [11/29] of the cases with a maximum total number of <50 lesions; no individuals with >300 lesions). This represents an 81% reduction in the expected number of varicella cases utilizing the historical attack rate of 87% following household exposure to varicella in unvaccinated individuals in the calculation of efficacy.

In later clinical trials, a total of 1114 children 1 to 12 years of age received 2900 to 9000 PFU of attenuated virus per dose of VARIVAX and have been actively followed for up to 10 years post single-dose vaccination. It was observed that 0.2% to 2.3% of vaccinees per year reported breakthrough varicella for up to 10 years post single-dose vaccination. This represents an estimated efficacy of 94% (95% CI, 93%, 96%), compared with the age-adjusted expected incidence rates in susceptible subjects over the same period {1,12,16}. In those who developed breakthrough varicella postvaccination, the majority experienced mild disease, with the median of the maximum total number of lesions <50. The severity of reported breakthrough varicella, as measured by number of lesions and maximum temperature, appeared not to increase with time since vaccination.

Among a subset of vaccinees who were actively followed in these later trials for up to 10 years postvaccination, 95 individuals were exposed to an unvaccinated individual with wild-type varicella in a household setting. There were no reports of breakthrough varicella in 92% (87/95) of exposed children, while 8% (8/95) reported a mild form of varicella (maximum total number of lesions <50; observed range, 10 to 34). This represents an estimated efficacy of 90% (95% CI, 82%, 96%) based on the historical attack rate of 87% following household exposure to varicella in unvaccinated individuals in the calculation of efficacy.

Two-Dose Regimen in Children

In a clinical trial, a total of 2216 children 12 months to 12 years of age with a negative history of varicella were randomized to receive either 1 dose of VARIVAX (n=1114) or 2 doses of VARIVAX (n=1102) given 3 months apart. Subjects were actively followed for varicella, any varicella-like illness, or herpes zoster and any exposures to varicella or herpes zoster on an annual basis for 10 years after vaccination. Persistence of VZV antibody was measured annually for 9 years. Most cases of varicella reported in recipients of 1 dose or 2 doses of vaccine were mild {13}. The estimated vaccine efficacy for the 10-year observation period was 94% for 1 dose and 98% for 2 doses ($p<0.001$). This translates to a 3.4-fold lower risk of developing varicella >42 days postvaccination during the 10-year observation period in children who received 2 doses than in those who received 1 dose (2.2% vs. 7.5%, respectively).

Clinical Data in Adolescents and Adults

Two-Dose Regimen in Adolescents and Adults

In early clinical trials, a total of 796 adolescents and adults received 905 to 1230 PFU of attenuated virus per dose of VARIVAX and have been followed for up to six years following 2-dose vaccination. A total of 50 clinical varicella cases were reported >42 days following 2-dose vaccination. Based on passive follow-up, the annual varicella breakthrough event rate ranged from <0.1 to 1.9%. The median of the maximum total number of lesions ranged from 15 to 42 per year.

Although no placebo-controlled trial was carried out in adolescents and adults, the protective efficacy of VARIVAX was determined by evaluation of protection when vaccinees received 2 doses of VARIVAX 4 or 8 weeks apart and were subsequently exposed to varicella in a household setting. Among the subset of vaccinees who were actively followed in these early trials for up to six years, 76 individuals had household exposure to varicella. There were no reports of breakthrough varicella in 83% (63/76) of exposed vaccinees, while 17% (13/76) reported a mild form of varicella. Among 13 vaccinated individuals who developed breakthrough varicella after a household exposure, 62% (8/13) of the cases reported maximum total number of lesions <50, while no individual reported >75 lesions. The attack rate of unvaccinated adults exposed to a single contact in a household has not been previously studied. Utilizing the previously reported historical attack rate of 87% for wild-type varicella following household exposure to varicella among unvaccinated children in the calculation of efficacy, this represents an approximate 80% reduction in the expected number of cases in the household setting.

In later clinical trials, a total of 220 adolescents and adults received 3315 to 9000 PFU of attenuated virus per dose of VARIVAX and have been actively followed for up to six years following 2-dose vaccination. A total of 3 clinical varicella cases were reported >42 days following 2-dose vaccination. Two cases reported <50 lesions and none reported >75. The annual varicella breakthrough event rate ranged from 0 to 1.2%. Among the subset of vaccinees who were actively followed in these later trials for up to five years, 16 individuals were exposed to an unvaccinated individual with wild-type varicella in a household setting. There were no reports of breakthrough varicella among the exposed vaccinees.

There are insufficient data to assess the rate of protective efficacy of VARIVAX against the serious complications of varicella in adults (*e.g.*, encephalitis, hepatitis, pneumonitis) and during pregnancy (congenital varicella syndrome).

14.2 Immunogenicity

In clinical trials, varicella antibodies have been evaluated following vaccination with formulations of VARIVAX containing attenuated virus ranging from 1000 to 50,000 PFU per dose in healthy individuals ranging from 12 months to 55 years of age {1,8}.

One-Dose Regimen in Children

In prelicensure efficacy studies, seroconversion was observed in 97% of vaccinees at approximately 4 to 6 weeks postvaccination in 6889 susceptible children 12 months to 12 years of age. Titers ≥5 gpELISA units/mL were induced in approximately 76% of children vaccinated with a single dose of vaccine at 1000 to 17,000 PFU per dose. Rates of breakthrough disease were significantly lower among children with VZV antibody titers ≥5 gpELISA units/mL compared with children with titers <5 gpELISA units/mL.

Two-Dose Regimen in Children

In a multicenter study, 2216 healthy children 12 months to 12 years of age received either 1 dose of VARIVAX or 2 doses administered 3 months apart. The immunogenicity results are shown in Table 3.

Table 3: Summary of VZV Antibody Responses at 6 Weeks Postdose 1 and 6 Weeks Postdose 2 in Initially Seronegative Children 12 Months to 12 Years of Age (Vaccinations 3 Months Apart)

	VARIVAX 1-Dose Regimen	VARIVAX 2-Dose Regimen (3 months apart)

	(N=1114)	(N=1102)	
	6 Weeks Postvaccination (n=892)	6 Weeks Postdose 1 (n=851)	6 Weeks Postdose 2 (n=769)
Seroconversion Rate	98.9%	99.5%	99.9%
Percent with VZV Antibody Titer ≥5 gpELISA units/mL	84.9%	87.3%	99.5%
Geometric mean titers in gpELISA units/mL (95% CI)	12.0 (11.2, 12.8)	12.8 (11.9, 13.7)	141.5 (132.3, 151.3)

N = Number of subjects vaccinated.
n = Number of subjects included in immunogenicity analysis.

The results from this study and other studies in which a second dose of VARIVAX was administered 3 to 6 years after the initial dose demonstrate significant boosting of the VZV antibodies with a second dose. VZV antibody levels after 2 doses given 3 to 6 years apart are comparable to those obtained when the 2 doses are given 3 months apart.

Two-Dose Regimen in Adolescents and Adults

In a multicenter study involving susceptible adolescents and adults 13 years of age and older, 2 doses of VARIVAX administered 4 to 8 weeks apart induced a seroconversion rate of approximately 75% in 539 individuals 4 weeks after the first dose and of 99% in 479 individuals 4 weeks after the second dose. The average antibody response in vaccinees who received the second dose 8 weeks after the first dose was higher than that in vaccinees who received the second dose 4 weeks after the first dose. In another multicenter study involving adolescents and adults, 2 doses of VARIVAX administered 8 weeks apart induced a seroconversion rate of 94% in 142 individuals 6 weeks after the first dose and 99% in 122 individuals 6 weeks after the second dose.

14.3 Persistence of Immune Response

One-Dose Regimen in Children

In clinical studies involving healthy children who received 1 dose of vaccine, detectable VZV antibodies were present in 99.0% (3886/3926) at 1 year, 99.3% (1555/1566) at 2 years, 98.6% (1106/1122) at 3 years, 99.4% (1168/1175) at 4 years, 99.2% (737/743) at 5 years, 100% (142/142) at 6 years, 97.4% (38/39) at 7 years, 100% (34/34) at 8 years, and 100% (16/16) at 10 years postvaccination.

Two-Dose Regimen in Children

In recipients of 1 dose of VARIVAX over 9 years of follow-up, the geometric mean titers (GMTs) and the percent of subjects with VZV antibody titers ≥5 gpELISA units/mL generally increased. The GMTs and percent of subjects with VZV antibody titers ≥5 gpELISA units/mL in the 2-dose recipients were higher than those in the 1-dose recipients for the first year of follow-up and generally comparable thereafter. The cumulative rate of VZV antibody persistence with both regimens remained very high at year 9 (99.0% for the 1-dose group and 98.8% for the 2-dose group).

Two-Dose Regimen in Adolescents and Adults

In clinical studies involving healthy adolescents and adults who received 2 doses of vaccine, detectable VZV antibodies were present in 97.9% (568/580) at 1 year, 97.1% (34/35) at 2 years, 100% (144/144) at 3 years, 97.0% (98/101) at 4 years, 97.4% (76/78) at 5 years, and 100% (34/34) at 6 years postvaccination.

A boost in antibody levels has been observed in vaccinees following exposure to wild-type varicella, which could account for the apparent long-term persistence of antibody levels in these studies.

14.4 Studies with Other Vaccines

Concomitant Administration with M-M-R II

In combined clinical studies involving 1080 children 12 to 36 months of age, 653 received VARIVAX and M-M-R II concomitantly at separate injection sites and 427 received the vaccines six weeks apart. Seroconversion rates and antibody levels to measles, mumps, rubella, and varicella were comparable between the two groups at approximately six weeks postvaccination.

Concomitant Administration with Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine Adsorbed (DTaP) and Oral Poliovirus Vaccine (OPV)

In a clinical study involving 318 children 12 months to 42 months of age, 160 received an investigational varicella-containing vaccine (a formulation combining measles, mumps, rubella, and varicella in one syringe) concomitantly with booster doses of DTaP and OPV (no longer licensed in the United States). The comparator group of 144 children received M-M-R II concomitantly with booster doses of DTaP and OPV followed by VARIVAX six weeks later. At six weeks postvaccination, seroconversion rates for measles, mumps, rubella, and VZV and the percentage of vaccinees whose titers were boosted for diphtheria,

tetanus, pertussis, and polio were comparable between the two groups. Anti-VZV levels were decreased when the investigational vaccine containing varicella was administered concomitantly with DTaP {17}. No clinically significant differences were noted in adverse reactions between the two groups.

Concomitant Administration with PedvaxHIB®

In a clinical study involving 307 children 12 to 18 months of age, 150 received an investigational varicella-containing vaccine (a formulation combining measles, mumps, rubella, and varicella in one syringe) concomitantly with a booster dose of PedvaxHIB [Haemophilus b Conjugate Vaccine (Meningococcal Protein Conjugate)], while 130 received M-M-R II concomitantly with a booster dose of PedvaxHIB followed by VARIVAX 6 weeks later. At six weeks postvaccination, seroconversion rates for measles, mumps, rubella, and VZV, and GMTs for PedvaxHIB were comparable between the two groups. Anti-VZV levels were decreased when the investigational vaccine containing varicella was administered concomitantly with PedvaxHIB {18}. No clinically significant differences in adverse reactions were seen between the two groups.

Concomitant Administration with M-M-R II and COMVAX

In a clinical study involving 822 children 12 to 15 months of age, 410 received COMVAX [Haemophilus b Conjugate (Meningococcal Protein Conjugate) and Hepatitis B (Recombinant) Vaccine] (no longer licensed in the US), M-M-R II, and VARIVAX concomitantly at separate injection sites, and 412 received COMVAX followed by M-M-R II and VARIVAX given concomitantly at separate injection sites, 6 weeks later. At 6 weeks postvaccination, the immune responses for the subjects who received the concomitant doses of COMVAX, M-M-R II, and VARIVAX were similar to those of the subjects who received COMVAX followed 6 weeks later by M-M-R II and VARIVAX with respect to all antigens administered. There were no clinically important differences in reaction rates when the three vaccines were administered concomitantly versus six weeks apart.

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16 HOW SUPPLIED/STORAGE AND HANDLING

No. 4827/4309 —VARIVAX is supplied as follows:
(1) a box of 10 single-dose vials of lyophilized vaccine (package A), NDC 0006-4827-00
(2) a box of 10 vials of diluent (package B).

Storage

Vaccine Vial

During shipment, maintain the vaccine at a temperature between −58°F and +5°F (−50°C and −15°C). Use of dry ice may subject VARIVAX to temperatures colder than −58°F (−50°C).

Before reconstitution, store the lyophilized vaccine in a freezer at a temperature between −58°F and +5°F (−50°C and −15°C). Any freezer (*e.g.*, chest, frost-free) that reliably maintains an average temperature between −58°F and +5°F (−50°C and −15°C) and has a separate sealed freezer door is acceptable for storing VARIVAX. Routine defrost cycling of a frost-free freezer is acceptable.

VARIVAX may be stored at refrigerator temperature (36°F to 46°F, 2°C to 8°C) for up to 72 continuous hours prior to reconstitution. Vaccine stored at 2°C to 8°C which is not used within 72 hours of removal from +5°F (−15°C) storage should be discarded.

Before reconstitution, protect from light.

DISCARD IF RECONSTITUTED VACCINE IS NOT USED WITHIN 30 MINUTES.

Diluent Vial

The vial of diluent should be stored separately at room temperature (68°F to 77°F, 20°C to 25°C), or in the refrigerator.

For information regarding the product or questions regarding storage conditions, call 1-800-9-VARIVAX (1-800-982-7482).

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Discuss the following with the patient:

- Question the patient, parent, or guardian about reactions to previous vaccines.
- Provide a copy of the patient information (PPI) located at the end of this insert and discuss any questions or concerns.
- Inform patient, parent, or guardian that vaccination with VARIVAX may not result in protection of all healthy, susceptible children, adolescents, and adults.
- Inform female patients to avoid pregnancy for three months following vaccination.
- Inform patient, parent, or guardian of the benefits and risks of VARIVAX.
- Instruct patient, parent, or guardian to report any adverse reactions or any symptoms of concern to their healthcare professional.

The U.S. Department of Health and Human Services has established a Vaccine Adverse Event Reporting System (VAERS) to accept all reports of suspected adverse events after the administration of any vaccine. For information or a copy of the vaccine reporting form, call the VAERS toll-free number at 1-800-822-7967, or report online at www.vaers.hhs.gov .

Dist. by: Merck Sharp & Dohme Corp., a subsidiary of
MERCK & CO., INC., Whitehouse Station, NJ 08889, USA

For patent information: www.merck.com/product/patent/home.html

uspi-v210-i-fro-2211r720

Vitamin K (Phytonadione)

VITAMIN K1- phytonadione injection, emulsion
Hospira, Inc.

VITAMIN K_1
INJECTION

Phytonadione

Injectable Emulsion, USP

Aqueous Dispersion of Vitamin K_1

Ampul

R_x only

Protect from light. Keep ampuls

in tray until time of use.

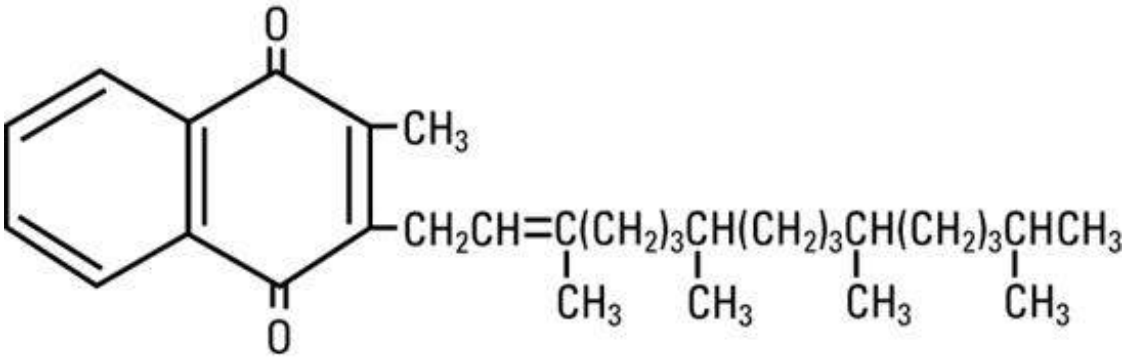
WARNING — INTRAVENOUS AND INTRAMUSCULAR USE

Severe reactions, including fatalities, have occurred during and immediately after INTRAVENOUS injection of phytonadione, even when precautions have been taken to dilute the phytonadione and to avoid rapid infusion. Severe reactions, including fatalities, have also been reported following INTRAMUSCULAR administration. Typically these severe reactions have resembled hypersensitivity or anaphylaxis, including shock and cardiac and/or respiratory arrest. Some patients have exhibited these severe reactions on receiving phytonadione for the first time. Therefore the INTRAVENOUS and INTRAMUSCULAR routes should be restricted to those situations where the subcutaneous route is not feasible and the serious risk involved is considered justified.

DESCRIPTION

Phytonadione is a vitamin, which is a clear, yellow to amber, viscous, odorless or nearly odorless liquid. It is insoluble in water, soluble in chloroform and slightly soluble in ethanol. It has a molecular weight of 450.70.

Phytonadione is 2-methyl-3-phytyl-1, 4-naphthoquinone. Its empirical formula is $C_{31}H_{46}O_2$ and its structural formula is:



Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) is a yellow, sterile, nonpyrogenic aqueous dispersion available for injection by the intravenous, intramuscular and subcutaneous routes. Each milliliter contains phytonadione 2 or 10 mg, polyoxyethylated fatty acid derivative 70 mg, dextrose, hydrous 37.5 mg in water for injection; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH is 6.3 (5.0 to 7.0). Phytonadione is oxygen sensitive.

CLINICAL PHARMACOLOGY

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) aqueous dispersion of vitamin K_1 for parenteral injection, possesses the same type and degree of activity as does naturally-occurring vitamin K, which is necessary for the production via the liver of active prothrombin (factor II), proconvertin (factor VII), plasma thromboplastin component (factor IX), and Stuart factor (factor X). The prothrombin test is sensitive to the levels of three of these four factors−II, VII, and X. Vitamin K is an essential cofactor for a microsomal enzyme that catalyzes the post-translational carboxylation of multiple, specific, peptide-bound glutamic acid residues in inactive hepatic precursors of factors II, VII, IX, and X. The resulting gamma-carboxy-glutamic acid residues convert the precursors into active coagulation factors that are subsequently secreted by liver cells into the blood.

Phytonadione is readily absorbed following intramuscular administration. After absorption, phytonadione is initially concentrated in the liver, but the concentration declines rapidly. Very little vitamin K accumulates in tissues. Little is known about the metabolic fate of vitamin K. Almost no free unmetabolized vitamin K appears in bile or urine.

In normal animals and humans, phytonadione is virtually devoid of pharmacodynamic activity. However, in animals and humans deficient in vitamin K, the pharmacological action of vitamin K is related to its normal physiological function, that is, to promote the hepatic biosynthesis of vitamin K dependent clotting factors.

The action of the aqueous dispersion, when administered intravenously, is generally detectable within an hour or two and hemorrhage is usually controlled within 3 to 6 hours. A normal prothrombin level may often be obtained in 12 to 14 hours.

In the prophylaxis and treatment of hemorrhagic disease of the newborn, phytonadione has demonstrated a greater margin of safety than that of the water-soluble vitamin K analogues.

INDICATIONS AND USAGE

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) is indicated in the following coagulation disorders which are due to faulty formation of factors II, VII, IX and X when caused by vitamin K deficiency or interference with vitamin K activity.

Vitamin K_1 Injection is indicated in:

- anticoagulant-induced prothrombin deficiency caused by coumarin or indanedione derivatives;
- prophylaxis and therapy of hemorrhagic disease of the newborn;
- hypoprothrombinemia due to antibacterial therapy;
- hypoprothrombinemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, celiac disease, intestinal resection, cystic fibrosis of the pancreas, and regional enteritis;
- other drug-induced hypoprothrombinemia where it is definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates.

CONTRAINDICATION

Hypersensitivity to any component of this medication.

WARNINGS

Benzyl alcohol as a preservative in Bacteriostatic Sodium Chloride Injection has been associated with toxicity in newborns. Data are unavailable on the toxicity of other preservatives in this age group. There is no evidence to suggest that the small amount of benzyl alcohol contained in Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP), when used as recommended, is associated with toxicity.

An immediate coagulant effect should not be expected after administration of phytonadione. It takes a minimum of 1 to 2 hours for measurable improvement in the prothrombin time. Whole blood or component therapy may also be necessary if bleeding is severe.

Phytonadione will not counteract the anticoagulant action of heparin.

When vitamin K_1 is used to correct excessive anticoagulant-induced hypoprothrombinemia, anticoagulant therapy still being indicated, the patient is again faced with the clotting hazards existing prior to starting the anticoagulant therapy. Phytonadione is not a clotting agent, but overzealous therapy with vitamin K_1 may restore conditions which originally permitted thromboembolic phenomena. Dosage should be kept as low as possible, and prothrombin time should be checked regularly as clinical conditions indicate.

Repeated large doses of vitamin K are not warranted in liver disease if the response to initial use of the vitamin is unsatisfactory. Failure to respond to vitamin K may indicate that the condition being treated is inherently unresponsive to vitamin K.

Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants.

WARNING: This product contains aluminum that may be toxic. Aluminum may reach toxic levels with prolonged parenteral administration if kidney function is impaired.

Premature neonates are particularly at risk because their kidneys are immature, and they required large amounts of calcium and phosphate solutions, which contain aluminum.

Research indicates that patients with impaired kidney function, including premature neonates, who receive parenteral levels of aluminum at greater than 4 to 5 mcg/kg/day accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates of administration.

PRECAUTIONS

Drug Interactions

Temporary resistance to prothrombin-depressing anticoagulants may result, especially when larger doses of phytonadione are used. If relatively large doses have been employed, it may be necessary when reinstituting anticoagulant therapy to use somewhat larger doses of the prothrombin-depressing anticoagulant, or to use one which acts on a different principle, such as heparin sodium.

Laboratory Tests

Prothrombin time should be checked regularly as clinical conditions indicate.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies of carcinogenicity, mutagenesis or impairment of fertility have not been conducted with Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP).

Pregnancy

Animal reproduction studies have not been conducted with Vitamin K_1 Injection. It is also not known whether Vitamin K_1 Injection can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Vitamin K_1 Injection should be given to a pregnant woman only if clearly needed.

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Vitamin K_1 Injection is administered to a nursing woman.

Pediatric Use

Hemolysis, jaundice, and hyperbilirubinemia in neonates, particularly those that are premature, may be related to the dose of Vitamin K_1 Injection. Therefore, the recommended dose should not be exceeded (See ADVERSE REACTIONS and DOSAGE AND ADMINISTRATION).

ADVERSE REACTIONS

Deaths have occurred after intravenous and intramuscular administration. (See Box Warning.)

Transient "flushing sensations" and "peculiar" sensations of taste have been observed, as well as rare instances of dizziness, rapid and weak pulse, profuse sweating, brief hypotension, dyspnea, and cyanosis.

Pain, swelling, and tenderness at the injection site may occur.

The possibility of allergic sensitivity including an anaphylactoid reaction, should be kept in mind.

Infrequently, usually after repeated injection, erythematous, indurated, pruritic plaques have occurred; rarely, these have progressed to scleroderma-like lesions that have persisted for long periods. In other cases, these lesions have resembled erythema perstans.

Hyperbilirubinemia has been observed in the newborn following administration of phytonadione. This has occurred rarely and primarily with doses above those recommended (See PRECAUTIONS, *Pediatric Use*).

OVERDOSAGE

The intravenous LD_{50} of Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) in the mouse is 41.5 and 52 mL/kg for the 0.2% and 1% concentrations, respectively.

DOSAGE AND ADMINISTRATION

Whenever possible, Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) should be given by the subcutaneous route (See Box Warning). When intravenous administration is considered unavoidable, the drug should be injected very slowly, not exceeding 1 mg per minute.

Protect from light at all times.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

Directions for Dilution

Vitamin K_1 Injection may be diluted with 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or 5% Dextrose and Sodium Chloride Injection. Benzyl alcohol as a preservative has been associated with toxicity in newborns. *Therefore, all of the above diluents should be preservative-free* (See WARNINGS). *Other diluents should not be used.* When dilutions are indicated, administration should be started immediately after mixture with the diluent, and unused portions of the dilution should be discarded, as well as unused contents of the ampul.

Prophylaxis of Hemorrhagic Disease of the Newborn

The American Academy of Pediatrics recommends that vitamin K_1 be given to the newborn. A single intramuscular dose of Vitamin K_1 Injection 0.5 to 1 mg within one hour of birth is recommended.

Treatment of Hemorrhagic Disease of the Newborn

Empiric administration of vitamin K_1 should not replace proper laboratory evaluation of the coagulation mechanism. A prompt response (shortening of the prothrombin time in

2 to 4 hours) following administration of vitamin K_1 is usually diagnostic of hemorrhagic disease of the newborn, and failure to respond indicates another diagnosis or coagulation disorder.

Vitamin K_1 Injection 1 mg should be given either subcutaneously or intramuscularly. Higher doses may be necessary if the mother has been receiving oral anticoagulants.

Whole blood or component therapy may be indicated if bleeding is excessive. This therapy, however, does not correct the underlying disorder and Vitamin K_1 Injection should be given concurrently.

Anticoagulant-Induced Prothrombin Deficiency in Adults

To correct excessively prolonged prothrombin time caused by oral anticoagulant therapy—2.5 to 10 mg or up to 25 mg initially is recommended. In rare instances 50 mg may be required. Frequency and amount of subsequent doses should be determined by prothrombin time response or clinical condition (See WARNINGS). If in 6 to 8 hours after parenteral administration the prothrombin time has not been shortened satisfactorily, the dose should be repeated.

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) Summary of Dosage Guidelines (See circular text for details)

Newborns	**Dosage**
Hemorrhagic Disease of the Newborn Prophylaxis	0.5 to 1 mg IM within 1 hour of birth
Treatment	1 mg SC or IM (Higher doses may be necessary if the mother has been receiving oral anticoagulants)
Adults	**Initial Dosage**
Anticoagulant-Induced Prothrombin Deficiency (caused by coumarin or indanedione derivatives)	2.5 mg to 10 mg or up to 25 mg (rarely 50 mg)
Hypoprothrombinemia Due to other causes (Antibiotics; Salicylates or other drugs; Factors limiting absorption or synthesis)	2.5 mg to 25 mg or more (rarely up to 50 mg)

In the event of shock or excessive blood loss, the use of whole blood or component therapy is indicated.

Hypoprothrombinemia Due to Other Causes in Adults

A dosage of 2.5 to 25 mg or more (rarely up to 50 mg) is recommended, the amount and route of administration depending upon the severity of the condition and response obtained.

If possible, discontinuation or reduction of the dosage of drugs interfering with coagulation mechanisms (such as salicylates; antibiotics) is suggested as an alternative

to administering concurrent Vitamin K_1 Injection. The severity of the coagulation disorder should determine whether the immediate administration of Vitamin K_1 Injection is required in addition to discontinuation or reduction of interfering drugs.

HOW SUPPLIED

Vitamin K_1 Injection (Phytonadione Injectable Emulsion, USP) is supplied as follows:

Unit of Sale	Concentration
NDC 0409-9157-01 Bundle of 5 clamcells containing 5 single-dose ampuls	1 mg/0.5 mL
NDC 0409-9158-01 Bundle of 5 clamcells containing 5 single-dose ampuls	10 mg/mL

Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

Protect from light. Keep ampuls in tray until time of use.

Distributed by Hospira, Inc., Lake Forest, IL 60045 USA

RL-7876

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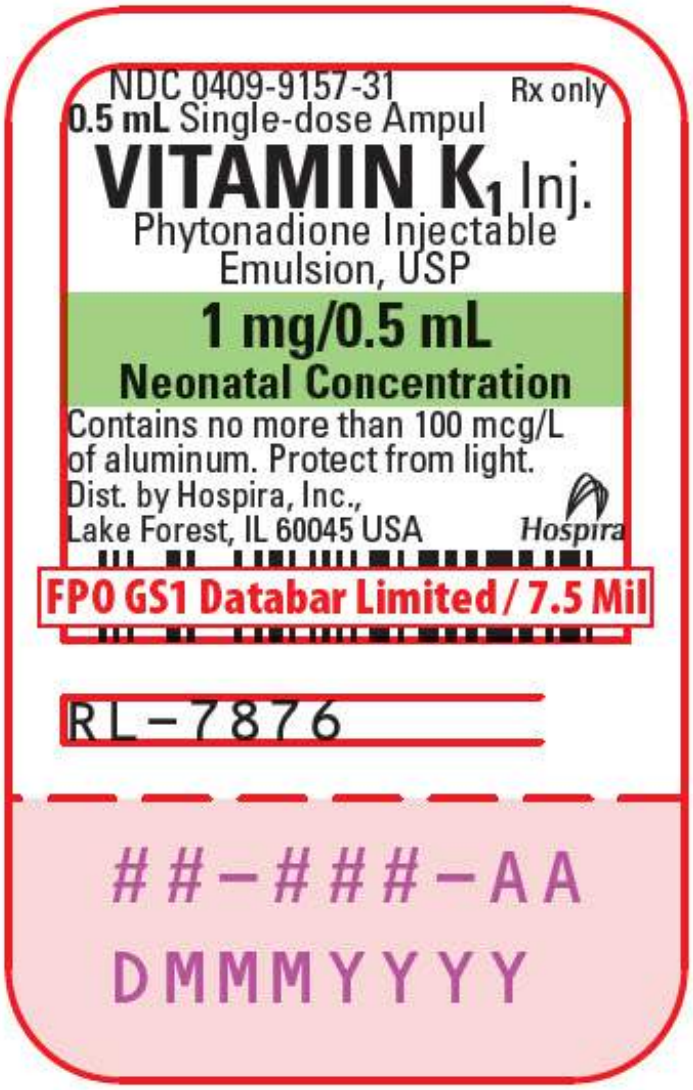
DMMMYYYY



Contains no more than 100 **mcg/L** Aluminum
(100 micrograms aluminum per **liter** of Vitamin K1 emulsion)

Neonatal dose: up to 1mg (milligram)
Concentration of emulsion: 1mg per 0.5mL (milliliter)

Amount of aluminum in 0.5mL dose:

1 Liter = 1000 milliliters
100mcg per 1000mL = 0.1mcg/1mL
0.1mcg/mL divided in half for 0.5mL dose
= **0.05mcg Aluminum** per 1mg dose

PRINCIPAL DISPLAY PANEL - 0.5 mL Ampul Tray Label - RL-7129

0.5 mL Single-dose Ampul

Rx only NDC 0409-9157-50
Contains 5 of NDC 0409-9157-31

VITAMIN K_1 Injection
Phytonadione Injectable Emulsion, USP

1 mg/0.5 mL
Neonatal Concentration

Protect from light. Keep ampuls in tray until time of use. For Intramuscular, Subcutaneous, or Intravenous (with caution).

Each mL contains phytonadione 2 mg; polyoxyethylated fatty acid

derivative 70 mg; dextrose, hydrous 37.5 mg; benzyl alcohol 9 mg added as preservative. May contain hydrochloric acid for pH adjustment. pH 6.3 (5.0 to 7.0). Usual dosage: See insert. Store at 20 to 25°C (68 to 77°F). [See USP Controlled Room Temperature.]

Distributed by Hospira, Inc.,
Lake Forest, IL 60045 USA

Hospira

RL-7129

PHYTONADIONE- phytonadione injection, emulsion
International Medication Systems, Limited

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use PHYTONADIONE INJECTABLE EMULSION, USP safely and effectively. See full prescribing information for PHYTONADIONE INJECTABLE EMULSION, USP.
PHYTONADIONE Injection, for intravenous, intramuscular, and subcutaneous use.Initial U.S. Approval: 1960

WARNING – HYPERSENSITIVITY REACTIONS WITH INTRAVENOUS AND INTRAMUSCULAR USE

***See full prescribing information for complete boxed warning*.**

Fatal hypersensitivity reactions, including anaphylaxis, have occurred during and immediately after INTRAVENOUS and INTRAMUSCULAR injection of Phytonadione Injectable Emulsion, USP. Reactions have occurred despite dilution to avoid rapid infusion and upon first and subsequent doses. Avoid the intravenous and intramuscular routes of administration unless the subcutaneous route is not feasible and the serious risk is justified (5.1)

RECENT MAJOR CHANGES

Warnings and Precautions, Cutaneous Reactions (5.3) 04/2018

INDICATIONS AND USAGE

Phytonadione Injectable Emulsion, USP is a vitamin K replacement indicated for the treatment of the following coagulation disorders which are due to faulty formation of factors II, VII, IX and X when caused by vitamin K deficiency or interference with vitamin K activity.

- Anticoagulant-induced hypoprothrombinemia deficiency caused by coumarin or indane-dione derivatives; (1.1)
- Hypoprothrombinemia due to antibacterial therapy; (1.1)
- Hypoprothrombinemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, celiac disease, intestinal resection, cystic fibrosis of the pancreas, and regional enteritis; (1.1)
- Other drug-induced hypoprothrombinemia where is it definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates. (1.1)

Phytonadione Injectable Emulsion, USP is indicated for prophylaxis and treatment of vitamin K-deficiency bleeding in neonates. (1.2)

DOSAGE AND ADMINISTRATION

- Administer Phytonadione Injectable Emulsion, USP by the subcutaneous route, whenever possible. (2.1)
- When intravenous administration is unavoidable, inject the drug very slowly, not exceeding 1 mg per minute. (2.1)

DOSAGE FORMS AND STRENGTHS

Injection: 1 mg/0.5 mL single-dose vial and a SAF-T-Jet® vial injector. (3)

CONTRAINDICATIONS

Hypersensitivity to any component of this medication. (4)

WARNINGS AND PRECAUTIONS

- Risk of Serious Adverse Reactions in Infants due to Benzyl Alcohol Preservative: Use benzyl alcohol-free formulations in neonates and infants, if available. (5.1)
- Cutaneous Reactions: May occur with parenteral use. Discontinue drug and manage medically. (5.3)

ADVERSE REACTIONS

Most common adverse reactions are cyanosis, diaphoresis, dizziness, dysgeusia, dyspnea, flushing, hypotension and tachycardia. (6)

To report SUSPECTED ADVERSE REACTIONS, contact Amphastar Pharmaceuticals, Inc. at 1-800-423-4136, or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

DRUG INTERACTIONS

Anticoagulants: May induce temporary resistance to prothrombin-depressing anticoagulants. (7)

USE IN SPECIFIC POPULATIONS

- Pregnancy: If available, use the preservative-free formulation in pregnant women. (8.1)

• Lactation: If available, use the preservative-free formulation in lactating women. (8.2)
• Pediatric Use: The safety and effectiveness of Phytonadione Injectable Emulsion, USP in pediatric patients from 6 months to 17 years have not been established. (8.4)

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 12/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

* Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

WARNING – HYPERSENSITIVITY REACTIONS WITH INTRAVENOUS AND INTRAMUSCULAR USE

Fatal hypersensitivity reactions, including anaphylaxis, have occurred during and immediately after intravenous and intramuscular injection of Phytonadione Injectable Emulsion, USP. Reactions have occurred despite dilution to avoid rapid intravenous infusion and upon first dose. Avoid the intravenous and intramuscular routes of administration unless the subcutaneous route is not feasible and the serious risk is justified [see *Warnings and Precautions* **(5.1)].**

1 INDICATIONS AND USAGE

1.1 Treatment of Hypoprothrombinemia Due to Vitamin K Deficiency or Interference

Phytonadione Injectable Emulsion, USP is indicated for the treatment of the following coagulation disorders which are due to faulty formation of factors II, VII, IX and X when caused by vitamin K deficiency or interference with vitamin K activity:

• anticoagulant-induced hypoprothrombinemia caused by coumarin or indanedione derivatives;

• hypoprothrombinemia due to antibacterial therapy;

• hypoprothrombinemia secondary to factors limiting absorption or synthesis of vitamin K, e.g., obstructive jaundice, biliary fistula, sprue, ulcerative colitis, celiac disease, intestinal resection, cystic fibrosis of the pancreas, and regional enteritis;

• other drug-induced hypoprothrombinemia where it is definitely shown that the result is due to interference with vitamin K metabolism, e.g., salicylates.

1.2 Prophylaxis and Treatment of Vitamin K-Deficiency Bleeding in Neonates

Phytonadione Injectable Emulsion, USP is indicated for prophylaxis and treatment of vitamin K-deficiency bleeding in neonates.

2 DOSAGE AND ADMINISTRATION

2.1 Dosing Considerations

Whenever possible, administer Phytonadione Injectable Emulsion, USP by the subcutaneous route *[see Boxed Warning]*. When intravenous administration is unavoidable, inject the drug very slowly, not exceeding 1 mg per minute *[see Warnings and Precautions (5.1)]*.

Monitor international normalized ratio (INR) regularly and as clinical conditions indicate. Use the lowest effective dose of Phytonadione Injectable Emulsion, USP.

The coagulant effects of Phytonadione Injectable Emulsion, USP are not immediate; improvement of INR may take 1-8 hours. Interim use of whole blood or component therapy may also be necessary if bleeding is severe.

Whenever possible, administer benzyl alcohol-free formulations in pediatric patients *[see Warnings and Precautions (5.2), Use in Specific Populations (8.4)]*.

When Phytonadione Injectable Emulsion, USP is used to correct excessive anticoagulant-induced hypoprothrombinemia, anticoagulant therapy still being indicated, the patient is again faced with the clotting hazards existing prior to starting the anticoagulant therapy. Phytonadione Injectable Emulsion, USP is not a clotting agent, but overzealous therapy with Phytonadione Injectable Emulsion, USP may restore conditions which originally permitted thromboembolic phenomena. Dosage should be kept as low as possible, and INR should be checked regularly as clinical conditions indicate.

2.2 Recommended Dosage for Coagulation Disorders from Vitamin K Deficiency of Interference

The recommended dosage of Phytonadione Injectable Emulsion, USP is based on whether the hypoprothrombinemia is anticoagulant-induced (e.g., due to coumarin or indanedione derivatives) or non-anticoagulant-induced (e.g., due to antibiotics; salicylates or other drugs; factors limiting absorption or synthesis) as follows:

• Anticoagulant-Induced Hypoprothrombinemia: Phytonadione Injectable Emulsion, USP 2.5 mg to 10 mg or more subcutaneously, intramuscularly, or intravenously. Up to 25 mg to 50 mg may be administered as a single dose.

Repeated large doses of Phytonadione Injectable Emulsion, USP are not warranted in liver disease if the initial response is unsatisfactory. Failure to respond to Phytonadione Injectable Emulsion, USP may indicate that the condition being treated is inherently unresponsive to Phytonadione Injectable Emulsion, USP.

• Hypoprothrombinemia Due to Other Causes (Non-Anticoagulation-Induced Hypoprothrombinemia): Phytonadione Injectable Emulsion, USP 2.5 mg to 25 mg or more intravenously, intramuscularly, or subcutaneously. Up to 50 mg may be administered as a single dose.

Evaluate INR after 6-8 hours, and repeat dose if INR remains prolonged. Modify subsequent dosage (amount and frequency) based on the INR or clinical condition.

2.3 Recommended Dosage for Prophylaxis and Treatment of Vitamin K Deficiency Bleeding in Neonates

Prophylaxis of Vitamin K-Deficiency Bleeding in Neonates
The recommended dosage of Phytonadione Injectable Emulsion, USP is 0.5 mg to 1 mg within one hour of birth for a single dose.

Treatment of Vitamin K Deficiency Bleeding in Neonates
The recommended dosage of Phytonadione Injectable Emulsion, USP is 1 mg given either subcutaneously or intramuscularly.
Consider higher doses if the mother has been receiving oral anticoagulants.

A failure to respond (shortening of the INR in 2 to 4 hours) may indicate another diagnosis or coagulation disorder.

2.4 Directions for Dilution

Dilute Phytonadione Injectable Emulsion, USP with 0.9% Sodium Chloride Injection, 5% Dextrose Injection, or 5% Dextrose and Sodium Chloride Injection. Avoid use of other diluents that may contain benzyl alcohol, which can cause serious toxicity in newborns or low birth weight infants *[see Warnings and Precautions (5.2) and Use in Specific Populations (8.4)]*.

When diluted, start administration of Phytonadione Injectable Emulsion, USP immediately after dilution.

Discard unused portions of diluted solution as well as unused contents of the vial.

Protect Phytonadione Injectable Emulsion, USP from light at all times.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

3 DOSAGE FORMS AND STRENGTHS

Injection: 1 mg/0.5 mL single-dose vial and a SAF-T-Jet® vial injector.

4 CONTRAINDICATIONS

Hypersensitivity to phytonadione or any other component of this medication *[see Warnings and Precautions (5.1)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Hypersensitivity Reactions

Fatal and severe hypersensitivity reactions, including anaphylaxis, have occurred with intravenous or intramuscular administration of Phytonadione Injectable Emulsion, USP. Reactions have occurred despite dilution to avoid rapid intravenous infusion and upon first dose. These reactions have included shock, cardiorespiratory arrest, flushing, diaphoresis, chest pain, tachycardia, cyanosis, weakness, and dyspnea. Administer Phytonadione Injectable Emulsion, USP subcutaneously whenever feasible. Avoid the intravenous and intramuscular routes of administration unless the subcutaneous route is not feasible and the serious risk is justified *[see Dosage and Administration (2.1)]*.

5.2 Risk of Serious Adverse Reaction in Infants due to Benzyl Alcohol Preservative

Use benzyl alcohol-free formulations in neonates and infants, if available. Serious and fatal adverse reactions including “gasping syndrome” can occur in neonates and infants treated with benzyl alcohol-preserved drugs, including Phytonadione. The “gasping syndrome” is characterized by central nervous system depression, metabolic acidosis, and gasping respirations.

When prescribing Phytonadione in infants, consider the combined daily metabolic load of benzyl alcohol from all sources including Phytonadione and other drugs containing

benzyl alcohol. The minimum amount of benzyl alcohol at which serious adverse reactions may occur is not known *[see Use in Specific Populations (8.1, 8.2 and 8.4)].*

5.3 Cutaneous Reactions

Parenteral administration of vitamin K replacements (including Phytonadione Injectable Emulsion, USP) may cause cutaneous reactions. Reactions have included eczematous reactions, scleroderma-like patches, urticaria, and delayed-type hypersensitivity reactions. Time of onset ranged from 1 day to a year after parenteral administration. Discontinue Phytonadione Injectable Emulsion, USP for skin reactions and institute medical management.

6 ADVERSE REACTIONS

The following serious adverse reactions are described elsewhere in the labeling:
• Hypersensitivity Reactions *[see Warnings and Precautions (5.1)]*
• Cutaneous Reactions *[see Warnings and Precautions (5.3)]*

6.3 Clinical Trials and Post-Marketing Experience

The following adverse reactions associated with the use of Phytonadione Injectable Emulsion, USP were identified in clinical studies or postmarketing reports. Because some of these reactions were reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

Cardiac Disorders: Tachycardia, hypotension.
General disorders and administration site conditions: Generalized flushing; pain, swelling, and tenderness at injection site.
Hepatobiliary Disorders: Hyperbilirubinemia
Immune System Disorders: Fatal hypersensitivity reactions, anaphylactic reactions.
Neurologic: Dysgeusia, dizziness.
Pulmonary: Dyspnea.
Skin and Subcutaneous Tissue Disorders: Erythema, pruritic plaques, scleroderma-like lesions, erythema perstans.
Vascular: Cyanosis.

7 DRUG INTERACTIONS

Anticoagulants
Phytonadione Injectable Emulsion, USP may induce temporary resistance to prothrombin-depressing anticoagulants, especially when larger doses of Phytonadione Injectable Emulsion, USP are used. Should this occur, higher doses of anticoagulant therapy may be needed when resuming anticoagulant therapy, or a change in therapy to a different class of anticoagulant may be necessary (i.e., heparin sodium).

Phytonadione Injectable Emulsion, USP does not affect the anticoagulant action of heparin.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

The preservative benzyl alcohol can cause serious adverse events and death when administered intravenously to neonates and infants. If Phytonadione is needed during pregnancy, consider using a benzyl alcohol-free formulation *[see Warnings and Precautions (5.2), Use in Specific Populations (8.4)].*

Published studies with the use of phytonadione during pregnancy have not reported a clear association with phytonadione and adverse developmental outcomes *(see Data)*. There are maternal and fetal risks associated with vitamin K deficiency during pregnancy *(see Clinical Considerations)*. Animal reproduction studies have not been conducted with phytonadione.

The estimated background risk for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2-4% and 15-20%, respectively.

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

Pregnant women with vitamin K deficiency hypoprothrombinemia may be at an increased risk for bleeding diatheses during pregnancy and hemorrhagic events at delivery. Subclinical maternal vitamin K deficiency during pregnancy has been implicated in rare cases of fetal intracranial hemorrhage.

Data

Human Data

Phytonadione has been measured in cord blood of infants whose mothers were treated with phytonadione during pregnancy in concentrations lower than seen in maternal plasma. Administration of vitamin K_1 to pregnant women shortly before delivery increased both maternal and cord blood concentrations. Published data do not report a clear association with phytonadione and adverse maternal or fetal outcomes when used during pregnancy. However, these studies cannot definitively establish the absence of any risk because of methodologic limitations including small sample size and lack of blinding.

Animal Data

In pregnant rats receiving vitamin K_1 orally, fetal plasma and liver concentrations increased following administration, supporting placental transfer.

8.2 Lactation

Risk Summary

If available, preservative-free Phytonadione is recommended when Phytonadione is needed during lactation *[see Warnings and Precautions (5.2), Use in Specific Populations (8.4)]*.

Phytonadione is present in breastmilk. There are no data on the effects of Phytonadione

Injectable Emulsion, USP on the breastfed child or on milk production. The developmental and health benefits of breastfeeding should be considered along with the clinical need for Phytonadione Injectable Emulsion, USP and any potential adverse effects on the breastfed child from Phytonadione Injectable Emulsion, USP or from the underlying maternal condition.

8.4 Pediatric Use

The safety and effectiveness of Phytonadione Injectable Emulsion, USP for prophylaxis and treatment of vitamin K deficiency have been established in neonates. Use of phytonadione injection for prophylaxis and treatment of vitamin K deficiency is based on published clinical studies.

Serious adverse reactions including fatal reactions and the “gasping syndrome” occurred in premature neonates and infants in the intensive care unit who received drugs containing benzyl alcohol as a preservative. In these cases, benzyl alcohol dosages of 99 to 234 mg/kg/day produced high levels of benzyl alcohol and its metabolites in the blood and urine (blood levels of benzyl alcohol were 0.61 to 1.378 mmol/L). Additional adverse reactions included gradual neurological deterioration, seizures, intracranial hemorrhage, hematologic abnormalities, skin breakdown, hepatic and renal failure, hypotension, bradycardia, and cardiovascular collapse. Preterm, low-birth weight infants may be more likely to develop these reactions because they may be less able to metabolize benzyl alcohol.

When prescribing Phytonadione in infants consider the combined daily metabolic load of benzyl alcohol from all sources including Phytonadione and other drugs containing benzyl alcohol. The minimum amount of benzyl alcohol at which serious adverse reactions may occur is not known *[see Warnings and Precautions (5.2)]*.

Whenever possible, use preservative-free phytonadione formulations in neonates. The preservative benzyl alcohol has been associated with serious adverse events and death in pediatric patients. Premature and low-birth weight infants may be more likely to develop toxicity.

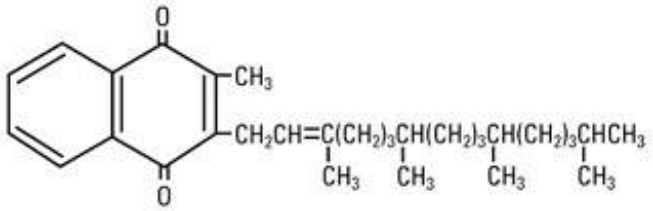
10 OVERDOSAGE

Hemolysis, jaundice, and hyperbilirubinemia in newborns, particularly in premature infants, may result from Phytonadione Injectable Emulsion, USP overdose.

11 DESCRIPTION

Phytonadione is a vitamin K replacement, which is a clear, yellow to amber, viscous, odorless or nearly odorless liquid. It is insoluble in water, soluble in chloroform and slightly soluble in ethanol. It has a molecular weight of 450.70.

Phytonadione is 2-methyl-3-phytyl-1, 4-naphthoquinone. Its empirical formula is $C_{31}H_{46}O_2$ and its molecular structure is:



Phytonadione Injectable Emulsion, USP injection is a yellow, sterile, aqueous colloidal solution of vitamin K_1, with a pH of 3.5 to 7.0, available for injection by the intravenous, intramuscular, and subcutaneous routes. Phytonadione Injectable Emulsion, USP is available in 1 mg (1 mg/0.5 mL) single-dose vials. Each 0.5 mL of Phytonadione Injectable Emulsion, USP contains the following inactive ingredients: 10 mg polysorbate 80, 10.4 mg propylene glycol, 0.17 mg sodium acetate anhydrous, and 0.00002 mL glacial acetic acid. Additional glacial acetic acid or sodium acetate anhydrous may have been added to adjust pH to meet USP limits of 3.5 to 7.0. The air above the liquid in the individual containers has been displaced by flushing with nitrogen during the filling operation.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Phytonadione Injectable Emulsion, USP aqueous colloidal solution of vitamin K_1 for parenteral injection, possesses the same type and degree of activity as does naturally-occurring vitamin K, which is necessary for the production via the liver of active prothrombin (factor II), proconvertin (factor VII), plasma thromboplastin component (factor IX), and Stuart factor (factor X). Vitamin K is an essential cofactor for a microsomal enzyme that catalyzes the posttranslational carboxylation of multiple, specific, peptide-bound glutamic acid residues in inactive hepatic precursors of factors II, VII, IX, and X. The resulting gamma-carboxy-glutamic acid residues convert the precursors into active coagulation factors that are subsequently secreted by liver cells into the blood.

In normal animals and humans, phytonadione is virtually devoid of activity. However, in animals and humans deficient in vitamin K, the pharmacological action of vitamin K is related to its normal physiological function, that is, to promote the hepatic biosynthesis of vitamin K dependent clotting factors.

12.2 Pharmacodynamics

The action of the aqueous dispersion, when administered intravenously, is generally detectable within an hour or two and hemorrhage is usually controlled within 3 to 6 hours. A normal INR may often be obtained in 12 to 14 hours.

12.3 Pharmacokinetics

Absorption:
Phytonadione is readily absorbed following intramuscular administration.

Distribution:
After absorption, phytonadione is initially concentrated in the liver, but the concentration declines rapidly. Very little vitamin K accumulates in tissues.

Elimination:

Little is known about the metabolic fate of vitamin K. Almost no free unmetabolized vitamin K appears in bile or urine.

13 NONCLINICAL TOXICOLOGY SECTION

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Studies of carcinogenicity, genotoxicity or impairment of fertility have not been conducted with phytonadione.

16 HOW SUPPLIED/STORAGE AND HANDLING

Phytonadione Injectable Emulsion, USP is a yellow, sterile, aqueous colloidal solution and is supplied in unit use packages containing one single-dose vial and a SAF-T-Jet® vial injector, 27 G. x ½" needle.

Phytonadione Injectable Emulsion USP, 1 mg in 0.5 mL
Stock No. 1240 NDC 76329-1240-1

10 individual cartons shrink wrapped as a group of 10 cartons.

Syringe Assembly Directions:
See User Guide
USE ASEPTIC TECHNIQUE
Do not remove from carton or assemble until ready to use.

1) Remove protective caps from vial and injector.

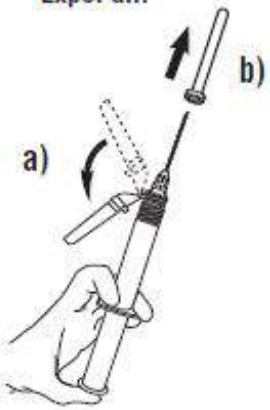
2) Align vial such that the injector needle is centered on the stopper.

Thread vial into injector 3 half turns; this will allow the needle to penetrate the stopper.*

DO NOT PUSH VIAL INTO INJECTOR; THIS MAY CAUSE MISALIGNMENT OF THE INJECTOR NEEDLE.

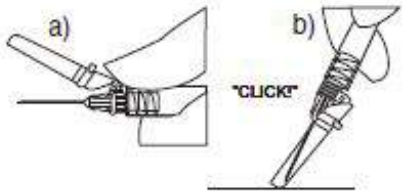
3) a. Flip shield down.

b. Remove needle cover PULLING STRAIGHT UP (DO NOT TWIST). Expel air.



4) a. After injection, push tab forward with thumb to flip shield toward needle.

b. With a FIRM, QUICK motion, press down against a flat surface until an audible "click" is heard. This sound indicates shield activation. Note: Audible click may not be heard on small needle sizes: visual confirmation is required.



*CAUTION: IMPROPER ENGAGING MAY CAUSE GLASS BREAKAGE AND SUBSEQUENT INJURY.

Store at 20° to 25°C (68° to 77°F); excursions permitted to 15° to 30°C (59° to 86°F) [see USP Controlled Room Temperature].

Protect Phytonadione Injectable Emulsion, USP from light. Store container in closed original carton until contents have been used.

17 PATIENT COUNSELING INFORMATION

Inform the patient of the following important risks of Phytonadione Injectable Emulsion, USP:

Serious Hypersensitivity Reactions
Advise the patient and caregivers to immediately report signs of hypersensitivity after receiving Phytonadione Injectable Emulsion, USP *[see Warnings and Precautions (5.1)].*

Risk of Gasping Syndrome Due to Benzyl Alcohol
Advise the patient and caregivers of the risk of gasping syndrome associated with the use of products that contain benzyl alcohol (including Phytonadione) in neonates,

infants, and pregnant women *[see Warnings and Precautions (5.2)]*.

Cutaneous Reactions

Advise the patient and caregivers to report the occurrence of new rashes after receiving Phytonadione Injectable Emulsion, USP. These reactions may be delayed for up to a year after treatment *[see Warnings and Precautions (5.3)]*.

Rx Only

INTERNATIONAL MEDICATION SYSTEMS, LIMITED
So. El Monte, CA 91733 U.S.A.
An Amphastar Pharmaceuticals Company
 Rev. 4-18

6912400N

PRINCIPLE DISPLAY PANEL: Carton

SAF-T-JET®

NDC 76329-1240-1

STOCK NO. 1240

Rx Only

SAF-T-JET®

27 G. X 1/2" NEEDLE

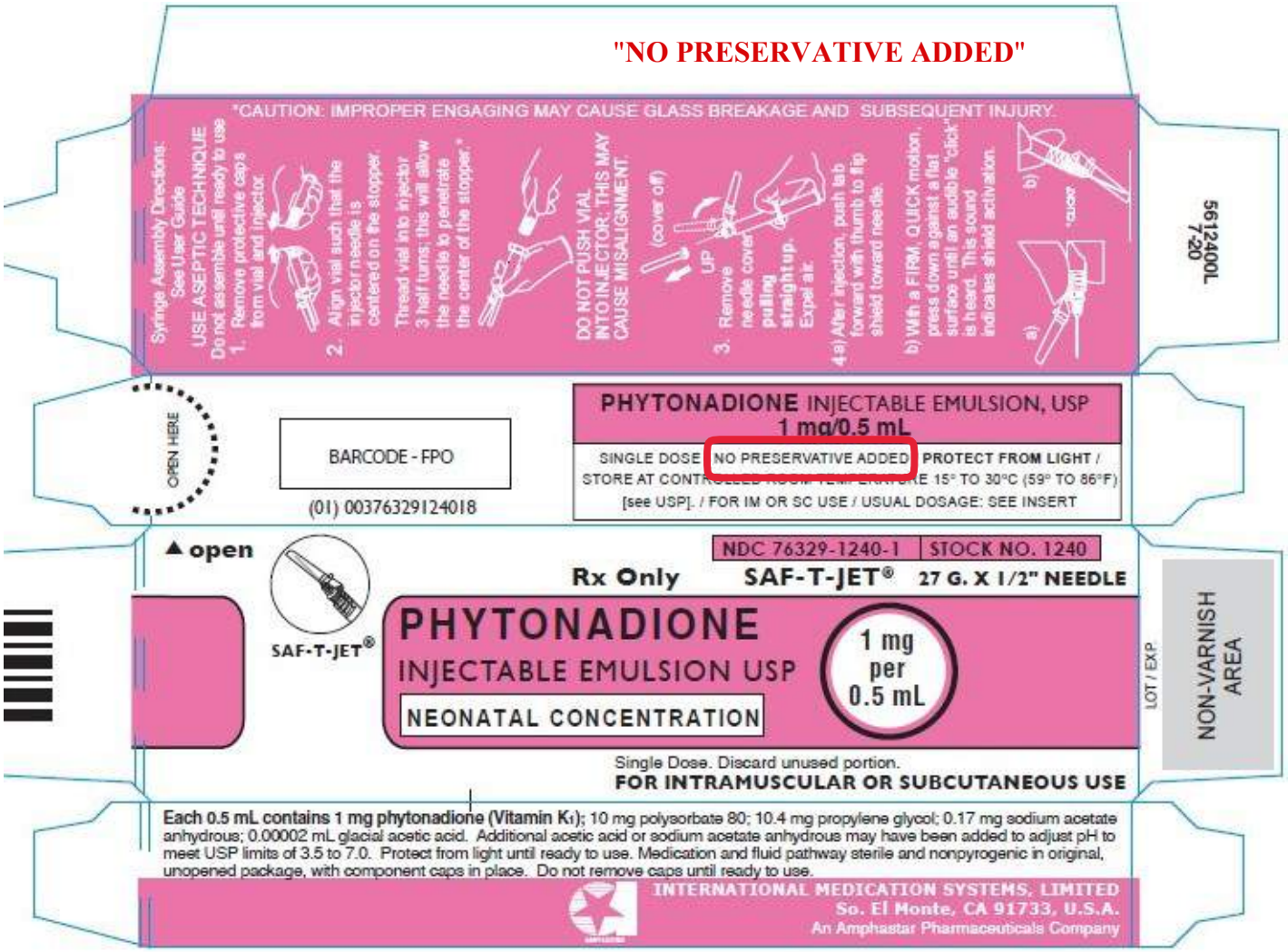
PHYTONADIONE INJECTABLE EMULSION USP

NEONATAL CONCENTRATION

1 mg per 0.5 mL

Single dose. Discard unused portion.

FOR INTRAMUSCULAR OR SUBCUTANEOUS USE



PRINCIPLE DISPLAY PANEL: User Guide

IMS Saf-T-Jet® Safety Needle

USER GUIDE

NOTE: USE ASEPTIC TECHNIQUE

Do not assemble until ready to use

1 Ensure shield is in the UP position (see inset), then remove protective caps from vial and injector.

2 Align vial such that the injector needle is centered on the stopper. Thread vial into injection 3 half turns to ensure the needle penetrates stopper. Do not push the injector needle into stopper.

3 a) Flip shield down.

b) Remove needle cover PULLING STRAIGHT UP (DO NOT TWIST). Expel air.

4 Administer injection following the established aseptic technique.

5 Position shield in preparation for device activation: Using a one-handed technique, push the tab forward with your finger or thumb so that the shield is less than 90

degrees from the needle. NOTE: Keep your finger or thumb behind the tab at all times.

6 Activate shield: Position the shield approximately 45 degrees to flat surface. Press down with a GENTLE, QUICK MOTION until a distinct AUDIBLE CLICK is heard. Note: Audible click may not be heard on small needle sizes: visual confirmation is required.

7VISUALLY CONFIRM that needle is fully engaged under lock.

8 Following activation of the needle shield, immediately discard the unit into an approved sharps container.

For additional questions or to request a traning video, contact Customer Service at (800) 423-4136.

7012400E 9-09

Zoster (Shingles)

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use SHINGRIX safely and effectively. See full prescribing information for SHINGRIX.

SHINGRIX (Zoster Vaccine Recombinant, Adjuvanted), suspension for intramuscular injection
Initial U.S. Approval: 2017

----------RECENT MAJOR CHANGES----------

Indications and Usage (1)	07/2021
Dosage and Administration, Dose and Schedule (2.3)	07/2021
Warnings and Precautions, Guillain-Barré syndrome (5.2)	03/2021
Warnings and Precautions, Syncope (5.3)	07/2021

----------INDICATIONS AND USAGE----------

SHINGRIX is a vaccine indicated for prevention of herpes zoster (HZ) (shingles):

- in adults aged 50 years and older.
- in adults aged 18 years and older who are or will be at increased risk of HZ due to immunodeficiency or immunosuppression caused by known disease or therapy.

Limitations of Use (1):

- SHINGRIX is not indicated for prevention of primary varicella infection (chickenpox).

----------DOSAGE AND ADMINISTRATION----------

For intramuscular administration only.

Two doses (0.5 mL each) administered intramuscularly according to the following schedules:

- A first dose at Month 0 followed by a second dose administered 2 to 6 months later. (2.3)
- For individuals who are or will be immunodeficient or immunosuppressed and who would benefit from a shorter vaccination schedule: A first dose at Month 0 followed by a second dose administered 1 to 2 months later. (2.3)

----------DOSAGE FORMS AND STRENGTHS----------

Suspension for injection supplied as a single-dose vial of lyophilized varicella zoster virus glycoprotein E (gE) antigen component to be reconstituted with the accompanying vial of $AS01_B$ adjuvant suspension component. After reconstitution, a single dose of SHINGRIX is 0.5 mL. (3)

----------CONTRAINDICATIONS----------

History of severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine or after a previous dose of SHINGRIX. (4)

----------WARNINGS AND PRECAUTIONS----------

- In a postmarketing observational study, an increased risk of Guillain-Barré syndrome was observed during the 42 days following vaccination with SHINGRIX. (5.2, 6.2)
- Syncope (fainting) can be associated with the administration of injectable vaccines, including SHINGRIX. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope. (5.3)

----------ADVERSE REACTIONS----------

- Solicited local adverse reactions reported in individuals aged 50 years and older were pain (78%), redness (38%), and swelling (26%). (6.1)
- Solicited general adverse reactions reported in individuals aged 50 years and older were myalgia (45%), fatigue (45%), headache (38%), shivering (27%), fever (21%), and gastrointestinal symptoms (17%). (6.1)
- Solicited local adverse reactions reported in autologous hematopoietic stem cell transplant recipients (aged 18 to 49 and ≥50 years of age) were pain (88% and 83%), redness (30% and 35%), and swelling (21% and 18%). (6.1)
- Solicited general adverse reactions reported in autologous hematopoietic stem cell transplant recipients (aged 18 to 49 and ≥50 years of age) were fatigue (64% and 54%), myalgia (58% and 52%), headache (44% and 30%), gastrointestinal symptoms (21% and 28%), shivering (31% and 25%), and fever (28% and 18%). (6.1)

To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or VAERS at 1-800-822-7967 or www.vaers.hhs.gov.

See 17 for PATIENT COUNSELING INFORMATION.

Revised: 07/2021

FULL PRESCRIBING INFORMATION: CONTENTS*

*Sections or subsections omitted from the full prescribing information are not listed.

FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

SHINGRIX is a vaccine indicated for prevention of herpes zoster (HZ) (shingles):

- in adults aged 50 years and older.

- in adults aged 18 years and older who are or will be at increased risk of HZ due to immunodeficiency or immunosuppression caused by known disease or therapy.

Limitations of Use:

- SHINGRIX is not indicated for prevention of primary varicella infection (chickenpox).

2 DOSAGE AND ADMINISTRATION

For intramuscular injection only.

2.1 Reconstitution

SHINGRIX is supplied in 2 vials that must be combined prior to administration. Prepare SHINGRIX by reconstituting the lyophilized varicella zoster virus glycoprotein E (gE) antigen component (powder) with the accompanying $AS01_B$ adjuvant suspension component (liquid). Use only the supplied adjuvant suspension component (liquid) for reconstitution. The reconstituted vaccine should be an opalescent, colorless to pale brownish liquid. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. If either of these conditions exists, the vaccine should not be administered.

Figure 1. Cleanse both vial stoppers. Using a sterile needle and sterile syringe, withdraw the entire contents of the vial containing the adjuvant suspension component (liquid) by slightly tilting the vial. Vial 1 of 2.

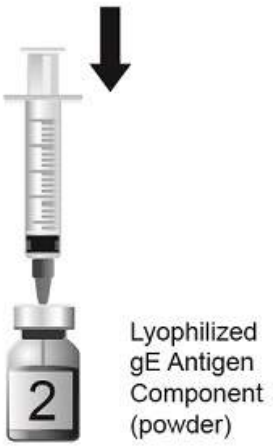


Figure 2. Slowly transfer entire contents of syringe into the lyophilized gE antigen component vial (powder). Vial 2 of 2.

Figure 3. Gently swirl the vial until powder is completely dissolved. **Do not shake vigorously.**

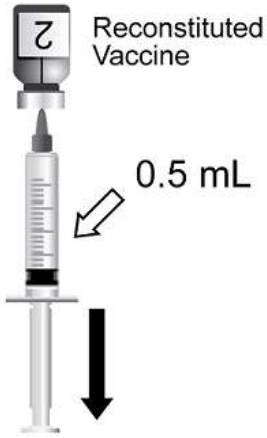


Figure 4. After reconstitution, withdraw 0.5 mL from the vial containing the reconstituted vaccine and administer **intramuscularly**.

2.2 Administration Instructions

For intramuscular injection only.

After reconstitution, administer SHINGRIX immediately or store refrigerated between 2° and 8°C (36° and 46°F) and use within 6 hours. Discard reconstituted vaccine if not used within 6 hours.

Use a separate sterile needle and sterile syringe for each individual. The preferred site for intramuscular injection is the deltoid region of the upper arm.

2.3 Dose and Schedule

Two doses (0.5 mL each) administered intramuscularly according to the following schedules:

- A first dose at Month 0 followed by a second dose administered 2 to 6 months later.
- For individuals who are or will be immunodeficient or immunosuppressed and who would benefit from a shorter vaccination schedule: A first dose at Month 0 followed by a second dose administered 1 to 2 months later.

3 DOSAGE FORMS AND STRENGTHS

SHINGRIX is a suspension for injection supplied as a single-dose vial of lyophilized gE antigen component to be reconstituted with the accompanying vial of $AS01_B$ adjuvant suspension component. A single dose after reconstitution is 0.5 mL.

4 CONTRAINDICATIONS

Do not administer SHINGRIX to anyone with a history of a severe allergic reaction (e.g., anaphylaxis) to any component of the vaccine or after a previous dose of SHINGRIX *[see Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Preventing and Managing Allergic Vaccine Reactions

Prior to administration, the healthcare provider should review the immunization history for possible vaccine sensitivity and previous vaccination-related adverse reactions. Appropriate medical treatment and supervision must be available to manage possible anaphylactic reactions following administration of SHINGRIX.

5.2 Guillain-Barré Syndrome (GBS)

In a postmarketing observational study, an increased risk of GBS was observed during the 42 days following vaccination with SHINGRIX *[see Adverse Reactions (6.2)]*.

5.3 Syncope

Syncope (fainting) can be associated with the administration of injectable vaccines, including

SHINGRIX. Syncope can be accompanied by transient neurological signs such as visual disturbance, paresthesia, and tonic-clonic limb movements. Procedures should be in place to avoid falling injury and to restore cerebral perfusion following syncope.

6 ADVERSE REACTIONS

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a vaccine cannot be directly compared with rates in the clinical trials of another vaccine and may not reflect the rates observed in practice. There is the possibility that broad use of SHINGRIX could reveal adverse reactions not observed in clinical trials.

Adults Aged 50 Years and Older

Overall, 17,041 adults aged 50 years and older received at least 1 dose of SHINGRIX in 17 clinical studies.

The safety of SHINGRIX was evaluated by pooling data from 2 placebo-controlled clinical studies (Studies 1 and 2) involving 29,305 subjects aged 50 years and older who received at least 1 dose of SHINGRIX (n = 14,645) or saline placebo (n = 14,660) administered according to a 0- and 2-month schedule. At the time of vaccination, the mean age of the population was 69 years; 7,286 (25%) subjects were aged 50 to 59 years, 4,488 (15%) subjects were aged 60 to 69 years, and 17,531 (60%) subjects were aged 70 years and older. Both studies were conducted in North America, Latin America, Europe, Asia, and Australia. In the overall population, the majority of subjects were White (74%), followed by Asian (18%), Black (1.4%), and other racial/ethnic groups (6%); 58% were female.

Solicited Adverse Reactions: In Studies 1 and 2, data on solicited local and general adverse reactions were collected using standardized diary cards for 7 days following each vaccine dose or placebo (i.e., day of vaccination and the next 6 days) in a subset of subjects (n = 4,886 receiving SHINGRIX, n = 4,881 receiving placebo with at least 1 documented dose). Across both studies, the percentages of subjects aged 50 years and older reporting each solicited local and general adverse reaction following administration of SHINGRIX (both doses combined) were pain (78%), redness (38%), and swelling (26%); and myalgia (45%), fatigue (45%), headache (38%), shivering (27%), fever (21%), and gastrointestinal symptoms (17%).

The reported frequencies of specific solicited local adverse reactions and general adverse reactions (overall per subject), by age group, from the 2 studies are presented in Table 1.

Table 1. Percentage of Subjects with Solicited Local and General Adverse Reactions within 7 Days[a] of Vaccination in Adults Aged 50 to 59 Years, 60 to 69 Years, and 70 Years and Older[b] (Total Vaccinated Cohort with 7-Day Diary Card)

Adverse Reactions	Aged 50-59 Years		Aged 60-69 Years		Aged ≥70 Years	
	SHINGRIX	Placebo[c]	SHINGRIX	Placebo[c]	SHINGRIX	Placebo[c]
Local Adverse Reactions	**n = 1,315 %**	**n = 1,312 %**	**n = 1,311 %**	**n = 1,305 %**	**n = 2,258 %**	**n = 2,263 %**
Pain	88	14	83	11	69	9
Pain, Grade 3[d]	10	1	7	1	4	0.2
Redness	39	1	38	2	38	1
Redness, >100 mm	3	0	3	0	3	0
Swelling	31	1	27	1	23	1
Swelling, >100 mm	1	0	1	0	1	0
General Adverse Reactions	**n = 1,315 %**	**n = 1,312 %**	**n = 1,309 %**	**n = 1,305 %**	**n =2,252 %**	**n = 2,264 %**
Myalgia	57	15	49	11	35	10
Myalgia, Grade 3[e]	9	1	5	1	3	0.4
Fatigue	57	20	46	17	37	14
Fatigue, Grade 3[e]	9	2	5	1	4	1
Headache	51	22	40	16	29	12
Headache, Grade 3[e]	6	2	4	0.2	2	0.4
Shivering	36	7	30	6	20	5
Shivering, Grade 3[e]	7	0.2	5	0.3	2	0.3
Fever	28	3	24	3	14	3
Fever, Grade 3[f]	0.4	0.2	1	0.2	0.1	0.1
GI[g]	24	11	17	9	14	8
GI, Grade 3[e]	2	1	1	1	1	0.4

Total vaccinated cohort for safety included all subjects with at least 1 documented dose (n).

[a] 7 days included day of vaccination and the subsequent 6 days.

[b] Data for subjects aged 50 to 59 years and 60 to 69 years are based on Study 1. Data for subjects 70 years and older are based on pooled data from Study 1: NCT01165177 and Study 2: NCT01165229.

[c] Placebo was a saline solution.

[d] Grade 3 pain: Defined as significant pain at rest; prevents normal everyday activities.

[e] Grade 3 myalgia, fatigue, headache, shivering, and GI: Defined as preventing normal activity.

[f] Fever defined as ≥37.5°C/99.5°F for oral, axillary, or tympanic route, or ≥38°C/100.4°F for rectal route; Grade 3 fever defined as >39.0°C/102.2°F.

[g] GI = Gastrointestinal symptoms including nausea, vomiting, diarrhea, and/or abdominal pain.

The incidence of solicited local and general reactions was lower in subjects aged 70 years and older compared with those aged 50 to 69 years.

The local and general adverse reactions seen with SHINGRIX had a median duration of 2 to 3 days.

There were no differences in the proportions of subjects reporting any or Grade 3 solicited local reactions between Dose 1 and Dose 2. Headache and shivering were reported more frequently by subjects after Dose 2 (28% and 21%, respectively) compared with Dose 1 (24% and 14%, respectively). Grade 3 solicited general adverse reactions (headache, shivering, myalgia, and fatigue) were reported more frequently by subjects after Dose 2 (2.3%, 3%, 4%, and 4%, respectively) compared with Dose 1 (1.4%, 1.4%, 2.3%, and 2.4%, respectively).

Unsolicited Adverse Events: Unsolicited adverse events that occurred within 30 days following each vaccination (Day 0 to 29) were recorded on a diary card by all subjects. In the 2 studies, unsolicited adverse events occurring within 30 days of vaccination were reported in 51% and 32% of subjects who received SHINGRIX (n = 14,645) or placebo (n = 14,660), respectively (Total Vaccinated Cohort). Unsolicited adverse events that occurred in ≥1% of recipients of SHINGRIX and at a rate at least 1.5-fold higher than placebo included chills (4% versus 0.2%), injection site pruritus (2.2% versus 0.2%), malaise (1.7% versus 0.3%), arthralgia (1.7% versus 1.2%), nausea (1.4% versus 0.5%), and dizziness (1.2% versus 0.8%).

Gout (including gouty arthritis) was reported by 0.18% (n = 27) versus 0.05% (n = 8) of subjects who received SHINGRIX or placebo, respectively, within 30 days of vaccination; available information is insufficient to determine a causal relationship with SHINGRIX.

Serious Adverse Events (SAEs): In the 2 studies, SAEs were reported at similar rates in subjects who received SHINGRIX (2.3%) or placebo (2.2%) from the first administered dose up to 30 days post-last vaccination. SAEs were reported for 10.1% of subjects who received SHINGRIX and for 10.4% of subjects who received placebo from the first administered dose up to 1 year post-last vaccination. One subject (<0.01%) reported lymphadenitis and 1 subject (<0.01%) reported fever greater than 39°C; there was a basis for a causal relationship with SHINGRIX.

Optic ischemic neuropathy was reported in 3 subjects (0.02%) who received SHINGRIX (all within 50 days after vaccination) and 0 subjects who received placebo; available information is insufficient to determine a causal relationship with SHINGRIX.

Deaths: From the first administered dose up to 30 days post-last vaccination, deaths were reported for 0.04% of subjects who received SHINGRIX and 0.05% of subjects who received placebo in the 2 studies. From the first administered dose up to 1 year post-last vaccination, deaths were reported for 0.8% of subjects who received SHINGRIX and for 0.9% of subjects who received placebo. Causes of death among subjects were consistent with those generally reported in adult and elderly populations.

Potential Immune-Mediated Diseases: In the 2 studies, new onset potential immune-mediated diseases (pIMDs) or exacerbation of existing pIMDs were reported for 0.6% of subjects who received SHINGRIX and 0.7% of subjects who received placebo from the first administered dose up to 1 year post-last vaccination. The most frequently reported pIMDs occurred with comparable frequencies in the group receiving SHINGRIX and the placebo group.

Dosing Schedule: In an open-label clinical study, 238 subjects 50 years and older received SHINGRIX as a 0- and 2-month or 0- and 6-month schedule. The safety profile of SHINGRIX was similar when administered according to a 0- and 2-month or 0- and 6-month schedule and was consistent with that observed in Studies 1 and 2.

Immunocompromised Adults Aged 18 Years and Older

The safety of SHINGRIX was evaluated in 6 placebo-controlled clinical studies that enrolled 3,116 subjects aged 18 years and older from 5 different immunodeficient or immunosuppressed (referred to as immunocompromised) populations, in which a total of 1,587 received SHINGRIX. In all studies, subjects received Doses 1 and 2 of SHINGRIX 1 to 2 months apart. Safety monitoring for these studies was similar to Studies 1 and 2. In addition, subjects were monitored for events relevant to their specific disease or condition.

At the time of receipt of SHINGRIX or placebo, the mean age of the population was 55 years; 28% subjects were aged 18 to 49 years and 72% subjects were aged 50 years and older. Each of the studies was conducted in one or more of the following regions: North America, Latin America, Europe, Asia, Africa and Australia/New Zealand. The majority of subjects were White (77%), followed by Asian (17%), Black (2%), and other racial groups (3%); 4% were of American Hispanic or Latino ethnicity; 37% were female.

Table 2. Clinical Studies with SHINGRIX in Immunocompromised Adults Aged ≥18 Years

Clinical Studies	Number of Subjects Vaccinated		Study Population	Safety Follow-up Period
	SHINGRIX	Placebo		
auHSCT (NCT01610414)	922	924	Autologous hematopoietic stem cell transplant recipients[a]	29 months median safety follow-up[b]
Hematologic Malignancies (NCT01767467)	283	279	Hematologic malignancies[c,d]	12 months post last vaccination
Renal Transplant (NCT02058589)	132	132	Renal transplant recipients[e]	12 months post last vaccination
Solid Malignant Tumors (NCT01798056)	117	115	Solid tumors receiving chemotherapy[f,g]	12 months post last vaccination
HIV (NCT01165203)	74	49	HIV-infected subjects	12 months post last vaccination
auHSCT (NCT00920218)	59	30	Autologous hematopoietic stem cell transplant recipients[a]	12 months post last vaccination

[a] The first dose was administered within 50 to 70 days after autologous hematopoietic stem cell transplantation.

[b] Safety follow-up was driven by HZ case accrual and ranged from a minimum of 12 months post last vaccination to 4 years at subject level.

[c] For subjects who were vaccinated during a cancer therapy course, each dose was administered with at least 10 days between vaccination and cancer therapy cycles.

[d] For subjects who received the vaccination after a full cancer therapy course, the first dose was administered from 10 days to 6 months after cancer therapy had ended.

[e] The first dose was administered between 4 to 18 months after renal transplantation.

[f] In the PreChemo group (TVC: SHINGRIX [n = 90], placebo [n = 91]), the first dose was administered a maximum of 1 month to a minimum of 10 days before the start of a chemotherapy cycle, and the second dose was administered on the first day of a chemotherapy cycle.

[g] In the OnChemo group (TVC: SHINGRIX [n = 27], placebo [n = 24]), each dose was administered on the first day of a chemotherapy cycle.

In the auHSCT study (NCT01610414), at the time of receipt of SHINGRIX or placebo, the mean age of the population was 55 years; 25% of subjects were aged 18 to 49 years and 75% subjects were aged 50 years and older. The majority of subjects were White (78%), followed by Asian (16%), Black (2%), and other racial groups (3%); 3% were of American Hispanic or Latino ethnicity; 37% were female.

Solicited Adverse Reactions: Solicited local adverse reactions reported within 7 days following administration of SHINGRIX (both doses combined) in auHSCT recipients (aged 18 to 49 and ≥50 years of age) were pain (88% and 83%), redness (30% and 35%), and swelling (21% and 18%). Solicited general adverse reactions reported within 7 days following administration of SHINGRIX (both doses combined) in auHSCT recipients (aged 18 to 49 and ≥50 years of age) were fatigue (64% and 54%), myalgia (58% and 52%), headache (44% and 30%), gastrointestinal symptoms (21% and 28%), shivering (31% and 25%), and fever (28% and 18%). The percentages of subjects aged 18 years and older reporting each solicited local and general adverse reaction following administration of each dose of SHINGRIX or placebo in the auHSCT study (NCT01610414) are presented in Table 3.

Table 3. Adult auHSCT Recipients (NCT01610414): Percentage of Subjects with Solicited Local and General Adverse Reactions within 7 Days[a] of Vaccination in Adults Aged 18 to 49 Years and 50 Years and Older by Dose (Total Vaccinated Cohort)

Adverse Reactions	Aged 18-49 Years				Aged ≥50 Years			
	SHINGRIX		Placebo[b]		SHINGRIX		Placebo[b]	
	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2	Dose 1	Dose 2
Local Adverse Reactions	**n = 223 %**	**n = 205 %**	**n = 217 %**	**n = 207 %**	**n = 673 %**	**n = 635 %**	**n = 673 %**	**n = 627 %**
Pain	81	82	8	6	75	74	6	5
Pain, Grade 3[c]	11	11	1	0	5	7	0.3	0
Redness	20	25	0	0	21	28	1	1
Redness, >100 mm	1	2	0	0	1	3	0	0
Swelling	14	17	0	0	10	15	1	1
Swelling, >100 mm	0	2	0	0	0.1	1	0	0
General Adverse Reactions	**n = 222 %**	**n = 203 %**	**n = 218 %**	**n = 207 %**	**n = 674 %**	**n = 633 %**	**n = 674 %**	**n = 628 %**
Myalgia	41	51	22	21	37	43	18	17
Myalgia, Grade 3[d]	4	8	2	2	2	4	1	1
Fatigue	49	51	34	25	37	46	31	26
Fatigue, Grade 3[d]	6	10	1	2	3	4	2	3
Headache	23	38	17	17	15	25	13	8
Headache, Grade 3[d]	1	5	0	2	0.1	2	0.4	1
Shivering	20	26	12	6	11	21	7	7
Shivering, Grade 3[d]	1	6	0	0	0.4	3	1	0.2
Fever, ≥37.5°C/99.5°F	9	28	4	2	6	15	3	4
Fever, Grade 3 >39.5°C/103.1°F	0	1	0	0	0.1	0.2	0	0.2
GI[e]	14	13	13	12	18	18	16	12
GI, Grade 3[d]	1	1	0	1	1	2	1	2

Total vaccinated cohort (TVC) for safety included all subjects with at least 1 documented dose (n).

% = Percentage of subjects reporting the symptom at least once.
[a] 7 days included day of vaccination and the subsequent 6 days.
[b] Placebo was sucrose reconstituted with saline solution.
[c] Grade 3 pain: defined as significant pain at rest preventing normal everyday activities.
[d] Grade 3 myalgia, fatigue, headache, shivering, and GI: defined as preventing normal activity.
[e] GI = Gastrointestinal symptoms including nausea, vomiting, diarrhea, and/or abdominal pain.

In general, the reported frequencies of solicited local and general adverse reactions in the other studies in immunocompromised populations were similar to that in the auHSCT study (NCT01610414). The local and general adverse reactions seen with SHINGRIX had a median duration of 1 to 3 days across all studies enrolling immunocompromised subjects.

Unsolicited Adverse Events: Across all 6 studies enrolling immunocompromised subjects, unsolicited adverse events, including both serious and non-serious events, occurring within 30 days following each vaccination were reported in 46% and 44% of subjects who received SHINGRIX or placebo. Adverse events of arthralgia, infective pneumonia, and influenza-like illness occurred in ≥1% of recipients of SHINGRIX and at a rate at least 1.5-fold higher than placebo (1.5% versus 1.0%, 1.5% versus 0.9%, and 1.3% versus 0.6%, respectively).

Serious Adverse Events: Across all 6 studies enrolling immunocompromised subjects, SAEs were reported at similar rates in subjects who received SHINGRIX (7%) or placebo (8%) from the first administered dose up to 30 days post-last vaccination. SAEs were reported for 26% of subjects who received SHINGRIX and for 27% of subjects who received placebo from the first administered dose up to 1 year post-last vaccination. SAEs of infective pneumonia were reported for 21 subjects (1.3%) who received SHINGRIX and for 11 subjects (0.7%) who received placebo up to 30 days post-last vaccination. Available information is insufficient to determine a causal relationship to vaccination.

Deaths: Across all 6 studies enrolling immunocompromised subjects, from the first administered dose up to 30 days post-last vaccination, deaths were reported for 2 subjects (0.1%) who received SHINGRIX and 7 subjects (0.5%) who received placebo. From the first administered dose up to 1 year post-last vaccination, deaths were reported for 6% of subjects who received SHINGRIX and for 6% of subjects who received placebo. Causes of death among subjects were consistent with those expected in the populations evaluated.

Potential Immune-Mediated Diseases: Across all 6 studies enrolling immunocompromised subjects, new onset pIMDs or exacerbation of existing pIMDs were reported for 1.3% of subjects who received SHINGRIX and 1.0% of subjects who received placebo from the first administered dose up to 1 year post-last vaccination. There were no notable imbalances in specific pIMDs between treatment groups.

Other Medically Relevant Events: In the auHSCT study (NCT01610414), relapse or progression was reported by 315 of 922 subjects (34%) who received at least one dose of SHINGRIX and 331 of 924 subjects (36%) who received placebo from the first vaccination to study end.

In the auHSCT study (NCT00920218), relapse or progression was reported by 17 of 59 subjects (29%) who received at least one dose of SHINGRIX and 8 of 30 subjects (27%) who received placebo from the first vaccination to study end.

In the hematologic malignancy study, relapse or progression was reported by 45 of 283 subjects (16%) who received at least one dose of SHINGRIX and 58 of 279 subjects (21%) who received placebo from the first vaccination to study end.

In the renal transplant study, biopsy-confirmed allograft rejection was reported by 4 of 132 (3%) of subjects who received SHINGRIX and by 7 of 132 (5%) of subjects who received placebo from the first vaccination to study end (approximately 13 months later). Creatinine as a measure of graft function and changes in alloimmunity post-vaccination were not systematically evaluated.

In the HIV study, at least 1 event of worsening HIV condition was reported by 9 of 74 (12%) of subjects who received SHINGRIX and by 5 of 49 (10%) of subjects who received placebo from the first vaccination to study end.

6.2 Postmarketing Experience

The following adverse reactions have been identified during postapproval use of SHINGRIX. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to the vaccine.

General Disorders and Administration Site Conditions

Decreased mobility of the injected arm which may persist for 1 or more weeks.

Immune System Disorders

Hypersensitivity reactions, including angioedema, rash, and urticaria.

Nervous System Disorders

Guillain-Barré syndrome.

Postmarketing Observational Study of the Risk of Guillain-Barré Syndrome following Vaccination with SHINGRIX

The association between vaccination with SHINGRIX and GBS was evaluated among Medicare beneficiaries aged 65 years or older. Using Medicare claims data, from October 2017 through February 2020, vaccinations with SHINGRIX among beneficiaries were identified through National Drug Codes, and potential cases of hospitalized GBS among recipients of SHINGRIX were identified through International Classification of Diseases codes.

The risk of GBS following vaccination with SHINGRIX was assessed in self-controlled case series analyses using a risk window of 1 to 42 days post-vaccination and a control window of 43 to 183 days post-vaccination. The primary analysis (claims-based, all doses) found an increased risk of GBS during the 42 days following vaccination with SHINGRIX, with an estimated 3 excess cases of GBS per million doses administered to adults aged 65 years or older. In secondary analyses, an increased risk of GBS was observed during the 42 days following the first dose of SHINGRIX, with an estimated 6 excess cases of GBS per million doses administered to adults aged 65 years or older, and no increased risk of GBS was observed following the second dose of SHINGRIX. These analyses of GBS diagnoses in claims data were supported by analyses of GBS cases confirmed by medical record review. While the results of this observational study suggest a causal association of GBS with SHINGRIX, available evidence is insufficient to establish a causal relationship.

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

All pregnancies have a risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively. The data are insufficient to establish if there is vaccine-associated risk with SHINGRIX in pregnant women.

A developmental toxicity study was performed in female rats administered SHINGRIX or the $AS01_B$ adjuvant alone prior to mating, during gestation, and during lactation. The total dose was 0.2 mL on each occasion (a single human dose of SHINGRIX is 0.5 mL). This study revealed no adverse effects on fetal or pre-weaning development due to SHINGRIX *(see Data)*.

Data

Animal Data: In a developmental toxicity study, female rats were administered SHINGRIX or the $AS01_B$ adjuvant alone by intramuscular injection 28 and 14 days prior to mating, on gestation Days 3, 8, 11, and 15, and on lactation Day 7. The total dose was 0.2 mL on each occasion (a single human dose of SHINGRIX is 0.5 mL). No adverse effects on pre-weaning development up to post-natal Day 25 were observed. There were no vaccine-related fetal malformations or variations.

8.2 Lactation

Risk Summary

It is not known whether SHINGRIX is excreted in human milk. Data are not available to assess the effects of SHINGRIX on the breastfed infant or on milk production/excretion.

The developmental and health benefits of breastfeeding should be considered along with the mother's clinical need for SHINGRIX and any potential adverse effects on the breastfed child

from SHINGRIX or from the underlying maternal condition. For preventive vaccines, the underlying maternal condition is susceptibility to disease prevented by the vaccine.

8.4 Pediatric Use

Safety and effectiveness in individuals younger than 18 years have not been established. SHINGRIX is not indicated for prevention of primary varicella infection (chickenpox).

8.5 Geriatric Use

Adults Aged 60 Years and Older

Of the total number of subjects who received at least 1 dose of SHINGRIX in Studies 1 and 2 (n = 14,645), 2,243 (15%) were aged 60 to 69 years, 6,837 (47%) were aged 70 to 79 years, and 1,921 (13%) were 80 years and older. There were no clinically meaningful differences in efficacy across the age groups. *[See Clinical Studies (14.1, 14.2, 14.3).]*

The frequencies of solicited local and general adverse reactions in subjects aged 70 years and older were lower than in younger adults (aged 50 through 69 years). *[See Adverse Reactions (6.1).]*

Immunocompromised Adults Aged 65 Years and Older

Of the total number of subjects who received at least 1 dose of SHINGRIX in the auHSCT study (n = 922), 172 (18.7%) were aged 65 years and older *[see Clinical Studies (14.6)]*. There were no clinically meaningful differences in efficacy between these subjects and younger adults (aged 18 through 64 years).

Of the total number of subjects who received at least 1 dose of SHINGRIX across the 6 studies in immunocompromised subjects (n = 1,587), 337 (21.2%) were aged 65 years and older. The frequencies of solicited local and general adverse reactions in subjects aged 65 years and older were generally similar to or lower than those reported by younger adults (aged 18 through 64 years).

11 DESCRIPTION

SHINGRIX (Zoster Vaccine Recombinant, Adjuvanted) is a sterile suspension for intramuscular injection. The vaccine is supplied as a vial of lyophilized recombinant varicella zoster virus surface glycoprotein E (gE) antigen component, which must be reconstituted at the time of use with the accompanying vial of $AS01_B$ adjuvant suspension component. The lyophilized gE antigen component is presented in the form of a sterile white powder. The $AS01_B$ adjuvant suspension component is an opalescent, colorless to pale brownish liquid supplied in vials.

The gE antigen is obtained by culturing genetically engineered Chinese Hamster Ovary cells, which carry a truncated gE gene, in media containing amino acids, with no albumin, antibiotics, or animal-derived proteins. The gE protein is purified by several chromatographic steps, formulated with excipients, filled into vials, and lyophilized.

The adjuvant suspension component is $AS01_B$ which is composed of 3-*O*-desacyl-4'-monophosphoryl lipid A (MPL) from *Salmonella minnesota* and QS-21, a saponin purified from plant extract *Quillaja saponaria* Molina, combined in a liposomal formulation. The liposomes are composed of dioleoyl phosphatidylcholine (DOPC) and cholesterol in phosphate-buffered saline solution containing disodium phosphate anhydrous, potassium dihydrogen phosphate, sodium chloride, and water for injection.

After reconstitution, each 0.5-mL dose is formulated to contain 50 mcg of the recombinant gE antigen, 50 mcg of MPL, and 50 mcg of QS-21. Each dose also contains 20 mg of sucrose (as stabilizer), 4.385 mg of sodium chloride, 1 mg of DOPC, 0.54 mg of potassium dihydrogen phosphate, 0.25 mg of cholesterol, 0.160 mg of sodium dihydrogen phosphate dihydrate, 0.15 mg of disodium phosphate anhydrous, 0.116 mg of dipotassium phosphate, and 0.08 mg of polysorbate 80. After reconstitution, SHINGRIX is a sterile, opalescent, colorless to pale brownish liquid.

SHINGRIX does not contain preservatives. Each dose may also contain residual amounts of host cell proteins (≤3.0%) and DNA (≤2.1 picograms) from the manufacturing process.

The vial stoppers are not made with natural rubber latex.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

The risk of developing HZ, which increases with age and with immunosuppression due to disease and/or therapy, appears to be related to a decline in varicella zoster virus (VZV)-specific immunity. SHINGRIX was shown to boost VZV-specific immune response, which is thought to be the mechanism by which it protects against zoster disease *[see Clinical Studies (14)]*.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

SHINGRIX has not been evaluated for its carcinogenic or mutagenic potential. Vaccination of female rats with SHINGRIX had no effect on fertility *[see Use in Specific Populations (8.1)]*. In a male fertility study, rats were vaccinated with 0.1 mL of SHINGRIX (a single human dose is 0.5 mL) on 42, 28, and 14 days prior to mating. There were no effects on male fertility.

14 CLINICAL STUDIES

14.1 Efficacy in Subjects Aged 50 Years and Older

Study 1 was a randomized, placebo-controlled, observer-blind clinical study conducted in 18 countries. Randomization was stratified (8:5:3:1) by age: 50 to 59 years, 60 to 69 years, 70 to 79 years, and ≥80 years. The study excluded, among others, subjects who were immunocompromised, had a history of previous HZ, were vaccinated against varicella or HZ,

and patients whose survival was not expected to be at least 4 years or with conditions that might interfere with study evaluations. Subjects were followed for the development of HZ and postherpetic neuralgia (PHN) for a median of 3.1 years (range: 0 to 3.7 years). Suspected HZ cases were followed prospectively for the development of PHN, an HZ-related complication defined as HZ-associated pain (rated as 3 or greater on a 0- to 10-point scale by the study subject) occurring or persisting at least 90 days following the onset of rash in confirmed cases of HZ.

The primary efficacy analysis population (referred to as the modified Total Vaccinated Cohort [mTVC]) included 14,759 subjects aged 50 years and older who received 2 doses (0 and 2 months) of either SHINGRIX (n = 7,344) or placebo (n = 7,415) and did not develop a confirmed case of HZ within 1 month after the second dose. In the mTVC population, 61% were female; 72% were White, 19% were Asian, 1.7% were Black, and 7% were of other racial/ethnic groups. The mean age of subjects was 62.3 years.

Confirmed HZ cases were determined by either Polymerase Chain Reaction (PCR) (89.4%) or by a Clinical Evaluation Committee (10.6%).

Efficacy against Herpes Zoster

Compared with placebo, SHINGRIX significantly reduced the risk of developing HZ by 97.2% (95% CI: 93.7, 99.0) in subjects aged 50 years and older (Table 4).

Table 4. Efficacy of SHINGRIX on Incidence of Herpes Zoster Compared with Placebo in Study 1[a] (mTVC[b])

Age Group (Years)	SHINGRIX			Placebo			% Efficacy (95% CI)
	N	n	Incidence Rate of HZ per 1,000 Person-Years	N	n	Incidence Rate of HZ per 1,000 Person-Years	
Overall (≥50)[c]	7,344	6	0.3	7,415	210	9.1	97.2 (93.7, 99.0)
50-59	3,492	3	0.3	3,525	87	7.8	96.6 (89.6, 99.3)
60-69	2,141	2	0.3	2,166	75	10.8	97.4 (90.1, 99.7)
≥70	1,711	1	0.2	1,724	48	9.4	97.9 (87.9, 100.0)

N = Number of subjects included in each group; n = Number of subjects having at least 1

confirmed HZ episode; HZ = Herpes zoster; CI = Confidence Interval.

[a] Study 1: NCT01165177.

[b] mTVC = Modified Total Vaccinated Cohort defined as subjects who received 2 doses (0 and 2 months) of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose.

[c] Primary study endpoint was based on confirmed HZ cases in subjects aged 50 years and older.

In a descriptive analysis, vaccine efficacy against HZ in subjects aged 50 years and older was 93.1% (95% CI: 81.3, 98.2) in the fourth year post-vaccination.

Occurrence of Postherpetic Neuralgia

Among all subjects aged 50 years or older in the mTVC, no cases of PHN were reported in the vaccine group compared with 18 cases reported in the placebo group.

14.2 Efficacy in Subjects Aged 70 Years and Older

Study 2 was a randomized, placebo-controlled, observer-blind clinical study conducted in 18 countries. Randomization was stratified (3:1) by age: 70 to 79 years and ≥80 years. With the exception of age, the study exclusion criteria were the same as for Study 1. Subjects were followed for the development of HZ and PHN for a median of 3.9 years (range: 0 to 4.5 years). Suspected HZ cases were followed prospectively for the development of PHN as for Study 1.

The primary efficacy analysis population (mTVC) included 13,163 subjects aged 70 years and older who received 2 doses (0 and 2 months) of either SHINGRIX (n = 6,541) or placebo (n = 6,622) and did not develop a confirmed case of HZ within 1 month after the second dose. In the mTVC population, 55% were female; 78% were White, 17% were Asian, 1% were Black, and 4% were of other racial/ethnic groups. The mean age of subjects was 75.5 years.

Confirmed HZ cases were determined by either PCR (92.3%) or by a Clinical Evaluation Committee (7.7%).

Efficacy against Herpes Zoster

Vaccine efficacy results against HZ in subjects aged 70 years and older are shown in Table 5.

Table 5. Efficacy of SHINGRIX on Incidence of Herpes Zoster Compared with Placebo in Study 2[a] (mTVC[b])

Age Group (Years)	SHINGRIX			Placebo			% Efficacy (95% CI)
	N	n	Incidence Rate of HZ per 1,000 Person-Years	N	n	Incidence Rate of HZ per 1,000 Person-Years	
Overall (≥70)[c]	6,541	23	0.9	6,622	223	9.2	89.8 (84.3, 93.7)
70-79	5,114	17	0.9	5,189	169	8.8	90.0 (83.5, 94.3)
≥80	1,427	6	1.2	1,433	54	11.0	89.1 (74.7, 96.2)

N = Number of subjects included in each group; n = Number of subjects having at least 1 confirmed HZ episode; HZ = Herpes zoster; CI = Confidence Interval.

[a] Study 2: NCT01165229.

[b] mTVC = Modified Total Vaccinated Cohort defined as subjects who received 2 doses (0 and 2 months) of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose.

[c] Primary study endpoint was based on confirmed HZ cases in subjects aged 70 years and older.

In a descriptive analysis, vaccine efficacy against HZ in subjects aged 70 years and older was 85.1% (95% CI: 64.5, 94.8) in the fourth year after vaccination.

Efficacy against Postherpetic Neuralgia

Among all subjects aged 70 years or older in the mTVC, 4 cases of PHN were reported in the vaccine group compared with 28 cases reported in the placebo group. Vaccine efficacy against PHN was 85.5% (95% CI: [58.5; 96.3]). The benefit of SHINGRIX in the prevention of PHN can be attributed to the effect of the vaccine on the prevention of HZ.

Reduction of Use of Pain Medication

Among subjects with confirmed HZ, the use of HZ-associated pain medications was reported for 10 of 23 subjects (43.5%) who received SHINGRIX and for 160 of 223 subjects (71.7%) who received placebo.

14.3 Pooled Efficacy Analyses across Studies 1 and 2

The efficacy of SHINGRIX to prevent HZ and PHN in subjects aged 70 years and older was evaluated by combining the results from Studies 1 and 2 through a pre-specified pooled analysis in the mTVC. A total of 8,250 and 8,346 subjects who received SHINGRIX and placebo, respectively, were included in the pooled mTVC analysis.

Efficacy against Herpes Zoster

Compared with placebo, SHINGRIX significantly reduced the risk of developing HZ by 91.3% (95% CI: 86.9, 94.5) in subjects 70 years and older (Table 6).

Table 6. Efficacy of SHINGRIX on Incidence of Herpes Zoster Compared with Placebo in Studies 1 and 2 (Pooled Data[a]) (mTVC[b])

Age Group (Years)	SHINGRIX			Placebo			% Efficacy (95% CI)
	N	n	Incidence Rate of HZ per 1,000 Person-Years	N	n	Incidence Rate of HZ per 1,000 Person-Years	
Overall (≥70)[c]	8,250	25	0.8	8,346	284	9.3	91.3 (86.9, 94.5)
70-79	6,468	19	0.8	6,554	216	8.9	91.3 (86.0, 94.9)
≥80	1,782	6	1.0	1,792	68	11.1	91.4 (80.2, 96.9)

N = Number of subjects included in each group; n = Number of subjects having at least 1 confirmed HZ episode; HZ = Herpes zoster; CI = Confidence Interval.

[a] Pooled data from Study 1: NCT01165177 (subjects ≥50 years) and Study 2: NCT01165229 (subjects ≥70 years).

[b] mTVC = Modified Total Vaccinated Cohort defined as subjects who received 2 doses (0 and 2 months) of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose.

[c] Primary endpoint of pooled analysis was based on confirmed HZ cases in subjects 70 years and older.

Efficacy against Postherpetic Neuralgia

Table 7 compares the overall rates of PHN in the vaccine and placebo groups across both studies.

Table 7. Efficacy of SHINGRIX on Overall Incidence of Postherpetic Neuralgia Compared with Placebo in Studies 1 and 2 (Pooled Data[a]) (mTVC[b])

Age Group (Years)	SHINGRIX			Placebo			% Efficacy (95% CI)
	N	n	Incidence Rate of PHN[c] per 1,000 Person-Years	N	n	Incidence Rate of PHN per 1,000 Person-Years	
Overall (≥70)	8,250	4	0.1	8,346	36	1.2	88.8 (68.7, 97.1)
70-79	6,468	2	0.1	6,554	29	1.2	93.0 (72.5, 99.2)
≥80	1,782	2	0.3	1,792	7	1.1	71.2 (-51.5, 97.1)

N = Number of subjects included in each group; n = Number of subjects having at least 1 PHN; CI = Confidence Interval.

[a] Pooled data from Study 1: NCT01165177 (subjects ≥50 years) and Study 2: NCT01165229 (subjects ≥70 years).

[b] mTVC = Modified Total Vaccinated Cohort defined as subjects who received 2 doses (0 and 2 months) of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose.

[c] PHN = Postherpetic neuralgia defined as HZ-associated pain rated as 3 or greater (on a 0- to 10-point scale) occurring or persisting at least 90 days following the onset of rash using Zoster Brief Pain Inventory questionnaire.

The benefit of SHINGRIX in the prevention of PHN can be attributed to the effect of the vaccine on the prevention of HZ. The efficacy of SHINGRIX in the prevention of PHN in subjects with confirmed HZ could not be demonstrated.

14.4 Immunological Evaluation to Support Dosing Schedule

A measure of the immune response that confers protection against HZ is unknown. Anti-gE antibody levels were measured by anti-gE enzyme-linked immunosorbent assay (gE ELISA) and were used to support the dosing schedule.

In an open-label clinical study, 238 subjects 50 years and older received SHINGRIX on either a 0- and 2-month or 0- and 6-month schedule. Non-inferiority of the 0- and 6-month schedule compared with the 0- and 2-month schedule based on anti-gE ELISA GMCs 1 month after the second dose was demonstrated.

14.5 Concomitant Administration with Influenza Vaccine

In an open-label clinical study, subjects 50 years and older received 1 dose each of SHINGRIX and FLUARIX QUADRIVALENT (QIV) at Month 0 and 1 dose of SHINGRIX at Month 2 (n = 413), or 1 dose of QIV at Month 0 and 1 dose of SHINGRIX at Months 2 and 4 (n = 415).

There was no evidence for interference in the immune response to any of the antigens contained in SHINGRIX or the coadministered vaccine.

14.6 Efficacy in Immunocompromised Adults Aged 18 Years and Older

The efficacy of SHINGRIX was evaluated in one Phase 3 randomized, placebo-controlled, observer-blind clinical study in immunocompromised adults aged ≥18 years who received an auHSCT 50 to 70 days prior to Dose 1 and who were expected to receive prophylactic antiviral therapy for ≤6 months post-transplant. The efficacy of SHINGRIX was calculated post-hoc in another randomized, placebo-controlled, observer-blind study in subjects with hematologic malignancies who received Dose 1 of SHINGRIX or placebo during or within 6 months of completing immunosuppressive chemotherapy. Each of these studies was conducted in the following regions: North America, Latin America, Europe, Asia, Africa (auHSCT study only), and Australia/New Zealand.

Efficacy in Subjects Aged 18 Years and Older: auHSCT Recipients

In the auHSCT study, subjects were followed for the development of HZ and PHN for a median of 21 months (range: 0 to 49.4 months). Suspected HZ cases were followed prospectively for the development of PHN as in Studies 1 and 2.

The primary efficacy analysis population (mTVC) for the auHSCT study included 1,721 subjects who received 2 doses of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose. Confirmed HZ cases were determined by either PCR (83.7%) or by a Clinical Evaluation Committee (16.3%).

Efficacy against Herpes Zoster: Compared with placebo, SHINGRIX significantly reduced the risk of developing HZ in auHSCT recipients aged 18 years and older (Table 8).

Table 8. Efficacy of SHINGRIX on Incidence of Herpes Zoster Compared with Placebo in Immunocompromised Adults Aged ≥18 Years (mTVC[a])

Clinical Studies	Age Group (Years)	SHINGRIX			Placebo			% Efficacy (95% CI)
		N	n	Incidence Rate of HZ per 1,000 Person-Years	N	n	Incidence Rate of HZ per 1,000 Person-Years	
auHSCT[b]	Overall (≥18)[c]	870	49	30.0	851	135	94.3	68.2 (55.5, 77.6)
	18-49	213	9	21.5	212	29	76.0	71.8 (38.7, 88.3)
	≥50	657	40	33.0	639	106	100.9	67.3 (52.6, 77.9)

auHSCT = Autologous, hematopoietic, stem cell transplant.

N = Number of subjects included in each group; n = Number of subjects having at least 1 confirmed HZ episode; HZ = Herpes zoster; CI = Confidence Interval.

[a] mTVC = Modified Total Vaccinated Cohort, defined as subjects who received 2 doses (0 and 1 to 2 months) of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose. Follow-up was censored at the time of treatment for relapse.

[b] NCT01610414.

[c] Primary study endpoint was based on confirmed HZ cases in subjects aged ≥18 years.

Efficacy in Subjects Aged 18 Years and Older with Hematologic Malignancies

In the study of hematologic malignancies, the mean age was 57 years. The majority of subjects were White (71%), followed by Asian (25%), Black (0.4%), and other racial groups (4%); 5% were of American Hispanic or Latino ethnicity; and 41% were female. Subjects were followed for the development of HZ for a median of 11.1 months (range: 0 to 15.6 months). PHN was not assessed as a study endpoint.

In the hematologic malignancy study, the population for the post hoc efficacy analysis included 515 subjects who received 2 doses of either SHINGRIX or placebo and did not develop a confirmed case of HZ within 1 month after the second dose. Confirmed HZ cases were determined by either PCR (81.3%) or by a Clinical Evaluation Committee (18.7%). The post hoc analysis showed SHINGRIX was 87.2% (95% CI [44.2; 98.6]) effective against development of HZ. The incidence rate of HZ per 1,000 person-years was 8.5 versus 66.2 in the SHINGRIX and placebo groups, respectively.

Additional Efficacy Endpoints Evaluated in the auHSCT Study

Efficacy against Postherpetic Neuralgia: In a descriptive analysis, including all subjects aged ≥18 years in the mTVC, 1 case of PHN was reported in the vaccine group compared with 9 cases reported in the placebo group. Vaccine efficacy against PHN was 89.3% (95% CI: [22.5; 99.8]). The benefit of SHINGRIX in the prevention of PHN can be attributed to the effect of the vaccine on the prevention of HZ.

Herpes Zoster-Associated Pain: Subjects with suspected HZ rated their "worst" HZ-associated pain on a 10-point scale. Among subjects with confirmed HZ, 37 out of 49 subjects (75.5%) receiving SHINGRIX and 120 out of 135 subjects (88.9%) receiving placebo rated their "worst" HZ-associated pain as 3 or greater. In this subset of subjects, the median duration of "worst" HZ associated pain was 14 and 24 days, among SHINGRIX and placebo recipients, respectively.

16 HOW SUPPLIED/STORAGE AND HANDLING

SHINGRIX is supplied as 2 components: A single-dose vial of lyophilized gE antigen component (powder) and a single-dose vial of adjuvant suspension component (liquid) (packaged without syringes or needles).

Table 9. Product Presentations for SHINGRIX

Presentation	Carton NDC Number	Components	
		Adjuvant Suspension Component (liquid)	Lyophilized gE Antigen Component (powder)
An outer carton of 1 dose	58160-819-12	Vial 1 of 2 NDC 58160-829-01	Vial 2 of 2 NDC 58160-828-01
An outer carton of 10 doses	58160-823-11	10 vials NDC 58160-829-03	10 vials NDC 58160-828-03

16.1 Storage before Reconstitution

Adjuvant suspension component vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light. Do not freeze. Discard if the adjuvant suspension has been frozen.

Lyophilized gE antigen component vials: Store refrigerated between 2° and 8°C (36° and 46°F). Protect vials from light. Do not freeze. Discard if the antigen component has been frozen.

16.2 Storage after Reconstitution

- Administer immediately or store refrigerated between 2° and 8°C (36° and 46°F) for up to 6 hours prior to use.
- Discard reconstituted vaccine if not used within 6 hours.
- Do not freeze. Discard if the vaccine has been frozen.

17 PATIENT COUNSELING INFORMATION

- Inform patients of the potential benefits and risks of immunization with SHINGRIX and of the importance of completing the 2-dose immunization series according to the schedule.
- Inform patients about the potential for adverse reactions that have been temporally associated with administration of SHINGRIX.
- Provide the Vaccine Information Statements, which are available free of charge at the Centers for Disease Control and Prevention (CDC) website (www.cdc.gov/vaccines).

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Research Triangle Park, NC 27709

SHX:6PI

VACCINE INGREDIENTS / EXCIPIENTS / CONTAMINANTS

Overview

Vaccine Excipient Summary

Excipients Included in U.S. Vaccines, by Vaccine

In addition to weakened or killed disease antigens (such as weakened, killed, or parts of viruses or bacteria), vaccines contain very small amounts of other ingredients – excipients.

Some excipients are added to a vaccine for a specific purpose. These include:

- **Preservatives**, to prevent contamination. For example, thimerosal.
- **Adjuvants**, to help stimulate a stronger immune response. For example, aluminum salts.
- **Stabilizers**, to keep the vaccine potent during transportation and storage. For example, sugars or gelatin.

Others are residual trace amounts of materials that were used during the manufacturing process and removed. These can include:

- **Cell culture materials**, used to grow the vaccine antigens. For example, egg protein, various culture media.
- **Inactivating ingredients**, used to kill viruses or inactivate toxins. For example, formaldehyde.
- **Antibiotics**, used to prevent contamination by bacteria. For example, neomycin.

The following table lists substances, other than active ingredients (i.e., antigens), shown in the manufacturers' package insert (PI) as being contained in the final formulation of each vaccine. **Substances used in the manufacture of a vaccine but not listed as contained in the final product (e.g., culture media) can be found in each PI, but are not shown on this table.** Each PI, which can be found on the FDA's website (see below) contains a description of that vaccine's manufacturing process, including the amount and purpose of each substance. In most PIs, this information is found in Section 11: "Description." Please refer to the PI for a complete list of ingredients or excipients. A table listing vaccine excipients and media by excipient is published by the Institute for Vaccine Safety at Johns Hopkins University, and can be found at http://www.vaccinesafety.edu/components-Excipients.htm.

B

Appendix B

Vaccine Excipient Table

Vaccine (Trade Name)	Package Insert Date	Contains[a]
Adenovirus	10/2019	monosodium glutamate, sucrose, D-mannose, D-fructose, dextrose, human serum albumin, potassium phosphate, plasdone C, anhydrous lactose, microcrystalline cellulose, polacrilin potassium, magnesium stearate, cellulose acetate phthalate, alcohol, acetone, castor oil, FD&C Yellow #6 aluminum lake dye
Anthrax (Biothrax)	11/2015	aluminum hydroxide, sodium chloride, benzethonium chloride, formaldehyde
BCG (Tice)	02/2009	glycerin, asparagine, citric acid, potassium phosphate, magnesium sulfate, iron ammonium citrate, lactose
Cholera (Vaxchora)	06/2016	ascorbic acid, hydrolyzed casein, sodium chloride, sucrose, dried lactose, sodium bicarbonate, sodium carbonate
Dengue (Dengvaxia)	06/2019	sodium chloride, essential amino acids (including L-phenylalanine), non-essential amino acids, L-arginine hydrochloride, sucrose, D-trehalose dihydrate, D-sorbitol, trometamol, urea
DT (Sanofi)	06/2018	aluminum phosphate, isotonic sodium chloride, formaldehyde
DTaP (Daptacel)	01/2021[b]	aluminum phosphate, formaldehyde, glutaraldehyde, 2-phenoxyethanol
DTaP (Infanrix)	01/2021[b]	formaldehyde, aluminum hydroxide, sodium chloride, polysorbate 80 (Tween 80)
DTaP-IPV (Kinrix)	01/2021[b]	formaldehyde, aluminum hydroxide, sodium chloride, polysorbate 80 (Tween 80), neomycin sulfate, polymyxin B
DTaP-IPV (Quadracel)	02/2021	formaldehyde, aluminum phosphate, 2-phenoxyethanol, polysorbate 80, glutaraldehyde, neomycin, polymyxin B sulfate, bovine serum albumin
DTaP-HepB-IPV (Pediarix)	01/2021[b]	formaldehyde, aluminum hydroxide, aluminum phosphate, sodium chloride, polysorbate 80 (Tween 80), neomycin sulfate, polymyxin B, yeast protein
DTaP-IPV/Hib (Pentacel)	12/2019	aluminum phosphate, polysorbate 80, sucrose, formaldehyde, glutaraldehyde, bovine serum albumin, 2-phenoxyethanol, neomycin, polymyxin B sulfate
DTaP-IPV-Hib-HepB (Vaxelis)	10/2020	polysorbate 80, formaldehyde, glutaraldehyde, bovine serum albumin, neomycin, streptomycin sulfate, polymyxin B sulfate, ammonium thiocyanate, yeast protein, aluminum
Ebola Zaire (ERVEBO)	01/2021[b]	Tromethamine, rice-derived recombinant human serum albumin, host cell DNA, benzonase, rice protein
Hib (ActHIB)	05/2019	sodium chloride, formaldehyde, sucrose
Hib (Hiberix)	04/2018	formaldehyde, sodium chloride, lactose
Hib (PedvaxHIB)	01/2021[b]	amorphous aluminum hydroxyphosphate sulfate, sodium chloride
Hep A (Havrix)	01/2021[b]	MRC-5 cellular proteins, formalin, aluminum hydroxide, amino acid supplement, phosphate-buffered saline solution, polysorbate 20, neomycin sulfate, aminoglycoside antibiotic
Hep A (Vaqta)	01/2021[b]	amorphous aluminum hydroxyphosphate sulfate, non-viral protein, DNA, bovine albumin, formaldehyde, neomycin, sodium borate, sodium chloride, other process chemical residuals
Hep B (Engerix-B)	01/2021[b]	aluminum hydroxide, yeast protein, sodium chloride, disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate
Hep B (Recombivax)	12/2018	formaldehyde, potassium aluminum sulfate, amorphous aluminum hydroxyphosphate sulfate, yeast protein
Hep B (Heplisav-B)	05/2020	yeast protein, yeast DNA, deoxycholate, phosphorothioate linked oligodeoxynucleotide, sodium phosphate, dibasic dodecahydrate, sodium chloride, monobasic dehydrate, polysorbate 80
Hep A/Hep B (Twinrix)	01/2021[b]	MRC-5 cellular proteins, formalin, aluminum phosphate, aluminum hydroxide, amino acids, sodium chloride, phosphate buffer, polysorbate 20, neomycin sulfate, yeast protein
HPV (Gardasil 9)	08/2020	amorphous aluminum hydroxyphosphate sulfate, sodium chloride, L-histidine, polysorbate 80, sodium borate, yeast protein

Vaccine (Trade Name)	Package Insert Date	Contains[a]
Influenza (Afluria) Quadrivalent[c]	03/2021	sodium chloride, monobasic sodium phosphate, dibasic sodium phosphate, monobasic potassium phosphate, potassium chloride, calcium chloride, sodium taurodeoxycholate, ovalbumin, sucrose, neomycin sulfate, polymyxin B, beta-propiolactone, hydrocortisone, thimerosal (multi-dose vials)
Influenza (Fluad) Quadrivalent[c]	03/2021	squalene, polysorbate 80, sorbitan trioleate, sodium citrate dihydrate, citric acid monohydrate, neomycin, kanamycin, hydrocortisone, egg protein, formaldehyde
Influenza (Fluarix) Quadrivalent[c]	2021	octoxynol-10 (TRITON X-100), α-tocopheryl hydrogen succinate, polysorbate 80 (Tween 80), hydrocortisone, gentamicin sulfate, ovalbumin, formaldehyde, sodium deoxycholate, sodium phosphate-buffered isotonic sodium chloride
Influenza (Flublok) Quadrivalent[c]	03/2021	sodium chloride, monobasic sodium phosphate, dibasic sodium phosphate, polysorbate 20 (Tween 20), baculovirus and *Spodoptera frugiperda* cell proteins, baculovirus and cellular DNA, Triton X-100
Influenza (Flucelvax) Quadrivalent[c]	10/2021[b]	Madin Darby Canine Kidney (MDCK) cell protein, phosphate buffered saline, protein other than HA, MDCK cell DNA, polysorbate 80, cetyltrimethlyammonium bromide, and β-propiolactone, thimerosal (multi-dose vials)
Influenza (Flulaval) Quadrivalent[c]	2021	ovalbumin, formaldehyde, sodium deoxycholate, α-tocopheryl hydrogen succinate, polysorbate 80, phosphate-buffered saline solution
Influenza (Fluzone) Quadrivalent[c]	2021	formaldehyde, egg protein, octylphenol ethoxylate (Triton X-100), sodium phosphate-buffered isotonic sodium chloride solution, thimerosal (multi-dose vials)
Influenza (Fluzone) High Dose[c]	07/2021	egg protein, octylphenol ethoxylate (Triton X-100), sodium phosphate-buffered isotonic sodium chloride solution, formaldehyde
Influenza (FluMist) Quadrivalent[c]	08/2021	monosodium glutamate, hydrolyzed porcine gelatin, arginine, sucrose, dibasic potassium phosphate, monobasic potassium phosphate, ovalbumin, gentamicin sulfate, ethylenediaminetetraacetic acid (EDTA)
IPV (Ipol)	01/2021[b]	calf bovine serum albumin, 2-phenoxyethanol, formaldehyde, neomycin, streptomycin, polymyxin B, M-199 medium
Japanese Encephalitis (Ixiaro)	09/2018	aluminum hydroxide, protamine sulfate, formaldehyde, bovine serum albumin, host cell DNA, sodium metabisulphite, host cell protein
MenACWY (Menactra)	04/2018	sodium phosphate buffered isotonic sodium chloride solution, formaldehyde, diphtheria toxoid protein carrier
MenACWY (MenQuadfi)	01/2021[b]	sodium chloride, sodium acetate, formaldehyde
MenACWY (Menveo)	07/2020	formaldehyde, CRM_{197} protein
MenB (Bexsero)	01/2021[b]	aluminum hydroxide, sodium chloride, histidine, sucrose, kanamycin
MenB (Trumenba)	2018	polysorbate 80, aluminum phosphate, histidine buffered saline
MMR (MMR-II)	12/2020	sorbitol, sucrose, hydrolyzed gelatin, recombinant human albumin, neomycin, fetal bovine serum, WI-38 human diploid lung fibroblasts
MMRV (ProQuad) (Frozen: Recombinant Albumin)	01/2021[b]	MRC-5 cells including DNA and protein, sucrose, hydrolyzed gelatin, sodium chloride, sorbitol, monosodium L-glutamate, sodium phosphate dibasic, recombinant human albumin, sodium bicarbonate, potassium phosphate monobasic, potassium chloride, potassium phosphate dibasic, neomycin, bovine calf serum, other buffer and media ingredients
PCV13 (Prevnar 13)	08/2017	CRM_{197} carrier protein, polysorbate 80, succinate buffer, aluminum phosphate
PPSV-23 (Pneumovax)	09/2020	isotonic saline solution, phenol
Rabies (Imovax)	10/2019	human albumin, neomycin sulfate, phenol red, beta-propiolactone
Rabies (RabAvert)	2018	chicken protein, polygeline (processed bovine gelatin), human serum albumin, potassium glutamate, sodium EDTA, ovalbumin, neomycin, chlortetracycline, amphotericin B
Rotavirus (RotaTeq)	01/2021[b]	sucrose, sodium citrate, sodium phosphate monobasic monohydrate, sodium hydroxide, polysorbate 80, cell culture media, fetal bovine serum

Appendix B

Vaccine (Trade Name)	Package Insert Date	Contains[a]
Rotavirus (Rotarix)	01/2021[b]	dextran, Dulbecco's Modified Eagle Medium (sodium chloride, potassium chloride, magnesium sulfate, ferric (III) nitrate, sodium phosphate, sodium pyruvate, D-glucose, concentrated vitamin solution, L-cystine, L-tyrosine, amino acids, L-glutamine, calcium chloride, sodium hydrogenocarbonate, and phenol red), sorbitol, sucrose, calcium carbonate, sterile water, xanthan [Porcine circovirus type 1 (PCV1) is present in Rotarix. PCV-1 is not known to cause disease in humans.]
Smallpox (Vaccinia) (ACAM2000)	03/2018	HEPES, 2% human serum albumin, 0.5 - 0.7% sodium chloride USP, 5% Mannitol USP, neomycin, polymyxin B, 50% Glycerin USP, 0.25% phenol USP
Td (Tenivac)	11/2019	aluminum phosphate, formaldehyde, sodium chloride
Td (TDVAX)	09/2018	aluminum phosphate, formaldehyde, thimerosal
Tdap (Adacel)	12/2020	aluminum phosphate, formaldehyde, 2-phenoxyethanol, glutaraldehyde
Tdap (Boostrix)	09/2020	formaldehyde, aluminum hydroxide, sodium chloride, polysorbate 80
Typhoid (Typhim Vi)	03/2020	formaldehyde, phenol, polydimethylsiloxane, disodium phosphate, monosodium phosphate, sodium chloride
Typhoid (Vivotif Ty21a)	9/2013	sucrose, ascorbic acid, amino acids, lactose, magnesium stearate, gelatin
Varicella (Varivax) Frozen	01/2021[b]	sucrose, hydrolyzed gelatin, sodium chloride, monosodium L-glutamate, sodium phosphate dibasic, potassium phosphate monobasic, potassium chloride, MRC-5 human diploid cells including DNA & protein, sodium phosphate monobasic, EDTA, neomycin, fetal bovine serum
Yellow Fever (YF-Vax)	2/2019	sorbitol, gelatin, sodium chloride
Zoster (Shingles) (Shingrix)	01/2021[b]	sucrose, sodium chloride, dioleoyl phosphatidylcholine (DOPC), 3-*O*-desacl-4'monophosphoryl lipid A (MPL), QS-21 (a saponin purified from plant extract *Quillaja saponaria* Molina), potassium dihydrogen phosphate, cholesterol, sodium dihydrogen phosphate dihydrate, disodium phosphate anhydrous, dipotassium phosphate, polysorbate 80, host cell protein and DNA

Abbreviations: DT = diphtheria and tetanus toxoids; DTaP = diphtheria and tetanus toxoids and acellular pertussis; Hep A = Hepatitis A; Hep B = Hepatitis B; Hib = *Haemophilus influenzae* type b; HPV = human papillomavirus; IPV = inactivated poliovirus; LAIV = live, attenuated influenza vaccine; MenACWY = quadrivalent meningococcal conjugate vaccine; MenB = serogroup B meningococcal vaccine; MMR = measles, mumps, and rubella; MMRV = measles, mumps, rubella, varicella; PCV13 = pneumococcal conjugate vaccine; PPSV23= pneumococcal polysaccharide vaccine; Td = tetanus and diphtheria toxoids; Tdap = tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis.

[a]All information was extracted from manufacturers' package inserts. The date shown in the Date column of the table is the edition date of the PI in use in January 2021 by month and year. In some cases, only a year was printed on the PI. If in doubt about whether a PI has been updated since this table was prepared, check the FDA's website at:

http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm093833.htm

[b]The PI was not dated and this is the date the PI was reviewed for this table.

[c]All influenza vaccine in this table are 2021-22 northern hemisphere formulation.

November 2021

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Vaccine Ingredients and Manufacturer Information

Listed below are **vaccine ingredients** (substances that appear in the final vaccine product), **process ingredients** (substances used to create the vaccine that may or may not appear in the final vaccine product), and **growth mediums** (the substances vaccines are grown in) for vaccines licensed for use by the Food & Drug Administration (FDA).

Three vaccines licensed for use by the FDA (plague, poliovax, and rabies vaccine adsorbed) are listed on the FDA site, but do not have available package inserts or other information, and thus are not included below.

Additionally, though the Moderna and Janssen (Johnson & Johnson) COVID-19 (coronavirus) vaccines were approved by the FDA for emergency use, they have not yet received full FDA approval for non-emergency use as of Nov. 17, 2021, and thus are not included below. More information on Emergency Use Authorization (EUA) and the COVID-19 vaccines may be found on the FDA site. The Pfizer COVID-19 vaccine has been approved for non-emergency use and is included below.

Controversial products used to make vaccines include but are not limited to: African Green Monkey (Vero) cells, aluminum, cow products, Cocker Spaniel cells, formaldehyde, human fetal lung tissue cells, insect products, and mouse brains. More information on some controversial products may be found in the **glossary** on this page.

Though not listed, each vaccine contains strains of the virus being vaccinated against. Each vaccine entry links to the manufacturer's package insert that contains information about dosage, ingredient quantity, and how the vaccine is made. Some vaccines, like influenza (flu) vaccines, are modified frequently and you may wish to consult your doctor's office or pharmacy for the most current information.

Glossary

Product	Explanation of Product
2-Phenoxyethanol	2-Phenoxyethanol is a glycol ether used as a preservative in vaccines.
Aluminum	Aluminum is used in vaccines as an adjuvant, which helps the vaccine work more quickly and more powerfully.
Bovine casein	A casein is a family of phosphoproteins commonly found in mammalian milk. 80% of the proteins in cow's milk are casein.
Bovine serum	"[T]he centrifuged fluid component of either clotted or defibrinated whole blood. Bovine serum comes from blood taken from domestic cattle. Serum from other animals is also collected and processed but bovine serum is processed in the greatest volume." "Bovine serum is categorized according to the age of the animal from which the blood was collected as follows: •'Fetal bovine serum' comes from fetuses •'Newborn calf serum' comes from calves less than three weeks old •'Calf serum' comes from calves aged between three weeks and 12 months •'Adult bovine serum' comes from cattle older than 12 months Serum processed from donor blood is termed 'donor bovine serum'. Donor animals can be up to three years old."
Chicken Eggs	Viruses can be grown in chicken eggs before being used in vaccinations.
CMRL-1969	Common Ingredients: L-alanine, L-arginine (free base)[b], L-aspartic acid, L-cysteine-HCL, L-cystine, L-glutamic acid-H20, L-glutamine, glycine, L-histidine (free base)[b], L-hydroxyproline, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, *p*-aminobenzoic acid, ascorbic acid, *d*-biotin, calcium pantothenate, cholesterol, choline chloride, ethanol, folic acid, glutathione, *i*-inositol, menadione, nicotinamide, nicotinic acid, pyridoxal-HCL, pyridoxine-HCL, riboflavine, riboflavine-5-phosphate, sodium acetate-3H2O, thiamine-HCL, Tween 80, vitamin A acetate, vitamin D (calciferol), vitamin E (a-tocopherol phosphate), D-glucose, phenol red, sodium chloride, potassium chloride, calcium chloride, magnesium culphate heptahydrate, sodium phosphate dibasic, sodium dihydrogen phosphate, monopotassium phosphate, sodium bicarbonate, iron nitrate nonahydrate
Dulbecco's Modified Eagle's Serum	Common Ingredients: glucose, sodium bicarbonate, L-glutamine, pyridoxine HCl, pyridocal HCl, folic acid, phenol red, HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid), L-methionine, L-cystine, sodium phosphate mono-basic, sodium pyruvate, vitamins

Product	Explanation of Product
Earle's Balanced Salt Medium	Common Ingredients: inorganic salts, D-glucose, phenol red, calcium, magnesium salts
Fenton Medium	bovine extract
Formaldehyde	Formaldehyde is used in vaccines to inactivate the virus so the person being inoculated does not contract the disease.
Human albumin	Human albumin is a blood plasma protein produced in the liver that, among other functions, transports hormones, fatty acids, and other compounds, and buffers pH.
Insect Cells	Cabbage moth and fall armyworm cells are used to grow viruses for vaccines.
Latham Medium	bovine casein
MDCK (Madin-Carby canine kidney cells)	Common Ingredients: Cells from normal female adult Cocker Spaniel (harvested in 1958 by SH Madin and NB Darby), EMEM(EBSS) (Eagle's Minimum Essential Medium with Earle's Balanced Salt Solution), glutamine, non essential amino acids, foetal bovine serum
Mouse Brains	Live mice brains are inoculated with the Japanese encephalitis virus to grow the virus used in the vaccine.
MRC-5	Common Ingredients: Medical Research Council 5, human diploid cells (cells containing two sets of chromosomes) derived from the normal lung tissues of a 14-week-old male fetus aborted for "psychiatric reasons" in 1966 in the United Kingdom, Eagle's Basal Medium in Earle's balanced salt solution with bovine serum.
Mueller Hinton Agar	Common Ingredients: beef extract, acid hydrolysate of casein, starch, agar
Mueller-Miller Medium	Common Ingredients: glucose, sodium chloride, sodium phosphate dibasic, monopotassium, phosphate, magnesium sulfate hydrate, ferrous sulfate heptaphydrate, cystine hydrochloride, tyrosine hydrochloride, urasil hydrochloride, Ca-pantothenate in ethanol, thiamine in ethanol, pyridoxin-hydrochloride in ethanol, riboflavin in ethanol, biotin in ethanol, sodium hydroxide, beef heart infusion (de-fatted beef heart and distilled water), casein solution
Polysorbate 80	Also called Tween 80, Alkest 80, or Canarcel 80 (brand names), Polysorbate 80 is used as an excipient (something to basically thicken a vaccine for proper dosing) and an emulsifier (something to bond the ingredients).
Porcine gelatin	Gelatin is used to protect viruses in vaccines from freeze-drying or heat and to stabilize vaccines so they stay stable.
Stainer-Scholte Liquid Medium	Common Ingredients: tris hydrochloride, tris base, glutamate (monosodium salt), proline, salt, monopotassium phosphate, potassium chloride, magnesium chloride, calcium chloride, ferrous sulfate, ascorbic acid, niacin, glutathione

Product	Explanation of Product
Thimerosal	Thimerosal is an organomercury compound used as a preservative.
Vero Cells (African Green Monkey Cells)	Vero cells were derived from the kidney of a normal, adult African Green monkey in 1962 by Y. Yasumura and Y. Kawakita.
WI-38 human diploid cells	Winstar Institute 38 is the human diploid lung fibroblasts derived from the lung tissues of a female fetus aborted because the family felt they had too many children in 1964 in the United States.

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4 Assessment of Toxicity

Introduction

This chapter discusses the methods used to evaluate the toxicity of a substance for the purpose of health risk assessment. Evaluation of toxicity involves two steps: hazard identification and dose-response evaluation. Hazard identification includes a description of the specific forms of toxicity (neurotoxicity, carcinogenicity, etc.) that can be caused by a chemical and an evaluation of the conditions under which these forms of toxicity might appear in exposed humans. Data used in hazard identification typically are derived from animal studies and other types of experimental work, but can also come from epidemiologic studies. Dose-response evaluation is a more complex examination of the conditions under which the toxic properties of a chemical might be evidenced in exposed people, with particular emphasis on the quantitative relationship between dose and toxic response. This step also includes study of how response can vary from one population subgroup to another.

Principles Of Toxicity Assessment

The basic principles guiding the assessment of a substance's toxicity are outlined in the *Guidelines for Carcinogen Risk Assessment* (EPA, 1987a) (currently being updated), *Chemical Carcinogens: A Review of the Science and Its Associated Principles* (OSTP, 1985), *Guidelines for Developmental Toxicity Risk Assessment* (EPA, 1991a) and have recently been summarized by the NRC (1993a). In addition, guidelines for the assessment of acute toxicity have recently been developed by NRC (1993b). The developmental-toxicity guidelines are used in this chapter to illustrate EPA's approach to health effects that involve noncancer end points. They constitute the first completed noncancer risk-assessment guidelines in a series that EPA plans to issue.

Hazard Identification

The first of the two questions typically considered in the assessment of chemical toxicity concerns the types of toxic effects that the chemical can cause. Can it damage the liver, the kidney, the lung, or the reproductive system? Can it cause birth defects, neurotoxic effects, or cancer? This type of *hazard* information is obtained principally through studies in groups of people who happen to be exposed to the chemical (epidemiologic studies) and through controlled laboratory experiments involving various animal species. Several other types of experimental data can also be used to assist in identifying the toxic hazards of a chemical.

Epidemiologic Studies

Epidemiologic studies clearly provide the most relevant kind of information for hazard identification, simply because they involve observations of human beings, not laboratory animals. That obvious and substantial advantage is offset to various degrees by the difficulties associated with obtaining and interpreting epidemiologic information. It is often not possible to identify appropriate populations for study or to obtain the necessary medical information on the health status of individuals in them. Information on the magnitude and duration of chemical exposure, especially that experienced in the distant past, is often available in only qualitative or semiquantitative form (e.g., the number of years worked at low, medium, and high exposure). Identifying other factors that might influence the health status of a population is often not possible. Epidemiologic studies are not controlled experiments. The investigator identifies an exposure situation and attempts to identify appropriate "control" groups (i.e., unexposed parallel populations), but the ease with which this can be accomplished is largely beyond the investigator's control. For those and several other

reasons, it is difficult or impossible to identify cause-effect relationships clearly with epidemiologic methods (OSTP, 1985).

It is rare that convincing causal relationships are identified with a single study. Epidemiologists usually weigh the results from several studies, ideally involving different populations and investigative methods, to determine whether there is a consistent pattern of responses among them. Some of the other factors that are often considered are the strength of the statistical association between a particular disease and exposure to the suspect chemical; whether the risk of the disease increases with increasing exposure to the suspect agent; and the degree to which other possible causative factors can be ruled out. Epidemiologists attempt to reach consensus regarding causality by weighing the evidence. Needless to say, different experts will weigh such data differently, and consensus typically is not easily achieved (IARC, 1987).

In the case of chemicals suspected of causing cancer in humans, expert groups ("working groups") are regularly convened by the International Agency for Research on Cancer (IARC) to consider and evaluate epidemiologic evidence. These groups have published their conclusions regarding the "degrees" of strength of the evidence on specific chemicals (sometimes chemical mixtures or even industrial processes when individual causative agents cannot be identified). The highest degree of evidence—sufficient evidence of carcinogenicity—is applied only when a working group agrees that the total body of evidence is convincing with respect to the issue of a cause-effect relationship.

No similar consensus-building procedure has been established regarding other forms of toxicity. Some epidemiologists disagree with IARC's cancer classification judgments in particular cases, and there seems to be even greater potential for scientific controversy regarding the strength of the epidemiologic evidence of non-cancer (e.g., reproductive, developmental, etc.) effects. There has been much less epidemiologic study of other toxic effects, in part because of lack of adequate medical documentation.

Animal Studies

When epidemiologic studies are not available or not suitable, risk assessment may be based on studies of laboratory animals. One advantage of animal studies is that they can be controlled, so establishing causation (assuming that the experiments are well conducted) is not in general difficult. Another advantage is that animals can be used to collect toxicity information on chemicals before their marketing, whereas epidemiologic data can be collected only after human exposure. Indeed, laws in many countries require that some classes of chemicals (e.g., pesticides, food additives, and drugs) be subjected to toxicity testing in animals before marketing. Other advantages of animal tests include the facts that

- The quantitative relationship between exposure (or dose) and extent of toxic response can be established.
- The animals and animal tissues can be thoroughly examined by toxicologists and pathologists, so the full range of toxic effects produced by a chemical can be identified.
- The exposure duration and routes can be designed to match those experienced by the human population of concern.

But laboratory animals are not human beings, and this obvious fact is one clear disadvantage of animal studies. Another is the relatively high cost of animal studies containing enough animals to detect an effect of interest. Thus, interpreting observations of toxicity in laboratory animals as generally applicable to humans usually requires two acts of extrapolation: interspecies extrapolation and extrapolation from high test doses to lower environmental doses. There are reasons based on both biologic principles and empirical observations to support the hypothesis that many forms of biologic responses, including toxic responses, can be extrapolated across mammalian species, including

Homo sapiens, but the scientific basis of such extrapolation is not established with sufficient rigor to allow broad and definitive generalizations to be made (NRC, 1993b).

One of the most important reasons for species differences in response to chemical exposures is that toxicity is very often a function of chemical metabolism. Differences among animal species, or even among strains of the same species, in metabolic handling of a chemical, are not uncommon and can account for toxicity differences (NRC, 1986). Because in most cases information on a chemical's metabolic profile in humans is lacking (and often unobtainable), identifying the animal species and toxic response most likely to predict the human response accurately is generally not possible. It has become customary to assume, under these circumstances, that in the absence of clear evidence that a particular toxic response is not relevant to human beings, any observation of toxicity in an animal species is potentially predictive of response in at least some humans (EPA, 1987a). This is not unreasonable, given the great variation among humans in genetic composition, prior sensitizing events, and concurrent exposures to other agents.

As in the case of epidemiologic data, IARC expert panels rank evidence of carcinogenicity from animal studies. It is generally recognized by experts that evidence of carcinogenicity is most convincing when a chemical produces excess malignancies in several species and strains of laboratory animals and in both sexes. The observation that a much higher proportion of treated animals than untreated (control) animals develops malignancies adds weight to the evidence of carcinogenicity as a result of the exposure. At the other extreme, the observation that a chemical produces only a relatively small increase in incidence of mostly benign tumors, at a single site of the body, in a single species and sex of test animal does not make a very convincing case for carcinogenicity, although any excess of tumors raises some concern.

EPA combines human and animal evidence, as shown in Table 4-1, to categorize evidence of carcinogenicity; the agency's evaluations of data on individual carcinogens generally match those of IARC. For noncancer health effects, EPA uses categories like those outlined in Table 4-2. Animal data on other forms of toxicity are generally evaluated in the same way as carcinogenicity data, although this classification looks at hazard identification (qualitative) and dose-response relationships (quantitative) together. No risk or hazard ranking schemes similar to those used for carcinogens have been adopted.

The hazard-identification step of a risk assessment generally concludes with a qualitative narrative of the types of toxic responses, if any, that can be caused by the chemical under review, the strength of the supporting evidence, and the scientific merits of the data and their value for predicting human toxicity. In addition to the epidemiologic and animal data, information on metabolism and on the behavior of the chemical in tissues and cells (i.e., on its mechanism of toxic action) might be evaluated, because clues to the reliability of interspecies extrapolation can often be found here.

Identifying the potential of a chemical to cause particular forms of toxicity in humans does not reveal whether the substance poses a risk in specific exposed populations. The latter determination requires three further analytic steps: emission characterization and exposure assessment (discussed in Chapter 3), dose-response assessment (discussed next), and risk characterization (discussed in Chapter 5).

Dose-Response Assessment

In the United States and many other countries, two forms of dose-response assessment involving extrapolation to low doses are used, depending on the nature of the toxic effect under consideration. One form is used for cancer, the other for toxic effects other than cancer.

Toxic Effects Other Than Cancer

For all types of toxic effects other than cancer, the standard procedure used by regulatory agencies for evaluating the dose-response aspects of toxicity involves identifying the highest exposure among all the available experimental studies at which no toxic effect was observed, the "no-observed-effect level" (NOEL) or "no-observed-adverse-effect level" (NOAEL). The difference between the two values is related to the definition of adverse effect. The NOAEL is the highest exposure at which there is no statistically or biologically significant increase in the frequency of an adverse effect when compared with a control group. A similar value used is the lowest-observed-adverse-effect level (LOAEL), which is the lowest exposure at which there is a significant increase in an observable effect. All are used in a similar fashion relative to the regulatory need. The NOAEL is more conservative than the LOAEL (NRC, 1986).

For example, if a chemical caused signs of liver damage in rats at a dosage of 5 mg/kg per day, but no observable effect at 1 mg/kg per day and no other study indicated adverse effects at 1 mg/kg per day or less, then 5 mg/kg per day would be the LOAEL and 1 mg/kg per day would be the NOAEL under the conditions tested in that study. For human risk assessment, the ratio of the NOAEL to the estimated human dose gives an indication of the margin of safety for the potential risk. In general, the smaller the ratio, the greater the likelihood that some people will be adversely affected by the exposure.

The uncertainty-factor approach is used to set exposure limits for a chemical when there is reason to believe that a safe exposure exists; that is, that its toxic effects are likely to be expressed in a person only if that person's exposure is above some minimum, or threshold. At exposures below the threshold, toxic effects are unlikely. The experimental NOAEL is assumed to approximate the threshold. To establish limits for human exposure, the experimental NOAEL is divided by one or more uncertainty factors, which are intended to account for the uncertainty associated with interspecies and intraspecies extrapolation and other factors. Depending on how close the experimental threshold is thought to be to the exposure of a human population, perhaps modified by the particular conditions of exposure, a larger or smaller uncertainty factor might be required to ensure adequate protection. For example, if the NOAEL is derived from high-quality data in (necessarily limited groups of) humans, even a small safety factor (10 or less) might ensure safety, provided that the NOAEL was derived under conditions of exposure similar to those in the exposed population of interest and the study is otherwise sound. If, however, the NOAEL was derived from a less similar or less reliable laboratory-animal study, a larger uncertainty factor would be required (NRC, 1986).

There is no strong scientific basis for using the same constant uncertainty factor for all situations, but there are strong precedents for the use of some values (NRC, 1986). The regulatory agencies usually require values of 10,100, or 1,000 in different situations. For example, a factor of 100 is usually applied when the NOAEL is derived from chronic toxicity studies (typically 2-year studies) that are considered to be of high quality and when the purpose is to protect members of the general population who could be exposed daily for a full lifetime (10 to account for interspecies differences and 10 to account for intraspecies differences).

Using the NOAEL/LOAEL/uncertainty-factor procedure yields an estimate of an exposure that is thought to "have a reasonable certainty of no harm." Depending on the regulatory agency involved, the resulting estimate of "safe" exposure can be termed an acceptable daily intake, or ADI (Food and Drug Administration, FDA); a reference dose, or RfD (EPA); or a permissible exposure level, or PEL (Occupational Safety and Health Administration, OSHA). For risk assessments, the dose received by humans is compared with the ADI, RfD, or PEL to determine whether a health risk is likely.

The requirement for uncertainty factors stems in part from the belief that humans could be more sensitive to the toxic effects of a chemical than laboratory animals and the belief that variations in sensitivity are likely to exist within the human population (NRC, 1980a). Those beliefs are plausible, but the magnitudes of interspecies and intraspecies differences for every chemical and toxic end point are not often known. Uncertainty factors are intended to accommodate scientific uncertainty, as well as uncertainties about dose delivered, human variations in sensitivity, and other matters (Dourson and Stara, 1983).

EPA's approaches to risk assessment for chemically induced reproductive and developmental end points rely on the threshold assumption. The EPA (1987a) guidelines for health-risk assessment for suspected developmental toxicants states that, "owing primarily to a lack of understanding of the biological mechanisms underlying developmental toxicity, intra/interspecies differences in the types of developmental events, the influence of maternal effects on the dose-response curve, and whether or not a threshold exists below which no effect will be produced by an agent," many developmental toxicologists assume a threshold for most developmental effects, because "the embryo is known to have some capacity for repair of the damage or insult" and "most developmental deviations are probably multifactorial."

EPA (1988a,b) later proposed guidelines for assessing male and female reproductive risks that incorporate the threshold default assumption "usually assumed for noncarcinogenic/nonmutagenic health effects," as well as the agency's new RfD approach to deriving acceptable intakes. The RfD is obtained as described above. The total adjustment or uncertainty factor referred to in the proposed guidelines for use in obtaining an RfD from toxicity data "usually ranges" from 10 to 1,000. The adjustment incorporates (as needed) uncertainty factors ("often" 10) for "(1) situations in which the LOAEL must be used because a NOAEL was not established, (2) interspecies extrapolation, and (3) intraspecies adjustment for variable sensitivity among individuals." An additional modifying factor may be used to account for extrapolating between exposure durations (e.g., from acute to subchronic) or for NOAEL-LOAEL inadequacy due to scientific uncertainties in the available database.

EPA's 1992 revision of its guidelines for developmental-toxicity risk assessment state that "human data are preferred for risk assessment" and that the "most relevant information" is provided by good epidemiologic studies. When these data are not available, however, reproductive risk assessment and developmental-agent risk assessment, according to EPA, are based on four key assumptions:

- An agent that causes adverse developmental effects in animals will do so in humans, with sufficient exposure during development, although the types of effects might not be the same in humans as in animals.
- Any significant increase in any of the expressions of developmental toxicants (e.g., death, structural abnormalities, growth alterations, and functional deficits) indicates a likelihood that the agent is a developmental hazard.
- Although the types of effects in humans and animals might not be the same, the use of the most sensitive animal species to estimate human hazards is justified.
- A threshold is assumed in dose-response relationships on the basis of current knowledge, although some experts believe that current science does not fully support this position.

The new guidelines state that "the existence of a NOAEL in an animal study does not prove or disprove the existence or level of a biological threshold." The guidelines also address statistical deficiencies and improvements in the NOAEL-based uncertainty-factor approach (Crump, 1984; Kimmel and Gaylor, 1988; Brown and Erdreich, 1989; Chen and Kodell, 1989; Gaylor, 1989; Kodell et al., 1991a). The guidelines also discuss EPA's plans to move toward a more quantitative "benchmark dose" (BD) for risk assessment for developmental end points "when sufficient data are available"; the BD approach would be consistent with the uncertainty-factor approach now in use (EPA, 1991a). Like the NOAEL and LOAEL, the BD is based on the most sensitive developmental effect observed in the most appropriate or most sensitive mammalian species. It would be derived by modeling the data in the observed range, selecting an incidence rate at a preset low observed response (e.g., 1% or 10%), and determining the corresponding lower confidence limit on dose that would yield that level of excess response. A BD thus calculated would then be divided by uncertainty factors to derive corresponding acceptable intake (e.g., RfD) values (EPA, 1991a). Thus, the traditional uncertainty-factor approach is retained in the 1991 developmental-toxicity guidelines, as well as in the proposed BD approach. However, the new guidelines are unique, in that they emphasize both the possible effect of

interindividual variability in the interpretation of acceptable exposures and the improvements that biologically based models could bring to developmental risk assessment (EPA, 1991a):

> It has generally been assumed that there is a biological threshold for developmental toxicity; however, a threshold for a population of individuals may or may not exist because of other endogenous or exogenous factors that may increase the sensitivity of some individuals in the population. Thus, the addition of a toxicant may result in an increased risk for the population, but not necessarily for all individuals in the population. … Models that are biologically based should provide a more accurate estimation of low-dose risk to humans. … The Agency is currently supporting several major efforts to develop biologically based dose-response models for developmental toxicity risk assessment that include the consideration of threshold.

Cancer

For some toxic effects, notably cancer, there are reasons to believe either that no threshold for dose-response relationships exists or that, if one does exist, it is very low and cannot be reliably identified (OSTP, 1985; NRC, 1986). This approach is taken on the basis not of human experience with chemical-induced cancer, but rather of radiation-induced cancer in humans and radiologic theory of tissue damage. Risk estimation for carcinogens therefore follows a different procedure from that for noncarcinogens: the relationship between cancer incidence and the dose of a chemical observed in an epidemiologic or experimental study is extrapolated to the lower doses at which humans (e.g., neighboring population) might be exposed (e.g., due to emissions from a plant) to predict an excess lifetime risk of cancer—that is, the added risk of cancer resulting from lifetime exposure to that chemical at a particular dose. In this procedure, there is no "safe" dose with a risk of zero (except at zero dose), although at sufficiently low doses the risk becomes very low and is generally regarded as without publichealth significance.

The procedure used by EPA is typical of those used by the other regulatory agencies. The observed relationship between lifetime daily dose and observed tumor incidence is fitted to a mathematical model to predict the incidence at low doses. Several such models are in wide use. The so-called linearized multistage model (LMS) is favored by EPA for this purpose (EPA, 1987a). FDA uses a somewhat different procedure that nevertheless yields a similar result. An important feature of the LMS is that the dose-response curve is linear at low doses, even if it displays nonlinear behavior in the region of observation.

EPA applies a statistical confidence-limit procedure to the linear multistage no-threshold model to generate what is sometimes considered an upper bound on cancer risk. Although the actual risk cannot be known, it is thought that it will not exceed the upper bound, might be lower, and could be zero. The result of a dose-response assessment for a carcinogen is a potency factor. EPA also uses the term *unit risk factor* for cancer potency. This value is the plausible upper bound on excess lifetime risk of cancer per unit of dose. In the absence of strong evidence to the contrary, it is generally assumed that such a potency factor estimated from animal data can be applied to humans to estimate an upper bound on the human cancer risk associated with lifetime exposure to a specified dosage.

The dose-response step involves considerable uncertainty, because the shape of the dose-response curve at low doses is not derived from empirical observation, but must be inferred from theories that predict the shape of the curve at the low doses anticipated for human exposure. The adoption of linear models is based largely on the science-policy choice that calls for caution in the face of scientific uncertainty. Models that yield lower risks, indeed models incorporating a threshold dose, are plausible for many carcinogens, especially chemicals that do not directly interact with DNA and produce genetic alterations. For example, some chemicals, such as chloroform, are thought to produce cancers in laboratory animals as a result of their cell-killing effects and related stimulation of cell division. However, in the absence of compelling mechanistic data to support such models, regulators are reluctant to use them, because of a fear that risk will be understated. For other substances (e.g., vinyl chloride), evidence shows that the human cancer risk at low doses could be substantially higher than would be estimated by the usual procedures from animal data.

Models that yield higher potency estimates at lower doses than the LMS model might also be plausible, but are rarely used (Bailar et al., 1988).

New Trends In Toxicity Assessment

With respect to carcinogenic agents, two types of information are beginning to influence the conduct of risk assessment.

For any given chemical, a multitude of steps can occur between intake and the occurrence of adverse effects. Those events can occur dynamically over an extended period, in some cases decades. One approach to understanding the complex interrelationships is to divide the overall scheme into two pieces, the linkages between exposure and dose and between dose and response. *Pharmacokinetics* has often been used to describe the linkage between exposure (or intake) and dose, and *pharmacodynamics* to describe the linkage between dose and response. Use of the root *pharmaco* (for drug) reflects the origin of those terms. When applied to the study and evaluation of toxic materials, the corresponding terms might more appropriately be *toxicokinetics* and *toxicodynamics.*

Exploration of the use of pharmacokinetic data is especially vigorous. Risk assessors are seeking to understand the quantitative relationships between chemical exposures and target-site doses over a wide range of doses. Because the target-site dose is the ultimate determinant of risk, any nonlinearity in the relationship between administered dose and target-site dose or any quantitative differences in the ratio of the two quantities between humans and test animals could greatly influence the outcome of a risk assessment (which now generally relies on an assumed proportional relationship between administered and target doses). The problem of obtaining adequate pharmacokinetic data in humans is being attacked by the construction of physiologically based pharmacokinetic (PBPK) models, whose forms depend on the physiology of humans and test animals, solubilities of chemicals in various tissues, and relative rates of metabolism (NRC, 1989). Several relatively successful attempts at predicting tissue dose in humans and other species have been made with PBPK modeling, and greater uses of this tool are being encouraged by the regulatory community (NRC, 1987).

A second major trend in risk assessment stems from investigations indicating that some chemicals that increase tumor incidence might do so only indirectly, either by causing first cell-killing and then compensatory cell proliferation or by increasing rates of cell proliferation through mitogenesis. In either case, increasing cell proliferation rates puts cells at increased risk of carcinogenesis from spontaneous mutation. Until a dose of such a carcinogen sufficient to cause the necessary toxicity or intracellular response is reached, no significant risk of cancer can exist. Such carcinogens, or their metabolites, show little or no propensity to damage genes (they are nongenotoxic).

Tables

TABLE 4-1 Categorization of Evidence of Carcinogenicity

Group		Criteria for Classification
A	Human carcinogen	Sufficient evidence from epidemiologic studies
B	Probable human carcinogen (two subgroups)	Limited evidence from epidemiologic studies and sufficient evidence from animal studies (B1); *or* inadequate evidence from epidemiologic studies (or no data) and sufficient evidence from animal studies (B2)
C	Possible human carcinogen	Limited evidence from animal studies and no human data
D	Not classifiable as to human carcinogenicity	Inadequate human and animal data or no data
E	Evidence of noncarcinogenicity in humans	No evidence of carcinogenicity from adequate human and animal studies

SOURCE: Adapted from EPA, 1987a.

TABLE 4-2 Weight-of-Evidence Classification Methods for Noncancer Health Effects

Sufficient Evidence
The sufficient-evidence category includes data that collectively provide enough information to judge whether a human developmental hazard could exist within the context of dose, duration, timing, and route of exposure. This category includes both human and experimental-animal evidence.
Sufficient Human Evidence: This category includes data from epidemiologic studies (e.g., case-control and cohort studies) that provide convincing evidence for the scientific community to judge that a causal relationship is or is not supported. A case series in conjunction with strong supporting evidence may also be used. Supporting animal data might or might not be available.
Sufficient Experimental Animal Evidence or Limited Human Data: This category includes data from experimental-animal studies or limited human data that provide convincing evidence for the scientific community to judge whether the potential for developmental toxicity exists. The minimal evidence necessary to judge that a potential hazard exists generally would be data demonstrating an adverse developmental effect in a single appropriate, well-conducted study in a single experimental-animal species. The minimal evidence needed to judge that a potential hazard does not exist would include data from appropriate, well-conducted laboratory-animal studies in several species (at least two) that evaluated a variety of the potential manifestations of developmental toxicity and showed no developmental effects at doses that were minimally toxic to adults.
Insufficient Evidence
This category includes situations for which there is less than the minimal sufficient evidence necessary for assessing the potential for developmental toxicity, such as when no data are available on developmental toxicity, when the available data are from studies in animals or humans that have a limited design (e.g., small numbers, inappropriate dose selection or exposure information, or other uncontrolled factors), when the data are from a single species reported to have no adverse developmental effects, or when the data are limited to information on structure/activity relationships, short-term tests. pharmacokinetics, or metabolic precursors.

SOURCE: EPA, 1987a.

Bookshelf ID: NBK208246

Aborted Fetal Cell DNA

MRC-5

CCL-171™

MRC-5 is a diploid cell line d in the production of vaccines, including hepatitis A, polio, and MCR-5, and is made up of fibroblasts isolated from the lung tissue derived from a White, male, 14-week-old embryo by J.P. Jacobs in 1966. The cells are capable of 42 to 46 population doublings.

Product category

Human cells

Organism

Homo sapiens, human

Cell type

fibroblast

Morphology

fibroblast

Tissue

Lung

Disease

Normal

Applications

3D cell culture
Bioproduction

Product format

Frozen

Storage conditions

Vapor phase of liquid nitrogen

Required Products

These products are vital for the proper use of this item and have been confirmed as effective in supporting functionality. If you use alternative products, the quality and effectiveness of the item may be affected.

Eagle's Minimum Essential Medium (EMEM)
30-2003

Price: **$24.00** ea

Fetal Bovine Serum (FBS)
30-2020

Price: **$704.00** ea

Dimethylsulfoxide (DMSO)
4-X

Price: **$52.00** ea

WI-38

CCL-75™

WI-38 cell line is the first human diploid cell line to be used in human vaccine preparation. WI-38 cells were isolated from the lung tissue of a 3-month-old, female, embryo. WI-38 is used in viruscide testing.

Product category
Human cells

Organism
Homo sapiens, human

Cell type
fibroblast

Morphology
fibroblast

Tissue
Lung

Disease
Normal

Applications
3D cell culture
Bioproduction

Product format
Frozen

Storage conditions
Vapor phase of liquid nitrogen

Required Products

These products are vital for the proper use of this item and have been confirmed as effective in supporting functionality. If you use alternative products, the quality and effectiveness of the item may be affected.

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Dimethylsulfoxide (DMSO)
4-X

Price: **$52.00** ea

Title: Spontaneous Integration of Human DNA Fragments into Host Genome

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Introduction

A trio of recent publications in the journal NEURON reports the presence of hundreds of diverse de novo gene mutations indicating that autism spectrum disorder (ASD) may be a disease of genomic instability, with a significant environmental component. Altered double strand break formation and repair pathways (DSB) may be a commonality among the diverse genetic mutations that have been documented in ASD. US birth year change points in AD are apparent in 1980, 1988 and 1996, coinciding with the switch to or introduction of childhood vaccines contaminated with human endogenous retrovirus K (HERVK) and human fetal DNA fragments (6). We hypothesize that the HERVK and human fetal DNA contaminants could contribute to the genomic instability of ASD as demonstrated by de novo mutations.

Cell free DNA can be taken up by healthy cells via receptor mediated uptake or may spontaneously penetrate cell membranes that have altered permeability, for instance, during inflammatory reactions. Nuclear uptake of cell free DNA fragments is thought to provide a source for maintenance of DNA integrity during rescue of collapsed replication forks or base lesion repair. Spontaneous extracellular DNA uptake has also been exploited for gene therapy as well as for cellular gene correction (2,4,5,7,8, and 9). While free DNA uptake has been used advantageously, the process has also been associated with generation of mutations and chromosomal aberrations (3).

Vaccines manufactured using human fetal cells contain residual DNA fragments (50-500 bp) (Table I). It is possible that these contaminating fragments could be incorporated into a child's genome and disrupt normal gene function, leading to autistic phenotypes. In this study we demonstrate foreign DNA uptake in human cells and genomic integration by incubating the cells with Cy3-labeled human Cot1 (placental) DNA fragments which represents contaminating residual human fetal DNA in vaccines.

Table 1. Levels of residual human double stranded DNA (Picogreen assay) and human single stranded DNA (Oligreen assay) in Rubella vaccine (MeruvaxII) and Hepatitis A vaccine (HAVRIX).

Vaccine name	Double Stranded DNA (ng/vial)	Single Stranded DNA (ng/vial)	Length (bps)
Meruvax II (Rubella)	142.05	35.00	240
HAVRIX (Hepatitis A)	276.00	35.74	Not measurable

Materials and Methods: Human Cot1 DNA (Invitrogen) was labeled with Mirus *Label* IT Cy™3 *Labeling Kit* (Mirus).

U937 cells (monocytes) were grown in Dublecco's Mofication of Eagle's Medium (DMEM) supplemented with 15% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution at 37°C under a humidified atmosphere containing 5% CO^2/95% air. HL-60 cells (myeloblast) were grown in Iscove's Modified Dulbecco's Medium (IMDM) supplemented with 20% FBS and 1% antibiotic-antimycotic solution at 37°C under the same condition. 750ng of Cy3 labeled Human Cot1 DNA was incubated per1.0×10^7 cells for 24 hours and 48 hours.

Cellular and nuclear DNA uptake was analyzed under fluorescent microscope. Genomic DNA of U937 cells was purified by ethanol precipitation removing short fragment of nucleic acids including unincorporated Cy3 labeled Human Cot1 DNA. The amount of Cy3 labeled human Cot1 DNA incorporated into U937 chromosomes was calculated with relative fluorescent unit (RFU) measured by a fluorimeter.

Loosely adherent NCCIT (teratocarcinoma) cells were grown with a cell density 3×10^4 per well of a 24-well plate which a German glass cover slips was placed in each well at 37°C under a humidified atmosphere containing 5% CO^2/95% air. HFF1 (Human Foreskin Fibroblast 1) cells were grown with the same condition except DMEM supplemented with 15% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution was used as a medium.

Methods and Results

BE (2)-C (neuroblastoma) cells were grown in the same condition except medium used was a 1:1mixture of Eagle's Minimum Essential Medium (EMEM) and F12 Medium supplemented with 10%FBS and 1% antibiotic-antimycotic solution. M059K (Glioblastoma-Double Stranded Break repair proficient) and M059J (Glioblastoma-Double Stranded Break repair deficient) were also grown with the same condition except the medium used was a 1:1 mixture of DMEM and Ham's F12 Medium supplemented with 10% FBS, 0.05mM non-essential amino acids, and 1% antibiotic-antimycotic solution. After cells were cultured in each condition for 2 to 3 days 500ng Cy3 labeled Human Cot1 DNA was added and incubated at 37°C under a humidified atmosphere containing 5% CO^2/95% air by gently shaking for 24 hours and 48 hours. After incubation nucleus was stained with Hoechst, German glass cover slips were placed on glass slides, and cellular and nuclear DNA uptake was analyzed under fluorescent microscope.

To model inflammation, all adherent cell lines were activated with lipopolysaccharide (LPS). And, saponin permeabilization was also tested for HFF1 cells . Three concentrations of LPS, $1ng/10^4$cells, $10ng/10^4$cells, and $100ng/10^4$cells were tested in the wells of each cell line previously mentioned. Cells were incubated with Cy3 labeled Human Cot1 DNA and LPS at 37°C under a humidified atmosphere containing 5% CO^2/95% air by gently shaking for 24 hours and 48 hours. As well as cells incubated without LPS, these cells were also stained with Hoechst before cellular and nuclear DNA uptake was analyzed under fluorescent microscope.

HFF1 cells were incubated with 0.02% saponin , 300ng DAPI, and 500ng Cy3 labeled human Cot 1 DNA for 24 hours, 48 hours, and 72 hours. Cells were viewed under fluorescent microscope as well.

Results (Table 2):

Spontaneous cellular and nuclear DNA uptake was evident in HFF1, NCCIT and U937 (Fig1, 3, 7 and 8). DNA uptake in BE (2)-C and M059K was not measurable because of high auto fluorescence of the cells. No Cy3 signal was observed in HL-60. With inflammation caused by LPS cellular DNA uptake was observed in HFF1, NCCIT, M059J, and U937 (Fig 2, 4, 5 and 6).

The amount of labeled Cy3 human Cot1 DNA incorporation in U937 genomic DNA was 0.0111 +/- 0.0034pg (n=12) per cell in 24 hours, which was approximately 0.167% of total U937 genomic DNA. DNA incorporation in NCCIT cells was 0.0026pg/cell in 24 hours and 0.04pg/cell in 48 hours which is 0.6% of total NCCIT genomic DNA.

Table 2: DNA uptake in Various Cell lines

	Spontaneous Cellular uptake	Spontaneous Nuclear uptake	Incorporation in Genomic DNA	Cellular /Nuclear Uptake with LPS or saponin
HFF1	Yes	Yes	Not Done	Increase/Increase
NCCIT	Yes	Yes (variable)	0.0026pg per cell 24 hrs 0.04pg per cell 48 hrs	Same/Same
BE(2)-C	No	No	Not Done	No/No
M059K	No	No	No	No/No
M059J	No	No	Not Done	Yes/No
U937	Yes	Yes	0.011 +/- 0.003pg per cell 24 hrs	Same/Same
HL60	No	No	No	No

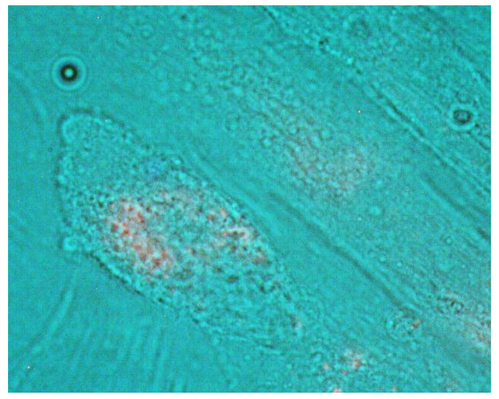

Fig 1. HFF1 spontaneous cellular and nuclear DNA uptake (bright field & Cy3 red combined).

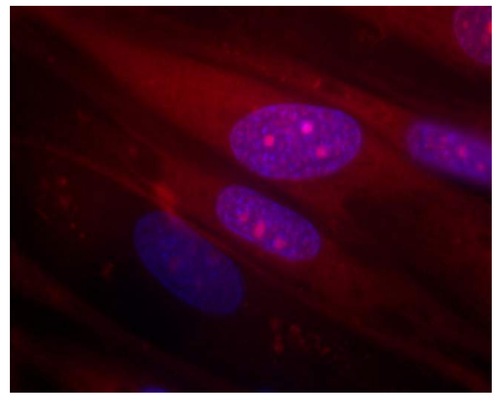

Fig 2. HFF1 cellular and nuclear DNA uptake after permeabilization with saponin. (Cy3 red & nucleus blue combined)

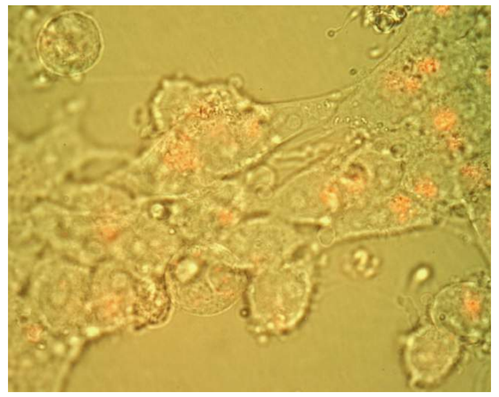

Fig 3. NCCIT spontaneous cellular DNA uptake (bright field & Cy3 red combined)

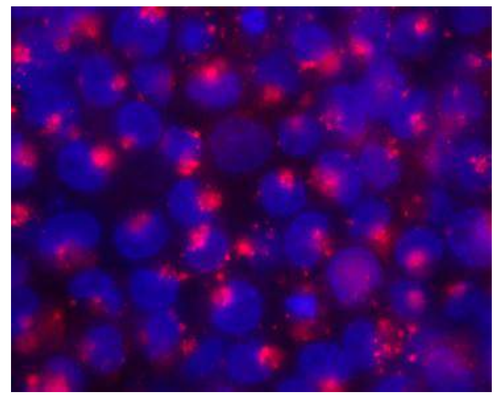

Fig 4. NCCIT cellular DNA uptake after lipopolysaccharide activation (Cy3 red & nucleus blue combined)

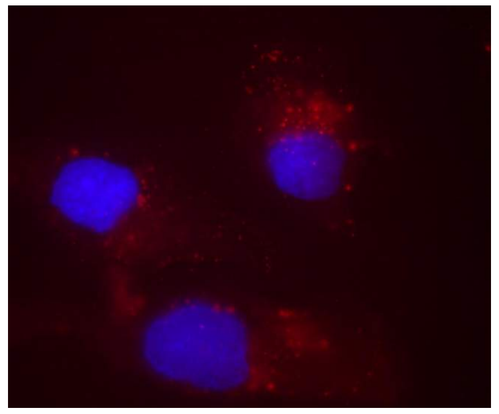

Fig 5. M059J cellular DNA uptake after lipopolysaccharide activation ($10ng/10^4$ cells).
(Cy3 red & nucleus blue combined).

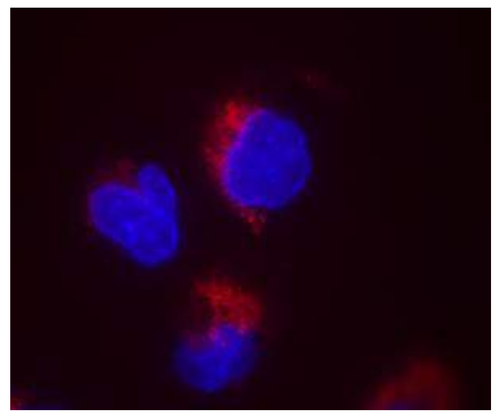

Fig 6. M059J cellular DNA uptake after lipopolysaccharide activation ($100ng/10^4$ cells).
(Cy3 red & nucleus blue combined).

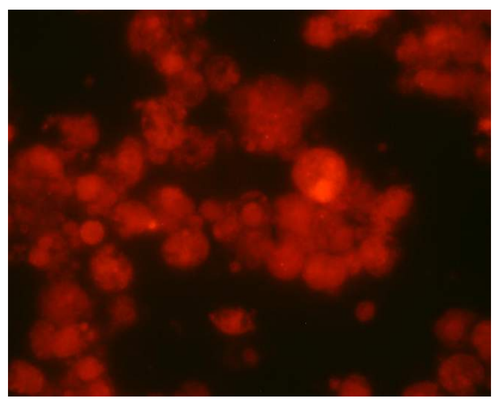

Fig 7. U937 spontaneous cellular/nuclear DNA uptake (Cy3 red)

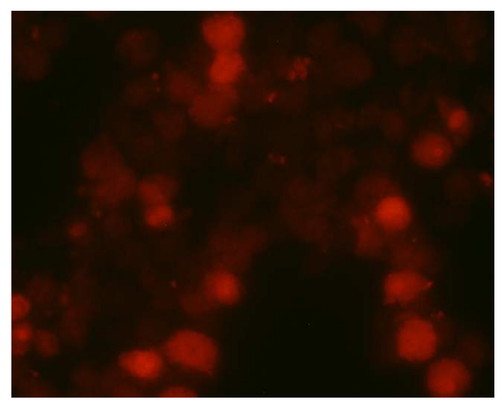

Fig 8. Purified U937 **nuclei** containing Cy3 labeled DNA before DNA purification (Cy3 Red)

Discussion

Our measured genomic incorporation (0.003 to 0.04 pgs) of 0.2% - 0.6% of the whole genome in 24 to 48 hours seems high at first glance. However, our numbers are consistent with previous reports showing that exogenous DNA replaced up to 1% of the whole genome within 30 minutes (6). Although HL-60 cells did not spontaneously take up exogenous DNA in our experiments, the cell line has been used in the past as a model for spontaneous DNA uptake (8).

Cellular and nuclear DNA uptake in human foreskin fibroblast (HFF1) cells and in NCCIT cells suggests that embryonic and neonatal cell are more susceptible to DNA uptake than cells from a more mature source. These results indicate the need for further study of DNA incorporation from exogenous sources to compare the susceptibility of infants and toddlers versus teens and adults.

Increased DNA uptake after LPS activation suggests that systemic inflammation or immune responses could increase susceptibility for exogenous DNA uptake. Human diploid cell produced vaccines are contaminated by exogenous DNA fragments and a retrovirus, and vaccines elicit systemic inflammation and immune activation. Our future research goals are to localize the sites of DNA integration, to demonstrate phenotype changes caused by foreign DNA integration in factor dependent cell lines, and to determine the biological and/or pathological activities of Human Endogenous Retrovirus K (HERVK) fragments in vaccines.

Table3: Cell Description

Cells	Source	Morphology	Transfection host
U937	Histocytic Lymphoma	Monocyte	Yes
HL60	Leukemia	Myeloblast	Yes
BE(2)C	Neuroblastoma	Neuroblast	No
M059K	Glioblastoma	Fibroblast	No
M059J	Glioblastoma	Fibroblast	No
HFF1	Foreskin	Fibroblast	No

Conclusion

Not only damaged human cells, but also healthy human cells can take up foreign DNA spontaneously. Foreign human DNA taken up by human cells will be transported into nuclei and be integrated into host genome, which will cause phenotype change. Hence, residual human fetal DNA fragments in vaccine can be one of causes of autism spectrum disorder in children through vaccination. Vaccine must be safe without any human DNA contaminations or reactivated viruses, and must be produced in ethically approved manufacturing processes.

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RESEARCH PAPER

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Characteristics and viral propagation properties of a new human diploid cell line, walvax-2, and its suitability as a candidate cell substrate for vaccine production

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Keywords: biological characteristics, cell substrate, human diploid cell strain (HDCSs), human diploid cell vaccines (HDCVs), viral sensitivities

Abbreviations: ATCC, American Type Culture Collection; CCID50, 50% cell culture infectious dose; CCTCC, China Center for Type Culture Collection; CPE, cytopathogenic effect; ELISA, enzyme-linked immuno sorbent Assay; FFU, fluorescent focus units; G6PD, glucose 6 phosphate dehydrogenase; GM, growth medium; HAV, hepatitis A virus; HDCSs, human diploid cell strains; HDCV, human diploid cell vaccine; LD, lactate dehydrogenase; MCB, master cell bank; MDCK, Madin–Darby canine kidney; MOI, multiplicity of infection; NIFDC, National Institute for Food and Drug Control; PAGE, polyacrylamide gelelectrophoresis; PCB, primary cell bank; PFU, plaque forming units; PPLO; pleuropneumonia-Like organisms; STR, Short tandem repeats; VZV, varicella zoster virus; WCB, Working cell bank

Human diploid cell strains (HDCSs), possessing identical chromosome sets known to be free of all known adventitious agents, are of great use in developing human vaccines. However it is extremely difficult to obtain qualified HDCSs that can satisfy the requirements for the mass production of vaccines. We have developed a new HDCS, Walvax-2, which we derived from the lung tissue of a 3-month-old fetus. We established primary, master and working cell banks successfully from reconstituted frozen cells. Observations during the concurrent propagation of Walvax-2 and MRC-5 cells revealed differences in terms of growth rate, cell viability and viral sensitivities. Specifically, Walvax-2 cells replicated more rapidly than MRC-5 cells, with Walvax-2 cells attaining the same degree of confluence in 48 hours as was reached by MRC-5 cells in 72 hours. Moreover, Walvax-2 cells attained 58 passages of cell doublings whereas MRC-5 reached 48 passages during this period. We also assessed the susceptibility of these cells to rabies, hepatitis A, and Varicella viruses. Analysis of virus titers showed the Walvax-2 cells to be equal or superior to MRC-5 cells for cultivating these viruses. Furthermore, in order to characterize the Walvax-2 cell banks, a series of tests including cell identification, chromosomal characterization, tumorigenicity, as well as tests for the presence of microbial agents, exogenous viruses, and retroviruses, were conducted according to standard international protocols. In conclusion, results from this study show that Walvax-2 cell banks are a promising cell substrate and could potentially be used for the manufacturing of HDCVs.

Introduction

The replication of viruses occurs only when the virus enters into host cells, often resulting in diseases that are difficult to treat. Currently, there are no widely accepted therapeutics available to treat such diseases, therefore prophylactic vaccines play an imperative role in the fight against viral diseases. Antibodies produced for most kinds of viral diseases when the immune system is stimulated by intact viral particles,.[1,2] Owing to this property, the vast majority of viral vaccines still adopt the traditional cell substrate culture method. Three cell substrates, human diploid cells, continuous cell lines and primary cell lines, are always used for developing vaccines.[3] However, continuous and primary cell lines used for vaccine production suffer from the limitation of

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being potentially strongly tumorigenic. Four Additionally the primary cell lines, which are obtained from animals, introduce potentially risky exogenous agents.[4] In contrast, human diploid cell strains (HDCSs), acquired from embryos or other tissue cells of human origin, possess identical chromosome sets that are free of all known adventitious agents.[5] These unique properties explain the value of such materials and the current interest in their use in the development of human viral vaccines.[6,7,8] Human diploid cell vaccines (HDCVs) have been licensed all over the world. Many studies have demonstrated superior immunogenicity and safety of HDCVs relative to those using any other tissue culture, such as hamster kidney cells or vero cell vaccines.[9] The WHO recommends HDCS as the safest cell culture substrate for the production of viral vaccines[10] and consequently they have become the preferred cell substrate for vaccine production worldwide.

Hayflick in 1961[8] and Jacobs in 1967[7] developed the 2 most well known HDCSs, Wistar Institute (WI)-38 and Medical Research Council (MRC)-5, respectively, that currently serve as international standardized cell strains. Since then, there has been continuing interest in the development of HDCSs. Eleven,[12] However, it is extremely hard to obtain human fetal tissue from which to derive qualified human diploid cell strains. This is due to issues that include the requirement for strict ethical review, the possibility of environmental degradation, and food safety hazards, all of which may lead to chromosomal aberrations such as the presence of aneuploidy and polyploidy for the karyotype.[13] Most importantly, strict requirements regarding the methods for obtaining suitable tissues from which to derive HDCS via abortion render the acquisition of appropriate material difficulty. Even if a new HDCS is derived successfully, it might not satisfy requirements for industrial production due to its inability to sustain multiple passages, the IMR-9 cell line being an example.[14,15] Due to the diminishing supply of WI-38[10] cells, the MRC-5 line has become the most widely used cell strain in the production of HDCS-derived human vaccines. China consequently confronts 2 key challenges for the production of viral vaccines from MRC-5 cells (which are mainly obtained from abroad): concerns about influences of limited passages, and the policies of the countries from which the cells are imported. More specifically, the numbers of passages of the imported MRC-5 cells are generally higher, generally later than the 20th passage, resulting in restricted mass production due to decreased growth vitality. Additionally, according to the standard for the Pharmacopoeia of the People's Republic of China (2010), Volume III, the use of the HDCSs is limited to generations within 2-thirds of the primary cell lifespan for the manufacture of vaccines. Due to the scarce HDCSs resources, the research and production of viral HDCVs in China are substantially restricted. For example, human diploid cell rabies vaccine, which is considered to be the gold standard for rabies vaccines, is not currently available in China.[16] Furthermore, the productive cell generations for the OKA-HDC on the Chinese market from 3 manufactures are MRC-5 cells in the 32nd and 33rd passages,[17] which have therefore already reached the limit required as described above in Chinese Pharmacopeia (the 33rd passage is the last cell doubling that could be used in the production for the MRC-5). Relying on imported HDCSs, may lead to unstable supply as well as unpredictable costs. Therefore the intention of this study is to develop a completely new HDCS of Chinese origin that could be used in manufacturing viral vaccines.

This study, therefore, aims to (i) establish and characterize Walvax-2, a totally new HDCS; and (ii) evaluate the susceptibility to 3 kinds of viral vaccine strains, namely the CTN-V/PV strain of rabies, the YN-5 strain of hepatitis A, and the Oka strain of Varicella virus in Walvax-2, thereby preparing for the industrial development of HDCVs in China.

Results

Source tissue material

We obtained 9 fetuses through rigorous screening based on carefully specified inclusion criteria (see Methods section). The Walvax-2 strain of cells met all of these criteria and proved to be the best cell line following careful evaluation. Therefore it was used for establishing a human diploid cell strain. Walvax-2 was derived from a fetal lung tissue, similar to WI-38 and MRC-5, and was obtained from a 3-month old female fetus aborted because of the presence of a uterine scar from a previous caesarean birth by a 27-year old healthy woman.

Primary cell stock and cell bank system

After incubation for 48 h, a confluent cell monolayer formed and then increased in density over the following 48 hours. After a series of successful cultures, these cells were specified as the primary cell seeds of the Walvax-2 human diploid cell line. Thereafter, a 3-tiered cell bank consisting of pre-master cell bank (PCB，P6), master cell bank (MCB, P14), and working cell bank (WCB, P20) was established.

Figure 1 shows a gradual growing procedure for cells after propagation. Initially, round cells with clear and relatively dark edges were observed; as time went by, the cells elongated to become spindle shaped and translucent fibroblasts (**Fig. 1A**). Over a period of 24 hours during which the cells divided and proliferated, the cells grew into flame shaped, typical plump diploid fibroblasts with good refractive properties, and rearranged into highly polarized areas with curling patterns (**Fig. 1B**). Finally, the cells formed a dense confluent monolayer after 48 hours (**Fig. 1C**).

Walvax-2 cells maintained excellent capabilities for growth and proliferation until the 50th passage, after which these abilities degraded. At passage 58, cells exhibited blurred edges and could not form a confluent monolayer after being cultured for 72 h. Also noted were increasing black spots in cells, as well as dead cells in the media. (**Fig. 1D**). Cell death was eventually observed after 20 d

Cryopreservation stability and recovery viability

The Walvax-2 3-tiered cell bank, composed of PCB (P6), MCB (P14), and WCB (P20), exhibited a homogeneous growth

pattern and attained identical population doublings (58) when compared with the primary cell seed. All the cells restored from frozen stock reached adherent growth in 2–8 h and formed a confluent monolayer in 24 h, with the percentage of viable cells in the range of 80–90% (**Fig. 2**). Each of the curves in **Figure 2** demonstrates the growth features for the Walvax-2 primary cell seed as well as the cell banks. Generally speaking, the typical diploid cell with limited replicative lifespan follows a "slow-logarithmic-slow" model. However, Walvax-2 cells show strong cell proliferation, with the missing "slow" pattern at the beginning for each curve, until the 50th passage, after which the viability of the cells decreases dramatically. Furthermore, comparative cell doubling times are summarized in **Table 1**. The results confirm that the Walvax-2 cells reconstituted from the frozen state do not alter their stability and viability, and could potentially be used as a cell substrate due to these crucial properties.

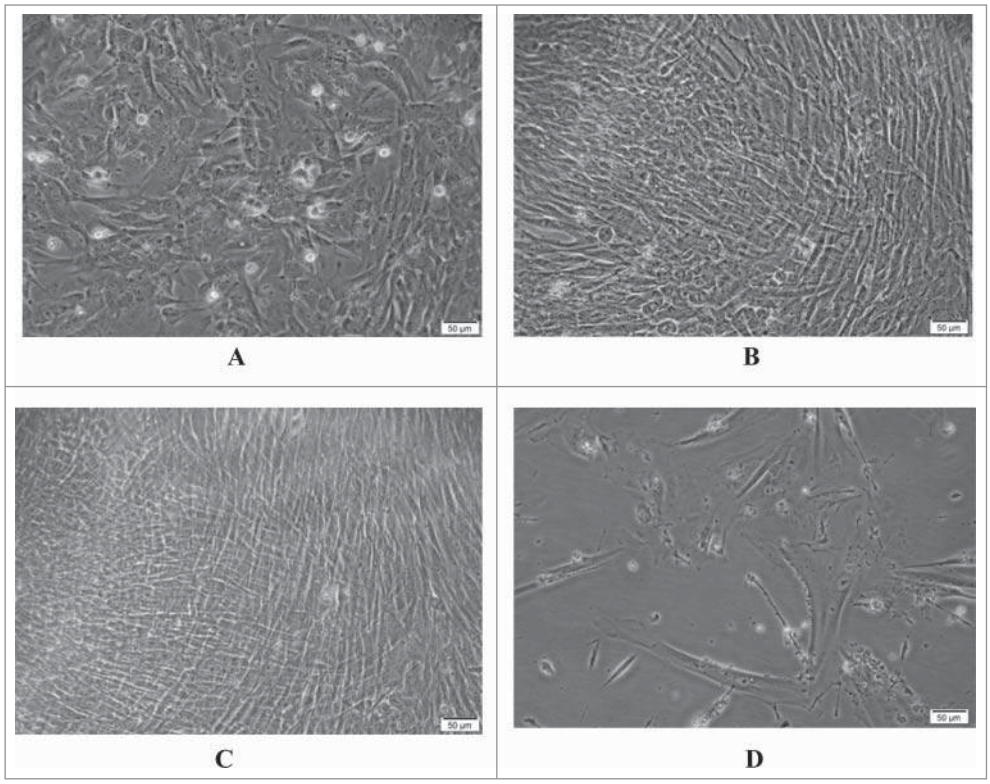

Figure 1. Morphology of the Walvax-2 cells. The cells were cultured and incubated at 37 °C. The photos were taken at 4 h (**A**), 24 h (**B**) and 48 h (**C**) and at 72 h post-subculture for the 58th passage (**D**).

Cell identification

As shown in **Figure 3**, the isozyme patterns of the Walvax-2 cells, using LD and G6PD as indicators, are identical to those of human diploid cells (MRC-5) and the human cervical cancer cell line (Hela) preserved in China Center for Type Culture Collection (CCTCC), whereas the mouse fibroblast cell line (L929) exhibits entirely different results. These findings confirm the fact that the Walvax-2 cell banks behave in a manner similar to other human-derived cell lines.

STR profiles of 16 DNA fragments of gene locus for Walvax-2 cells are shown in **Figure 4**, from which we see that they match the targeted alleles as expected. In **Table 2** the data from this study are compared with those of STR databases in ATCC of USA and DSMZ of Germany. Three qualified laboratories, CCTCC, NIFDC (National Institutes for Food and Drug Control) and Law School of Kunming Medical University, draw the

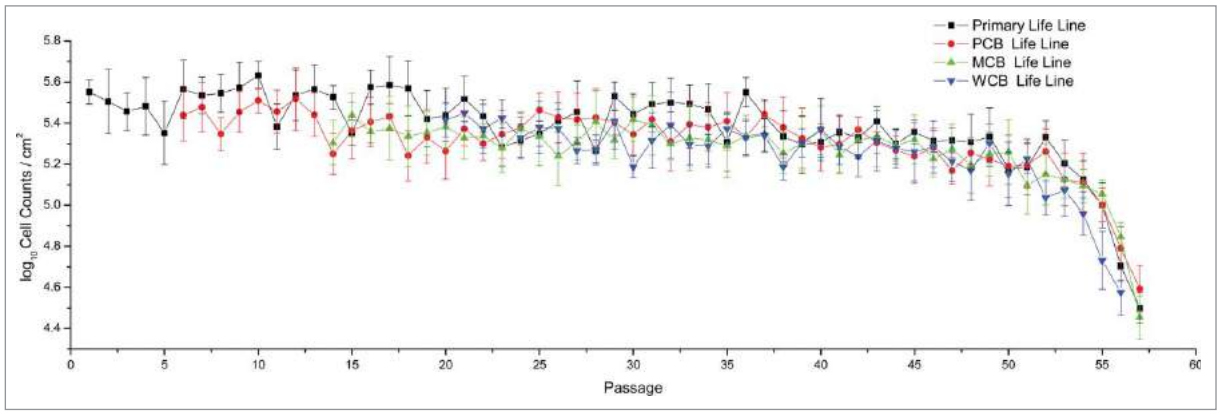

Figure 2. The growth patterns of Walvax-2 cell banks. Primary cells were isolated from fetal lung tissue, frozen at the 6th, 14th and 20th passages, and then recovered and subcultured continuously until cell senescence occurred.

Table 1. Population doubling times of the Walvax-2 cells with and without being subjected to freezing

Passage number	Without being subjected to freezing	Reconstituted from the frozen state	
	Population doubling time(h)	Cell origin	Population doubling time(h)
P 10	18–20	PCB,P6	18–20
P 20	29–31	MCB, P14	30–32
P25	30–32	WCB, P20	30–32
P32	38–40	The 28th passage from the WCB	39–41
P43	39–41	The 38th passage from the WCB	40–42
P55	55–60	The 48th passage from the WCB	57–62

same conclusions that the Walvax-2 cell line displays its own specific DNA profile of human individual origin distinct from the MRC-5 and the Hela cell lines.

Chromosomal characterization

The chromosomal characterization for PCB (P6), MCB (P14), WCB (P20) from the 38th passage, which is the last passage that could be used for producing viral vaccines according to the requirements of Chinese Pharmacopeia, are illustrated in **Figure 5**. They show clearly that the Walvax-2 cells are 46/XX, typical diploid type of human origin. The chromosomal analysis of the Walvax-2 cells as summarized in **Table 3** demonstrate that the karyological properties of Walvax-2 cells satisfy the requirements of diploid cells of human origin to be used for producing viral vaccines, with the frequencies of abnormalities being considerably lower than the corresponding national standards.

Microbial agent tests

No cultivable bacteria or fungi were found in broth and agar cultures. Mycoplasma tests using both the culture method and DNA staining technique, also met the corresponding requirements.

Exogenous virus agent tests

Results for the testing of general (non-specific) as well as specific adventitious viral agents were negative for all tested viruses as described in detail in the "materials and methods" section.

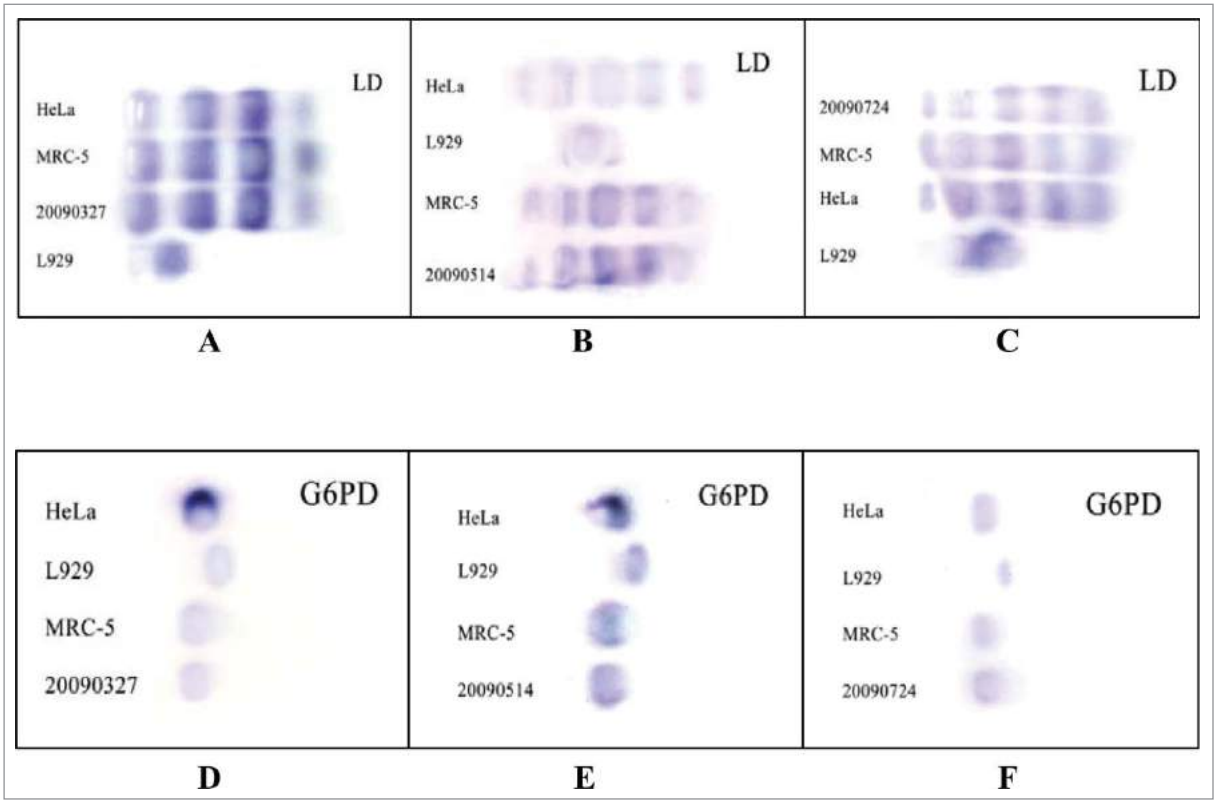

Figure 3. Isoenzyme tests for the Walvax-2 cells. Firstly, LD and G6PD, 2 isoenzymes used as indicators, were isolated from HeLa, L929, MRC-5 and Walvax-2 cells, and then subjected to PAGE and stained. The numbers of 20090327, 20090514 and 20090724 illustrated in the pictures represent Walvax-2 cells for the 18th, 30th and 50th passages.

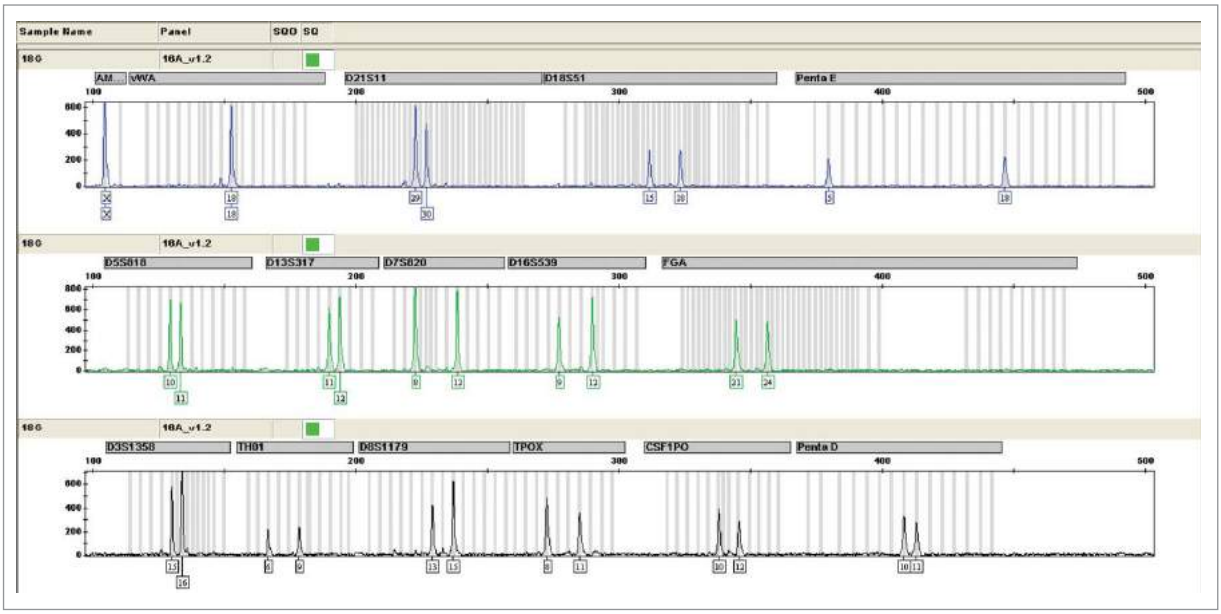

Figure 4. The Short Tandem Repeat （STR） map of Walvax-2 cells for the 18th passage. According to the instructions supplied with the Goldeneye 16A identification kit (people spot), the DNA of Walvax-2 cells at the 18th passage were isolated and amplified by multiplex PCR with primers of 16 STR sites. Then the STR map was obtained by analyzing the samples of PCR by capillary electrophoresis (CE). The STR maps of Walvax-2 cells at the 30th and 50th passages (not shown) were the same as shown.

Retrovirus test

The results in **Figure 6** make it clear that no retroviruses were found in the Walvax-2 cells, as well as the system control cells of MRC-5. However obvious retroviruses were found in the Sp2/0-Ag14 cells of the positive control group, seen as tiny black dots in the figure.

Tumorigenicity test

Tumorigenicity tests were conducted at 2 points following the inoculation of cells into the nude mice, 21 and 84 d All mice survived in all study groups. During the animal tests, no pathological abnormalities of nodule growths were found in the experimental as well as the parallel negative control group (MRC-5), and for both groups there was no pathological heterogeneous cell growth observed at the inoculating site or other sites including heart, liver, spleen, lung, kidney, brain and mesenteric lymph nodes after autopsy. In contrast, nodule and heterogeneous cell growth were easily found in the inoculating site in the positive control group (Hela). These results show that the Walvax-2 cells can be used for the production of vaccines with little risk of potential carcinogenesis.

Susceptibility to viruse tests

Infectivity titers of the CTN-1V strain for the rabies virus are presented in **Table 4**. CTN-1V virus was well adapted in Walvax-2 relative to MRC-5. Maximum infectivity titers of CTN-1V virus for Walvax-2 and MRC-5 were 8.14 and 7.41 FFU/ml, respectively. During the period for virus propagation, the titers in Walvax-2 cells were consistently higher than those of MRC-5 cells, although the differences were not always statistically significant. However, analysis of the overall situation of the adaptation of the CTN-1V in Walvax-2 cells relative to MRC-5 cells yielded a significant difference ($P < 0.001$) by a 2-tailed t-test. Similarly, the results for the PV strain adaptation in both human diploid cells demonstrated a consistent trend, which exhibited distinct differences for the titers.

Table 2. The STR mapping of the Walvax-2 cells

gene locus	Walvax-2	MRC-5*	HeLa*	gene locus	Walvax-2	MRC-5*	HeLa*
Amelogenin	X	X,Y	X	D16S539	09,12	9,11	9,10
vWA	18	15	16,18	FGA	21,24	—	—
D21S11	29,30	—	—	D3S1358	15,16	—	—
D18S51	15,18	—	—	THO1	06,09	8	7
PentaE	05,18	—	—	D8S1179	13,15	—	—
D5S818	10,11	11,12	11,12	TPOX	08,11	8	8,12
D13S317	11,12	11,14	12.13.3	CSF1PO	10,12	11,12	9,10
D7S820	08,12	10,11	8,12	PentaD	10,11	—	—

*Data from ATCC and DSMZ

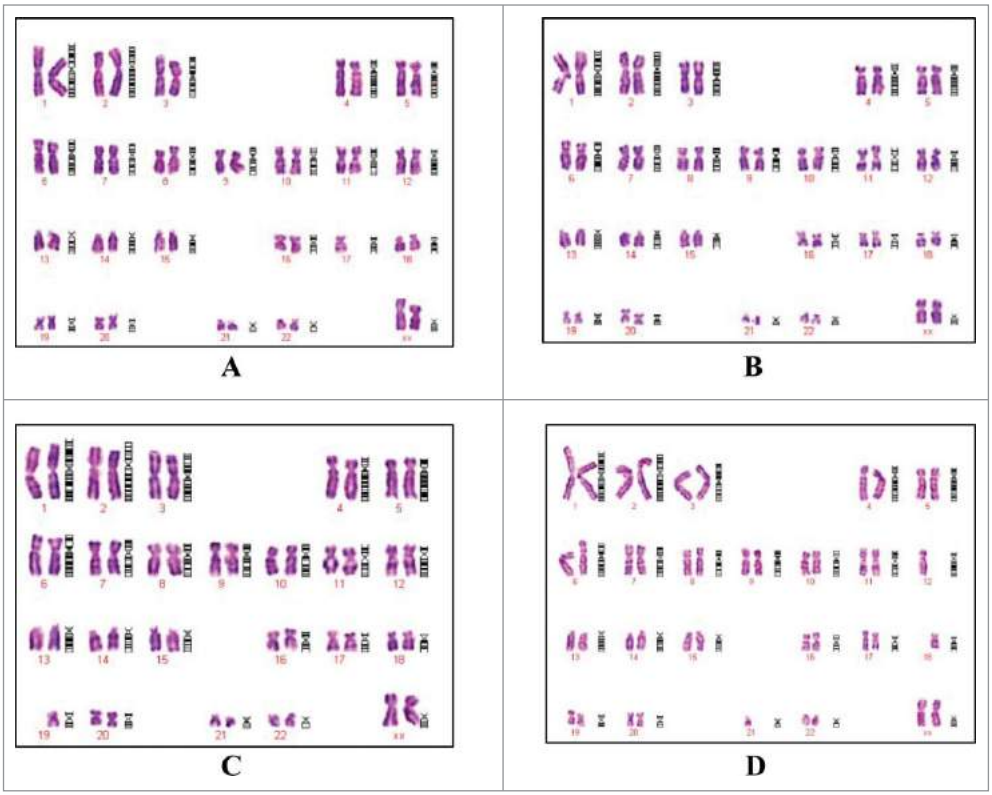

Figure 5. Chromosomes from Walvax-2 cell banks. Walvax-2 cells were incubated 1 day post-subculture, after which the colcemid and then Giemsa banded karyotype analyses were carried out. Pictures were the karyotype of Walvax-2 cells at the 6th (**A**), 14th (**B**), 20th (**C**) and 38th (**D**) passages

Data for the VZV virus propagating in Walvax-2 and MRC-5 cells is given in **Table 5**. In Walvax-2 cells the virus titer grew rapidly to 6.28 log PFU/ml, and reached a peak of 6.59 log PFU/ml at passage 41. In contrast, the virus titers in MRC-5 were much lower, with the overall numbers less than 6.0 log PFU/ml. All comparisons except that of the earliest generation were all statistically significant, indicating strong adaptation of the VZV strain for Walvax-2 cells relative to MRC-5 cells.

The comparative results for YN5 adaptability are listed in **Table 6**. The titer after one generation in Walvax-2 reached 7.32 log CCID50/ml, even higher than the value in the original cells (passage 23). During the course of 8 passages propagated continuously in the Walvax-2 cells, the infectious virus titers increased from 7.32 to 7.65 log CCID50/ml, which was marginally higher than those of MRC-5 cells (7.0 to 7.36 log CCID50/ml).

Discussion

HDCS, deemed as the safest cell substrate, play a vital role in the production of viral human vaccines. However, it is extremely hard to obtain qualified HDCSs that meet the requirements for mass production. It took us 4 y to successfully establish Walvax-2 cell lines and a 3-tiered cell bank, namely PCB, MCB and WCB. Complete records for the cell bank establishment, cell culture conditions, and tests are available. The criteria used for characterizing the Walvax-2 cell banks are those recommended internationally[18,19] and concurrent titrations were set up using MRC-5 cells (the most widely used human diploid cell substrate as a parallel control. Walvax-2 cells have received qualification test reports from the NIFDC and CCTCC, an important step in their use for the production of human viral vaccines in China. Given that the availability of HDCSs, and therefore the production of HDCVs, is currently subject to external forces, the development of an HDCS of Chinese origin has great implications for improving the stability of the supply of HDCVs in China.

Walvax-2 cells displayed a fibroblastic morphology similar to that of MRC-5 cells. However, observations during the concurrent propagation of Walvax-2 and MRC-5 cells revealed differences in terms of growth rates and cell viability. The Walvax-2 cells replicated more rapidly than MRC-5 –they attained the same degree of confluence in 48 hours as was reached by MRC-5 in 72 hours, and the results are in line with measured cell doubling times as listed in **Table 1**. After freezing and recovering, the growth characteristics and patterns of the 3 life lines (PCB, MCB, and WCB) are similar to those of the primary life line, and attained 58 passages of cell doublings whereas MRC-5 reached 48 passages, with the difference decreasing gradually

Table 3. The accumulated results of chromosomal analysis of Walvax-2 cells

Passage	Structural abnormalities	Aneuploidy	Polyploidy	Hyperdiploidy	Breaks or gaps
Standard*	≤2 %	≤18 %	≤4 %	≤2 %	≤8 %
10—19	0/3500	265/3500 (7.57%)	1/3500 (0.03%)	22/3500 (0.63%)	0/3500
20—29	0/6000	538/6000 (8.97%)	3/6000 (0.05%)	49/6000 (0.82%)	1/6000 (0.17%)
30—39	0/4500	423/4500 (9.4%)	1/4500 (0.02%)	40/4500 (0.89%)	1/4500 (0.02%)
40—50	0/9000	945/9000 (10.5%)	7/9000 (0.08%)	113/9000 (1.26%)	7/9000 (0.08%)

*Chinese pharmacopeia, volume III, 2010 edition

with increasing hours of freezing.[8] In conclusion, these results may indicate that Walvax-2 is a cell line with superior characteristic of high growth ability, as well as strong viability compared to MRC-5. It could be used as a host for the cultivation and inoculation of viruses, although different schedules for inoculation and propagation should be further studied based on the growth characteristics of particular viruses. Furthermore, the stability of the karyotype is another crucial issue when using the HDCS in the manufacture of vaccines. The results for karyological data on Walvax-2 cells, as summarized in **Table 3**, demonstrate increases of aneuploidy and hyperdiploidy with age. However, this is not a concern on the grounds that the 2 "middle groups," which are directly related to those to be used in the manufacture of vaccines according to the requirements of Chinese Pharmacopeia, have frequencies of aneuploidy and hyperdiploidy of 9.4% and 0.89% respectively, which are substantially lower than the national standards of 18% and 2%, respectively.

The susceptibility of the human fetal cell strain MRC-5 to viruses infectious in man has

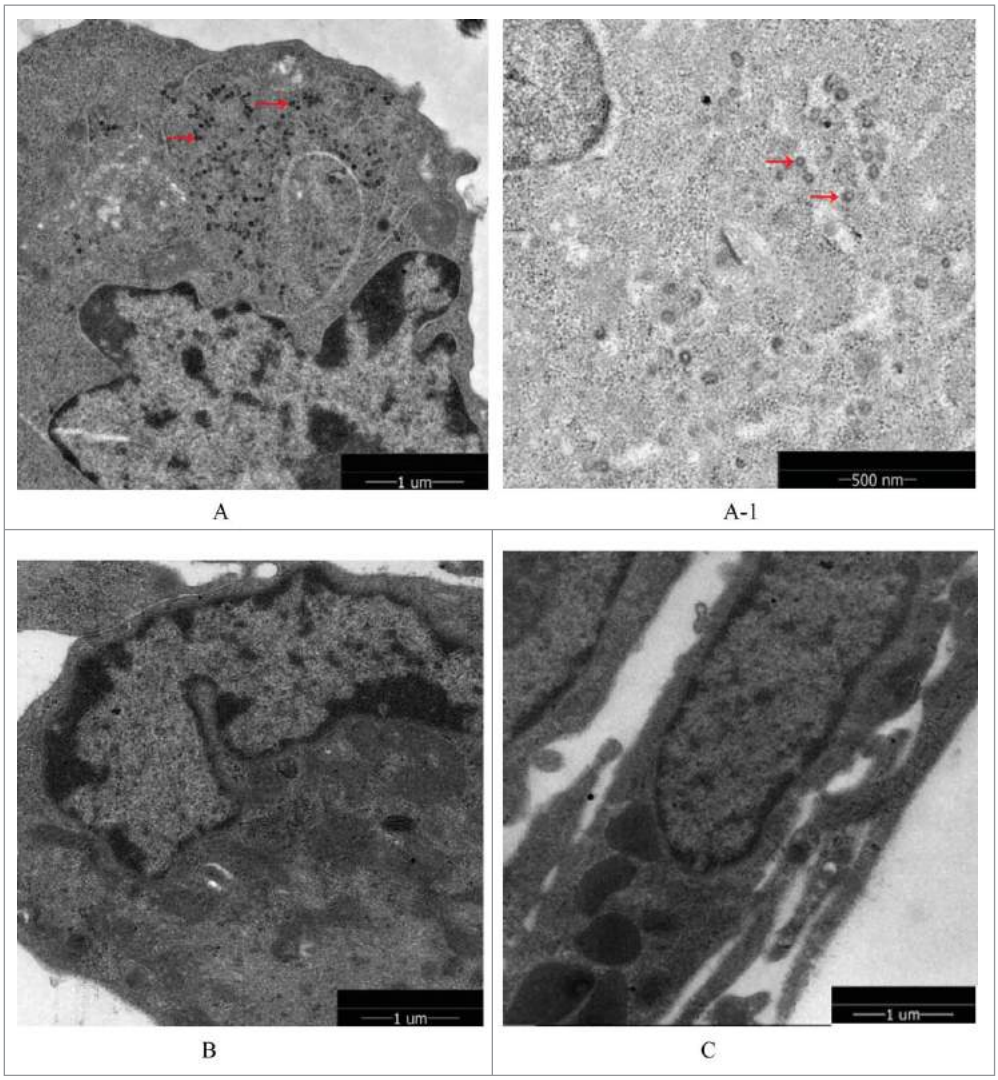

Figure 6. Retrovirus tests of Walvax-2. The results were observed by mirror electron microscopy(200Kv 5000x/160Kv 7800x). The arrows point to virus particles detected as shown in the picture. (**A**) and (**A-1**) were represented positive controls (Sp2/0-Ag14), (**A-1**) was partial enlarged detail of (**A**). (**B**) was represented negative control (MRC-5). (**C**) was represented the cells of Walvax-2 of the 24th passage.

Table 4. Propagation of CTN-1V or PM virus in the Walvax-2 or MRC-5 cells

Virus Passage NO.	CTN-1V virus (log FFU/ml)[a]			PVvirus (log FFU/ml)[a]		
	Walvax-2 cells	MRC-5 cells	P[b]	Walvax-2 cells	MRC-5 cells	P[b]
original	7.50	7.50	/	7.50	7.50	/
1	4.84 ± 0.62	4.58 ± 0.40	>0.05	4.40 ± 0.27	3.62 ± 0.23	>0.05
2	5.40 ± 0.21	5.02 ±0.34	<0.05	5.04 ±0.18	4.75 ± 0.24	<0.05
3	6.10 ± 0.37	5.41 ± 0.24	<0.05	5.30 ± 0.33	4.83 ± 0.25	<0.05
4	6.530.31	6.09 ± 0.17	<0.05	5.86 ± 0.10	5.02 ± 0.13	<0.05
5	6.78 ± 0.40	6.14 ± 0.16	<0.05	6.21 ± 0.21	5.63 ± 0.05	<0.05
6	7.08 ± 0.15	6.57 ± 0.42	>0.05	6.57 ± 0.53	6.02 ± 0.18	>0.05
7	7.34 ± 0.22	6.89 ± 0.21	<0.05	7.01 ± 0.70	6.00 ± 0.23	>0.05
8	7.51 ± 0.21	7.16 ± 0.08	>0.05	6.93 ± 0.19	6.28 ± 0.25	<0.05
9	7.67 ± 0.18	7.09 ± 0.10	<0.05	7.23 ± 0.23	6.59 ± 0.26	<0.05
10	8.14 ± 0.31	7.41 ± 0.35	<0.05	8.02 ± 0.19	7.11 ± 0.38	<0.05

Passages the 1th to 4th, subculture; Passages the 5th to 8th, cell-mixing; Passages the 9th to 10th, cell-free medium;
[a] ±SD.
[b] Significance of difference (P value) determined by 2-tailed t-test

Table 5. Propagation of VZV strain in the Walvax-2 or MRC-5 cells

Virus Passage No.	Virus Titer in Walvax-2 cell (log PFU/ml)[a]	Virus Titer in MRC-5 cell(log PFU/ml) [a]	P[b]
31(original)	5.0	5.0	
33	6.28 ± 0.28	5.42 ± 0.19	>0.05
35	6.13 ± 0.12	5.56 ± 0.11	<0.05
37	6.31 ± 0.28	5.52 ± 0.08	<0.05
39	6.27 ± 0.14	5.58 ± 0.12	<0.05
41	6.59 ± 0.06	5.74 ± 0.13	<0.05

[a] ±SD.
[b] Significance of difference (P value) determined by 2-tailed t-test

been well demonstrated over the past 10 years, indicating the value of such material for the isolation of viruses and the development of vaccines. In this study, the Walvax-2 cell line served as a host for the cultivation of the CTN-V/PV strain of rabies, the YN-5 strain of hepatitis A, the Oka strain of Varicella virus, with results that demonstrate good sensitivity to these viruses. Compared to the MRC-5 cells, titers for viruses in the Walvax-2 cells are higher, with the overall numbers achieving statistical significance. These discrepancies elucidate that Walvax-2, as a new human diploid cell line, is equal or superior to MRC-5 for the propagation of viruses. Generally speaking, as the cell passage number increases the viral titers will experience an initial decrease, and then increase gradually as the cell substrate adapts to the virus. This trend is observed for the propagation of rabies virus in our study. However, the results are not the same for the propagation of VZV and HAV strains in HDCSs, which exhibit increased titers after only one generation. To the best of our knowledge, this may be attributed to the fact that these 2 virus strains are quite sensitive to HDCSs, and particularly to the Walvax-2 cells. Alternatively, the higher titers for Walvax-2 may relate to the characteristics of high growth ability as well as strong viability compared with MRC-5, as described in the "Results" section. Nevertheless, more research needs to be done to investigate the susceptibility of Walvax-2 cells to a greater variety of viruses, and to develop fully the potential of Walvax-2 cells as a cell substrate platform for producing viral vaccines for human use in China.

Table 6. The titers of HAV (YN5) adapted in human diploid cells

Virus Passage NO.	Infectivity titer in Walvax-2 cells (log $CCID_{50}$/ml)[a]	Infectivity titer in MRC-5 cells (log $CCID_{50}$/ml) [a]	P[b]
23(original)	7.0	7.0	
24	7.32 ± 0.28	6.27 ± 0.27	<0.05
25	7.47 ± 0.09	7.01 ± 0.23	>0.05
26	7.50 ±0.17	7.35 ± 0.14	>0.05
27	7.62 ± 0.06	7.18 ± 0.38	>0.05
28	7.97 ± 0.09	7.50 ± 0.23	>0.05
29	8.21 ± 0.29	7.54 ± 0.24	<0.05
30	7.81 ± 0.17	7.35 ± 0.14	<0.05
31	7.65 ± 0.14	7.36 ± 0.34	>0.05

[a] ±SD.
[b] Significance of difference (P value) determined by 2-tailed t-test

The sensitivity to rabies virus of Walvax-2 has important implications for China. Human diploid cell rabies vaccine, which is free of complications but is highly immunogenic,[20] is considered to be the gold standard for rabies vaccine.[16] According to the report by the WHO, there are roughly 55000 human deaths caused by rabies annually.[21] Following India, China ranks in second place for the highest number of human cases in the world.[22] However, there is no such gold standard rabies vaccine on the Chinese market, where the disease burden is remarkably high. Possible reasons are as follows: the vaccine, regarded as liquid gold by the general public, represents a financial burden and hence has lower usage in developing countries. To minimize costs as well as make it affordable for Indians, the Serum Institute of India indigenously developed Ravivax (Pitman-Moore strain, MRC-5), decreasing the cost for the vaccine dramatically (from US $40 dropped to $7).[20] This is also one of the motivations for this study, to develop a totally new HDCS that could be used as a culture medium in manufacturing viral vaccines in China. Recently, a document from the Chinese pharmacopeia commission indicates that the current 2 kinds of cell substrate rabies vaccine presently on the international market, PVRV and PHK, may not be included in the updated Chinese pharmacopeia (2015).[23] The explanations for the removed vaccines are that they will no longer be manufactured or will be replaced by others. Human diploid cell rabies vaccine is gaining increased national attention in China. We tested the susceptibility of 2 rabies strains concurrently in our study, CTN-V and PV. We found that the titers of CTN-1V strain are higher than those of PV strain, independent of the effects of adaptation by the cell substrates. Both strains have been used for production in China over the years, and the safety and immunogenicity of the vaccines have been verified.[24] Consequently, considering the impact on future production, CTN-1V will be the preferred rabies strain for research and production in the future. Although we have reported results of the susceptibility of 3 viruses in this study, we prepared rabies vaccines using the preferred CTN-1V-HDC (Walvax-2) viral strain (15^{th} passage) and determined the potency to be higher than 6.0IU/dose, which was significantly greater than the WHO-recommended standard of 2.5 IU/ dose[16] (described in detail in another study[25]). The efficacies of the diploid rabies vaccines on animal tests would further confirm the use of the Walvax-2 cells in human viral vaccine production.

There are several limitations to this study. More work is required regarding the adaptation of a greater variety of viruses on the Walvax-2 cells and the possibility for the industrial development of appropriate vaccines. In recent years, a large number of research papers have reported the application of Gene chip technology and high-throughput sequencing PCR technology for detecting potential contaminations of viruses. Thus, further screening of human-derived viruses needs to be conducted, especially for tumorigenic DNA viruses, retroviruses et al. Currently, we have been conducting tests for the human

herpes simplex virus 6 and 7, and further screening will be carried out soon.

In conclusion, we have successfully established and characterized a new human diploid cell line designated Walvax-2, and evaluated its susceptibility to 3 kinds of viral vaccine strains. The Walvax-2 cells are equally susceptible, and in some cases superior to, the MRC-5 line for the cultivation of viruses. Results from this study suggest that the Walvax-2 cell banks are a promising cell substrate and could potentially be used for the manufacturing of HDCVs.

Materials and Methods

Cells and viruses

HeLa, MRC-5, L929, MDCK, VeroandSp2/0-Ag14 cells were obtained from the America Center for Type Culture Collection (CCL-2, CCL-171, CCL-1, CCL-34, CCL-81, andCRL-158). Rabies fixed virus CTN-1Vstrain[26,27] and Pasteur strain were provided by the National Institute for Food and Drug Control (NIFDC, P.R. China) and Jiangsu Simcere Vaxtec Bio-Pharmaceutical Co., Ltd, respectively. The Varicella zoster virus Oka strain[28] was provided by American Type Culture Collection (ATCC). The hepatitis A virus (HAV) YN5 strain was isolated in 2003 from a hepatitis A patient in Kunming, China.[29]

Laboratory animals

Kunming mice, guinea pigs and rabbits were supplied by Guangdong Medical Laboratory Animal Center (Guangdong Province, P.R. China). Specific-pathogen-free (SPF) eggs were purchased from Beijing Merial Vital Laboratory Animal Technology Co., Ltd. Nude mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd.

The care and use of laboratory animals were approved by the Animal Care and Use Committee of Yunnan Walvax Biotechnology Co., Ltd. All animals were treated humanely and euthanized by cervical dislocation at the end of the experimental period.

Culture medium and other reagents

The growth medium (GM) for all cells was Eagle's minimum essential medium (M0769; Sigma) supplemented with 10 percent calf serum, 2 percent 2 M Glutamine (G8540; Sigma) and 2 percent 0.83 M $NaHCO_3$. The cryopreservation solution was GM added with 10 percent DMSO (D8418, sigma). Inorganic salts were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, P.R. China).

Source tissue material

The fetal material was provided by the Department of Obstetrics and Gynecology of Yunnan Hospital, with legal and ethical agreements from the donator. Before the study, we made strict and comprehensive inclusion criteria in order to guarantee a high quality cell strain: 1) gestational age 2 to 4 months; 2) induction of labor with the water bag method; 3) the parents career should not involve contact with chemicals and radiation; 4) both parents are in good health without neoplastic and genetic diseases, and with no history of human tissue or organ transplantation in the families traced for 3 generations; and 5) no infectious diseases. The tissues from the freshly aborted fetuses were immediately sent to the laboratory for the preparation of the cells.

Preparation of primary cell stock and cell banks

The preparations of the primary cell stock and serial propagation of cells were carried out according to the methods of Jacobs in 1970[8] and Hayflick in 1961.[30] The selected primary Walvax-2 cell seed was used for passaging, with the inoculation concentration of 5×10^5 cells /ml. Subsequent subculture was conducted at a 1:2 split ratio immediately subsequent to the formation of a dense cell monolayer. When the cells reached the 6^{th}, 14^{th}, and 29^{th} cell doublings, cultures were harvested and frozen to form a pre-master cell bank (PCB), master cell bank (MCB) and working cell bank (WCB).

Cryopreservation stability and recovery viability

Cryopreservation was performed 6 times for Walvax-2 cells with the cell lines designated as P6, P14, P20, P28, P38 and P48. These particular cell lines were chosen on the grounds that they represented the corresponding cell banks of PCB, MCB, WCB, the major working passages, and the entire lifecycle of the Walvax-2 cells. The cells were centrifuged and re-suspended in cryopreservation solution, and the cell concentration was adjusted to $6\sim10\times10^6$ cells /ml. The suspension was dispensed in 1.0 ml to 2.0 ml Cryogenic Vials (#430659，Corning). Following the manufacturer's instructions for the use of programmed cooling boxes (Nalgene Mr. Frosty，Thermo Fisher), the cryogenic vials were then sealed and put into the boxes at −70C overnight. Then cryogenic vials were placed directly into liquid nitrogen for long term cryopreservation. The frozen cells were recovered according to the procedures given by Jacobs in 1970[8] and Hayflick in 1961.[30]

Cells reconstituted from the frozen state were taken immediately for the calculation of population doubling times by cell counting. The three-tiered banks were propagated serially for doubling time assessments. The experiments were repeated 8 times, and the doubling times were compared with that of cells that had not been frozen.

Cell identification

The cell identification was evaluated by a 2-step procedure: Firstly isoenzyme analysis was performed using lactate dehydrogenase (LD) and Glucose 6 phosphate dehydrogenase (G6PD) as indicators to confirm Walvax-2 cell banks were human-derived cells. Then, Short Tandem Repeat (STR) analysis was conducted using MRC-5 as a parallel control by 3 qualified laboratories: China Center for Type Culture Collection (CCTCC), National Institutes for Food and Drug Control (NIFDC), and Law School of Kunming Medical University to assure that the cells were derived from the tissue of a specific human individual and different from any other established human diploid cell lines.

Chromosomal characterization

Chromosome examinations were conducted for every 10 passages by counting percentages for 5 types of chromosomal aberrations, including structural abnormalities, aneuploidy, polyploidy, hyperploidy and breaks or gaps. Chromosome specimen slides were obtained using the method of Coburn and Leykauf,[31] and then stained with Giemsa. Giemsa-banded karyotypes were recorded by Applied Imaging Software—Karyotyping 3.0 (England).

Microbial agents tests

The presence of bacterium, fungus and mycoplasmas for Walvax-2 cells were tested according to the requirements of ATCC and WHO.[18,19] Bacillus subtilis (CMCC(B)63501), Clostridium sporogenes (CMCC(B)64941) and Candida albicans (CMCC(F)98001) were used as positive controls for the tests of bacteria and fungi. A total of 19 cell passages were tested for sterility. The cell samples were tested under different temperatures for 2 weeks to confirm that no bacterial and fungal contamination was present. The mycoplasma test was conducted as per requirement in Volume III of Chinese Pharmacopeia, using the culture method and DNA staining technique, and B6yh4 cells were used as a positive control. All positive controls were provided by the National Institute for Food and Drug Control (NIFDC, P.R. China).

Exogenous virus agents tests

Tests for adventitious viral agents of Walvax-2 cells were conducted as per requirements for Preparation and Control of Animal Cell Substrates Used for Production and Testing of Biologics in Volume III of Chinese Pharmacopeia, including testing for general adventitious viral agents (non-specific virus) and specific adventitious viral agents.

General adventitious agents included embryonated egg inoculation by the yolk sac, allantoic cavity; i.c. and i.p. inoculation of adult and suckling mice, i.p. inoculation of guinea pigs; monolayer cell culture using MRC-5, and vero cells for detection of various human viruses.

Tests for specific adventitious agents consisted of human derived virus, bovine derived virus and porcine virus. For the human derived virus test, 6 viruses including HBV, HCV, HIV, Human cytomegalovirus, human nasopharyngeal virus and human parvovirus B19, were carried out based on per testing kit, using ELISA and PCR methods, respectively. For the bovine derived virus test, 3 methods were used: (i) the microscopic CPE observation method; (ii) different cell culture conditions for hemadsorption activity, and (iii) fluorescence quantitative RT-PCR method (bovine adenovirus, bovine parvovirus, bovine diarrhea virus, bovine influenza virus, bovine parainfluenza virus, rabies virus and retrovirus). The possible swine viral contamination was examined using RT-PCR and PCR methods for classical swine fever virus, Japanese encephalitis virus and Pseudorabies virus.

Retrovirus test

The retrovirus test was performed according to procedures described in " Reverse transcriptase activity assay in attenuated live vaccine"(Yan Kong et al)[32] and "Development of an improved product enhanced reverse transcriptase assay" (Audrey Chang, et al).[33] More specifically, the testing methods included product-enhanced reverse transcriptase (PERT) assay, infection test and direct observation by transmission electron microscopy. The mouse bone marrow cell line Sp2/0-Ag14 served as a positive control while MRC-5 cells were used for the system control.

Tumorigenicity test

To ascertain whether the cells had any neoplastic properties, P10, P20, P28, P38 and P48 Walvax-2 cells were implanted into 10 nude mice aged 4–6 weeks, in the thigh of the right hind leg of each mouse according to the requirements of Chinese Pharmacopeia. MRC-5 cells served as the negative control, and Hela cells served as the positive control. All animals were examined after 21 and 84 d following the inoculation of the cells. Animals not surviving the full period were examined post mortem, and observations for neoplastic growth were conducted for all tested animals.

Susceptibility to viruses test

Particular cell generations that would potentially be used for producing viral vaccines, were used to determine susceptibility to viruses after 25 to 30 cell doublings. Three kinds of viral vaccine strains (rabies, Varicella zoster and Hepatitis A) were used for the assays. To determine the susceptibility of Walvax-2 cells relative to MRC-5 cells, concurrent titrations were compared for the same cell doublings.

Rabies Virus

Virus propagation

The CTN-1V and Pasteur strains were propagated in Walvax-2 and MRC-5 cells by the method of Wiktor et al.[34] The virus maintenance medium was consistent with GM with the addition of 2% (v/v) fetal calf serum. A multiplicity of infection (MOI) of 0.01 was used. The viruses were incubated at 34–35°C.

Virus titration

The rabies virus was titrated using a modified test as described by Smith et al.[35] Virus titer was expressed in fluorescent focus units (FFU)/ml. Briefly, a monolayer of BSR cells in 96-well plates was incubated with serial fold5- virus dilutions at 37°C in a 5% CO_2 humidified incubator for 24 h. The cells were then fixed with 80% cold acetone at -20°C for 30 minutes, and then stained with the Rabies DFA Reagent (5100; Millipore). The plates were examined by fluorescence microscopy (Olympus Corp., Tokyo, Japan), and the numbers of fluorescent foci presented in the wells were recorded. The highest dilutions with fluorescent foci less than 30 were defined as endpoints, and virus titers were calculated by the following formula: virus titer (FFU/ml) = (the mean foci number in the endpoint wells × 5 + the mean foci number in the wells with lower dilutions next to the

endpoint well) ÷2 × the dilution factor of the lower dilutions ÷ the volume of virus dilution inoculated into each well.

Varicella Zoster Virus (VZV)

Virus propagation

The Oka strain at passage 31 was inoculated into Walvax-2 and MRC-5 cells and grown into a confluent monolayer at an MOI of 0.01.[36] Infected cells were incubated at 36 °C for 48–52 hrs till the cytopathogenic effect (CPE) was estimated to be approximately 75% to 100%. The cells were then trypsinized and resuspended in cryopreservation solution and stored at -196°C. The virus was serially propagated 8 times as described above for Walvax-2 and MRC-5 cells.

Virus titration

A plaque assay[37,38] was used and virus titer was expressed in plaque forming units (PFU)/ml. When Walvax-2 and MRC-5 cells in 6-well plates grew to a near confluent monolayer, the old medium was poured off and the monolayer was infected with cell-associated virus in fresh medium (with 2% fetal calf serum and 1% penicillin-streptomycin). Infections were allowed to proceed for 8–9 days, at which point the first signs of CPE was visible, the cells were stained and plaques counted.

Hepatitis A Virus

Virus propagation

According to the method of Wang et al,[39] the HAV YN5 strain was propagated in Walvax-2 cells and MRC-5. Briefly, Walvax-2 cells were trypsinized and inoculated with HAV at a MOI of 0.01 and stirred gently with a magnetic stirrer for 2 h at 37°C. The cells were then seeded in T225 flasks filled with GM at 37°C for 3–4 d until a confluent monolayer was formed. The GM was replaced by virus maintenance medium, consisting of MEM supplemented with 2% (v/v) fetal calf serum, 0.35% (m/v) $NaHCO_3$, 2% (v/v) and 2 M Glutamine; Cells were incubated at 35°C for 25 d Afterwards the cells were harvested and stored at −80°C.

Virus titration

An enzyme-linked immunosorbent assay (ELISA) was used to determine the virus infectivity titer of HAV.[40] The monolayers of Walvax-2 and MRC-5 were inoculated with serial fold5- cell-associated virus dilutions and incubated at 37°C for 1 h. Each dilution was assayed in quadruplicate. Then the inoculums were removed and replaced with a 1 ml nutrient MEM overlay containing 2 % fetal calf serum and incubated at 35°C for 25 d. The infected cells were harvested and sonicated. The presence of HAV-Ag was tested by ELISA. The $CCID_{50}$ value was calculated by a modified Reed-Muench's method.[41]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Aluminum Adjuvants

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Commentary

An aluminium adjuvant in a vaccine is an acute exposure to aluminium

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1. Introduction

Aluminium salts are common adjuvants in vaccines given to children. Their physical, chemical and biological properties have recently been reviewed [1]. However, a debate continues as to whether neonate and infant exposure to aluminium through vaccination is biologically significant with respect to their exposure to aluminium through other routes and especially diet. For example, paediatricians, responsible for administering the vaccine schedule for children, seem in particular, to be uninformed about the properties of aluminium adjuvants and their mode of action in vaccines. This apparent ignorance of the published scientific literature is unexpected in those charged with the wellbeing of neonates and infants and especially in the light of Janeway's description of alum adjuvant as 'the immunologist's dirty little secret' [2]. Paediatricians such as recently (07/04/2019) Andrew Pollard in The Sunday Times, have a habit of reverting to pure 'baby talk' when for example; describing how much aluminium is present in an infant vaccine. They use terms such as 'minuscule' and 'teeny-weeny' to tell anyone, who asks, how little aluminium there is in a vaccine. They usually then proceed to compare the amount of aluminium in a vaccine with the amount of aluminium in (an adult's) diet. There are, of course, more accurate, understandable ways to inform parents and other interested parties how much aluminium is present in a vaccine, and I shall endeavour to achieve this herein. An appreciation of how much aluminium is present in a single injection of a vaccine is critical to understanding how aluminium adjuvants are effective in stimulating the immune response.

2. How much aluminium is found in vaccines?

Currently about 20 childhood vaccines include an aluminium adjuvant. Vaccine industry literature (for example; https://www.medicines.org.uk/emc/product/2586/smpc) expresses the aluminium content of an individual vaccine as an amount (weight) of aluminium (not aluminium salt) per unit volume of a vaccine (usually 0.5 mL). Industry does this to account for the fact that there are no strict molecular weights for the polymeric aluminium salts that are used as adjuvants in vaccinations. They prepare acid digests of the adjuvants and measure their total aluminium using ICP MS. This is not explained in the literature they provide with vaccines and can cause confusion for some as the actual weight of hydrated aluminium salt (e.g. aluminium oxyhydroxide, aluminium hydroxyphosphate and aluminium hydroxyphosphatesulphate) in any vaccine preparation is actually approximately ten fold higher. The aluminium salt is the major component of a vaccine (after water) and its high content is why vaccine preparations are invariably cloudy in appearance [1]. As an example, GlaxoSmithKline's Infanrix Hexa vaccine is reported by the manufacturer to contain 0.82 mg of aluminium per vaccine (0.5 mL). Thus, the weight of aluminium salt in this vaccine is approximately 8 mg, which is approximately ten times the weight of all of the other components of the vaccine when combined. An aluminium-adjuvanted vaccine is essentially a very high concentration of an aluminium salt (8 mg/0.5 mL or 16 mg/mL or 16 g/L) in which just μg of other vaccine components including antigens and other excipients are occluded.

3. Is the amount of aluminium in a vaccine 'minuscule'?

Generally, in the United Kingdom the first dose of Infanrix Hexa vaccine is injected into muscle when an infant is 8 weeks old. All 8 mg of the aluminium salt (or 0.82 mg of aluminium) will immediately be systemic; it is inside the infant's body. The repercussions of this being that the injected aluminium may only leave the body through its excretion in either the infant's urine or sweat. What is the immediate biological response to this exposure to aluminium adjuvant? Aluminium is described as a silent visitor to the human body. What this means is that in the evolution of life on Earth and latterly human evolution, no historic signature is found as evidence for previous exposure to aluminium [3]. By way of comparison with another toxic and non-essential metal, if the adjuvant used in a vaccine was composed of a cadmium salt its injection would immediately initiate a counter-response by the body in an attempt to remove the toxicant. Proteins known to bind and help in the detoxification of cadmium are produced and this is a sure sign that biochemistry had previously encountered non-essential cadmium and selected it out of essential biochemical pathways. Such restorative attempts at detoxification are not triggered for biologically available aluminium and so the 'processing' of aluminium adjuvant at the injection site of a vaccine is completely adventitious and one might suggest, random and chaotic. The latter because the fate of aluminium in the body, unlike essential and other non-essential metals, is not subject to any form of homeostasis. Myriad

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chemical and biological processes will initiate the slow redistribution of the injected aluminium throughout the infant's body. These steps will involve the processes of disaggregation, dissolution, complexation, precipitation, distribution, cellular uptake and translocation. The description of each one of these processes is an essay in itself and we have addressed them all in many complementary publications [1]. An important and vaccination-specific distinction to make at this point and to carry forward to the following discussion is that aluminium injected into muscle as an adjuvant in a vaccine potentially has uninterrupted access to the infant brain. This is because there is no prerequisite for its passage via the liver, the most prominent organ of detoxification in humans.

We asked if 0.82 mg of systemically available aluminium administered as a single dose in a vaccine is, as some paediatricians would suggest, a minuscule amount of aluminium, for example, as compared to aluminium in the diet. Infants receiving Infanrix Hexa vaccine at 8 weeks of age are concurrently either being breast or formula fed. Data show that the former is likely to result in an 8 week old infant ingesting up to 0.1 mg of aluminium each day [4,5]. On the day an infant receives 8 mg of an aluminium salt, or 0.82 mg of aluminium, in a vaccine it will also ingest 0.1 mg of aluminium in breast milk. However, what proportion of this 0.1 mg of dietary aluminium will be absorbed across the infant gut? Previous research has asked a similar question [6]. The reality is that data for the absorption of aluminium across the infant gut do not presently exist and one has to apply gastrointestinal absorption data obtained for adults. The oft-cited value for adults is that less than 0.1% of ingested aluminium in diet is actually absorbed [7]. The infant gut at 8 weeks is incomplete [8] and is likely to be much more permeable to dietary aluminium, perhaps as much as 100 times more permeable. Applying such clearly conditional criteria it can be estimated that 10% of ingested aluminium or 0.01 mg/day of aluminium in breast milk is absorbed across the infant gastrointestinal tract. However, the blood carrying nutrients and toxins that have been absorbed from the gut, to the rest of the body must first pass through the liver, the major detoxification system of the body. Data on the efficiency of the liver in removing aluminium from the blood is, at best, incomplete in adults [9] and completely unknown in infants. If it is estimated that the liver is 75% efficient in this respect for adults then it is probably only 50% efficient in an infant. When these various conditional factors are accounted for it can be estimated that an infant's exposure to systemically available aluminium from breast-feeding is approximately 0.005 mg of aluminium each day. In essence during the first 8 weeks or 56 days of life, breast-feeding ostensibly drip feeds an infant with a combined total of 0.28 mg of systemically available aluminium. On day 56 the infant receives a single dose of 0.82 mg of aluminium in the Infanrix Hexa vaccine, a dose equivalent to 3 times the amount of aluminium the infant received during the entire 55 days of life prior to its vaccination. It is well known, if highly unfortunate, that infant formulas are heavily contaminated with aluminium [10,11] and in a worst-case scenario an infant only being formula-fed from birth might be exposed to 0.030 mg of aluminium each day up to vaccination on day 56. Even in this worst-case scenario, the exposure to systemically available aluminium on vaccination day is 25 times higher through the vaccine than through the diet.

4. Acute versus chronic exposure to aluminium

Breast or formula feeding in an infant is a chronic exposure to aluminium. The infant is exposed to a small but continuous supply of systemically available aluminium, aluminium that has the potential to be stored in the infant's body and excreted from the infant's body in the urine. Perhaps, at no point during continuous chronic (drip feed) exposure in infancy (0–12 months of age) does the concentration of aluminium in any one physiological compartment increase to bring about overt toxicity. How does dietary exposure to aluminium in infants compare to exposure through vaccination, for example, a single Infanrix Hexa vaccine at 8 weeks of age? The concentration of aluminium (not aluminium salt) in an Infanrix Hexa vaccine upon its injection into muscle is, according to the manufacturer, 0.82 mg/0.5 mL or 1.64 mg/mL or 1.64 g/L or approximately 60 mmol/L. This is the concentration of total systemically available aluminium immediately present at the injection site of the vaccine and available to bring about biological effect. Aluminium adjuvants are not inert depots at the vaccine injection site; they are sources of biologically reactive aluminium [1]. This concentration of total aluminium at the injection site of a vaccine can be put into context by examining the cellular toxicity of aluminium [12] and specifically as identified in recent scientific publications. We can ask the question if we would expect this concentration of aluminium to produce biological effects including cell death at the vaccine injection site. A relevant cell to investigate are lymphocytes and research has demonstrated significant genotoxicity in lymphocytes exposed to only 0.020 mmol/L total aluminium [13]. Similarly, in another study using lymphocytes 0.6 mmol/L total aluminium resulted in significant immunosuppression in both T and B-lymphocytes [14]. Clearly, we would expect profound effects on lymphocytes at the injection site of a vaccine where the total aluminium concentration is 60 mmol/L. Macrophages, a characteristically robust cell, are susceptible to aluminium toxicity demonstrating 50% cell death at a total aluminium concentration of 10 mmol/L [15]. Other more sensitive cell lines would include neuroblastoma where cell viability is reduced by 50% by less than 1 mmol/L total aluminium [16] and similarly for primary hippocampal neurons exposed to only 0.05 mmol/L total aluminium [17]. The concentration of systemically available aluminium immediately present at the injection site of a vaccine is very high in comparison to studies on cell cytotoxicity in the scientific literature. It is an acute exposure to aluminium and it results in significant cytotoxicity including necrotic cell death [1]. The resulting tissue inflammation is the characteristic red mark on the skin at the injection point. This acute toxicity in the immediate vicinity of the injection site underlies the success of aluminium salts as adjuvants in vaccinations [1]. However, while some cells, both present at and infiltrating the injection site, are compromised and especially immediately, other cells act to remedy the situation by taking up aluminium adjuvant into their cytoplasm [18]. This action reduces the concentration of biologically reactive (toxic) aluminium at the injection site and locks away potentially cytotoxic aluminium in intracellular vesicles. Herein may be the real issue linking aluminium adjuvants and severe adverse events following a vaccine. These aluminium-loaded cells remain viable for days, potentially weeks, which means that they can transport their cargo of aluminium anywhere in the body including the infant brain. The recruitment of systemic cells including macrophages to the central nervous system is a widely documented phenomenon [19]. There is now a viable mechanism for the accelerated loading of an infant's brain with aluminium and evidence to support such a mechanism was demonstrated in our recent paper on aluminium in brain tissue in autism [20].

5. Conclusion: is the amount of aluminium in a vaccine 'minuscule'?

Simply by looking at just one dose of a vaccine given at 8 weeks of age it is abundantly clear that science does not support this contention, as espoused regularly by many infant paediatricians. In fact, just a single dose of Infanrix Hexa vaccine represents a severe acute exposure to systemically available aluminium. A single dose of this vaccine is equivalent to the exposure to aluminium that an infant would receive from 150 days breast-feeding. It is equivalent to 25 times the daily dose of aluminium received from the most contaminated of infant formulas. It is pertinent to emphasise that an infant would receive a further two doses of this vaccine during the aforementioned 150 day period. It is also highly relevant that other aluminium adjuvanted vaccines, for example Prevenar 13 (https://www.medicines.org.uk/emc/product/453/smpc) and Men B (https://www.medicines.org.uk/emc/product/

5168/smpc) are also part of the infant vaccine schedule for this same period. In the United Kingdom it is not uncommon for an infant to receive all three of these aluminium adjuvanted vaccines on the same day. A combined daily exposure of 1.445 mg of aluminium (according to the manufacturer's data), equivalent to 260 days exposure to aluminium through breast feeding. Exposure to aluminium through a vaccine is, in comparison to diet, an acute exposure and an infant's physiology will respond differently to exposure to a high concentration of aluminium over a very short time period. The latter, acute versus chronic exposure, while not yet being taken into account in infant vaccination programmes, must now be considered to help to ensure that future vaccination schedules are safe. Currently the EMA and the FDA limit the aluminium content of a vaccine to 1.25 mg (See for example, https://www.ecfr.gov/cgi-bin/text-idx?SID=832c22988b6c802fe810e16ea34ace1a&mc=true&node=se21.7.610_115&rgn=div8). This limit is based upon the aluminium adjuvant's efficacy in inducing antibody titres. Perhaps now is the time to revise this limit based upon additional factors of vaccine safety.

Author contributions

CE conceived and wrote the manuscript.

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Declaration of Competing Interest

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Research Article

Aluminium Involvement in Neurotoxicity

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The aetiology of neurodegenerative diseases (ND) seems to involve susceptibility genes and environmental factors. Toxic metals are considered major environmental pollutants. Following our study of a case of multiple sclerosis (MS) improvement due to removal of aluminium (Al) and other toxic metals, we have examined the possible relationship between Al intoxication and ND. We used the slow intravenous treatment with the chelating agent EDTA (calcium disodium ethylene diamine tetraacetic acid) (chelation test) to remove Al and detected it in the urine collected from the patients for 12 hours. Patients affected by MS represented 85.6% of total ND. Al was present in 44.8% of cases comprehensive of ND and healthy patients. Al levels were significantly higher in ND patients than in healthy subjects. We here show that treatment of patients affected by Al burden with ten EDTA chelation therapies (EDTA intravenous administration once a week) was able to significantly reduce Al intoxication.

1. Introduction

Exposure of human populations to toxic metals can result in damage to a variety of organ systems.

One of the most commonly toxic metals studied, aluminum (Al), is implicated in many diseases. Al is a highly abundant and ubiquitously distributed as environmental and industrial toxicant and is also contained in many food products, being involved in skeletal, haematological, and neurological diseases [1]. Al toxicity is caused by disruption of homeostasis of metals such as magnesium, calcium, and iron (Fe): in fact, Al mimics these metals in their biological functions and triggers many biochemical alterations [2]. In particular, Al both exerts direct genotoxicity in primary human neural cells [3] and induces neurodegeneration, through an increase in Fe accumulation and oxygen reactive species (ROS) production [4]. Al-induced oxidative damage to DNA has been previously associated with neurodegeneration in different regions of rat brain [5]. In addition, more recently Al^{3+} has been shown to provoke transporter-mediated dopamine neuron degeneration in the nematode *Caenorhabditis elegans* [6].

The removal of toxic metal from human body can represent a useful tool to avoid the beginning or progression of many diseases related to metal intoxication.

The methods useful to determine some metal content in biological samples for monitoring purposes were developed some years ago. Indeed, both toxic and essential metals have been assayed in blood, urine, and hair by atomic absorption spectroscopy [7]. Successively, methods for trace-element analysis in human biological materials have been developed and inductively coupled plasma mass spectrometry (ICP-MS) was considered preferable for screening of multiple elements [8]. However, it seems difficult to show metal excess in blood and urine in conditions different from acute metal intoxication. In fact, blood toxic metal increase reflects only recent exposure to metals [9]. After acute exposure, toxic metals rapidly move from blood to many tissues, where they are sequestered, as in central nervous system (CNS). The only way able to remove accumulated toxic metals from human organs is to bind these metals by means of chelating agents, with the aim of forming complexes able to be excreted in the urine. Toxic metal levels can be examined in the urine samples collected from patients, following "challenge" with a chelating agent ("chelation test"). We have selected, among known chelating agents, calcium disodium ethylenediamine tetraacetic acid ($CaNa_2EDTA$ or EDTA), which was intravenously administered. The stability constants of aluminium and other metals of biochemical

interest with various chelating agents including EDTA have been previously studied [10]. The development of a set of metal complex constants served to correlate chemical and functional properties of the metals and suggested that EDTA was able to mobilize aluminium.

In the past, toxic levels of Al have been associated with neurodegenerative diseases (ND). A possible link between Al and Alzheimer's disease has been highlighted [11]. In 1991, treatment with low dose intramuscular desferrioxamine (DFO), a trivalent chelator that can remove excessive iron and/or aluminium from the body, was reported to slow the progression of Alzheimer's disease [12].

In the present work we have decided to study whether Al was involved in neurotoxicity. Indeed we evaluated the Al body burden in patients affected or not by ND. We studied also the possible reduction of this burden following treatments with the chelating agent EDTA.

2. Materials and Methods

2.1. Study Design and Patient Recruitment. Out of 471 consecutive subjects who had undergone a medical checkup in an outpatient medical center, only 211 were selected and enrolled for this study due to evidence of their Al burden and compliance in following the protocol, for example, receiving chelation therapy once a week by personal choice. The ND examined in this study were multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD). Many MS patients had been previously treated with conventional drugs used in such pathology (e.g., immunosuppressant agents, as mitoxantrone and azathioprine, broad-spectrum immunomodulatory agents, as glatiramer acetate and interferon β, and monoclonal antibodies, as rituximab and natalizumab). Some MS patients had never been previously treated with drugs. Patients not affected by known diseases (healthy subject or controls) as well as patients affected by nonneurodegenerative pathologies (not ND, which refers to diseases not classified as ND as fibromyalgia) have also been recruited. Some healthy patients who had been previously exposed to environmental or working toxic metals preferred to examine their possible intoxication by evaluating the presence of such metals in hair samples. Indeed, they were excluded from the present study. All patients provided informed consent to participate in this study. They were between 18 and 75 years old.

2.2. Chelation Test and Evaluation of Urine Al. Patients have been subjected to the chelation test to show possible Al intoxication. Indeed, they were invited to collect the urine samples before and after the intravenous treatment with the chelating agent EDTA (ethylenediamine tetraacetic acid, e.g., calcium disodium edetate, 2 g/10 mL diluted in 500 mL physiological saline, Farmax srl, Brescia, Italy). EDTA was intravenously slowly administered (the infusion lasted about 2 hours) to the patients. The time of urine collection following chelation lasted 12 h. Samples recovered from such collection were accurately enveloped in sterile vials and transported to the Laboratory of Toxicology (Doctor's Data Inc., St. Charles, IL, USA), where they have been processed. Samples were acid-digested with certified metal-free acids; digestion took place in a closed-vessel microwave digestion system. For sample dilution ultrapure water was used.

To avoid contamination, only plastic materials were used. All laboratory ware (pipette tips, volumetric flasks, etc.) was immersed for at least 48 h in a 10% (v/v) HNO_3/ethanol solution and, shortly before use, washed with Milli-Q purified water. To avoid contamination from the air, all steps in the preparation of samples and reagents were carried out on a class 100 clean bench [13, 14].

Testing was performed via inductively coupled plasma mass spectrometry (ICP-MS) utilizing collision/reaction cell methods coupled with ion-molecule chemistry, a new reliable method for interference reduction. The method has been recently used for biomonitoring of 20 trace elements in blood and urine of occupationally exposed workers [15]. Certified urine standards and in-house standards were used for quality control and to validate results. To avoid the potentially great margin of error that can result from fluid intake and sample volume, results were reported in micrograms (μg) per g creatinine. Creatinine was measured by reverse-phase high-performance liquid chromatography and was used to correct the total volume of urinary Al for differences in the glomerular filtration rates of individuals at the time of the spot sample [16]. The research program entitled "Effects of Chelation Therapy with EDTA in Patients Affected by Pathologies Related to Exposition (Acute or Chronic) to Toxic Metals" has been approved by Ethical Commitment of The University of Milan (Italy) (number 64/2014).

2.3. Clinical Evaluation of Patient's Symptom Improvement in MS. In the absence of a diagnostic test specific for MS, the neurological community has adopted diagnostic criteria which were replaced in the time [17]. Magnetic resonance imaging (MRI), analysis of cerebrospinal fluid, and visual evoked potential, added to clinical diagnosis, have been considered to present limitations of sensitivity and specificity. Successively MRI has gained an importance. However, the diagnosis of improvement in patient's symptoms is currently based on clinical criteria, as reduction of neurological disability (paresthesia, gait ataxia, spasticity, optic neuritis, and bladder dysfunction) and fatigue. Sometimes, symptoms of ALS, as paresis, muscle atrophy, and dysarthria, are associated with MRI and cerebrospinal fluid abnormalities typical of MS. Indeed, we have considered the improvement of patient's symptoms the recover from clinical disability, for example, ability to work, reduction of spasticity, relapse delay, and/or fatigue disappearance.

2.4. Effect of EDTA Chelation Therapy on Al Intoxication. Patients who revealed Al intoxication (by examination of its levels in urine samples) were subjected to EDTA chelation therapy. EDTA (2 g in 500 mL physiological saline) was intravenously infused in each patient in about 2 hours. Treatment was given once a week and lasted ten weeks. At the end of treatments urine Al levels were analysed, as previously described.

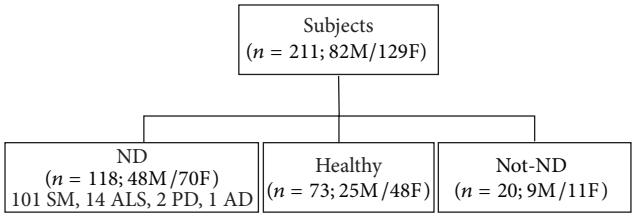

Figure 1: Scheme of enrolled subject's characteristics.

2.5. Data Analysis. Statistical analysis was performed using Microsoft Excel 2010 and IBM SPSS Statistics 20 (IBM Armonk, New York, USA). A logistic regression analysis was used to examine the relative contributions of several variables to the operation outcomes. $P < 0.05$ was considered significant.

3. Results

3.1. Patient's Characteristics. Figure 1 reports the distribution of patients who displayed Al intoxication.

The most represented patients affected by ND were those with MS (85.6% of total ND). Indeed, we compared both the group of MS patients and the group of ND patients with the group of healthy patients.

3.2. Al Intoxication. All patients did not show Al intoxication before EDTA challenge (data not shown). All patients affected by ND displayed intoxication by different toxic metals (data not shown). After challenge with EDTA, Al was present in 44.8% of cases comprehensive of ND and healthy patients. The levels of Al intoxication, as obtained from evaluation of μg/g creatinine content of Al in the urine samples collected following the first intravenous treatment with EDTA (chelation test), are reported in Figure 2. The data indicate that Al values were significantly higher in the urine samples of SM and ND patients than in those healthy patients.

3.3. Usefulness of EDTA Chelation Therapy. The effect of EDTA chelation therapy is reported also in Figure 2. Indeed, the patients who have shown Al intoxication following chelation test underwent chelation therapy (EDTA intravenous administration once a week). After ten therapies, the levels of Al in the urine samples were further evaluated and compared with that obtained following chelation test. EDTA administration was demonstrated to be significantly efficient in removing Al burden, as shown in Figure 2. Our results showed that reduction in the time of Al intoxication well related with improved clinical conditions of the patients. In fact they presented, at different extent, reduction of neurological disability and fatigue.

Noteworthily, the efficacy of EDTA chelation therapy was more evident in ND than in healthy patients.

4. Discussion

Toxic metals, pesticides, and phenols are considered major environmental pollutants [18]. Toxic metals are classified as

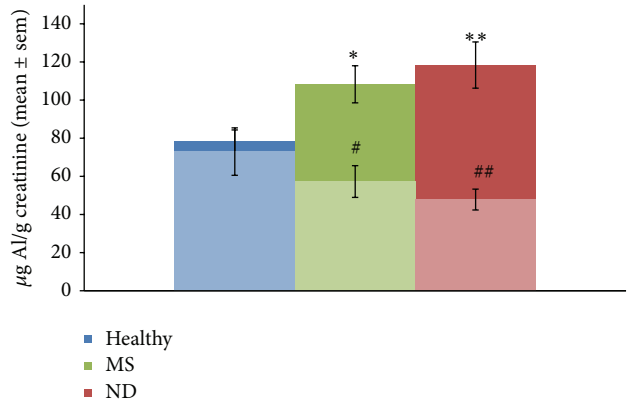

Figure 2: Aluminium (Al) levels evaluated in the urine samples of examinated subjects, following chelation test (dark) and after ten chelation therapies with EDTA (light), expressed as mean ± SEM of μg/g creatinine. The studied subjects were healthy patients, patients affected by multiple sclerosis (MS), and patients affected by all neurodegenerative diseases (ND). The levels of Al in both MS and ND patients were significantly higher with respect to those obtained in healthy subjects following chelation test ($^{*}P/^{**}P < 0.05$ versus healthy). After chelation therapies with EDTA, the levels of Al were significantly lower than that obtained following chelation test ($^{\#}P < 0.05$ versus $^{*}P$ and $^{\#\#}P < 0.05$ versus $^{**}P$).

nonbiodegradable substances, as well as plastics and detergents, because they are not degraded by microorganisms. They represent a global health risk because of their ability to contribute to a variety of diseases. In this context, Al (which is a highly reactive element and ubiquitous environmental contaminant) has been associated with some diseases [1]. In fact, osteomalacia is a skeletal disease related to Al toxic effects, such as phosphate deficiency, Ca-uptake impairment, and dysfunctional osteoblast proliferation [19]. Moreover, Al exposure can impair Fe intestinal absorption, promoting an anemic state [20]. In addition, Al may play an active role in the pathogenetic mechanisms of neurological diseases. In particular, Al has been shown to be responsible for critical neuropathologic lesions in AD and other related disorders for its ability to cross-link hyperphosphorylated proteins [21]. Al has been detected in amyloid fibers in the cores of senile plaques in brains of AD patients [22]. The presence of Al in biological systems could lead to an important prooxidant activity, by promoting superoxide generation through Fenton reaction [23]. More recently, Al removal in AD patients by treatment with DFO has been further proposed [24]. Successful treatment with DFO (both at low and at standard dose) has been performed for Al overload among haemodialysis patients [25]. Moreover, DFO has been shown to be able to exert protective effects in the brain tissue of mice against Al-induced structural and metabolic alterations [26]. However, since some patients can have intolerance to DFO or develop DFO side effects such as allergic reactions, neurological symptoms, or obvious gastrointestinal upset, we decided to use EDTA as a chelating agent. The chelator N-(2-hydroxyethyl) ethylenediamine triacetic acid (HEDTA), similar to EDTA, has been shown to be efficient, also

in association with selenium, against Al-induced oxidative stress in rat brain [27].

Elevated urinary excretion of Al and Fe has been previously shown in MS patients [28].

We have previously studied the case of a young man affected by MS, who has been unsuccessfully treated for some years with current therapies [29]. Symptoms revealed by the patient were subacute vision loss, diplopia, and pain with eye movements as the first symptoms of optic neuritis; disturbance of fine motor skills; paresthesia and gait ataxia; bladder dysfunction; and significant tiredness. We examined his levels of toxic metals in the urine, following intravenous "challenge" with EDTA. The patient displayed elevated levels of Al, Pb, and Hg in the urine. Indeed, he was subjected to treatment with EDTA twice a month. Under treatment, the patient revealed in time improved symptoms suggestive of MS remission. In fact, he recovered eye vision and bladder function and paresthesia disappeared as well as tiredness. Because the most represented toxic metal in this patient was Al, we decided to examine the possible relationship of Al intoxication with ND.

Our results show that Al levels measured in urine samples of patients affected by both MS separately studied and total ND studied were significantly higher than that of healthy patients, as reported in Figure 2. Healthy patients displayed about 80 μg/g creatinine, as mean Al levels, even if normal values are 35 μg/g creatinine. These data suggest that Al intoxication is not necessarily related to onset of ND clinical symptoms. Moreover, control patients are possibly able to limit further Al burden through neuroprotective or antioxidant mechanisms which are absent in ND patients. Clinical evaluations of each patient suggested the presence of an important relation between Al intoxication and impairment of movements, paresthesia, ataxia, and other symptoms displayed by subjects affected by ND. Indeed, the patients who displayed maximal values of Al in the urine sample displayed also the most serious features of disease at clinical level. The objection that mobilizing (by chelating agents) Al from relatively safe sites such as bone and depositing this highly neurotoxic metal in the CNS can be dangerous is opposed by the consideration that patients affected by ND were affected by Al burden (responsible for the pathogenesis of the disease) in CNS before chelation. Moreover, the complexes formed by toxic metal with chelating agents are well removed by kidneys. Recent studies demonstrated that severe behavioural motor deficits and loss of the motor neurons through the nervous system resulted when an Al vaccine adjuvant was applied to an animal model. Indeed, mice injected with Al hydroxide showed a significant increase in cell death in the spinal cord and motor cortex, primarily affecting the motor neurons and inducing neuroinflammation. The effects closely resembled the damage seen in human ALS [30].

As recently reported, the immune system also appears to be sensitive to Al exposure [31]. Effects of Al on autoimmunity, oral tolerance, CD4+ and CD8+ expression, hypersensitivity, and erythrocyte immune function are suggestive of its immunotoxicologic activity. It has been suggested that many of the features of Al-induced neurotoxicity may arise in part from autoimmune reactions [30].

Finally, in a recent report by Exley C [32] Al is considered a potential contributor to the onset, progression, and aggressiveness of ND, even if it appears to be difficult to establish when it contributes to disease etiology. However, since Al represents a risk to human health, it is necessary to implement measures to reduce its body burden to the lowest practical limit.

Which strategy for common therapy of injury provoked by toxic metals can be proposed? Intracellular uptake of toxic metals would be adequately prevented by relevant inhibitors (chelators), whereas the ROS generation and ROS-mediated processes would be prevented or ameliorated by relevant antioxidant and scavengers of free radicals and Fe.

In our experience, as shown in previous studies and in the present, removal of toxic metals has induced beneficial effects by improving patient symptoms [29, 33, 34]. No adverse effects were observed from EDTA treatments. Metal removal appeared gradual in the time, and suggested many chelation therapies. In conclusion, in the present study we show that EDTA chelation therapy was able to reduce Al burden in patients affected by ND by ameliorating their clinical conditions. We hope that in the future such treatment will be considered as a useful tool to improve ND patient's symptoms.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Contact allergy to aluminium induced by commonly used pediatric vaccines

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We would like to complete the paragraph on Adjuvants (page 6) in the review Vaccination in children with allergies to non active vaccine components by Francheschini et al. [1] which was initiated by the Italian Pediatric Society of Allergy and Immunology in 2013 and published in Clinical and Translational Medicine in 2015.

As mentioned in the review, aluminium (Al) salts are widely used as adjuvants in diphtheria-tetanus-pertussis (DTP) and hepatitis A and B vaccines. The list can be completed with pneumococcal and meningococcal conjugate vaccines, which today are included in the national vaccination schedules in most countries in Europe and the Americas, and also in vaccines against human papilloma virus (HPV) and tick-bore encephalitis (TBE).

According to the authors, the most known and frequent reaction to Al salts is “a palpable nodule at the injection site”. This sounds harmless enough—but in typical cases the nodules are most annoying to the child due to severe pruritus for a very long time [2, 3]. Besides, most children with persistent itching vaccination granulomas become sensitized against Al [4].

Itching vaccination granulomas are described since 1960 [5] but considered very rare [6] until the 1990s when they were reported in 745 of 76,000 children participating in studies on a monocomponent acellular pertussis vaccine in Sweden [7]. Since then, another 102 children in Sweden who received commercial DTaP-polio-Hib-(HepB) combinations (Infanrix®, Pentavac®) and/or pneumococcal vaccines (Prevenar, Synflorix) are described [4, 8, 9]. The vaccines were given intramuscularly in three doses at 3, 5 and 12 months. In a prospective cohort study on 4758 toddlers the frequency of granulomas was 0.63% in those who received a DTaP combination vaccine alone and 1.18% in those who received an Al adsorbed pneumococcal vaccine at the same time. The risk for granulomas increased with the number of Al-containing vaccine doses [4].

The itching nodules appear remarkably late (months or even years) after the vaccination. Histopathological examination shows granuloma formations in which Al crystals can be demonstrated [10]. Clinically, pruritus is the dominating symptom with intense local itching in the vaccination area on the thigh, often causing skin alterations like eczema, hypertrichosis and hyperpigmentation. Intensified itching and swelling of the nodules is often reported when the child has a cold or another infection. After a duration of ½–12 years (median 3–4 years) the nodules eventually disappear and the pruritus ceases.

In some cases nodules were mistaken as tumours leading to unnecessary anxiety, investigations and surgery [11, 12].

Contact allergy to aluminium was verified in 77–95% of children with itching vaccination granulomas by epicutaneous testing with Al Chloride hexahydrate 2% and metallic Al (4, 7, 9). Sensitized individuals have reported contact dermatitis after the use of Al containing deodorants, pharmaceutics (ear drops, antiseptics), sun protectors, tattooing pigments and metallic aluminium [13]. Fortunately, and contrary to earlier belief, the sensitization to aluminium seems to vane with time [14].

The consequences of future vaccination with Al adsorbed vaccines in children who once reacted with itching granulomas and/or contact allergy to Al is only partially studied. Our clinical experience so far is that the risk for new granulomas diminishes with time and is very low when the original one has vanished and the itching ceased. In case of on-going severe pruritus the next dose may be postponed 6–12 months. The Al allergy is a delayed type IV reaction not associated with increased risk for anaphylaxis.

We want to point out that itching granulomas* are benign and self-limiting and no cause to refrain from vaccination in consideration of the risk for a serious infectious disease. They are poorly known but easy to recognize once you are aware of them. They should be familiar to all health care staff working with children to avoid mistrust and anxiety in the parents and unnecessary investigations of the child.

*see MMF studies under Adverse Reactions

Authors' contributions

EB participated in the pertussis vaccine study, organized the follow-up and testing of children with itching vaccination granulomas, performed the prospective cohort study and drafted the manuscript. BT organized and performed the clinical study on the monocomponent acellular pertussis vaccine in Göteborg and participated in the follow-up of the itching children. AI performed the testing of the children in the pertussis vaccine study. AGL performed the re-testing study where aluminium sensitization was showed to vane with time. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Aluminum hydroxide injections lead to motor deficits and motor neuron degeneration

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Abstract

Gulf War Syndrome is a multi-system disorder afflicting many veterans of Western armies in the 1990–1991 Gulf War. A number of those afflicted may show neurological deficits including various cognitive dysfunctions and motor neuron disease, the latter expression virtually indistinguishable from classical amyotrophic lateral sclerosis (ALS) except for the age of onset. This ALS “cluster” represents the second such ALS cluster described in the literature to date. Possible causes of GWS include several of the adjuvants in the anthrax vaccine and others. The most likely culprit appears to be aluminum hydroxide. In an initial series of experiments, we examined the potential toxicity of aluminum hydroxide in male, outbred CD-1 mice injected subcutaneously in two equivalent-to-human doses. After sacrifice, spinal cord and motor cortex samples were examined by immunohistochemistry. Aluminum-treated mice showed significantly increased apoptosis of motor neurons and increases in reactive astrocytes and microglial proliferation within the spinal cord and cortex. Morin stain detected the presence of aluminum in the cytoplasm of motor neurons with some neurons also testing positive for the presence of hyper-phosphorylated tau protein, a pathological hallmark of various neurological diseases, including Alzheimer's disease and frontotemporal dementia. A second series of experiments was conducted on mice injected with six doses of aluminum hydroxide. Behavioural analyses in these mice revealed significant impairments in a number of motor functions as well as diminished spatial memory capacity. The demonstrated neurotoxicity of aluminum hydroxide and its relative ubiquity as an adjuvant suggest that greater scrutiny by the scientific community is warranted.

Keywords

Aluminum hydroxide; Adjuvant; Neurotoxicity; Gulf War Syndrome; Amyotrophic lateral sclerosis

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Animal ethics approval: Protocols governing the use of animals were approved by review committees of the University of British Columbia and were in compliance with guidelines published by the Canadian Council on Animal Care and are in accordance with the international guidelines including the NIH Guide for the Care and Use of Laboratory Animals, as well as the EEC Council Directive.

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1. Introduction

Various studies have established a correlation between Gulf War service (1990–1991) and a multi-system disorder commonly termed Gulf War Syndrome. Included in GWS are various neurological disorders, including an apparent cluster of cases of amyotrophic lateral sclerosis [1–4]. Haley [3] described classical ALS symptoms such as muscle weakness and wasting, impaired speech and swallowing, difficulty in breathing, and fasciculation in Gulf War veterans years after they first developed other symptoms of GWS. Seventeen of the 20 servicemen diagnosed with Gulf War illness and definite ALS were less than 45 years of age with the youngest of these 20 years old. All 20 of these patients presented with signs of upper (motor cortex or bulbar region) and lower (spinal cord) motor neuron degeneration. None of these patients had a family history of ALS or of other neurodegenerative disorders. Horner et al. [2] conducted a nationwide case study performed to identify incidence levels of ALS for the decade after August 1990 amongst active duty members of the military. One hundred and seven confirmed cases of ALS were identified from approximately 2.5 million eligible military personnel. When standardized to the average 1990 US general population, the average annual incidence of ALS among non-deployed military population was 1.4 per 100 000 persons per year compared to the generally accepted overall population incidence of 1.5 cases of ALS per 100 000. The incidence rate of ALS among the deployed military population was 3.6 per 100 000 persons/year. Weisskopf et al. [4] noted a general increase in ALS in US military populations going back a number of decades regardless of the conflict.

ALS–GWS is one of only two ALS disease clusters currently accepted as satisfying the definition of a cluster. The other is the Guamanian variant of ALS first described after World War 2 termed amyotropic lateral sclerosis parkinsonism dementia complex (ALS–PDC). This spectrum of disorders, once present with an incidence levels hundreds of times higher than in the continental United States [5] (see Kurland, 1988, for review), expressed in one of two ways. The first was as a nearly classical form of ALS; the second was a form of parkinsonism associated with an Alzheimer's disease-like dementia (PDC). About 10% of the victims developed both disorders, with the ALS phenotype typically appearing first. Studies into potential etiologies focused on environmental factors with most attention eventually directed at the consumption of toxin-containing seeds of the local variety of cycad palm [6] and the presence of high aluminum in the soil on southern Guam [7].

In regard to the GWS-ALS AVA vaccine, attention has recently been directed at the anthrax vaccine adsorbed (AVA) and various vaccine ingredients, in particular the known and suspected adjuvants, aluminum hydroxide and squalene [8]. An adjuvant is a substance added during vaccine production designed to non-specifically increase the immune response to an antigen [9]. Aluminum compounds were first identified as adjuvants over 90 years ago. Currently aluminum, in various forms (aluminum hydroxide, aluminum phosphate and aluminum sulfate), is the most commonly licensed adjuvant whose use is generally regarded by both the pharmaceutical industry and the various governmental regulatory agencies as safe [10]. Various studies have found no adverse or long-term health effects due to aluminum adjuvants [11–13] and the Food and Drug Administration (FDA) has continued its longstanding approval for the use of aluminum in this fashion.

In spite of the long history of widespread use, the physicochemical interactions between aluminum compounds and antigens are relatively poorly understood and their underlying mechanisms remain relatively unstudied [14]. It also seems that there have been no rigorous animal studies of potential aluminum adjuvant toxicity. The absence of such studies is peculiar given the well known observation that aluminum in general can be neurotoxic under a number of conditions [15,16] and adjuvants in particular have previously been implicated in neurological disease [17–19]. Table 1 shows the results from previous studies that treated

animals with aluminum hydroxide, listing the resulting impacts on the nervous system. In context to the use of aluminum in vaccines, LD_{50} values for aluminum hydroxide have not been published to date to the best of our knowledge (J.T. Baker Material Safety Data Sheets).

The potential for aluminum injections to induce macrophagic myofasciitis has also been noted in the literature [20–22].

A previous publication looked at the potential neurotoxicity of several known or suspected vaccine adjuvants [8]. In the current study, we will focus exclusively on the impact of aluminum hydroxide injections on motor and cognitive behaviours and on the expression of different forms of neuropathology in an *in vivo* mouse model.

2. Experimental procedures

2.1. Experimental animals

In our initial study [8], young adult (3 month old) CD-1 male mice were used (approx. 35 g at experiment onset). Younger animals were deliberately chosen to mimic the typical age of service during the Gulf War [3]. Four subcutaneous injection groups (two injections spaced 2 weeks apart) were used: control saline/phosphate buffered solution (PBS) ($n = 10$); aluminum hydroxide ($n = 11$); squalene ($n = 10$); and aluminum hydroxide and squalene ($n = 10$). The current study will report only on the aluminum treated and control groups from this experimental series. A second series of experiments was conducted on 9 month old CD-1 males that received six aluminum hydroxide injections over a 2 weeks period. These mice, along with controls and other treatment groups (to be reported elsewhere), were subjected to a more rigorous behavioural testing regime to be described below. Histological analyses of the spinal cords and brains of these mice are in progress.

All animals in both experiments were singly caged at the Jack Bell Research Centre animal care facility in Vancouver, B.C., Canada. An ambient temperature of 22 °C and a 12/12 h light cycle were maintained throughout the experiment. All mice were fed Purina® mouse chow and given access to both food and water *ad libitum*.

Mice from both studies were sacrificed with an overdose of halothane and transcardially perfused with 4% paraformaldehyde (PFA). CNS tissues were collected for histological examination. Fixed brains and spinal cords from all mice were transferred to a 30% sucrose/PBS solution overnight and then frozen and stored at −80 °C until sectioning. All brain/cord tissue blocks were mounted in Tissue-Tek optimum cutting temperature (O.C.T) compound (Sakura, Zoeterwoude, Netherlands), and then sectioned by cryostat into 30 μm coronal slices. Spinal cords were sectioned at 25 μm in the transverse plane. The sections were cryoprotected in 30% ethylene glycol–20% glycerol–dibasic and monobasic sodium phosphate solution and kept frozen at −20 °C until use.

2.2. Adjuvants

Alhydrogel®, an aluminum hydroxide ($Al(OH)_3$) gel suspension, was used as a source of aluminum hydroxide. Alhydrogel is manufactured by Superfos Biosector a/s (Denmark) and was purchased from SIGMA Canada.

2.2.1. Doses—To calculate approximate human dosages of aluminum hydroxide for our experiments, we used the following information: The AVA vaccine for human use is made by Bioport Corporation, of Lansing, Michigan. According to product data sheets from the Michigan Biologic Products Institute (MBPI, Lansing, Michigan, USA; Bioport's predecessor), a single dose of AVA vaccine contains 2.4 mg of aluminum hydroxide (equivalent to 0.83 mg aluminum). Based on an assumed average human body weight of 70–80 kg, the amount per

kg body weight would be approximately 30–34 μg/kg. Soldiers or civilians receiving the vaccine would have received between 30–34 μg/kg (1 injection) and up to approx. 200 μg/kg if six injections were received.

The adjuvant injections in the treated mice were calibrated based on average animal weight for both experiments. At 3-month-old male CD-1 mice weigh approx. 35 g; at 9 months, the weight is approx. 50 g. In Experiment 1, we performed two injections of a suspension of aluminum hydroxide of (50 μg/kg) in a total volume of 200 μL sterile PBS (0.9%) spaced 2 weeks apart. The mice in this experiment would therefore have received 100 μg/kg versus a probable 68 μg/kg in humans. In Experiment 2, mice received six injections for a total of 300 μg/kg aluminum hydroxide over 2 weeks. Controls in both studies were injected with 200 μL PBS.

The injection site for human administration is typically subcutaneous over the deltoid muscle. For injections in mice we used a subcutaneous injection into the loose skin behind the neck (the “scruff”) to minimize discomfort and for ease of injection.

2.3. Behavioural tests

In the first study, mice were subjected at regular intervals to specific behavioral tests of motor and cognitive function, including wire mesh hang (2×/week), open field (1×/week), and water maze (1×/week) over a 6 months post injection period (see [22]). The order in which the animals were tested was randomized for each trial. In the second study, we conducted a more detailed behavioural examination based on the automated EthoVision system (Noldus Information Technology, Seattle, WA) employing a video camera and tracking software (Noldus EthoVision® 3.1). Individual movements of the mice were tracked for 5 min in an open field at weekly intervals. The software allowed for quantitative measurements of a variety of motor functions, including distance moved, percentage of time moving, velocity, and a variety of others. These latter experiments continued for 28 weeks following the last injections.

2.4. Histological measurements (Experiment 1)

2.4.1. NeuN and active caspase-3—As cited in Petrik et al. [8], five mice were used from each treatment group. In each, multiple brain ($n = 3$) and spinal cord ($n = 8$) sections at different levels were examined. Fluorescent intensity levels of NeuN and activated caspase-3 were used to identify neurons and cells dying by apoptosis, respectively. Regions of interest were defined using landmarks from mouse brain and spinal cord stereotaxic atlases [23,24]. All sections were counted in an unbiased manner under a 40× objective.

2.4.2. Choline acetyltransferase (ChAT) and Glial fibrillary acidic protein (GFAP) —As cited in Petrik et al. [8], the ChAT antibody was used to identify cholinergic motor neurons in the brain and spinal cord [25,26]. GFAP was used to label reactive astrocytes [27, 28].

2.4.3. Iba-1—A rabbit polyclonal antibody against the ionized calcium binding adapter molecule (Iba-1) (Wako, Richmond, VA, USA) was used to stain for activated microglia [29]. For Iba-1 fluorescent immunolableling, staining followed the same protocol used for GFAP labeling except for the following modification: Sections were incubated with primary rabbit-anit-Iba-1 (in PBST with 1%NGS + 1%BSA; 1:1000 dilution) overnight at 4 °C. Sections were then incubated in anti-rabbit AlexaFluor 546™ secondary antibody for 2 h at room temperature (Molecular Probes; Eugene, OR, 1:200).

2.4.4. Morin (3,5,7,2′,4′-pentahydroxyflavone, BDH)—Morin (M4008-2G, Sigma) is a fluorochrome which forms a fluorescent complex with aluminum fluorescing green (with an

excitation wavelength of 420 nm) [15,30] when it does so. The aluminum-Morin fluorescence assay was used for the visualization and detection of aluminum in lumbar spinal cord and other CNS tissues in the present experiments. The Morin stain was used as a 0.2% solution in 85% ethyl alcohol containing 0.5% acetic acid. All mounted sections were first washed with PBS twice for 5 min. Sections were then pretreated for 10 min in a 1% aqueous solution of hydrochloric acid, rinsed in double distilled water (ddH_2O) twice for 5 min, and immersed in 0.2% Morin stain for 10 min. The sections were then washed in ddH_2O twice for 5 min, dehydrated in 70%, 90%, and 100% ethyl alcohol (EtOH), and cleared with 100% xylene. All sections were then mounted using Vectashield mounting medium (Vector Laboratories), sealed with clear nail polish, and allowed to air dry.

2.4.5. Staining for hyper-phosphorylated tau protein—Hyper-phosphorylated tau (Anti-Human PHF-Tau, Pierce Biotechnology, Inc., Rockford, IL) labeling was determined using the non-fluorescent diaminobenzidine (DAB) method. Slides containing mounted sections of lumbar spinal cord were first rinsed twice PBS (2× 5 min) before performing antigen unmasking. Endogenous peroxidase activity was quenched using 0.3% hydrogen peroxide in methanol for 20 min. The sections were rinsed twice in PBS (2× 5 min) before blocking at room temperature for 1 h in M.O.M. blocking reagent (M.O.M. Kit – peroxidase, cat # PK 2200, Vector Laboratoraties, Inc., Burlingame CA) followed by a quick rinse in PBS and a 5 min incubation in M.O.M. diluent solution. The primary PHF-Tau antibody was diluted 100× in M.O.M. diluent solution and incubation was conducted at room temperature for 1 h. After the primary antibody incubation step, the slides were rinsed twice in PBS, and then incubated in the M.O.M. biotinylated anti-mouse IgG reagent for 10 min. The sections were rinsed in PBS before incubating with the secondary antibody (Vectastain ABC Elite Kit, cat # PK-6101) for 1 h at room temperature followed by incubation in the Vectorstain ABC Elite Reagents for another 30 min. The slides were rinsed again in 1× PBS. Color development was achieved using the Vector ImmPACT™ DAB solution (cat # SK-4105). When the desired color was achieved, the slides were rinsed in ddH_2O for 5 min and counter-stained in 0.1% methyl green for 5 min. After counter-staining, the slides were rinsed briefly in ddH_2O, two changes of 95% ethanol and two changes of 100% ethanol. The slides were allowed to dry before they were mounted in Permount® (Fisher Scientific, Fair Lawn, NJ).

2.5. Microscopy

Brain and spinal cord sections processed with fluorescent antibodies or DAB were viewed with a Zeiss Axiovert 200 M (Carl Zeiss Canada Limited, Toronto, ON, Canada) microscope at 40× and 100× (under oil) magnification. DAPI (blue fluorescence) was viewed with a 359/461 nm absorption/emission filter. Alexa Fluor 546™ (red), and rabbit IgG DuoLuX™ (red) were viewed with 556 557/572 573 nm filter. FITC was viewed with a 490 494/520 525 nm filter. Brain and lumbar spinal cord sections for histology were chosen randomly for each group. When counting using 40× magnification two images were captured per spinal cord section: ventral left, ventral right. 40× images were 350 × 275 μm and 100× images were 50 × 115 μm. Images were captured using AxioVision 4.3 software.

2.6. Criteria for determination and quantification of labeled cells

For quantification, only cells that were in focus and completely within the field of view were counted. To eliminate the likelihood that the same cell would be counted twice, slices for each histological experiment were drawn from only one well of the collection dish to ensure that sections were at least 250 μm apart. Regions of interest for cell counts were defined using landmarks and reference points from mouse spinal cord and brain stereotaxic atlases [39,40]. In the spinal cord, only cells which were anterior to the central canal and deep apex where the grey and white matters meet were considered as part of the ventral horns; conversely, only cells which were posterior to the central canal and the posterior deep apex were considered as

part of the dorsal horns. These criteria applied regardless of the spinal segments examined. In the brain, only cells found within the corresponding brain structures were counted. All sections were counted in an unbiased manner (a code key was assigned to the animals for tracking purposes, but did not reveal the identity of treatment the animal was prescribed).

2.7. Statistics

Values for each mouse on the individual tasks and in the cell counts were used to calculate mean ± S.E.M. for each group and condition. Behavioral scores and cell counts were normalized to the mean value of controls. The means were compared using one- or two-way ANOVA (Statistica, Statsoft Inc., Tulsa, OK; GraphPad Prism, San Diego, CA).

3. Results

Unlike the Petrik et al. [8] study which showed a loss of ChAT positive motor neurons in the lumbar cord of aluminum hydroxide treated mice, there was no significant difference in ChAT labeling or motor neuron counts in either the cervical or thoracic spinal cord segments (Fig. 1A and B). However, the aluminum injected group showed a highly significant increase in the expression of GFAP positive astrocytes (70%) are the control group (listed as 100% for all graphs; Fig. 1C) in the cervical segment of spinal cord. These GFAP results mirrored the outcomes previously reported in lumbar cord.

Iba-1 labeling demonstrated significantly increased levels of actived microglia in the lumbar spinal cord of animals injected with aluminum (111%) compared to controls (Fig. 1E). Other levels of cord were not tested for microglia in the present study.

Only mice injected with aluminum hydroxide showed significantly increased Morin labeling of cells in lumbar spinal cord compared to the other groups (Fig. 2A–E). Similarly, only aluminum-injected mice showed the presence of abnormal tau protein in motor neurons in lumbar cord (Fig. 3). Other regions of the cord were not tested in the current studies for either Morin or tau protein.

The multiple aluminum hydroxide injections of experiment 2 showed profound effects on motor and other behaviours as shown in Figs. 4 and 5. Multiple aluminum injections produced significant behavioural outcomes including changes in locomotive behaviour, (Fig. 4) and induced memory deficits on water maze tasks (Fig. 5). Other behavioural measures including muscle strength and endurance as measured by the wire hang and motor coordination and balance as measured by rotarod were not significantly affected.

4. Discussion

The current results extend the preliminary results reported by Petrik et al. [8] by showing that microglial activation is part of the underlying pathology in the lumbar cord. These data add to those previously reported, i.e., the loss of motor and other neurons and the activation of reactive astrocytes. Taken together with the current data, the overall activation of a glial inflammatory response in lumbar cord suggests that this process is a key early stage of the pathological events leading to motor neuron death. This interpretation is supported by an absence of motor neuron loss and astrocyte activation in the other levels of the spinal cord observed in the present study. In ALS and in animal models of the disease, glial activation followed by motor neuron death often appears to proceed in sequential manner along the ventral neuraxis with the first signs of pathology appearing first in lumbar cord [31]. Given this, it seems possible that an examination of later time points would show pathological responses in the thoracic and cervical cord as well. Alternatively, the aluminum shown to be present in lumbar cord motor neurons may not

have reached these other spinal cord segments. Studies now in progress will determine if motor neurons in these other segments stain positively for aluminum.

The positive Morin staining in lumbar cord clearly demonstrates that post injection aluminum finds entry into this part of the nervous system. One possibility is that it does so by retrograde transport from muscles to motor neurons in particular segments. This seems unlikely given that our paradigm of injecting *subcutaneous* should not have targeted any particular spinal cord segment. Another possibility is that aluminum can enter the CNS in a systemic manner if it enters the circulatory system. Experiments in progress are designed to distinguish between these possibilities.

The presence of hyper-phosphorylated tau protein, one of the hallmarks of both Alzheimer's disease and ALS–PDC of Guam, in motor neurons in lumbar spinal cord clearly suggests that additional pathological processes associated with aluminum are occurring.

The behavioural outcomes in the second experiment reported here reinforce the pathological outcomes seen in the first studies. While the histological measurements from these studies are still pending, the extent of the behavioural deficits strongly suggests that we will observe widespread neuronal pathologies. The greater extent of the behavioural outcomes in this experiment may be related to the experimental paradigm that tripled the number of aluminum hydroxide injections.

Overall, the results reported here mirror previous work that has clearly demonstrated that aluminum, in both oral and injected forms, can be neurotoxic [15,16,32,33]. Potential toxic mechanisms of action for aluminum may include enhancement of inflammation (i.e., microgliosis) and the interference with cholinergic projections [34], reduced glucose utilization [33], defective phosphorylation-dephosphorylation reactions [35], altered rate of transmembrane diffusion and selective changes in saturable transport systems in the blood brain barrier (BBB [36], and oxidative damage on cellular processes by the inhibition of the glutathione redox cycle [37].

Given the above, it is not surprising that aluminum has been widely proposed as a factor in neurodegenerative diseases and has been found in association with degenerating neurons in specific CNS regions [38–41]. In animal studies, aluminum has been linked to the accumulation of tau protein and amyloid-beta protein and observed to induce neuronal apoptosis *in vivo* as well as *in vitro*[30]. Aluminum injected animals show severe anterograde degeneration of cholinergic terminals in cortex and hippocampus [42].

Aluminum in its adjuvant form can gain access to the CNS [42–44], however, oral administration of aluminum hydroxide gel does not appear to be neurotoxic in humans [45], although aluminum chloride is, in rats [46]. The route of exposure, and perhaps the form of aluminum, may be important factors that determine the potential for toxicity.

We speculate that the observed neurotoxic effects of aluminum hydroxide in the present study arise by both ‘direct’ and ‘indirect’ pathways, some of which are cited above. Direct toxicity refers to the physical presence (or close proximity) of aluminum and its potential for initiating cell death pathways. Accumulation of aluminum into the cytoplasm via cellular uptake mechanisms or diffusion could cause alterations in glutaminase and glutamine synthetase and easily alter the availability of the neurotransmitter glutamate [47]. Aluminum acting to induce abnormal tau protein accumulation could also increase neurofibrillary tangles and impair cellular transport mechanisms [48]. Outside the cell, aluminum could affect neurons by altering synapses. For example, aluminum has been shown to decrease the thickness of post-synaptic density, increase the width of the synaptic cleft, and increase the number of flat synapses [49]. Aluminum could also block voltage-activated calcium channels [50], augment the activity

of acetylcholinesterase [51], or interfere with synaptic transmission by merely accumulating in the synaptic cleft [52]. Aluminum can also induce apoptosis in astrocytes [53]. Since astrocytes are essential for maintaining neuronal health, any loss of astrocyte function could prove toxic to neurons. Indirect toxicity of aluminum could occur in various ways, including by activating various cytokines [54], releasing glutamate in an excitotoxic cascade, or by modifying various enzymatic pathways [55].

In addition to the above actions specifically on neural cells, aluminum might act indirectly by stimulating abnormal, generalized immune responses. This is, in fact, what adjuvants are placed in vaccines to do in the first place. Adjuvant neurotoxicity could thus be the result of an imbalanced immune response. Rook and Zumla [56] hypothesized that multiple vaccinations, stress, and the method of vaccination could lead to a shift in immune response [56,57]. Aluminum hydroxide has previously been shown to stimulate a Th2-cytokine response [9, 58].

While the current results and our previous study have demonstrated significant behavioural and neuropathological outcomes with aluminum hydroxide and some additionally significant outcomes due to a combination of adjuvants, it is important to recognize that these were achieved under *minimal* conditions. Table 1 summarizes aspects of human ALS and GWS symptoms compared with outcomes observed in aluminum-injected mice. The likelihood exists that a synergistic effect between adjuvants and other variables such as stress, multiple vaccinations, and exposure to other toxins likely occurs. A recent study examining some of these factors in combination showed that stress, vaccination, and pyridostigmine bromide (a carbamate anticholinesterase (AchE) inhibitor), may synergistically act on multiples stress-activated kinases in the brain to induce neurological impairments in GWS [59]. In addition, a genetic background in context to aluminum exposure may play a crucial role and may be an important area for future research.

The demonstration of neuropathological outcomes and behavioural deficits in aluminum hydroxide injected mice may provide some insight into the causes of not only GWS–ALS, but may open avenues of investigation into other neurological diseases.

Acknowledgments

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Abbreviations

chE	Anticholinesterase
ALS–PDC	Amyotrophic lateral sclerosis- parkinsonism dementia complex
AVA	Anthrax vaccine adsorbed
BSA	Bovine serum albumin
GFAP	Glial fibrillary acidic protein
ChAT	Choline acetyltransferase
GWS	Gulf War Syndrome
NGS	normal goat serum
OCT	Optimum cutting temperature
PBST	Phosphate buffer saline – Tween 20
PFA	Paraformaldehyde

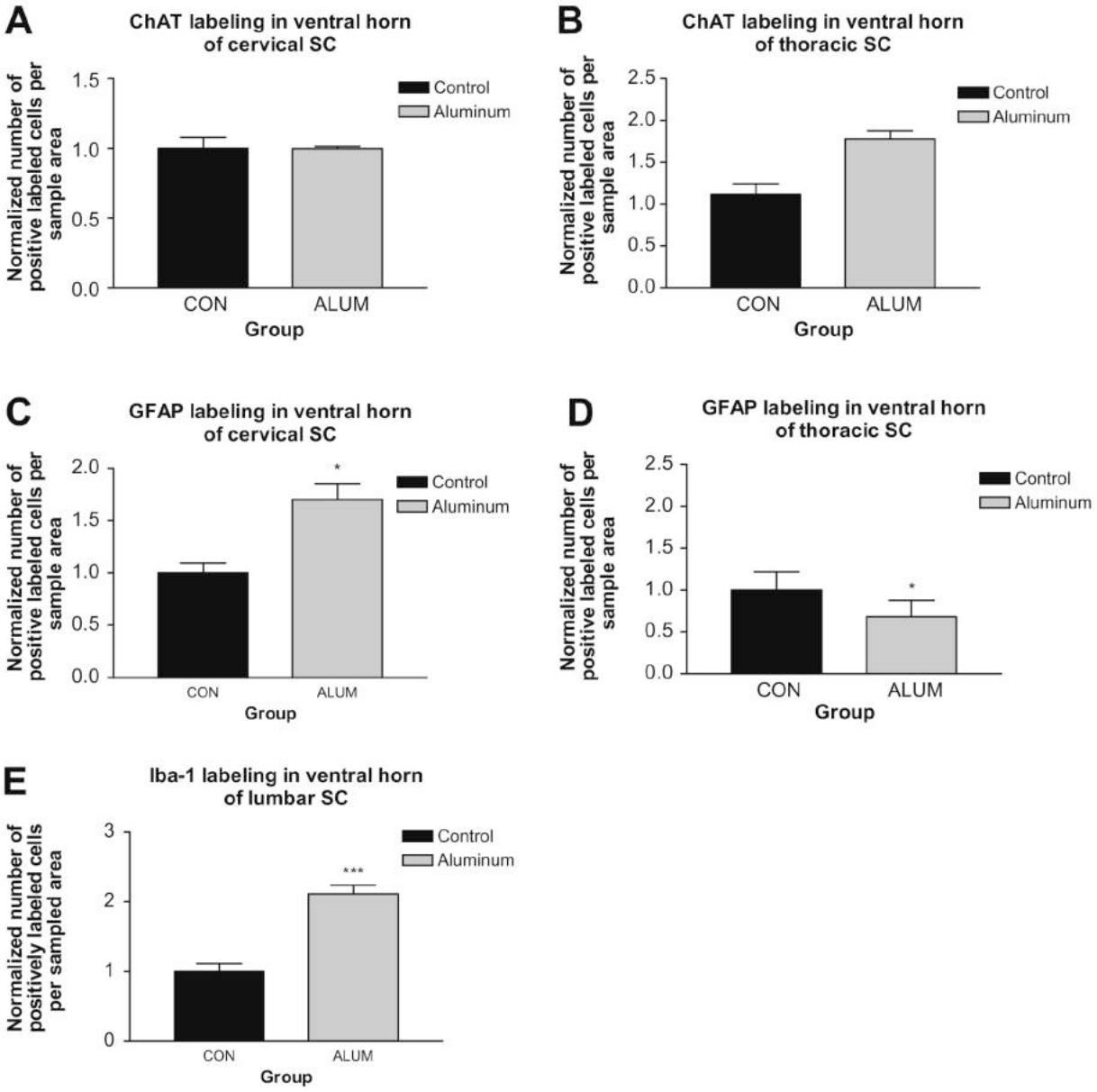

Fig. 1.

Impact of aluminum hydroxide on different levels of spinal cord (SC). (A and B) ChAT labeling in cervical and thoracic cords, respectively. (C and D) Normalized cell counts for GFAP labeling of reactive astrocytes in cervical and thoracic spinal cord, respectively. In cervical cord, the aluminum hydroxide treated groups showed higher levels of GFAP labeling with the aluminum alone group achieving statistical significance. (E) Iba-1 fluorescent labeling in the ventral horn of mouse lumbar cord showed that aluminum-injected mice had significantly increased numbers of activated microglia. Data are means ± S.E.M. ***$p < 0.001$, one-way ANOVA.

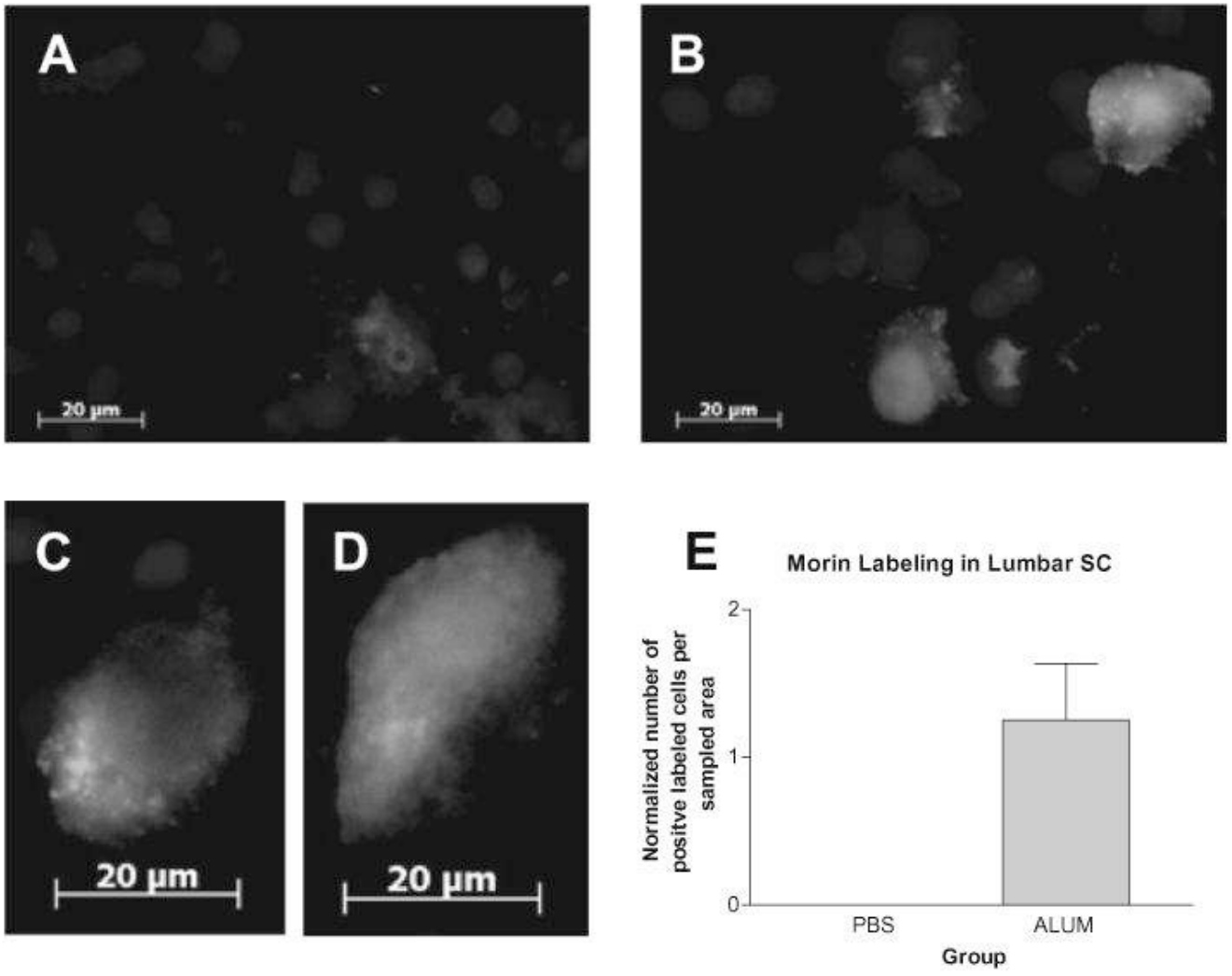

Fig. 2.

Morin fluorescent labeling in ventral horn of mouse lumbar spinal cord. Sections from control (A) mice showed no Morin fluorescent labeling. Scale bar = 20 µm. (B) Morin-positive motor neurons in aluminum hydroxide treated mice. (C and D) Higher power of motor neurons in aluminum-injected mice showing show high levels of cytoplasmic Morin labeling. Scale bar = 20 µm. (E) Cell counts for Morin positive cells in the different treatment groups (n = 4 mice/group, four sections each). Data are mean ± S.E.M. One-way ANOVA analysis revealed a significance level of $*p < 0.05$.

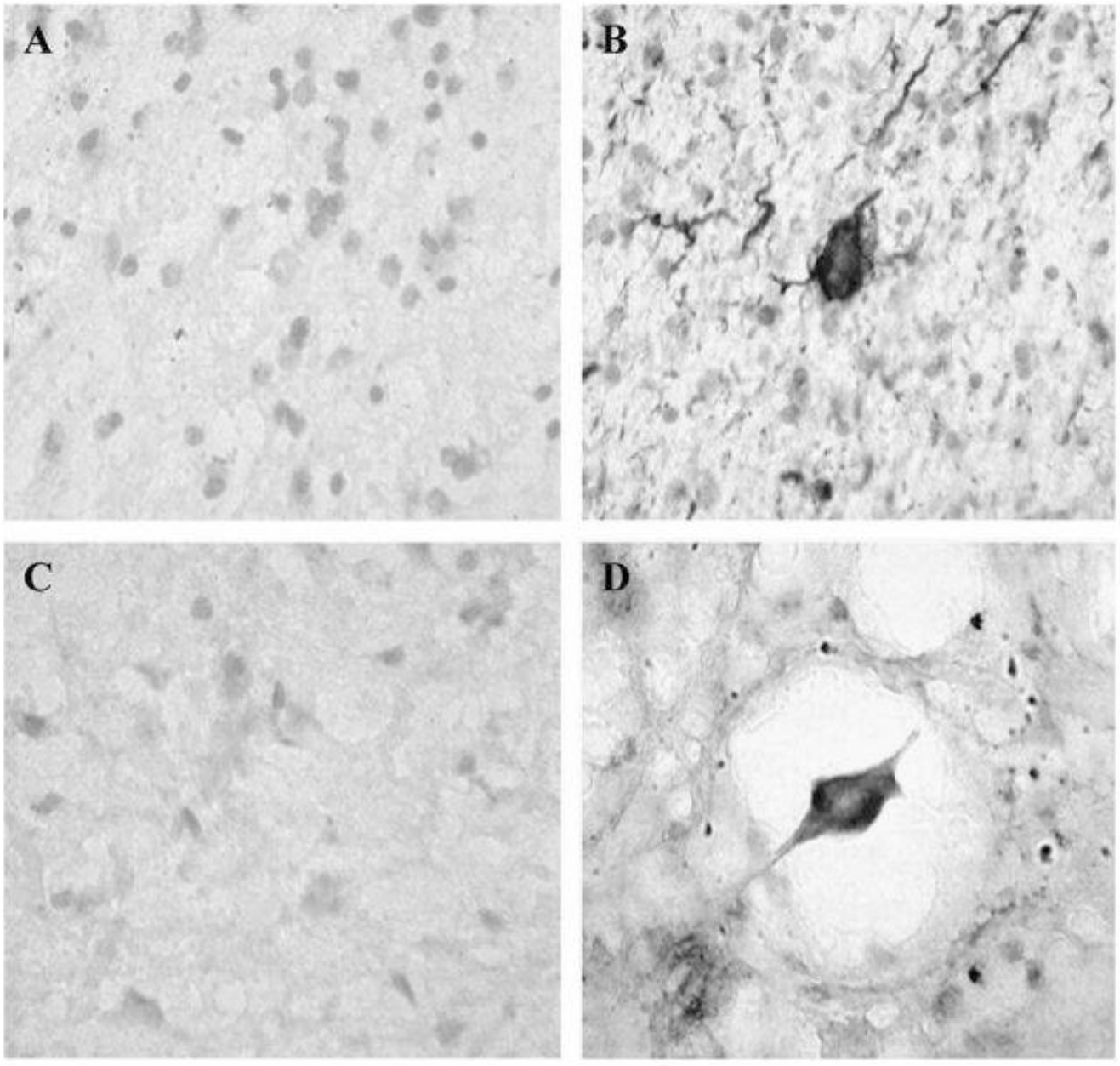

Fig. 3.

Hyper-phosphorylated tau immunostaining in the ventral horn of mouse lumbar spinal cord compared to Alzheimer's disease. (A) A section of human entorhinal cortex from a control patient. (B) Human entorhinal cortex section from a patient with Alzheimer's disease (sections kindly provided courtesy of Dr. P. McGeer). (C) Lumbar spinal cord sample from a saline injected mouse. (D) Equivalent section from a aluminum hydroxide injected mouse. All pictures are 100× magnification.

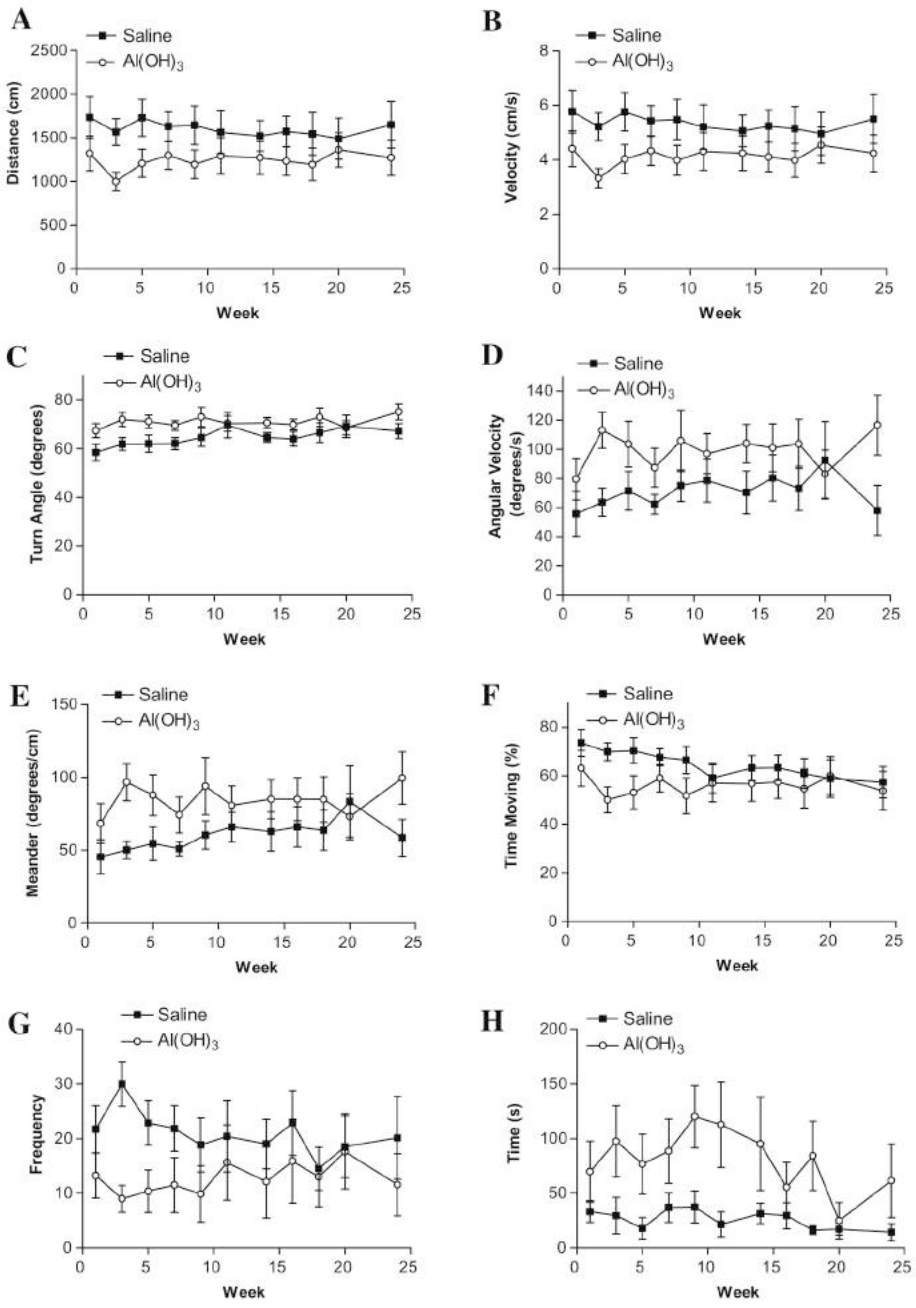

Fig. 4.

Open field movement analysis as an assessment of spontaneous activity and anxiety in control mice vs. mice injected six times with aluminum hydroxide. Aluminum hydroxide injected mice showed the following behavioural changes: (A) Shorter distances moved (***$p < 0.0001$). (B) Slower movement (***$p < 0.0001$). (C) Greater mean turn angle (***$p < 0.0001$). (D) More rapid turning (***$p < 0.0001$). (E) Greater meander (***$p < 0.0001$). (F) Smaller percentage of time in overall movement (**$p = 0.0030$). (G) Fewer entries into the centre of the open field (***$p < 0.001$). Late entry into centre (***$p < 0.0001$). (All measures, two-way ANOVA).

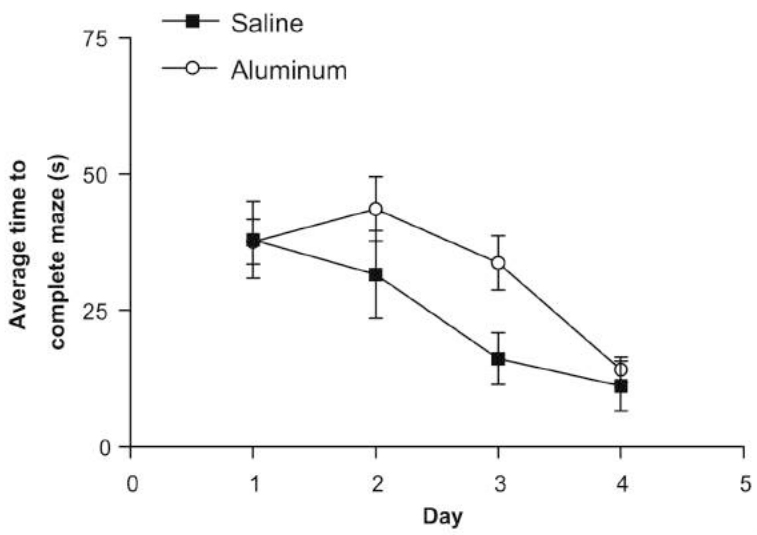

Fig. 5.

Water maze test as an evaluation of learning and memory. Mice injected 6× with aluminum hydroxide on average took significantly longer to complete the maze compared to saline injected mice (two-way ANOVA. *p = 0.0389).

Table 1

Summary of human ALS and GWI symptoms compared with symptoms observed in aluminum-treated mice and rats. This table also outlines the similarities between human ALS and Gulf War illness.

Animal	Age	Dose	Injection type	Result	Reference
Female NIH mice	4 week	315–335 μg/kg	i.p.	Significantly elevated levels of Al in brain	Redhead et al., 1991
Male and female Long Evan rats	2 month	100 or 300 mg/kg/day	Oral	Significantly reduced learning ability and elevated levels of Al in brain	Bilkei-Gorzo, 1993
Male Swiss albino mice	Not stated	~20 μg/kg/day	Oral	Significantly elevated levels of Al in brain, kidney and liver.	Sahin et al., 1994
Pzh:SFIS mice	Not stated	1.0 mg every 2 weeks or 0.1 mg 5 days/week	i.p.	Significantly elevated levels of Al in liver and tibia (bone), but not in brain.	Fiejka et al., 1996

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Administration of aluminium to neonatal mice in vaccine-relevant amounts is associated with adverse long term neurological outcomes

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Abstract

Our previous ecological studies of autism spectrum disorder (ASD) has demonstrated a correlation between increasing ASD rates and aluminium (Al) adjuvants in common use in paediatric vaccines in several Western countries. The correlation between ASD rate and Al adjuvant amounts appears to be dose-dependent and satisfies 8 of 9 Hill criteria for causality. We have now sought to provide an animal model to explore potential behavioural phenotypes and central nervous system (CNS) alterations using s.c. injections of Al hydroxide in early postnatal CD-1 mice of both sexes. Injections of a "high" and "low" Al adjuvant levels were designed to correlate to either the U.S. or Scandinavian paediatric vaccine schedules vs. control saline-injected mice. Both male and female mice in the "high Al" group showed significant weight gains following treatment up to sacrifice at 6 months of age. Male mice in the "high Al" group showed significant changes in light-dark box tests and in various measures of behaviour in an open field. Female mice showed significant changes in the light-dark box at both doses, but no significant changes in open field behaviours. These current data implicate Al injected in early postnatal life in some CNS alterations that may be relevant for a better understanding of the aetiology of ASD.

Keywords: Adjuvants; Aluminium; Autism; Neurodevelopmental disorders; Neurotoxicity; Vaccines.

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Biopersistence and brain translocation of aluminum adjuvants of vaccines

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Aluminum oxyhydroxide (alum) is a crystalline compound widely used as an immunological adjuvant of vaccines. Concerns linked to the use of alum particles emerged following recognition of their causative role in the so-called macrophagic myofasciitis (MMF) lesion detected in patients with myalgic encephalomyelitis/chronic fatigue/syndrome. MMF revealed an unexpectedly long-lasting biopersistence of alum within immune cells in presumably susceptible individuals, stressing the previous fundamental misconception of its biodisposition. We previously showed that poorly biodegradable aluminum-coated particles injected into muscle are promptly phagocytosed in muscle and the draining lymph nodes, and can disseminate within phagocytic cells throughout the body and slowly accumulate in brain. This strongly suggests that long-term adjuvant biopersistence within phagocytic cells is a prerequisite for slow brain translocation and delayed neurotoxicity. The understanding of basic mechanisms of particle biopersistence and brain translocation represents a major health challenge, since it could help to define susceptibility factors to develop chronic neurotoxic damage. Biopersistence of alum may be linked to its lysosome-destabilizing effect, which is likely due to direct crystal-induced rupture of phagolysosomal membranes. Macrophages that continuously perceive foreign particles in their cytosol will likely reiterate, with variable interindividual efficiency, a dedicated form of autophagy (xenophagy) until they dispose of alien materials. Successful compartmentalization of particles within double membrane autophagosomes and subsequent fusion with repaired and re-acidified lysosomes will expose alum to lysosomal acidic pH, the sole factor that can solubilize alum particles. Brain translocation of alum particles is linked to a Trojan horse mechanism previously described for infectious particles (HIV, HCV), that obeys to CCL2, signaling the major inflammatory monocyte chemoattractant.

Keywords: alum, vaccine adjuvants, macrophagic myofasciitis, neurotoxicity, genetics, monocytes, CCL2, MCP1

Billions of humans have been vaccinated and marked regression or eradication of several severe infectious diseases was observed. Nowadays, the potential applications of vaccines extend far beyond prevention of infectious diseases, and vaccination is considered to be a most promising weapon against a variety of different conditions. Vaccine safety has been regarded as excellent at the level of the population (1), but adverse effects have also been reported (2).

Concerns about the use of aluminum adjuvants have emerged following (i) recognition of their role at the origin of the so-called macrophagic myofasciitis (MMF) lesion in 2001 (3, 4), which revealed fundamental misconception of their adjuvant effect and pointed out their unexpectedly long-lasting biopersistence (4); and (ii) demonstration of their apparent capacity to migrate in lymphoid organs and then disseminate throughout the body within monocyte-lineage cells and progressively accumulate in the brain (5).

The present paper will review these emerging characteristics of alum adjuvant particles that raise concerns about innocuity of this widely used compound.

ALUM ADJUVANTS ARE LYSOSOME-DESTABILIZING PARTICULATE COMPOUNDS

Adjuvants have been used in vaccines for their ability to enhance the adaptive immune response to a co-administered antigen. Particulate aluminum salts (known as alum) have been the main approved adjuvants for use in human vaccines for more than 80 years (6). They are currently used in vaccines against tetanus, hepatitis A, hepatitis B, human papillomavirus, haemophilus influenzae B, pneumococcal and meningococcal infections, and anthrax. They mainly include aluminum oxyhydroxide, a crystalline compound, aluminum hydroxyphosphate, and amorphous aluminum phosphate. Alum is able to adsorb vaccine antigens on its surface. The strongest adsorption phenomenon results from ligand exchange, which involves the replacement of a surface hydroxyl on the adjuvant by a terminal phosphate group of the antigen (7).

Alum induces strong innate immune responses at the site of injection, as assessed by an influx of neutrophils, monocyte/macrophages, eosinophils, and MHC-II + antigen presenting cells, mainly dendritic cells (DCs) (8). Muscle-resident macrophages mainly located in fascias are among the first cells

to sense disturbance in muscle homeostasis (9). They alert the immune system through local production of chemokines, and recruit other myeloid cells, like neutrophils, and inflammatory monocytes that differentiate into inflammatory DCs (9). Specialized for antigen uptake, monocyte-derived inflammatory DCs have an immature phenotype in the muscle. However, they migrate to the lymph node T-cell paracortex upon contact with tissue debris or foreign material, and arrive there as mature cells expressing costimulatory molecules (10). Inflammatory DCs may be crucial for the alum adjuvant activity as assessed by selective depletion studies (11), but eosinophils also appear to play an important role (12).

Alum has been long believed to ensure a long-lasting immune response through formation of a depot slowly releasing the antigen under the influence of the interstitial fluid (13, 14). The view that the injected adjuvant remains extra-cellular has been challenged by muscle biopsy findings in immunized patients (4). In contrast to ancient belief, alum particles are avidly taken up by phagocytic cells (15). The strong binding of antigen to alum particles increases antigen uptake by DCs, reduces antigen degradation, and sustains antigen presentation *in vitro* (16). Macrophage survival may also be promoted by alum particle uptake (17). Alum injection induces *in vivo* the formation of persistent alum-induced granuloma at site of previous immunization (4, 18, 19). However, good immunization does not require local alum persistence, since no decrease of antigen-specific T- and B-cell responses were observed in case of removal of the injection site as early as 2 h after injection (20).

In spite of their long usage, the literature has pointed out that the adjuvanticity mechanisms of aluminum salts remain basically unknown despite most active investigation in the field in recent years (21, 22). Alum is deficient at initiating cell-mediated immunity and skews the immune response toward a T-helper type 2 (Th2) response associated with strong production of IL-4 and the IgG1 antibody subtype (23). Concerning the mechanisms of alum adjuvanticity, several explanations have been proposed, most of them being subsequently challenged (24). Notably, the NLRP3 inflammasome was shown to be strongly activated by alum (25, 26), but this finally appeared unessential to the adjuvant effect (27, 28). It remains true, however, that aluminum hydroxide and other crystals such as silica, urate sodium, and asbestos, strongly induce NLRP3 activation, IL1b release, and activation of the downstream inflammatory cascade. More recently, alternate models for alum-mediated immunity have been proposed on the basis of the link of alum adjuvant effects and the release of non-cytokine biomolecules, including uric acid (29), double-stranded DNA (30), and prostaglandin E2 (31). The specificity of crystal-induced signaling pathways has been proposed to explain why aluminum hydroxide particles exhibit a much more irritating effect than soluble aluminum (32). Consistently, alum crystals bind to and aggress the plasma membrane lipid bilayer (33), destabilizes lysosomes that degrade endocytosed, phagocytosed, or autophagocytosed materials (34, 35), and play important role in immunity. Highly controlled antigen processing functions of DCs use lysosomal proteases and pH changes optimal for the generation of peptides, rather than complete protein degradation (36). It is known that limitation of lysosomal proteolysis of antigenic proteins increases antigen presentation and immunogenicity (37), and that the stability of peptide:MHCII complexes allowing their accumulation on the DC surface is enhanced by lysosome activity inhibition (38). Alum adjuvant mechanisms may thus involve alum-induced blockade of lysosomes. Alum lysosomal destabilization remains still uncertain, but the physical rupture of the membrane may be directly caused by the crystalline structure of alum itself (39).

MMF IS A BIOMARKER ASSESSING LONG-TERM ALUM BIOPERSISTENCE IN A GIVEN INDIVIDUAL

In 1998, several French myopathologists described MMF as an emerging condition of unknown cause characterized by a pathognomonic lesion in muscle biopsy mixing large macrophages with submicron to micron-sized agglomerates of nanocrystals in their cytoplasm and lymphocytic infiltrates (3), distinct from other histiocytic diseases and always detected in the deltoid muscle of adults (40). Cytoplasmic inclusions were constantly found, surrounded or not by altered lysosomal membranes, and contained aluminum (4). Their crystalline structure was characteristic of aluminum hydroxide, and no exposure to aluminum other than that conferred by a prior immunization (100%) could be detected (4). It is now clear that the rapid emergence of MMF in France reflected the combination of (i) the replacement of the subcutaneous (s.c.) by the intramuscular (i.m.) route for vaccine injections in the early 1990s; (ii) the large-scale campaign of primo-vaccination of French adults against hepatitis B in the mid 1990s; and (iii) the preferential choice of the deltoid muscle for routine muscle biopsy in France, contrasting with the preferential use of the biceps brachialis and quadriceps muscles in other countries. Alum-containing vaccines may also induce skin pseudo-lymphoma in humans (41), and fibrosarcoma in cats (42).

Macrophagic myofasciitis has been reproduced experimentally by i.m. vaccination in mice, rats, and monkeys (4, 18, 19). The experimental lesion invariably shrinks over time (19), and, in monkeys, it begins to disappear completely from the muscle between 6 and 12 months after a DTP injection corresponding to 14- to 21-fold the human DTP-equivalent dose of alum (18).

Because of the unethical character of muscle biopsy in asymptomatic individuals, whether or not longstanding MMF may be commonly present in a hidden form in healthy individuals could not be directly determined. This seems very unlikely, however, as shown in a recent review of 130 consecutive deltoid muscle biopsies performed for diagnostic purposes in myalgic patients previously immunized with alum-containing vaccines. This study revealed that most alum receivers do not have long-lasting MMF. This could be reliably assessed whereas age, sex ratio, number of alum-adjuvanted injections, and delays elapsed from the last injection to deltoid muscle biopsy were similar in the MMF and non-MMF groups (43). This refutes non-documented belief that every vaccinee may have long-standing MMF lesions when biopsy is performed in the deltoid muscle (44). In addition, MMF and non-MMF patients had clinical differences as developed below.

In light of experimental models, it is important to check the individual vaccine record in each patient to assess the "unusually persistent" character of MMF. In a recent evaluation of 583 patients collected from 1994 to 2012 (45), the median time elapsed between the last alum administration and the biopsy was

65 months. Compared to our previous reports, this time had gradually increased from 36 months in 2001, i.e., shortly after the peak of French adult immunization, to 53 months in 2003 (46). An average number of 5.3 alum-containing shots had been administered during the 10 years prior to biopsy detecting MMF, mainly corresponding to vaccinations against hepatitis B (89.7%), tetanus (42.2%), and hepatitis A (8.8%). In practice, we consider that the MMF is unusually persistent when time elapsed from last immunization to the MMF detection exceeds 18 months. It is important to consider this point in young children who receive multiple vaccine injections in the first year of life, thus increasing the risk of coincidental association between a constitutive muscle disease and MMF detected in the quadriceps muscle used for pediatric immunizations. If the risk of such coincidental associations also potentially exists in adults, it is low in practice. For example, adult patients combining MMF and hereditary muscle disease is extremely rare, despite the intense immunization program of patients with muscular dystrophy.

Animal studies indicate that alum-induced granulomatous lesions considerably vary in size according to the genetic background (19), and the initial hypothesis made by WHO that MMF may reflect some individual inability to clear out alum from the body remains valid (47). In summary, the long-lasting MMF lesion should be considered as a biomarker assessing unusually long-term biopersistence of alum in affected individuals.

PATIENTS WITH MMF AT BIOPSY SUFFER MYALGIC ENCEPHALOMYELITIS/CHRONIC FATIGUE SYNDROME

Macrophagic myofasciitis is typically detected in patients with diffuse myalgias and chronic fatigue, as shown in both the French series (46) and the recently published series of 16 patients (48).

In both series, most patients are women (70–80%) with a mean age of 45 years at the time of the biopsy, that typically complain of myalgias, with or without arthralgia, and disabling chronic fatigue. The onset of these symptoms is typically delayed from the immunization.

Strong statistical association between myalgias and MMF was detected by general survey in different French neuromuscular centers (myalgias in 90% of patients with MMF vs. 44% without MMF, $p < 0.0001$) (4). Onset of myalgia may follow exercise. They usually begin in the lower limbs, and not at the site of previous immunization from 0.5 to 84 months in the French patients and 3 to 192 months in Portuguese patients. They gradually extend toward the top of the body, affect the paravertebral muscles, and become diffuse (46). Myopathic electromyogram and elevation of creatine kinase (CK) are, respectively, observed in less than half of patients. Comparison of myalgic vaccinees with and without MMF at deltoid muscle biopsy showed significant differences: patients with MMF rarely had fibromyalgia (the required 11 tender points of the ACR 1990 criteria for fibromyalgia present in 16.6 vs. 55.5%, $p < 0.04$), and more often had delayed evoked potentials suggestive of CNS demyelination (38.5 vs. 5.7%, $p < 0.01$) (43), which does not support coincidental association.

Chronic fatigue is another important symptom (48, 49). A case–control study conducted under the aegis of the French regulatory agency AFSSAPS yielded chronic fatigue as both significantly more frequent and more severe in patients with MMF compared to those without MMF in the deltoid muscle (http://ansm.sante.fr/var/ansm_site/storage/original/application/030593fa4e393af7cec8ff7092832215.pdf).

Cognitive alterations further assess CNS involvement that are disabling though often not detected by routine examination. Patients complain of memory loss, foggy brain, and mood changes. Cognitive tests almost constantly show alterations suggestive of organic cortico-subcortical impairment, impacting visual memory, working memory, and dichotic listening (50). These deficits usually remain stable with time (51).

Taken together, chronic muscle pain, chronic fatigue, and cognitive dysfunction are consistent with the so-called myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and about 50% of MMF patients meet international criteria for ME/CFS (48, 49). ME/CFS is a severe, complex, acquired illness classified as a neurological disorder in the WHO International Classification of Diseases since 1969 (ICD 10 G93.3), distinct from fibromyalgia and psychasthenia, which are classified as musculoskeletal (M79.7) and psychiatric (F48.8) disorders, respectively. International studies have estimated the prevalence of ME/CFS between 0.4 and 2.6% of the population, with a total annual cost burden to society of approximately $18.7–$24.0 billion in the USA (52). Symptoms of ME/CSF are closely similar to the post-infective chronic fatigue syndrome (53). The underlying cause of ME/CSF is currently unknown, but the illness is thought to be triggered by an abnormal immune response to an infectious or toxic agent, that results in chronic immune activation (54). Notably, ME/CFS patients have increased risk of developing diffuse large B-cell lymphoma and marginal zone B-cell lymphoma (55). Such a public health burden deserves continued efforts to investigate possible causes and to understand the pathological mechanisms of CFS.

PHAGOCYTES TRANSPORT ALUM PARTICLES TO THE LYMPHOID ORGANS AND THEN TO THE BRAIN

The conceptual link between long-term persistence of alum particles within macrophages at the site of previous immunization, and the occurrence of adverse systemic events, in particular neurological ones, has long remained an unsolved question. Aluminum has long been identified as a neurotoxic metal, affecting memory, cognition and psychomotor control, altering neurotransmission and synaptic activity, damaging the blood–brain barrier (BBB), exerting pro-oxidant effects, activating microglia and neuroinflammation, depressing the cerebral glucose metabolism and mitochondrial functions, interfering with transcriptional activity, and promoting beta-amyloid and neurofilament aggregation (56). In addition, alum particles impact the immune system through their adjuvant effect and by many other means. They adsorb vaccine antigens on their surface, which protect them from proteolysis thus forming a persistently immunogenic pseudo-pathogen (57). Alum particles may also bind undesirable residual products inherent to vaccine production procedures, as shown for HPV DNA sequences (58) or yeast proteins (59) that may be potentially hazardous (60). Finally, alum particles can directly induce allergy (61, 62) as other metals (63).

Concerns about long-term biopersistence of alum largely depend on the ability of alum particles to reach and exert toxicity in remote organs. This ability has been suggested by several

studies (64–67). The reference study on aluminum hydroxide biodisposition used isotopic ^{26}Al-enriched alum injected in the rabbit muscle: ^{26}Al was weakly eliminated in the urine (6% on day 28) and was detected in lymph nodes, spleen, liver, and brain (13). Whether ^{26}Al was still in particulate form or in soluble form was not explored. The fate of particulate material was explored in mice by our team. We successively performed i.m. injections of alum-containing vaccine, fluorescent latex beads, and fluorescent nanohybrids coated with precipitated alum (5). These materials were quickly captured by macrophages, a large proportion of which cleaved the injected muscle, mainly within immune cells, reaching the draining lymph nodes. Particle-laden cells then escaped the lymphatic system to reach the blood circulation, presumably via the thoracic duct. In so-doing, they were able to reach distant organs such as the spleen and liver and, much slowly, the brain. Recombinant chemokine injection and the use of genetically modified mice showed that systemic biodistribution of particles crucially depends on the monocyte chemoattractant MCP-1/CCL2. Into the brain, particles were mainly found in microglial cells. In accordance with good overall tolerance of alum, brain penetration was extremely low in normal conditions. However, brain translocation was significantly increased in case of altered BBB or after systemic and/or cerebral increase of the MCP-1/CCL2 signaling (5). Expression of this chemokine is subjected to significant interindividual variations related to age, genetic, and environmental factors. We have identified selective increase of circulating MCP-1/CCL2 in CFS/ME patients with MMF (45). The imbalance between the huge number of vaccinated individuals and the relatively low number of MMF cases suggests crucial involvement of individual susceptibility factors in intolerance to alum. Genetically driven MCP-1/CCL2 production might represent one of these factors (5).

Thus alum and other poorly biodegradable materials taken up at the periphery by phagocytes circulate in the lymphatic and blood circulation and can enter the brain using a Trojan horse mechanism similar to that used by infectious particles (68, 69). Previous experiments have shown that alum administration can cause CNS dysfunction and damage (70–72), casting doubts on the exact level of alum safety (73).

THE CONCEPT OF ASIA

Many CNS diseases likely result from gene–environment interactions. Some of them, such as idiopathic ME/SFC (74) and multiple sclerosis (MS) (75), have been previously associated with aluminum overload. An increased risk of developing MS in the long-term after alum-containing vaccine administration has been also reported (76, 77), and remains the subject of fierce debate.

Notably, about 10% of our MMF patients had concurrent MS-like disease (78), an additional 5–10% had another autoimmune disease, such as thyroiditis and diffuse inflammatory myopathies, and the remaining patients occasionally had low titers of various autoantibodies (46).

Yehuda Shoenfeld had delineated the "autoimmune (autoinflammatory) syndrome induced by adjuvants" (ASIA)(79), acknowledging that various combinations of (i) specific autoimmune diseases identified by well-established criteria, (ii) less-specific symptoms, such as myalgia, arthralgia, chronic fatigue, and cognitive impairment (the combination of which defines ME/CFS); and (iii) the appearance of circulating autoantibodies, can occur after exposure to a variety of chemical or natural products with immunological adjuvant properties. Discussion of the ASIA is very useful since it may alert physicians, when they encounter the above-mentioned symptoms, to check for prior vaccinations, and may help them to put a name on such conditions.

Symptoms associated with MMF are strikingly similar to those described as the Gulf war syndrome (GWS), a condition strongly associated with the administration of multiple vaccinations to soldiers (80, 81), especially the anthrax vaccine that contains alum, capable of inducing MMF (82), and possibly squalene (83). On these grounds, we proposed to delineate a vaccine adjuvant syndrome (84). Yehuda Shoenfeld reasoned similarly but added to GWS and MMF, his own experience on siliconosis, a disease complex observed in patients with leaky breast silicone implants attributed to deleterious adjuvanticity of silicone particles (85, 86). In so-doing, he enlarged the causal relationship to any compound with adjuvant properties. ASIA major and minor diagnostic criteria still need international validation but the ASIA concept already caught the attention of the international human and veterinary medical community, pointing out a need in the field (87, 88).

A LOT MUST BE DONE TO UNDERSTAND HOW, IN CERTAIN INDIVIDUALS, ALUM-CONTAINING VACCINES MAY BECOME INSIDIOUSLY UNSAFE

Alum has been used for decades to levels considered as an acceptable compromise between its role of adjuvant and its toxic effects by the industry and the regulatory agencies. However, the MMF story revealed several gaps in the knowledge on alum particles, including their exact mechanisms of action, their fate after injection, their systemic dissemination, and their safety on the long-term. Efforts have been done in the last years to develop novel adjuvants, but attempts to seriously examine safety concerns raised by the bio-persistent character and brain accumulation of alum particles have not been made.

The main questions that should be addressed concerning alum safety problems are listed in **Table 1**. It is important to look for genetic susceptibility factors that could explain why a given individual will appear intolerant to alum-containing vaccines whereas the vast majority of individuals vaccinated with the same vaccines remain healthy. Some patients with MMF are of the HLA-DRB1*01group, which is associated with an increased risk to develop autoimmune diseases (89). Genetic factors influencing alum biodistribution were also investigated. In keeping with experimental evidence that the CCL2/MCP-1 chemokine signaling governs brain translocation of phagocytosed particles (5), and that CCL2/MCP-1 serum levels are selectively increased in patients with MMF (45), genotyping of 252 symptomatic MMF patients and 516 healthy controls for 4 single nucleotide polymorphisms (SNPs) localized in the CCL2 gene showed that the AG haplotype of the SNP rs3760396C($-927G > C$) was associated with a slightly increased risk for disease (5). Interestingly, the rs 3760396 C allele is associated with a higher level of expression of CCL2 *in vitro* as assessed by transfection (90). These preliminary results deserve further investigations. Another axis of research consists in attempts to detect if subtle genetically determined defects in the

Table 1 | Main unsolved questions linked to alum adjuvants toxic effects.

WHAT IS THE MOST TOXIC?
Al^{3+} metal toxicity (or allergy to Al)
Particle toxicity due to elementary nanoparticles, e.g., mitochondrial toxicity, or to the micronic agglomerates they form, e.g., proinflammatory effects
Immune reactions against biopersistent biomolecules adsorbed on alum, and protected from degradation until complete particle solubilization (vaccine antigen or trace residual DNA sequences linked to vaccine production, or even self-antigens adsorbed on alum at time of injection-induced muscle necrosis)
WHAT FACTORS CONTRIBUTE TO BIOPERSISTENCE?
The quantity administered
Adsorbed molecules impeding extracellular solubilization and/or favoring phagocytosis of alum particles
Crystalline structure of the adjuvant damaging lipid bilayers (e.g., lysosomes)
WHAT FACTORS CONTRIBUTE TO BRAIN TRANSLOCATION?
Al^{3+} ion transport by transferrin (receptors present in CNS increase with iron deficiency)
Direct BBB damage by alum particles (proportion and kinetics in the circulation are unknown)
Monocyte cell transport of particles (the MCP1/CCL2-dependent Troian horse mechanism is increased in case of altered BBB and/or neuroinflammation)
WHAT ARE THE SUSCEPTIBILITY FACTORS?
Individual environment (other exposures to Al, exposure to other metals, exposure to other particles, chronic viral infection)
Age of immunization, including early age (low body weight, immature BBB, early neurodevelopmental stage) and old age (increased MCP-1/CCL2 production, progressive BBB weakness, hidden neuropathological processes)
Genetic factors impacting either immunologic responses (e.g., HLA genotypes) or intracellular persistence of particles (xeno/autophagy genes), or neuromigration (chimiokines and other inflammation genes)

cell machinery used to clear out particles, namely autophagy (91), could contribute to the long-standing biopersistence of alum particles, as previously reported to explain intracellular persistence of intestinal pathogens in Crohn's disease (92). Cells coping with microbes use a dedicated form of autophagy termed "xenophagy" as a host defense mechanism to engulf and degrade intracellular pathogens. The same holds true for inert particles subjected to phagocytosis/endocytosis (93). As mentioned above, crystal particles are likely toxic to membranes, which may destabilize phagosomes and lysosomes, trigger inflammasome assembly, and impede the autophagy pathways (32–35, 39). However, crystal particles instead of killing macrophages promote their survival (17). Thus, macrophages will continuously perceive as foreign particles in their cytosol, just like senescent organelles or bacteria, and will likely reiterate the autophagic process until they dispose of alien materials. The process includes compartmentalization of particles within double membrane autophagosomes and subsequent fusion with repaired and re-acidified lysosomes, exposing antigen-bound alum particles to lysosomal acidic pH, the sole factor that can solubilize alum crystal and acid hydrolases that will degrade the antigen. The process involves a conserved pathway in which particles decorated by ubiquitinated proteins, recruit the adaptor protein p62/SQSTM1 (sequestosome 1), which targets the whole to the autophagosome through binding to the autophagosomal membrane protein LC3/Atg8 (94, 95). Autophagosomes formation also involves other Atg molecules, such as the high molecular weight complex (Atg12–Atg5–Atg16L), Atg7, and many others, and is regulated by IRGM (immunity-related GTPase family-M1). The autophagosome external membrane eventually fuses with lysosomes. Genes of all molecules of the autophagy pathway are subjected to variations that are currently screened in patients with MMF.

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Aluminum in Childhood Vaccines Is Unsafe

Neil Z. Miller

ABSTRACT

Aluminum is a neurotoxin, yet infants and young children are repeatedly injected with aluminum adjuvants from multiple vaccines during critical periods of brain development. Numerous studies provide credible evidence that aluminum adversely affects important biological functions and may contribute to neurodegenerative and autoimmune disorders. It is impossible to predetermine which vaccinated babies will succumb to aluminum poisoning. Aluminum-free health options are needed.

Introduction

From 1999 through 2002, several vaccines containing mercury were phased out of the childhood immunization schedule. Manufacturing of childhood vaccines with thimerosal ceased in 2001, but those that were not past their expiration date remained on the market for sale until January 2003.[1] They were replaced with low-mercury or "thimerosal-free" vaccines. In the years that followed, autism rates continued to rise, prompting health authorities to assert that autism is not linked to mercury in vaccines and that vaccination policies are safe and appropriate.[2-4] (If mercury in vaccines contributed to autism, then rates should have dropped after mercury was removed.) However, in 2002, during this so-called phase-out period, the Centers for Disease Control and Prevention (CDC) actually added two doses of mercury-containing influenza vaccines to the list of inoculations urged for all babies 6 to 23 months of age.[5] Two years later, the CDC also added *pregnant women in their first trimester* to the list of people officially recommended and actively encouraged to receive influenza vaccines, even though a majority of available doses contained mercury.[6]

In addition to these questionable actions during this highly publicized "phase-out" of mercury, four doses of a new vaccine with high *aluminum* content were added to the childhood immunization schedule in February 2000 (for pneumococcus) and two doses of another aluminum-containing vaccine (for hepatitis A) were added in 2005.[7,8] These changes to the vaccine schedule resulted in a substantial increase of aluminum-containing vaccine doses—from 10 to 16 injections—that babies are still mandated to receive by 18 months of age.

Prior to the mercury phase-out (pre-2000), babies received 3,925 micrograms (mcg) of aluminum in their first year-and-a-half of life. After pneumococcal and hepatitis A vaccines were added to the immunization schedule, babies began receiving 4,925 mcg of aluminum during the same age period—a 25% increase (Figure 1).[9,10] In 2011, CDC recommended that pregnant women receive a pertussis vaccine (Tdap), which also contains aluminum.[11] Studies show that aluminum crosses the placenta and accumulates in fetal tissue.[12] Thus, millions of babies in utero, infants, and young children were injected with, and continue to receive, unnaturally high doses of neurotoxic substances—mercury and aluminum—long after unsuspecting parents were led to believe that vaccines were purified and made safe.

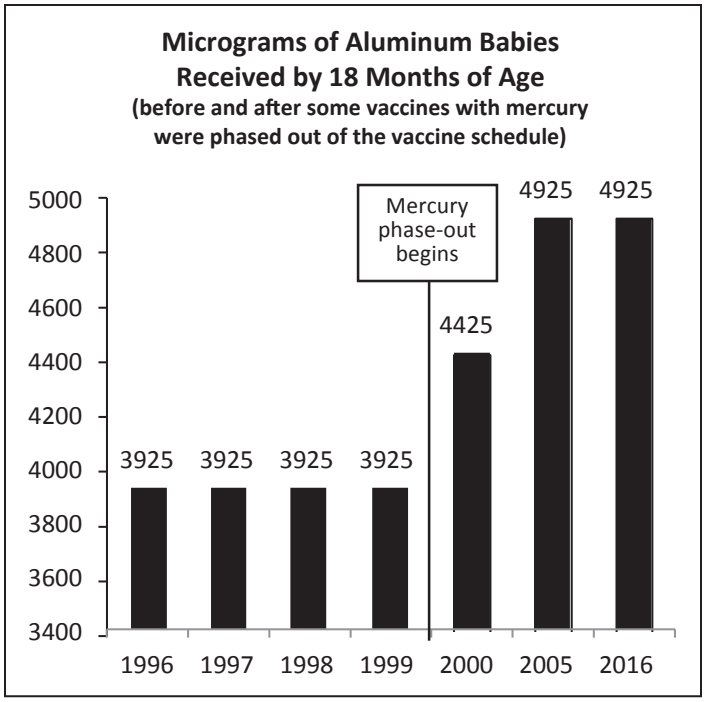

Figure 1. Aluminum Content from Childhood Vaccines

Vaccines containing aluminum were added to the childhood immunization schedule when some vaccines containing mercury were removed. Prior to the mercury phase-out (pre-2000), babies received 3,925 mcg of aluminum by 18 months of age. After pneumococcal and hepatitis A vaccines were added to the schedule, babies began receiving 4,925 mcg of aluminum during the same age period—a 25% increase.

Source: The vaccine manufacturers' product inserts and the CDC's annual childhood vaccination schedules.

Aluminum

Aluminum adjuvants are added to several vaccines to elicit a more robust immune response and increase vaccine efficacy. In the United States, Canada, Europe, Australia, and many other parts of the world, infants and young children receive high quantities of aluminum from multiple inoculations. For example, in the U.S. the hepatitis B, DTaP (for diphtheria, tetanus and pertussis), pneumococcal (PCV), *Haemophilus influenzae* type b (Hib), and hepatitis A vaccines are all administered during early childhood. Each of these

vaccines contains aluminum, and multiple doses (booster shots) are required (Table 1). Babies are injected with 1,225 mcg of aluminum instantaneously at age 2 months, and 4,925 mcg of accumulated aluminum by age 18 months (Figure 2).[9,10]

Table 1. Aluminum Exposures in Early Childhood from Recommended Vaccines

Vaccine	Aluminum Content	Vaccine Schedule
Hep B	250 mcg x 3 doses	Birth, 2, 6 months
DTaP	625 mcg x 4 doses	2, 4, 6, 15 months
PCV	125 mcg x 4 doses	2, 4, 6, 12 months
Hib	225 mcg x 3 doses	2, 4, 12 months
Hep A	250 mcg x 2 doses	12, 18 months

Source: The vaccine manufacturers' product inserts and the CDC's 2016 childhood vaccination schedule.

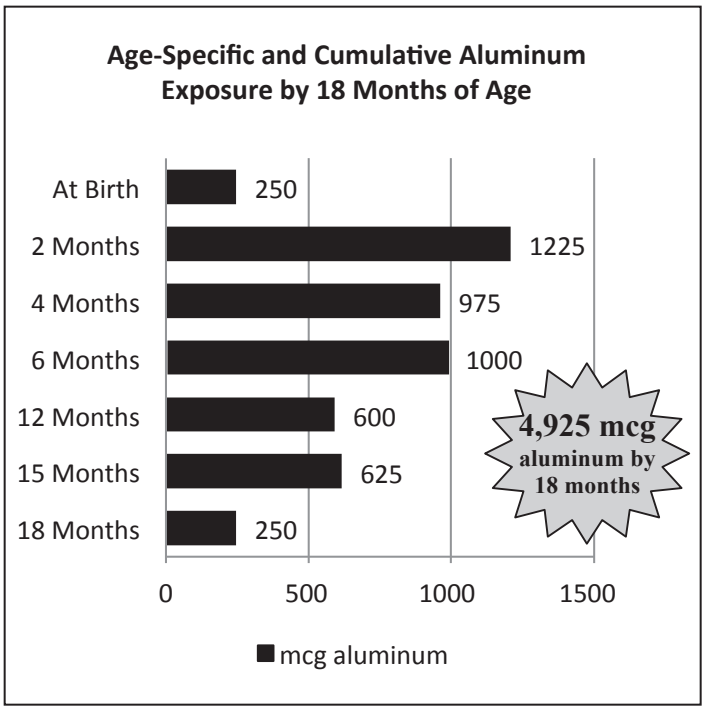

Figure 2. Cumulative Aluminum Exposure from Recommended Childhood Vaccines

Source: The vaccine manufacturers' product inserts and the CDC's 2016 childhood vaccination schedule.

Babies are not the only age group exposed to high quantities of aluminum from vaccines. The HPV vaccine (indicated for the prevention of cervical cancer and genital warts associated with some strains of human papillomavirus) is marketed to pre-teens and adolescents. Each dose in the three-dose series contains 500 mcg of aluminum. The Tdap vaccine (for tetanus, diphtheria, and pertussis) is given to pre-teens as well, and contains 390 mcg of aluminum.[13] Several adult vaccines also contain aluminum.

Aluminum is neurotoxic and has a long history of well-documented hazards.[14] For example, as early as 1921 The *Lancet* described a 46-year-old metal worker in whom "aluminium produced a rather slow intoxication. In this case it caused memory loss, tremor, jerky movements and incontinence of urine."[15] In 1927, Dr. Victor Vaughn, a toxicologist with the University of Michigan, testified before the Federal Trade Commission that "all salts of aluminum are poisonous when injected subcutaneously or intravenously."[16] By 1951, Chusid et al. showed that chronic epilepsy could be induced in monkeys through intra-cerebral administration of aluminum hydroxide cream.[17] In 1968, Driver et al. performed a similar experiment by placing aluminum hydroxide cream unilaterally on the posterior parietal cortex of six monkeys.[18] From 3 to 8 weeks after surgery, electrical abnormalities could be seen on an electroencephalogram and the monkeys exhibited "episodic twitching of the limbs and face." The animals were also impaired at learning new tasks and at re-learning tasks first learned prior to the intervention.

According to the American Academy of Pediatrics (AAP), "Aluminum is now being implicated as interfering with a variety of cellular and metabolic processes in the nervous system and in other tissues."[19] Bishop et al. published data showing that "aluminum accumulates in the body when protective gastrointestinal mechanisms are bypassed, renal function is impaired, and exposure is high."[20] For example, in premature infants, "prolonged intravenous feeding with solutions containing aluminum is associated with impaired neurologic development" by 18 months of age. More recently, Kawahara et al. published research confirming that "aluminum can cause severe health problems in particular populations, including infants."[21] The authors of this paper also declared that "whilst being environmentally abundant, aluminum is not essential for life. On the contrary, aluminum is a widely recognized neurotoxin that inhibits more than 200 biologically important functions and causes various adverse effects in plants, animals, and humans."

Neurologic and Autoimmune Disorders

Numerous studies provide compelling evidence that injected aluminum is detrimental to health. For example, a recent paper by Tomljenovic and Shaw affirmed that aluminum is a neurotoxin and may be a co-factor in several neurodegenerative disorders and diseases, including Alzheimer's, Parkinson's, multiple sclerosis, amyotrophic lateral sclerosis (ALS), autism, and epilepsy.[22] According to the authors, "The continued use of aluminum adjuvants in various vaccines for children as well as the general public may be of significant concern. In particular, aluminum presented in this form carries a risk for autoimmunity, long-term brain inflammation and associated neurological complications and may thus have profound and widespread adverse health consequences."

Recent data by Perricone et al. showed that aluminum adjuvants in vaccines have been linked to multiple sclerosis, systemic lupus erythematosus, chronic fatigue syndrome, Gulf War syndrome, macrophagic myofasciitis, arthritis, and autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome), an autoimmune disease with neurological and cognitive manifestations.[23] Clinical symptoms associated with vaccine-induced autoimmunity can take months or years to manifest, much longer than the time intervals utilized in most vaccine safety studies.

Although aluminum is a neurotoxin, pre-school children are repeatedly injected with aluminum adjuvants from multiple vaccines during critical periods of brain development. A recent paper published in the journal *Lupus* found that this may lead to neuro-developmental and autoimmune disorders.[24] During early development, the child's blood-brain barrier is more permeable to toxins, and the kidneys are less able to eliminate them. Thus, children have a greater risk than adults of adverse reactions to aluminum adjuvants in vaccines. The authors of this paper issued the following warning: "Because children may be most at risk of vaccine-induced complications, a rigorous evaluation of the vaccine-related adverse health impacts in the pediatric population is urgently needed."

Macrophagic Myofasciitis (MMF)

Some people develop macrophagic myofasciitis (MMF) after receiving an aluminum-containing vaccine.[25-39] MMF is characterized by an aluminum-filled lesion (wound) at the site of an earlier vaccination. MMF lesions occur when the aluminum adjuvant from a vaccine remains embedded in the muscle tissue and causes a continuous immune reaction. The lesions are persistent, long-term granulomas (or inflammatory tumors) found in the quadriceps in children and deltoid muscles of adults, common vaccination sites. Several vaccines contain aluminum hydroxide, which has been identified as the causal factor of MMF lesions.[25]

Although MMF is associated with a macrophagic lesion at the site of vaccination, it is a systemic ailment. Symptoms include chronic fatigue, chronic diffuse myalgia (muscle weakness), arthralgia (joint pain), and disabling headaches. Aluminum's toxic effects can also manifest as impaired psychomotor control, repetitive behavior, speech disorders, sleep disturbances, seizures, confusion, and anxiety, as well as deficits of concentration, learning, and memory. Nearly 20% of patients with MMF develop an autoimmune disease, including neuromuscular and multiple sclerosis-like demyelinating disorders.[26-28]

Several descriptive studies document MMF in pediatric populations. For example, Spanish scientists presented data on seven children younger than 3 years of age with lesions of macrophages on muscle biopsies at the site of vaccination.[29] In three of four cases tested, elevated levels of aluminum in muscle were detected (indicative of a reaction to aluminum adjuvants in vaccines). All of the children developed hypotonia (a lack of normal muscle tone) and motor or psychomotor delay. Six of the children also had abnormal neuro-imaging, associated with neurological anomalies, including atrophy and abnormal myelination.

In the U.S., Gruis et al. evaluated four cases of MMF in young children with hypotonia, motor delay and failure to thrive, likely due to intramuscular injections of aluminum-containing vaccines.[30] Another team of American physicians evaluated MMF in two fully vaccinated children. Both showed typical aluminum-filled macrophages at muscle biopsies.[31] One child had abnormal pupillary reflexes and urinary retention suggesting dysautonomia while the other child had developmental delay and hypotonia.

Israeli researchers documented MMF in six Arab children.[32] Reactions included hypotonia, seizures, motor delay, and developmental delay. The authors of this paper believe that genetic predisposition is a factor in determining the prevalence of MMF in different populations.

German researchers documented MMF in a 3-month-old East Indian child following his hepatitis B vaccine at birth, "after which he developed generalized hypotonia, and central nervous system and peripheral nervous system manifestations at one month of age."[33] The child also had respiratory failure, decreased spontaneous movements, apnea spells, and generalized seizures. Aluminum was detected in the muscle biopsy macrophages. The authors recommend that "after vaccination, children should be closely followed to detect these complications at early stages."

Italian researchers believe that MMF in children "is probably more common than reported. Diagnosis requires a high index of suspicion and can be missed if biopsy is performed outside the vaccination site."[34] According to Canadian MMF researchers, "aluminum has been demonstrated to impact the central nervous system at every level, including by changing gene expression. These outcomes should raise concerns about the increasing use of aluminum salts as vaccine adjuvants." Moreover, "based on the current and emerging literature, it seems unlikely that in the future aluminum will be considered safe for human use in any of the current medicinal applications."[28]

Animal Studies

A recent paper by Luján et al. found that sheep developed a new type of autoimmune and inflammatory disorder—ovine autoimmune/inflammatory syndrome induced by adjuvants (ASIA)—after receiving vaccines containing aluminum adjuvants.[40] The condition appears in some sheep two to six days after they are vaccinated. Symptoms of the acute phase include poor response to external stimuli and acute meningoencephalitis. The chronic phase causes muscular atrophy, neurodegeneration of the gray matter of the spinal cord, and death.

Khan et al. conducted several mouse experiments to determine the long-term biological distribution of vaccine-related aluminum nanoparticles.[41] They discovered that aluminum travels from the injection site to distant organs such as the spleen and brain, where aluminum deposits could still be detected one year later. Aluminum remains in monocyte-lineage cells long after vaccination and may cause neurologic and autoimmune disorders. According to these scientists, "Alum has high neurotoxic potential, and administration of continuously escalating doses of this poorly biodegradable adjuvant in the population should be carefully evaluated by regulatory agencies since the compound may be insidiously unsafe."

Scientists also looked at whether Gulf War Syndrome, which afflicted many veterans of Western militaries with cognitive and behavioral deficits similar to ALS (a progressive neurodegenerative disease that destroys nerve cells), could be related to the aluminum-containing anthrax vaccines they received. In a series of studies, mice were injected with adjuvants at doses equivalent to those given to vaccinated U.S. Gulf War veterans.[42,43] The aluminum-injected mice exhibited significant deficits in memory and motor functions. Testing showed motor neuron loss and progressive deficiencies in strength. The mice also had pathological abnormalities that are characteristic of neurological diseases such as Alzheimer's and dementia. According to the authors of these studies, "The demonstrated neurotoxicity of aluminum hydroxide and its relative ubiquity as an adjuvant suggest that greater scrutiny by the scientific community is warranted."[43]

Israeli scientists recently evaluated an aluminum adjuvant and the HPV vaccine Gardasil to determine behavioral and inflammatory effects.[44] Female mice were injected with either aluminum or Gardasil in amounts equivalent to human exposure, or they received a true placebo. (Vaccine safety trials for the HPV vaccine did not provide the control group with an inert substance or true placebo; the "control" group was injected with aluminum.) The Gardasil and aluminum-injected mice spent significantly more time exhibiting depressive behavior when compared to the placebo-injected mice. In addition, anti-HPV antibodies from the sera of Gardasil-injected mice showed cross-reactivity with the mouse brain protein extract. Analysis revealed microglial activation in the hippocampi of Gardasil-injected mice. According to the authors, "It appears that Gardasil via its aluminum adjuvant and HPV antigens has the ability to trigger neuroinflammation and autoimmune reactions, further leading to behavioral changes."

Autism

There is evidence that aluminum in vaccines may be linked to autism. For example, the *Journal of Inorganic Biochemistry* published data showing a highly significant positive linear correlation between the amount of aluminum infants receive from their vaccines and the rates of autism in several developed nations (Pearson r = 0.89-0.94).[45] The authors of this ecological study commented on their findings: "Our results...suggest that a causal relationship may exist between the amount of aluminum administered to preschool children at various ages through vaccination and the rising prevalence of autism spectrum disorders."

In another recently published paper, Shaw et al. found that genetic predispositions may sensitize some children to central nervous system damage induced by aluminum-containing pediatric vaccines.[46] Moreover, vaccines with aluminum adjuvants are *injected* into the body, bypassing protective barriers of the gastrointestinal tract and skin. Absorption of aluminum by this mode is more efficient than through ingestion, increasing the likelihood of a toxic outcome. The authors summarized their findings: "Evidence has now emerged showing that autism may in part result from early-life immune insults induced by environmental xenobiotics. One of the most common xenobiotic with immuno-stimulating as well as neurotoxic properties to which infants under two years of age are routinely exposed worldwide is the aluminum vaccine adjuvant."

Recent research published in the *Journal of Toxicology* found that aluminum exposure produces adverse effects in living organisms and is especially damaging to the central nervous system.[47] Aluminum from vaccine adjuvants crosses the blood-brain and blood-cerebrospinal fluid barriers, provoking harmful immuno-inflammatory responses in neural tissues. Yet, clinical studies on vaccine safety often give aluminum-containing injections to a "control" group as a harmless "placebo" despite evidence that aluminum is toxic to humans and animals. The use of aluminum as a placebo cannot be justified. According to the authors of this paper, "Studies on animal models and humans have shown that aluminum adjuvants by themselves cause autoimmune and inflammatory conditions. These findings plausibly implicate aluminum adjuvants in pediatric vaccines as causal factors contributing to increased rates of autism spectrum disorders in countries where multiple doses are almost universally administered."

In another recent animal study, young mice were injected with either high or low levels of aluminum adjuvants (designed to correlate with U.S. or Scandinavian childhood vaccine schedules).[48] Significant changes in the mice were observed, affirming the role of aluminum adjuvants in adversely altering the central nervous system. The authors commented on their findings: "These current data implicate aluminum injected in early postnatal life in some central nervous system alterations that may be relevant for a better understanding of the etiology of autism spectrum disorders."

Vaccine Industry Conferences and Concerns

In May 2000—3 months *after* the CDC added the aluminum-containing pneumococcal vaccine to the recommended immunization schedule for children—the U.S.

Department of Health and Human Services (HHS) sponsored a Workshop on Aluminum in Vaccines.[49,50] The workshop, given in San Juan, Puerto Rico, was attended by members of the vaccine industry, including government officials, immunologists, pathologists, vaccine manufacturers, metal ion specialists, and other interested people. It was organized to increase knowledge about aluminum as an adjuvant in vaccines, investigate potential adverse reactions associated with aluminum in vaccines, and develop a research agenda on the effect of aluminum in the human body. Experts from around the world were invited to give their presentations on vaccines and aluminum.

Dr. Romain Gherardi, a specialist in neuromuscular disease and professor at the Mondor Institute of Biomedical Research, showed that MMF without vaccination does not occur. In fact, it often begins after receiving a hepatitis B vaccine. Myalgia was present in 94% of patients with MMF, and 85% of these people were disabled. Although 30% of patients had their first myalgias within 3 months after their last vaccination, 20% of patients' symptoms took longer than 2 years to manifest. These myalgias begin in the calves and legs, then progress to diffuse myalgia. Fatigue was present in 93% of patients with MMF, and 87% of these people were disabled. In addition, 34% of MMF patients had autoimmune disease, including multiple sclerosis and arthritis.[50, pp 48-74]

In June 2000, the CDC sponsored a conference on thimerosal (mercury) in vaccines, although aluminum was discussed as well.[51] CDC scientists analyzed the agency's Vaccine Safety Datalink (VSD) database containing thousands of medical records of vaccinated children and found statistically significant relationships between mercury in vaccines and developmental delay, tics, and attention deficit disorder.[51, pp 40-41] However, Dr. Tom Verstraeten, CDC epidemiologist, analyzed the data and determined that the injuries could have been caused by aluminum in the vaccines.[51, p 77] It is also possible that the neurological damage was due to the synergistic effects of both aluminum and mercury in the vaccines given to the affected children.

Although millions of children every year are required to receive vaccines containing aluminum and mercury, evidence supporting the safety of this practice is lacking. For example, according to Dr. Richard Johnston, immunologist and professor of pediatrics at the University of Colorado School of Medicine, "Aluminum and mercury are often simultaneously administered to infants, both at the same site and at different sites. However...there is absolutely no data, including animal data, about the potential for synergy, additivity or antagonism, all of which can occur in binary metal mixtures."[51, p 20] Dr. Alison Maule, who attended the Workshop on Aluminum in Vaccines, voiced similar concerns: "We need to bear in mind that we are not only putting aluminum in here, we are putting in mercury.... Often these effects are additive but there is always the possibility of synergy. We know nothing about that."[50, p 106] Dr. Vito Caserta, chief medical officer for the Vaccine Injury Compensation Program, had this to say: "One of the things I learned at the aluminum conference in Puerto Rico…that I never really understood before, is the interactive effect of different metals when they are together in the same organism. It is not the same as when they are alone, and I think it would be foolish for us not to include aluminum as part of our thinking with this."[51, p 234] Dr. William Weil, pediatrician, former member of the National Institutes of Health, and representative for the AAP Committee on Environmental Health, was also present at the CDC conference and made his concerns known: "In relationship to aluminum, being a nephrologist for a long time, the potential for aluminum and central nervous system toxicity was well established by dialysis data. To think there isn't some possible problem here is unreal."[51, pp 24-25]

Some health authorities who oversee federal vaccine initiatives candidly acknowledge their limited understanding of metals—aluminum and mercury—that are added to several vaccines. For example, Dr. Martin Myers, director of the National Vaccine Program Office and host of the HHS-sponsored Workshop on Aluminum in Vaccines, made a frank admission: "Perhaps the most important thing that I took away from the last meeting was that those of us who deal with vaccines have really very little applicable background with metals and toxicological research."[49, pp 1-2] Dr. Neal Halsey, director of the Institute for Vaccine Safety, Johns Hopkins Bloomberg School of Public Health, and former member of the CDC's Advisory Committee on Immunization Practices (ACIP), was also present at the workshop on aluminum. He had concerns regarding missing data: "We do not seem to have information on the age-related toxicity of aluminum, especially when we are dealing with very young infants…. We do not know whether or not there is a difference in susceptibility by age, as there [is] with other metals."[50, pp 83-84]

Some health authorities seemed to admit that even if aluminum is dangerous, it would be burdensome to remove it. For example, according to Dr. John Clements with the World Health Organization's Expanded Programme on Immunization, "There are not easy and obvious substitutes to aluminum adjuvants…. The existing vaccines, if they change the adjuvant for any reason, would need to be resubmitted for clinical trials for safety and efficacy and it would take a great deal of time to do that."[50, p 75] Furthermore, "Aluminum is not perceived, I believe, by the public as a dangerous metal. Therefore, we are in a much more comfortable wicket in terms of defending its presence in vaccines."[49, p 64]

Note: In 2005, 5 years after conference attendees spoke out about a lack of data on the effects of mixing different metals in childhood vaccines, Dr. Boyd Haley, former professor of medicinal chemistry and chairman of the chemistry department at the University of Kentucky, published a study in which he investigated the effect of combining aluminum hydroxide with thimerosal.[52] In this study, cultured neurons showed no significant cell death six hours after they were exposed to just aluminum; more than 90% survived. Thimerosal alone also caused few neurons

to die after six hours of exposure. Again, more than 90% survived. However, when cultured neurons were exposed to aluminum and thimerosal, only about 40% survived after six hours, clearly demonstrating synergistic toxicity (Figure 3).

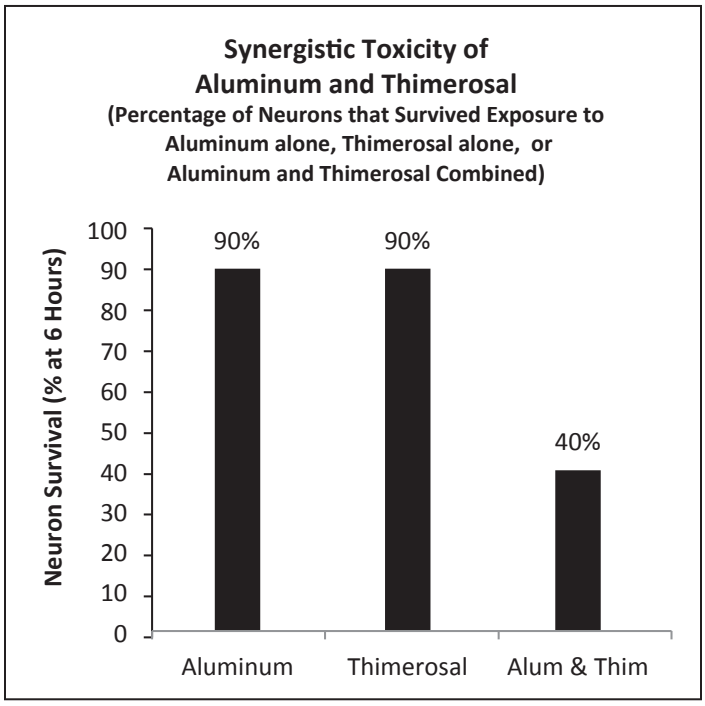

Figure 3. Survival of Neurons Exposed to Aluminum, Thimerosal, or Both

Unconvincing Evidence of Adjuvant Safety

Although several high-level representatives of the CDC, World Health Organization (WHO), American Academy of Pediatrics, Institute for Vaccine Safety, National Vaccine Program Office, and Vaccine Injury Compensation Program who attended the conferences on aluminum and thimerosal had serious concerns about the potential hazards associated with aluminum in vaccines, a conference report and workshop summary published in the journal *Vaccine* 2 years later declared that "the message from this conference for the global public should stress the safety of both these adjuvants and these vaccines," despite acknowledging that "we don't know" how aluminum adjuvants interact with the immune system and how it is processed by infants and children.[53] The conference report minimized risks by claiming that aluminum has been used as a vaccine adjuvant for more than 70 years and "has an established safety record with low incidence of reported adverse events." However, no one is warning vaccine recipients to consider the possibility that their adverse event could be related to aluminum in their vaccines nor encouraging them to report it to health authorities. Furthermore, research indicates that many people who have adverse reactions to aluminum-containing vaccines won't exhibit symptoms for several weeks, months, or years, so it's very difficult for vaccine recipients to recognize that the vaccines they received some time ago may be related to their current disabling autoimmune ailments.

A few years later, the FDA published a study, Mitkus et al., in which the authors concluded that "the benefits of using vaccines containing aluminum adjuvant outweigh any theoretical concerns."[54] This study is often cited as a confirmation that injecting babies with multiple doses of aluminum-containing vaccines is safe. However, there are major flaws in the FDA's analysis:

1. To determine an aluminum intake "minimal risk level" (MRL) for humans, a single animal study was used.[55] This study found that mice could receive up to 26 milligrams of aluminum per kilogram of body weight per day (26 mg/kg/day) with no adverse effects. After considering differences between mice and humans (and other factors), this number was reduced to create a margin of safety, and an MRL of 1 mg/kg/day was established for humans, including infants.[56] But there is a problem: 26 mg/kg/day is not a safe amount of aluminum for animals. Several studies confirm that animals are harmed by much lower quantities of aluminum—3.4 to 6.1 mg/kg/day—and at least three of these studies were published before the FDA paper in 2011, so the FDA study was fallacious at its inception.[57-60] Rats that were given just 6.1 mg/kg/day aluminum (30 mg/kg/day $AlCl_3$) needed significantly more repetitions to learn a maze when compared to a control group.[57] Rats that were given just 5.6 mg/kg/day aluminum (50 mg/kg/day $AlCl_3$-$6H_2O$) had significantly impaired spatial learning and memory abilities when compared to a control group. They also had cellular shrinking, plus behavioral, biochemical, and histological alterations.[58] Rats that were given just 3.4 mg/kg/day aluminum (17 mg/kg/day $AlCl_3$) "showed behavioral, biochemical, and histological changes similar to those associated with Alzheimer's disease."[60]

2. The MRL for humans is derived from dietary aluminum *fed* to mice. But infants are *injected* with aluminum. Injected aluminum bypasses the gastrointestinal tract and has unique toxic properties compared to aluminum that is ingested. To determine the safety of injected aluminum, scientists must conduct experiments with injected—not ingested—aluminum.

3. After vaccines containing aluminum adjuvants are injected into the body, aluminum nanoparticles can be transported by monocyte-lineage cells to draining lymph nodes, blood and spleen—and may also penetrate the brain.[41] Aluminum is unsafe even in trace quantities. For example, just 50 nanomolars of aluminum are sufficient to generate reactive oxygen species (ROS), or oxidative stress, in human primary neuronal-glial cell cultures and induce inflammatory gene expression.[61] In another study, just 10 nanomolars of aluminum increased C-reactive protein (CRP) levels four-fold, causing inflammation in human brain microvessel endothelial cells.[62] But the FDA assumes, without evidence, that these poorly biodegradable aluminum nanoparticles,

which have been detected in body organs up to a year after vaccination, are harmless, and they are not calculated by the FDA as part of the aluminum "body burden" until they dissolve.

4. The "retention function for aluminum," a mathematical equation that the FDA used to help estimate levels of aluminum in infants, was derived from data on only one person, an adult (rather than from numerous infants), and an estimate on the rate of absorption of aluminum hydroxide following injection was based on data from just two rabbits.

The FDA paper also falsely claimed that "occasional irritation (dermal) at the site of injection is the only adverse effect that has been reported in the published literature" following injections of aluminum-containing vaccines. And the clinical symptoms in patients diagnosed with MMF "are considered to be due to separate, coincidental immune or neurological disorders that are unrelated to the presence of aluminum in vaccines."[54] The Global Advisory Committee on Vaccine Safety, established by WHO, welcomed the FDA's analysis endorsing the safety of aluminum in vaccines.[63] The CDC vigorously defends the presence of aluminum in vaccines as well.[64] Clearly, FDA, CDC, and WHO agree on continuing indefinitely with their current policies of injecting babies with multiple doses of aluminum-containing vaccines.

Aluminum Toxicity Acknowledged for Parenteral Nutrition

Although the FDA's recent paper advocates the continued use of aluminum in childhood vaccines, FDA has known for many years that aluminum can be dangerous. For example, some infants require parenteral nourishment (administered by intravenous injection). All parenteral nutritional formulas contain aluminum. According to the FDA, "when medication and nutrition are administered orally, the gastrointestinal tract acts as an efficient barrier to the absorption of aluminum, and relatively little ingested aluminum actually reaches body tissues. However, parenterally administered drug products containing aluminum bypass the protective mechanism of the gastrointestinal tract and aluminum circulates and is deposited in human tissues."[65]

In a 1997 study published in the *New England Journal of Medicine*, scientists assessed 182 infants who received intravenous injections of nutritional formula that contained differing quantities of aluminum.[20] They calculated that infants who received aluminum at greater than 4 to 5 mcg/kg/day would lose 1 point per day on the Bayley Mental Development Index ($p = 0.03$). Babies who score low on this test are at risk for subsequent developmental and educational problems. This study contributed to FDA's decision to set limits on aluminum content in parenteral drug products and require warning labels on the package inserts—safety measures that were never required with aluminum-containing vaccines. In the Code of Federal Regulations, Title 21, published in the Federal Register, aluminum toxicity levels are revealed:

> WARNING: This product contains aluminum that may be toxic.... Research indicates that patients with impaired kidney function, including premature neonates, who receive [injections] of aluminum at greater than 4 to 5 mcg per kilogram of body weight per day, accumulate aluminum at levels associated with central nervous system and bone toxicity. Tissue loading may occur at even lower rates.[66]

This means that for a 6-pound baby with impaired kidney function, 11-14 mcg of injected aluminum would be toxic. The hepatitis B vaccine given at birth contains 250 mcg of aluminum—20 times higher than safety levels indicated for preemies. Babies weigh about 12 pounds at two months of age when they are injected with 1,225 mcg of aluminum from their CDC-recommended vaccines—50 times higher than safety levels for preemies.

Healthy babies may be able to handle quantities of aluminum above FDA toxicity levels indicated for patients with impaired kidney function. However, no one knows how much more aluminum is safe because adequate studies were never conducted. In addition, babies are not screened for renal function prior to vaccination. Therefore, it is impossible to know ahead of time which babies will succumb to aluminum poisoning. Instead, parents are expected to play Russian roulette with their children.

Summary

Aluminum adjuvants are added to several vaccines to elicit a more robust immune response and increase vaccine efficacy. Infants and young children throughout the world receive high quantities of aluminum from multiple inoculations. Incremental changes to the vaccination schedule during the past several years significantly increased the quantity of aluminum in childhood shots. Numerous studies provide compelling evidence that injected aluminum can be detrimental to health. Aluminum is capable of remaining in cells long after vaccination and may cause neurologic and autoimmune disorders. During early development, the child's brain is more susceptible to toxins and the kidneys are less able to eliminate them. Thus, children have a greater risk than adults of adverse reactions to aluminum in vaccines.

Millions of children every year are injected with vaccines containing mercury and aluminum despite well-established experimental evidence of the potential for additive or synergistic toxicity when an organism is exposed to two or more toxic metals. Dr. Haley's study in which cultured neurons died at an accelerated rate following concurrent exposure to aluminum and thimerosal provides evidence of an enhanced detrimental effect. In addition, aluminum toxicity levels published by FDA indicate that two-month-old babies who are vaccinated according to CDC guidelines may

be receiving quantities of aluminum that are significantly higher than safety levels.

Conclusion

Toxic metals such as aluminum do not belong in prophylactic medications administered to children, teenagers, or adults. Vaccines are normally recommended for healthy people, so safety (and efficacy) standards must be impeccable. Parents, especially, should not be compelled to permit their loved ones to receive multiple injections of toxic metals that could increase their risk of neurodevelopmental and autoimmune ailments. Safe alternatives to current disease prevention technologies are urgently needed.

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Fetal Bovine Serum

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The use of fetal bovine serum: ethical or scientific problem?

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Abstract

Fetal bovine serum (FBS) is a common component of animal cell culture media. It is harvested from bovine fetuses taken from pregnant cows during slaughter. FBS is commonly harvested by means of a cardiac puncture without any form of anaesthesia. Fetuses are probably exposed to pain and/or discomfort, so the current practice of fetal blood harvesting is inhumane. Apart from moral concerns, several scientific and technical problems exist with regard to the use of FBS in cell culture. Efforts should be made to reduce the use of FBS or, preferably, to replace it with synthetic alternatives.

Glyphosate

Glyphosate pathways to modern diseases VI: Prions, amyloidoses and autoimmune neurological diseases

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Usage of the herbicide glyphosate on core crops in the USA has increased exponentially over the past two decades, in step with the exponential increase in autoimmune diseases including autism, multiple sclerosis, inflammatory bowel disease, type 1 diabetes, coeliac disease, neuromyelitis optica and many others. In this paper we explain how glyphosate, acting as a non-coding amino acid analogue of glycine, could erroneously be integrated with or incorporated into protein synthesis in place of glycine, producing a defective product that resists proteolysis. Whether produced by a microbe or present in a food source, such a peptide could lead to autoimmune disease through molecular mimicry. We discuss similarities in other naturally produced disease-causing amino acid analogues, such as the herbicide glufosinate and the insecticide L-canavanine, and provide multiple examples of glycine-containing short peptides linked to autoimmune disease, particularly with respect to multiple sclerosis. Most disturbing is the presence of glyphosate in many popular vaccines including the measles, mumps and rubella (MMR) vaccine, which we have verified here for the first time. Contamination may come through bovine protein, bovine calf serum, bovine casein, egg protein and/or gelatin. Gelatin sourced from the skin and bones of pigs and cattle given glyphosate-contaminated feed contains the herbicide. Collagen, the principal component of gelatin, contains very high levels of glycine, as do the digestive enzymes: pepsin, trypsin and lipase. The live measles virus could produce glyphosate-containing haemagglutinin, which might induce an autoimmune attack on myelin basic protein, commonly observed in autism. Regulatory agencies urgently need to reconsider the risks associated with the indiscriminate use of glyphosate to control weeds.

Keywords: autism, autoimmune disease, collagen, glycine, glyphosate, multiple sclerosis, protein misfolding, vaccines

1. INTRODUCTION

At first glance, multiple sclerosis (MS) and autism appear to have little in common, aside from the fact that both are neurological diseases. Autism is a condition with prenatal or early childhood onset, characterized by repetitive behaviours, impaired social interaction and cognitive impairment. The male:female ratio for autism is 4:1, while multiple sclerosis is twice as common in women as in men; its first symptoms usually begin in early adulthood to involve impaired lower limb mobility, although in later stages it affects both mental and physical capabilities. Both conditions are, however, associated with inflammatory autoimmune features [1, 2], and both diseases are viewed as having an environmental and a genetic component [3–6].

A study comparing a population of 658 MS patients with the general population found an association between MS and increased rates of asthma, inflammatory bowel disease (IBD), type 1 diabetes mellitus, pernicious anaemia and autoimmune thyroid disease [7], all of which have also been linked to autism [8–11]. These conditions are all considered to be *autoimmune diseases*, which can be triggered through molecular mimicry, where an antibody responding to a foreign protein that resembles a native protein becomes sensitized to the native protein as well [12]. A paper by Shoenfeld and Aron-Maor in 2000 developed the argument that both autism and MS may be examples of an autoimmune reaction via mimicry following exposure to an antigenic stimulus, possibly from an infection or through vaccination [13]. They further propose specifically that myelin basic protein (MBP) and other proteins constituting the myelin sheath are attacked by the immune system in both autism and MS. This has been recognized by many others in autism [14, 15] and MS [16–20]. In 1982, Weizman et al. reported a cell-mediated autoimmune response to human MBP in 76% of the autistic children studied [16]. Immune sensitization to the myelin sheath proteins could arise either through mimicry as a consequence of exposure of the immune system to a foreign antigen with a similar peptide sequence that is

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resistant to clearance, or because the proteins themselves have been altered in some way that renders them defective, exposed and/or resistant to proteolysis.

Unlike DNA synthesis, protein synthesis is highly prone to error [21, 22]. It appears that biological systems have adopted a strategy of allowing coding errors to survive during active synthesis, but use protein misfolding as a criterion to mark a defective peptide for degradation and recycling through ubiquitination. It is estimated that 15% of average-length proteins will have at least one misincorporated amino acid. Typically, 10–15% of random substitutions disrupt protein function, mostly because of misfolding [22]. Such destabilization causes protein–protein aggregation, and can lead to multiple neurological diseases and amyloidoses. Drummond et al. propose that early-forming toxic oligomers of amyloidogenic proteins are enriched with missense errors [22].

Glyphosate is the active ingredient in the pervasive herbicide Roundup and in many other formulations of herbicides used to control weeds on agricultural, residential and public land worldwide. A recent study based in Germany involving 399 urine samples from adults not involved in agricultural work revealed glyphosate residues above the detection limit in the urine of 32% of the subjects, and residues of AMPA, a metabolite, in 40% [23]. In a paper published in 2014, Swanson et al. showed a remarkable correlation between the rising rate of glyphosate usage on corn (maize) and soy crops in the USA and an alarming rise in a number of different chronic diseases [24]. Additional strong correlations for other conditions and diseases are provided in two follow-on papers [25, 26]. While correlation does not necessarily mean causation, causation becomes much more likely if a plausible mechanism can be found. Swanson et al. found a remarkable 0.98 correlation coefficient between the rise in autism rates in the USA and the use of glyphosate on crops (P-value $\leq 9.6 \times 10^{-6}$). The correlation for multiple sclerosis was not as high, but still highly significant at 0.83 (P-value $\leq 1.1 \times 10^{-5}$). IBD had a correlation coefficient of 0.94 (P-value $\leq 7.1 \times 10^{-8}$) (see Table 1 for other diseases).

Table 1. Correlations between time trends in several diseases and conditions recorded by the US Centers for Disease Control (CDC) with glyphosate usage on corn (maize) and soy crops reported by the USDA. Data reproduced from [23] and [25].

Disease	Correlation coefficient (R)	P-value
Autism (prevalence)	0.98	9.6×10^{-6}
MS (deaths)	0.83	1.1×10^{-5}
IBD	0.94	7.1×10^{-8}
Anaemia	0.90	1.8×10^{-4}
Diabetes (prevalence)	0.97	9.2×10^{-9}
Thyroid cancer (incidence)	0.99	7.6×10^{-9}

IBD, especially among children, is an emerging global epidemic [27] that is linked to autism [28, 29]. Impairment of intestinal barrier function is a core feature of IBD [30]. Increased intestinal permeability promotes infiltration of unmetabolized peptides into the lymph system and general circulation. This provides an opportunity for an immune antigenic response, which by molecular mimicry can lead to an attack on crucial proteins in the brain and spinal column. Disturbances of collagen texture are a major factor leading to the onset of diverticular disease and IBD along with the disturbed wound-healing mechanisms seen in the pathogenesis of anastomatic leakage following large bowel surgery [31].

In a recent paper [32], we suggested that glyphosate, a non-coding amino acid analogue of glycine, could substitute for glycine in error during protein synthesis. Such misincorporation and disruption of proteostasis could explain the strong correlations observed between glyphosate usage and multiple modern diseases. *In this paper, we show that this could be one of the most important mechanisms by which glyphosate could induce multiple autoimmune diseases.*

A prime site for initiation of the disease process is the colon, where misfolded collagen, resistant to degradation, could lead to an autoimmune disease and, subsequently, a leaky gut. Autoantibodies against type VII collagen have been detected in up to 68% of IBD patients [33]. Glycine is the most common amino acid in collagen, making up one fourth of the residues in the protein. Proline is also a very common component of collagen and, as we discuss later in this paper, proline resists hydrolysis. Incomplete collagen degradation by matrix metalloproteinases in the gut could lead to the accumulation of short pro–gly–pro peptides that are resistant to proteolysis. These could then induce the infiltration of neutrophils or the activation of resident immune cells to induce an inflammatory response [34].

An unpublished study conducted by Monsanto and submitted to the US Environmental Protection Agency (EPA) traced the accumulation of radiolabeled glyphosate in various tissues of rats following low-dose oral administration (10 mg/kg body weight) [35]. By far the highest accumulation was found in the bones (Table 11 in [36]). Radioactive levels in the colon were 4–6 times as high as those in the stomach and small intestine.

The production of novel non-coding amino acids by plants and microbes wards off predators. The toxicity of these products may be due to the fact that they replace coding analogues during protein synthesis. Examples include: azetidine-2-carboxylic acid (Aze), a proline analogue [37, 38]; glufosinate, a glutamate analogue that is also a popular herbicide [39]; β-N-methylamino-L-alanine

(BMAA), an analogue of serine [40]; and L-canavanine, a natural analogue of L-arginine that is exploited as an insecticide [41, 42].

A remarkable true-life story involving a 119-day Alaskan wilderness experiment conducted by Christopher McCandless was recounted in the book *Into the Wild* by Jon Krakauer (later made into a popular movie) [43]. McCandless was thought to have died in the wilderness from starvation; however, Krakauer always suspected a toxin in the seeds of the wild potato, *Hedysarum alpinum*, which formed a staple of his diet in his last month of life. Krakauer had originally suspected a poisonous alkaloid but, through later research, was able to identify a significant level of L-canavanine in the wild potato seeds and published a paper on this analysis with several other authors in 2016 [42].

A key factor in L-canavanine's toxicity is its ability to insinuate itself into peptides in place of L-arginine. L-canavanine can be assimilated into essentially any protein to create aberrant canavanyl proteins that can disrupt many fundamentally important biochemical reactions across a broad spectrum of organisms [41, 44]. L-canavanine is exploited in agriculture as a potent insecticide against the tobacco hornworm [45], although the tobacco budworm has developed tolerance with a unique enzyme, canavanine hydrolase, which can quickly metabolize it [46]. Larvae exposed to L-canavanine incorporate it into the protein lysozyme, resulting in a 48% loss in catalytic activity [41]. Furthermore, diptericins B and C of *Protoformia terranovae*, but not diptericin A, are negatively impacted by L-canavanine. The distinction is that diptericin A has histidine at position 38 instead of the L-arginine found in the other two diptericins. Presciently, with respect to glyphosate, Rosenthal wrote: "These insect studies support the view that the biological effects of canavanine result from its incorporation into a protein, resulting in an alteration in protein conformation that leads ultimately to impairment of protein function" [41].

2. SHIKIMATE PATHWAY INHIBITION REVISITED

The shikimate pathway enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) is believed to be the main target of glyphosate's toxicity to plants [47]. A 1991 paper by Padgette et al. describes studies to gain insight into the mechanism by which glyphosate disrupts EPSPS [47]. Surprisingly, it is not understood exactly how glyphosate binds to the active site.

The microbes *Klebsiella pneumoniae, Escherichia coli* [47, 48] and *Agrobacterium sp.* strain CP4 [48, 49] have all evolved to produce versions of EPSPS that are glyphosate-resistant. The CP4 variant has been widely exploited by importing it into genetically modified glyphosate-resistant crops [48]. Insight can be gained by investigating the alterations to the peptide sequence that afforded resistance. All three mutations involved replacing a glycine residue at the active site with alanine [47, 48]. In the case of *E. coli*, the mutated enzyme is about 72 times *less* efficient than the wild-type enzyme, but 69 times *more* efficient in the presence of glyphosate. Changing the DNA code from glycine to alanine completely disables glyphosate's inhibiting effects on the enzyme [48].

Substitution of gly-96 at the active site in *E. coli* by serine leads to a version of the enzyme that is unable to bind PEP, most likely due to steric hindrance. The authors speculated that the hydroxymethyl group of serine displaces the phosphate of PEP and functions as a nucleophile. In fact, this mutated enzyme achieves a kind of reverse reaction, breaking EPSP down into shikimate-3-phosphate and pyruvate via hydrolysis.

We propose that substitution of gly-96 (gly-100 in the CP4 variant) by glyphosate during protein synthesis could explain its disruption of the enzyme's function. One can expect that the highly reactive and bulky glyphosate molecule, if substituted for gly-96, would behave more like serine than alanine. An additional disruptive factor is glyphosate's chelation of manganese, which would disrupt the catalytic action of EPSPS. A cell containing both wild-type and glyphosate-substituted forms of the enzyme would arguably circuitously convert PEP to pyruvate via EPSP without producing ATP from ADP; i.e., would waste the energy in the phosphate bond, as shown in Fig. 1, and end up with excess pyruvate and a deficiency in EPSP.

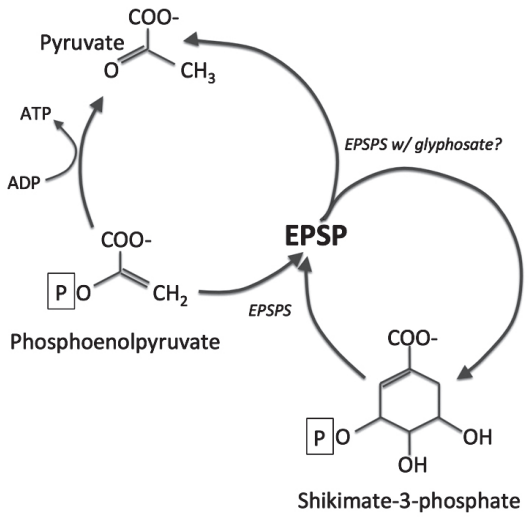

Figure 1. Diagram of the hypothetical pathway by which glyphosate substitution for glycine in EPSPS could result in the synthesis of pyruvate from PEP without generating ATP; i.e., wasting the energy in the phosphate group, as discussed in the text.

3. GLYPHOSATE AS A GLYCINE ANALOGUE

While glyphosate's main mechanism of toxicity to plants is considered to be disruption of the shikimate pathway, it is also likely that it disrupts other biological pathways where glycine is either a substrate or a ligand, due to the fact that it is a glycine analogue. It has been proposed that, through glycine mimicry, glyphosate's rôle as a ligand to NMDA receptors in the brain could explain its known ability to activate NMDA receptors and cause neuronal damage [49, 50]. In [51], acute exposure of rat hippocampal slices to Roundup (0.00005–0.1%) for 30 minutes caused oxidative stress and neuronal cell death, which was attributed to NMDA receptor activation. Glyphosate also interferes with the synthesis of porphyrin, a precursor to haem, by disrupting the first step in the pathway where glycine is substrate [52].

N-substituted glycine "peptoids" are an attractive class of synthetic molecules that can be constructed by linking component N-substituted glycines at sequential nitrogen–carbon bonds; they are directly analogous to the linking of amino acids into peptides [53]. Glyphosate is of course an N-substituted glycine, where the nitrogen side chain is a methyl phosphonyl group. Part of the attraction of peptoids is that they are highly resistant to proteolysis, just as is the amino acid proline, in which the carbon side chain circles back and binds to the peptide nitrogen. Impaired ability to break down proline-rich gliadin has been proposed as a contributing factor in coeliac disease and gluten intolerance [54]. This can explain why common cereals with high proline contents are especially problematic to gluten-sensitive individuals [55, 56].

Glyphosate is probably particularly problematic when it substitutes for N-terminal glycines in proteins where these glycines are highly conserved and play a significant rôle. Several proteins rely on an N-terminal glycine for anchoring to the plasma membrane (e.g., endothelial nitric oxide synthase (eNOS) [57]) or to the cytoskeleton (e.g., Kelch-like ECH-associated protein 1 (KEAP1) [58]). Protein N-myristoylation and prenylation depend on an amide bond to the N-terminal glycine residue [59]. For example, myristoylated G proteins involved in many signaling mechanisms depend on an N-terminal glycine residue [59]. This would be disrupted if the nitrogen atom has a side chain through glyphosate substitution for the terminal glycine.

N-nitrosoamino acids form a reasonable model for N-nitrosoglyphosate, a carcinogenic derivative of glyphosate that was of concern to the EPA during Monsanto's early studies. N-nitrosoproline is particularly relevant because proline, like glyphosate, has an extra carbon atom bound to the nitrogen atom. With respect to non-coding amino acids, and especially the incorporation of N-nitrosoamino acids into peptides and proteins, R.C. Massey remarked: "In addition to their presence as free N-nitrosoamino acids, species such as N-nitrosoproline (NPRO) and N-nitroso-4-hydroxyproline (HONPRO) may exist in a peptide- or protein-bound form as a result of N-nitrosation of an N-terminal imino acid residue" [62]. Tricker et al. [63] and Kubacki et al. [64] devised high performance liquid chromatography–thermal energy analyser (HPLC–TEA) techniques for analysis of multiple dipeptides with a nitrosylated N-terminal, including N-nitrosoprolylalanine (NPROALA), N-nitrosoprolyl-4-hydroxyproline (NPROHOPRO) and N-nitrosoprolylglycine (NPROGLY) [63, 64]. Tricker notes that the average recoveries for NPROALA, NPROHOPRO and NPROGLY, 200 μg of which was added to cured meat, were between 69 and 88%. Tricker also used the method to analyse the nitroso-tripeptide N-nitrosoprolylglycylglycine [65].

Nitrosamines of glyphosate (N-phosphonomethylglycine), its salts and esters include: N-nitrosoglyphosate (NNG) (Monsanto CP 76976), N-nitrosoiminodiacetic acid (NNIDA), N-nitrosoglyphosate sodium salt (NNGNa), N-nitrosoglyphosate isopropylamine ester (NNGIPA), N-nitrosoglyphosate potassium salt (NNGK), the metabolite N-nitrosoAMPA (NNAMPA), the metabolites N-nitrosodimethyl amine (NDMA) and N-nitrosarcosine (NSAR), which occur in glyphosate products or may be generated *in vivo* or in soils and waterways. N-nitroso compounds derived from secondary amines are considered carcinogenic.

Monsanto glyphosate documents reveal analysis and quantification of five nitrosamines of concern [61]. Out of six lots of Roundup analysed for NNG, four lots contained NNG residues of 0.61 to 0.78 ppm and two lots had residues from 0.22 to 0.40 ppm NNG. Analysis of six lots of Monsanto Rodeo revealed NNG residues in the range 0.13–0.49 ppm.

Recently, a powerful metatranscriptome study on bacterial gene expression following glyphosate treatment was conducted on microbes growing within the rhizosphere of glyphosate-tolerant corn [66]. RNA transcript abundance was compared between control and glyphosate-treated samples in order to characterize which protein genes were upregulated or downregulated. While they found many changes in gene expression, most striking to us was the upregulation of genes involved in both protein synthesis and protein hydrolysis. The ribosomal proteins L16p (L10e) and Firmicutes ribosomal L7Ae family proteins involved in the synthesis of the ribosomal large subunit increased 1.4- and two-fold, respectively, and the small subunit ribosomal protein S11p (S14e) increased 1.5-fold. Upregulation of genes involved in protein degradation was even more dramatic. For

example, transcripts for a proteasome β 2 subunit (EC 3.4.25.1) increased 4.3-fold and aminopeptidase YpdF increased threefold. An explanation could be an increase in the number of proteins that fail to fold properly due to glyphosate substitution for glycine in the protein. These authors also suggested a potential shift towards an increase in glyphosate-tolerant bacteria, a point that will become important later in this paper.

These results are corroborated by a study on pea plants grown in hydroponic culture, which revealed that glyphosate induced a significant increase in two major systems for proteolytic degradation: the ubiquitin-26 S proteasome system and papain-like cysteine proteases [67]. It also increased the total free amino acid content and decreased the soluble protein in the root system.

4. GLYPHOSATE-CONTAMINATED COLLAGEN AND PROTEOLYSIS RESISTANCE

We mentioned in the Introduction the gly–pro–gly peptide sequence that is common in collagen and linked to autoimmune disease. There are several enzymes in multiple organisms that are devoted to the proteolysis of peptide sequences containing proline, particularly the gly–pro sequence. These include enzymes that detach a terminal proline, enzymes that detach a dipeptide sequence where the second residue is a proline molecule and the first one is often glycine, and enzymes that break apart the X–pro dipeptide to release two free amino acids, one of which is proline. Certain pathogens have special modified versions of these enzymes, and there are genetic diseases related to pathologies in these enzymes. Substitution of glyphosate for glycine in this sequence is likely to cause extra stress to the enzymes that break down these sequences, potentially leading to autoimmune disease.

Prolyl aminopeptidase is an enzyme that detaches a terminal proline residue from a peptide. The enzyme is expressed predominantly by pathogenic bacteria in the gut, in particular *Serratia marcescens*, a common pathogen in the gut as well as in the urinary tract; it is often multiply antibiotic-resistant and is a serious threat in hospital-acquired infection [34]. This enzyme is especially important to the pathogens for degrading collagen, providing amino acids as fuel. It is conceivable that the pathogens are able to degrade glyphosate-contaminated peptides terminating in proline whereas the human form of the enzyme is not. It is intriguing that the *S. marcescens* version of prolyl aminopeptidase is unusual in having extra space at the active site [34], which could potentially accommodate the larger glyphosate molecule adjacent to the terminal proline residue. This might also contribute to glyphosate's observed effect on the gut microbiome: excessive growth of pathogens.

Multiple strains of the toxic mould *Aspergillus* secrete an X–prolyl dipeptidyl aminopeptidase (X-PDAP) that is important for digesting collagen because it can separate out an X–pro pair to bypass the difficult step of breaking the X–pro bond. Research has shown that this enzyme is essential for hydrolysing proline-containing peptides [69, 70]. It is likely that it becomes even more essential when X is glyphosate, as the peptoid sequence glyphosate–proline is likely almost impossible to break. Since gly–pro is a very common sequence in collagen, glyphosate–pro is likely to impede the breakdown of collagen fragments, which may then encourage *Aspergillus* infection in both plants and animals. Glyphosate has been shown to increase the growth rate of *Aspergillus* [71].

The most disturbing question is, what happens in the absence of pathogens that can effectively clear collagen peptides contaminated with glyphosate? As we will see later in this paper, antibodies to collagen are linked to antibodies to vaccines. A genetic defect in the enzyme prolidase, which can break apart the very common gly–pro dipeptide to release the individual amino acids, leads to a severe disease with mental deficiencies and multiple skin lesions [72]. Intriguingly, a common plant pathogen, *Xanthomonas campestris*, which causes blight on multiple plant species has a unique variant of prolidase with two mutations, a substitution of tyrosine for gly-385 and valine for tyr-387, two highly conserved residues in the peptide sequence [73]. Is it possible that swapping out glycine affords protection from glyphosate substitution for this residue? We hypothesize that peptides derived from multiple proline and glyphosate-contaminated proteins, which are highly resistant to proteolysis, are causing an autoimmmune epidemic that is an important contributor to autism and other autoimmune disorders.

5. BMAA AND ALS IN GUAM

β-N-methylamino-L-alanine (BMAA) is another noncoding amino acid and an analogue of serine [40]. BMAA is synthesized by cyanobacteria, the microbes responsible for the toxic algal blooms that occur in lakes experiencing an accumulation of nitrogen and phosphate nutrients following hot, rainy weather [74]. An *in vitro* study by Dunlop et al. in 2013 demonstrated that BMAA can be misincorporated into human proteins, causing protein misfolding that could lead to neurological diseases [40].

BMAA has, in fact, been linked to several neurodegenerative diseases, including Parkinson's, Alzheimer's and amyotrophic lateral sclerosis (ALS) [75]. A 2013 study linked an ALS cluster in Chesapeake Bay to consumption of BMAA-contaminated crabs [76]. A study in France investigated an ALS cluster near a lagoon that supplied oysters and mussels to the local

population. The authors demonstrated that the shellfish were contaminated with BMAA, but also remarked that there was intensive chemical-based agriculture in the region [77]. Interestingly, cyanobacteria have been found to be remarkably resistant to glyphosate [78, 79], and this could contribute to the recent record-setting algal blooms in the Great Lakes region, where glyphosate is extensively used on genetically modified (GM) Roundup-Ready crops [80].

One likely molecule that could be adversely affected by BMAA is the glutamate transporter, whose defective expression has been linked to ALS [81]. Glutamate excitotoxicity in motor neurons is associated with ALS, and this could be caused by an impaired glutamate transport system. Ordinarily, astrocytes quickly clear glutamate from the synapse, following its release by neurons, and the transporter is essential for this clearance. A conserved serine-rich motif in the glutamate transporter forms a reëntrant loop, similar to a structure found in many ion channels [82]. This loop is crucial for the enzyme's proper function, and would be disrupted by substitution of BMAA for serine.

An interesting detective story has evolved around an epidemic of a complex neurological condition termed amyotrophic lateral sclerosis–Parkinsonism dementia complex (ALS–PDC), which reached epidemic proportions during a short interval after World War II among the native Chamorro people on the small island of Guam in the South Pacific. At the peak of the epidemic, the natives had a hundredfold increased risk to ALS and Parkinson's disease compared to the risk in the general human population.

A plausible explanation for this epidemic relates to a popular native food source: seeds from the cycad trees [83–85]. Cycad seeds contain BMAA, likely derived from associated cyanobacteria. However, what is especially interesting is that the BMAA becomes concentrated in the skin of fruit bats that feed on the cycad seeds. Fruit bats were a popular delicacy among the natives, who ate every part of them, including the skin. Increased access to firearms from the USA during the war may have made it easier to kill the bats, on which the natives then feasted, ultimately leading to the natives' near-extinction through the accumulation of BMAA in their brains [86]. Meanwhile the near-extermination of the bats through the hunting removed the presumed source of the epidemic [83].

However, the warfare also led to the accumulation of many toxic chemicals in the soil, which could have encouraged the proliferation of cyanobacteria, which are especially resilient in the face of stressors. The bats' demise was undoubtedly hastened by the accumulation of excess BMAA in their tissues. A measurement of the amount of BMAA in three dried specimens of fruit bats from Guam taken from a museum in Berkeley found concentrations between 1200 and 7500 μg/g, which indicates up to hundredfold bioamplification over the level in the seeds of the cycad tree [87].

There have been inconsistent results in measuring the levels of BMAA in different tissue samples, but this has been explained recently by the realization that any BMAA incorporated into proteins may be missed in analysis without sufficient proteolysis. Ince et al. wrote: "When the insoluble, protein-containing fraction following TCA (trichloroacetic acid) extraction is further hydrolysed to release BMAA from protein, there is a further pool of protein-bound BMAA that is present in a ratio of between 60:1 and 120:1 compared with the pool of free BMAA" [84, p. 348]. We believe that this point has great significance when it comes to glyphosate: we highly suspect that different methodologies used to measure glyphosate contamination in any situation where there is a significant protein-bound component may yield different results depending on the degree to which protein hydrolysis is carried out.

6. GLYPHOSATE CONTAMINATION IN COLLAGEN, ENZYMES, GELATIN AND VACCINES

Gelatin is commonly used as an excipient stabilizer in vaccines, particularly the live virus vaccines. Gelatin is derived from animal skin and bone, especially of pigs and cattle; they may be fed glyphosate-contaminated forages, including GM Roundup-Ready corn and soy feed, which are sometimes supplemented with GM Roundup-Ready beet pulp. Gelatin is mainly derived by partial hydrolysis from the collagen in skin and bone. 26% of the amino acids in collagen are glycine; proline and hydroxyproline together make up 18% [88]; and glutamate constitutes 6%. All three of these components are problematic. The proline could be substituted by Aze from the sugar beet, the glycine could be substituted by residual glyphosate in the feed, and glutamate is a neurotransmitter but known to be neurotoxic at high concentrations; it works together with glycine to excite NMDA receptors in the brain. The vaccine virus may incorporate some of the noncoding amino acids into its own proteins to produce versions of them that resist proteolysis and induce autoimmunity through molecular mimicry.

One of us (Samsel) analysed a number of animal protein products for glyphosate. These included the bones of pigs, cows, horses' hooves, bees and bee products, collagen and gelatin products, vitamins, protein powders, enzymes and vaccines. Results are shown in Tables 2 and 3. Both high performance liquid

chromatography with tandem mass spectrometry (HPLC–MSMS) and enzyme-linked immunosorbent assay (ELISA) methods were utilized. It has been shown that both HPLC and ELISA are comparable in terms of accuracy and precision for detection and quantification of glyphosate in water-based analysis and including Nanopure, tap and river waters. Water-based solvents for glyphosate demonstrate a detection limit of 0.6 ng/mL and a linear functional range of 1–25 ng/mL [200]. However, HPLC was not able to achieve detection below 5 ppb;[1] hence, in cases including water-based vaccines, analysis using numerous sample runs was made including using two independent labs to test the same samples.

Table 2. Residues of glyphosate found in animal-based products that were reported to the US Food and Drug Administration (FDA) by Samsel Environmental & Public Health Services. The limit of detection for glyphosate using hot water extraction is 0.075 parts per billion (ppb).[1]

Protein substrate	Type	Test date	Glyphosate residue (ppb)[1]
GELATIN	JELL-O ORANGE #07 JAN 2018 DB02 02:36	29 July 2016	9.00
GELATIN	POWER-MAX PROTEIN POWDER ADVANCED NUTRITION	29 July 2016	14.94
GELATIN	DISNEY GUMMIES VITAMINS	9 August 2016	8.27
GELATIN	FLINTSTONES GUMMIES VITAMINS	9 August 2016	5.32
ORAGEL	CHILDREN'S ORAGEL 7.5% BENZOCAINE FORMULA	26 September 2016	2.81

HPLC–MSMS was also later used, where the method detection limit (MDL) permitted, for additional confirmation and quantification of glyphosate in digestive enzymes and collagens. Spiked sample recoveries were done for all samples tested. Freshly prepared glyphosate standard solutions were run as controls and results were calculated based on a standard curve.

In 1989, Monsanto researchers conducted an experiment on exposure of bluegill sunfish to ^{14}C-radiolabeled glyphosate [89]. One of us (Samsel) obtained the (unpublished) report from the EPA through the Freedom of Information Act. The researchers had found that, with EDTA extraction, the amount of radiolabel in tissue samples was much higher than the amount of detected glyphosate. They decided to apply a digestive enzyme, proteinase K, and discovered that this "caused a substantial improvement in extractability". It brought the yield from 17–20% in the case of EDTA to 57–70% following digestion with proteinase K. They summed up as follows: "Proteinase K hydrolyses proteins to amino acids and small oligopeptides, suggesting that a significant portion of the ^{14}C activity residing in the bluegill sunfish tissue was tightly associated with *or incorporated into* protein" (present authors' emphasis). In this context it is important to recall that a 60- to 120-fold higher detection level of BMAA was obtained following protein *hydrolysis* of contaminated proteins [84].

Since Monsanto found bioaccumulation of glyphosate in all animal tissues, with the highest levels in the bones and marrow [35, 36], one would expect that all tissues derived from animals fed a diet containing glyphosate residues and used for food by people around the globe would be contaminated. Knowing that the bioaccumulation of glyphosate would be evident in the vast majority of animals raised for market and fed a contaminated diet, as well as their products; and suspecting the possibility of contamination of even the digestive enzymes derived from these animals, one of us (Samsel) decided to analyse random samples.

Results from various gelatin-based products, along with the results for several different vaccines (discussed later) were reported to the FDA by Samsel Environmental & Public Health Services in August 2016. Table 2 shows results for glyphosate residues found in these gelatin-based products. The highest level found in a gelatin sample was almost 15 ppb.[1]

Having found glyphosate in animal gelatins, analysing the collagen at the source was a logical next step. Tissues from pork and cattle obtained from a local supermarket, commercially available collagen sourced from industrially-raised swine and oxen, as well as the purified digestive enzymes pepsin, lipase and trypsin, derived from pigs, were selected for evaluation. Three methods of laboratory analysis were used to determine if

[1] Parts per (US) billion. To put this into perspective, 1 ppb = 1 μg/kg, and 1 μg of glyphosate (N-phosphonomethylglycine) contains 3.561×10^{12} molecules of the substance, each one of which could integrate with a protein.

glyphosate was present in porcine pepsin and in the glycine-rich collagen from the tissues of pigs and cattle, protein sources that are regularly consumed by Americans. The results are given in Table 3.

Glyphosate integration with enzymes is a serious consideration, as glyphosate may serve as an enzyme inhibitor like other phosphonates [90–92]. Inhibition and immobilization of enzymes may occur via three basic categories: covalent linkage; adsorption on a carrier; or entrapment within macromolecules [93].

Inhibition of enzymes may be reversible or irreversible. Types of reversible enzyme inhibition include competitive, noncompetitive and uncompetitive. *Irreversible* inhibitors covalently bond to the functional groups of the active site, thus permanently inactivating catalytic activity. Irreversible inhibition includes two types: group-specific inhibition and "suicide" inhibition.

The importance of fully functional digestive enzymes cannot be understated. They are essential for metabolic function, as they convert food into nutrients and other molecules that are then available to cells for tissue and organ growth, maintenance and repair. The precursor trypsinogen, produced in the pancreas, is enzymatically transformed into the serine protease trypsin. Trypsin catalyses the hydrolysis of proteins into peptides and provides substrates for further enzymatic hydrolysis for protein absorption.

Pepsin, a primary protease of digestion, is also responsible for the metabolism of dietary protein. Pepsin's cleavage of peptide bonds is responsible for the availability of the aromatic amino acids phenylalanine, tyrosine and tryptophan. It is also responsible for the cleavage and release of several other amino acids, including valine, glycine, histamine, glutamine, alanine and leucine.

Lipase participates in cell signaling, inflammation and metabolism. Pancreatic lipase is the catalyst for the hydrolysis of dietary lipids, which include fats, oils, cholesterol esters and triglycerides [94]. Triglyceride triester is metabolized for utilization as glucose and three fatty acids. Glyphosate integration into and inhibition of lipase could induce excessive bioaccumulation of fatty material in the blood vessels, gut, liver, spleen and other organs, as well as mimic lysosomal acid lipase deficiency. It would also allow for an increase in triglycerides in the blood, leading to numerous disease cascades, including malabsorption, fatty liver disease, jaundice, failure to thrive in infants, calcification of the adrenal gland, anaemia, hypercholesterolaemia, biliary dysfunction, decreased HDL, increased LDL, blood clots, fat-enlarged hepatocytes and liver fibrosis and failure. Samsel found that radiolabeled glyphosate was not detectable by HPLC–MSMS in samples of lipase deliberately spiked for analysis, suggesting that glyphosate may irreversibly inhibit lipase. On the other hand, pepsin and trypsin had good spike recoveries, demonstrating reversibility as glyphosate was released from the protein.

Table 3. Integration of glyphosate residues in various proteins, assessed using three testing methods.[a]

Protein substrate (Method)	Type	Glyphosate residue (ppb)
Bone (ELISA)	Bovine leg	11.56
Bone marrow (ELISA)	Bovine leg marrow	4.22
Bone (ELISA)	Porcine foot	9.81
Skin (ELISA)	Porcine	0.325
Gelatin (ELISA)	Bovine, Sigma Aldrich, gel strength 225 Type B	2.04
Collagen (ELISA)	Bovine I & III	120.18
Collagen (GC-MS)	Bovine I & III	130 µg/kg
Collagen (HPLC-MSMS)	Bovine I & III	95 µg/kg
Pepsin (ELISA)	Purified porcine enzyme	< 40.00
Pepsin (GC-MS)	Purified porcine enzyme	430 µg/kg
Pepsin (HPLC-MSMS)	Purified porcine enzyme	290 µg/kg
Trypsin (ELISA)	Purified porcine enzyme	61.99
Lipase (ELISA)	Purified porcine enzyme	24.43
Bee bread (HPLC-MSMS)	Bee bread	2300 µg/kg
Bees (HPLC-MSMS)	*Apis mellifera*	< 10 µg/kg trace
Honey & comb (HPLC-MSMS)	Honey	< 10 µg/kg trace

[a] The trace amount found in the bee substrates appeared as a small peak, which directly corresponded to glyphosate, complete with retention time and molecular features confirming contamination using HPLC–MSMS.

Table 3 shows results for various bovine and porcine products, including enzymes, bone, bone marrow, skin, collagen and gelatin. Acid hydrolysis was used on the bovine and porcine skin, bones and marrow, which were shaken and digested with 0.15 M hydrochloric acid for 24 h. The analysis methods were ELISA, gas chromatography–mass spectrometry (GC–MS) and HPLC–MSMS. All of the tested products were contaminated, with the highest level detected being 430 μg/kg in porcine pepsin (via GC–MS).

Additional evidence of glyphosate accumulation was found by Samsel in 2015 in the bodies of dead bees, bee bread and honey from bee hives suspected of colony collapse disorder (CCD), and these are also shown in the table. Colony collapse disorder (CCD) is an ever-increasing problem threatening pollination of crops globally. It may share a similar aetiology to that of Alzheimer's disease with regard to learning and memory within the bee's brain. Integration of glyphosate with the structural proteins and enzymes of the bee may affect protein folding and function. Additionally, glyphosate may also affect the digestive enzymes and bacterial homeostasis within the digestive system, which in turn may affect the quality of the honey produced. Glyphosate in bees may become part of their chitin, which has a structural function, in their bodies, analogous to glyphosate becoming part of the collagens of humans and other animals.

The results in Table 3 show ubiquitous contamination of the bee and bee products. Honey is derived from nectar and is the source of carbohydrates in the bee diet, whereas pollen turned into bee bread supplies the fats and proteins. Royal jelly, made from the secretions of the glands found in the hypopharynx of the worker bees, is fed to the queen and developing larvae [96].

Results for nineteen different vaccines, from five manufacturers, are shown in Table 4. Some vaccines do not contain live viruses and do not involve gelatin in their preparation, but many involve the use of eggs, bovine calf serum, fetal bovine serum or bovine proteins [95]. Engerix Hepatitis B vaccine is manufactured through a novel procedure, which involves culturing genetically engineered *Saccharomyces cerevisiae* yeast cells that carry the surface antigen gene of the hepatitis B virus. The procedures result in a product that can contain up to 5% yeast proteins, which could be a source of glyphosate if the yeast is grown on broths or media that utilize glyphosate-contaminated nutrient sources such as animal or plant proteins.

Vaccines that tested negative for glyphosate included Merck's Hep-B vaccine, most of the pneumococcal vaccines and the sterile diluent included as a control. Gelatin is not listed as an ingredient in any of these vaccines, nor is bovine serum. In contrast, all of the vaccines that listed gelatin as an excipient tested positive for glyphosate, and nearly all of them also included bovine serum (including Varicella, MMR-II, MMRV and Zoster).

It is significant that MMR-II consistently contained the highest levels of glyphosate, significantly more than any of the other vaccines. This vaccine uses up to 12% hydrolysed gelatin as an excipient–stabilizer; as well as foetal bovine serum albumin, human serum albumin and residual chick embryo; all of which are contaminated by glyphosate during animal production.

7. EVIDENCE FOR A ROLE FOR COLLAGEN IN VACCINE ADVERSE REACTIONS

Post-vaccination allergic reactions to MMR and varicella vaccines have been linked to the gelatin excipient, and confirmed through observation of induced gelatin-specific IgE antibodies [97–100]. 24 out of 26 children with allergic reactions to vaccines (e.g., anaphylactic shock) had anti-gelatin IgE ranging from 1.2 to 250 μg/mL. Seven were allergic to gelatin-containing foods. A pool of 26 control children all tested negative for anti-gelatin IgE [99]. A study from 2009 that looked at gelatin sensitivity in children who were sensitive to cows' milk, beef and/or pork as determined by IgE antibody levels [101] found that 16% of beef-sensitized children and 38% of pork-sensitized children had IgE antibodies to beef- or pork-derived gelatins that were cross-reactive with each other.

In a published case study, a 2-month-old baby developed Kawasaki disease one day after receiving its first dose of Infanrix (DTaP-IPV-Hib) and Prevenar, a pneumococcal conjugate vaccine [102]. Kawasaki disease is an acute, multisystemic vasculitis whose occurrence very early in life is extremely rare. Extensive tests for the presence of infection with multiple bacteria and viruses were all negative. We suggest that glyphosate contamination in one or both of the vaccines may have contributed to the vasculitis through glyphosate uptake into common proteins such as collagen in the vasculature to induce the autoimmune reaction.

Kelso (1993) reported the case of a 17-year-old girl who experienced anaphylaxis within minutes of receiving an MMR vaccine [98]. The girl described the event as "kind of like what happens when I eat Jell-O[2]". Further testing found gelatin to be the component of the vaccine

[2] Jell-O is a proprietary brand of gelatin-based desserts, popular in the USA, and manufactured by Kraft Foods, part of the Kraft Heinz Company, headquartered in Chicago.

Table 4. Glyphosate levels in vaccines determined by ELISA reported to the US CDC, NIH, FDA and UN WHO of the Americas in September 2016 by Samsel Environmental & Public Health Services.[a]

Vaccine undiluted	Manufacturer	Lot number Exp date	Test date Lab #	Glyphosate residue (ppb)	% Recovery in spiked sample
DTaP ADACEL	SANOFI PASTEUR NDC	58160-820-43 3-30-2018	7-15-2016 LAB #1	0.109	82%
DTaP	SANOFI PASTEUR	C50418A 9-2-2018	5-11-2016 LAB #1	< 0.075	81%
DTaP ADACEL	SANOFI PASTEUR	NDC 58160-820-43 3-30-2018	7-12-2016 LAB #2	ND	-
HEPATITIS-B	MERCK	LO16427 4-13-2017	5-11-2016 LAB #1	< 0.075	97%
HEPATITIS ENGERIX-B	GLAXOSMITH- KLINE	NDC 58160-820-43 6-1-2018	7-15-2016 LAB #1	0.337	73%
INFLUENZA FLUZONE QUAD	SANOFI PASTEUR	6762 6-30-2016	7-15-2016 LAB #1	0.170	95%
INFLUENZA	NOVARTIS	1573 3P 05/2016	5-11-2016 LAB #1	0.227	106%
Pneumococcal PNEUMOVAX 23	MERCK	700281601 5-18-2017	9-19-2016 LAB #1	0.112	118%
MMR II	MERCK	7002151400 9-9-2017	7-15-2016 LAB #1	3.740	-
MMR II	MERCK	009545 3-19-2017	5-11-2016 LAB #1	2.963	-
MMR II	MERCK	7002151400 9-9-2017	9-19-2016 LAB #1	3.154	-
MMR II	MERCK	7002151400 9-9-2017	7-12-2016 LAB #2	2.90	-
MMRV PROQUAD	MERCK	7002305700 9-12-2017	9-19-2016 LAB #1	0.659	103%
MMRV PROQUAD	MERCK	7002305700 9-12-2017	7-15–2016 LAB #1	0.512	86%
MRV PROQUAD	MERCK	7002305700 9-12-2017	7-12-2016 LAB #2	0.43	-
Pneumococcal PNEUMOVAX 23	MERCK	700281601 5-18-2017	7-15-2016 LAB #1	< 0.075	77%
Pneumococcal PREVNAR 13	WYETH	73332 07/2017	5-11-2016 LAB #1	< 0.075	82%
Pneumococcal PNEUMOVAX 23	MERCK	7002681601 5-18-2017	7-12-2016 LAB #2	ND	-
STERILE DILUENT	MERCK, SHARP & DOHME	LO 40058 5-11-2018	7-15-2016 LAB #1	< 0.075	97%
VARICELLA VARIVAX	MERCK	7002025000 2-8-2018	7-15–2016 LAB #1	0.556	84%
MVARICELLA VARIVAX	MERCK	7002025000 2-8-2018	7-12-2016 LAB #2	0.41	-
ZOSTER ZOSTAVAX	MERCK	7002502401 6-1-2017	9-19-2016 LAB #1	0.620	95%
ZOSTER ZOSTAVAX	MERCK	7002602401 6-1-2017	7-15-2016 LAB #1	0.558	98%
ZOSTER ZOSTAVAX	MERCK	7002602401 6-1-2017	7-12-2016 LAB #2	0.42	-

[a] Limits of detection for glyphosate in vaccines in parts per billion (ppb):[1] 0.075 (LAB #1); 0.15 (LAB #2).

to which the girl was allergic. The connexion may be to misfolded proteins, which include the collagens and associated partially hydrolysed gelatins. Indeed, both Jell-O and vaccines have been contaminated by glyphosate, as we reported in the previous section.

Puppies immunized with the rabies vaccine and a multivalent canine vaccine were compared to unvaccinated control puppies [103]. The vaccinated puppies, but not the unvaccinated ones, developed autoantibodies to their own collagen. A follow-up study where either just the rabies vaccine or just the multivalent vaccine was administered produced a similar result. The authors suggested that this could explain issues of joint pain that are currently common among dogs, particularly as they age.

8. MULTIPLE SCLEROSIS (MS)

8.1 Sugar beet and MS

The world obtains 30% of its sugar supply from beet sugar. While sugar cane is grown in tropical regions, sugar beet requires a temperate climate. The highest incidences of MS worldwide are in the USA, Canada and western Europe [5], where most of the beet sugar is produced. MS rates are higher in the northern states of the USA compared to the south, corresponding to the distribution of sugar beet cultivation. MS rates in Canada are highest in the Alberta prairie region, at the centre of the Canadian sugar beet industry [104]. Studies on migrants have shown that those who move from a low-risk to a high-risk area tend to adopt high-risk only if they migrated during childhood [105]. This implicates local environmental factors acting before adolescence. Tokachi province in Japan hosts only 0.3% of the population, but produces 45% of the sugar beet consumed in Japan [37]; this province has the highest rate of MS among all Asian populations [106].

A fascinating proposition how sugar beet could cause MS implicates a unique noncoding amino acid that is produced by sugar beet, namely Aze. Both proline and Aze have a unique structure for an amino acid: the side chain loops back round to connect up to the nitrogen atom. In the case of Aze, there are only 3 carbons in the ring instead of the 4 carbons in proline (Fig. 2). It has been shown experimentally that Aze can be inserted by mistake into proteins in place of proline [38].

Myelin basic protein (MBP) is an essential protein for maintaining the myelin sheath, and it interacts with actin, tubulin, calmodulin and SH3 domains [107]. It assembles actin filaments and microtubules, binds actin filaments and SH3 domains to membrane surfaces, and participates in signal transduction in oligodendrocytes and myelin. A central proline-rich region in MBP is functionally significant [108–110] and, in particular, is a binding site for Fyn-SH3, a key regulatory protein [111]. Proline substitutions of the SH3 ligand decrease its affinity for the Fyn-SH3 domain [108]. Fyn is localized to the cytoplasmic leaflet of the oligodendrocyte plasma membrane, where it participates in numerous signaling pathways during development of the central nervous system [112, 113]. Phosphorylation at a polyproline structure in the Fyn-binding region of MBP affects its structure.

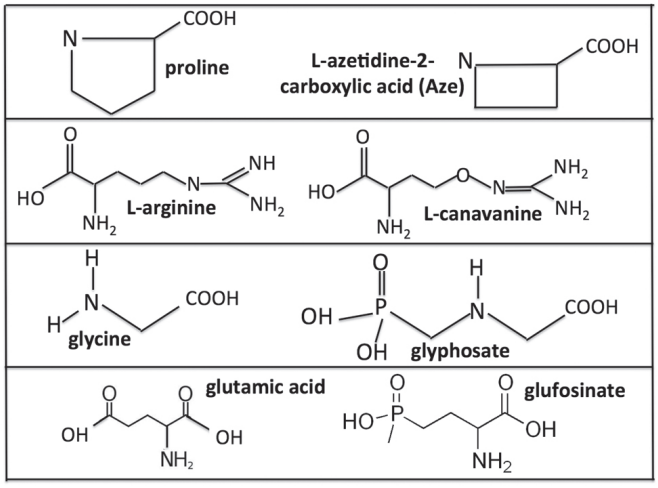

Figure 2. Molecular structures of the coding amino acids proline, L-arginine, glycine and glutamic acid; and their respective noncoding analogues Aze, L-canavanine, glyphosate and glufosinate.

A study using recombinant murine MBP inserted into *E. coli* strains demonstrated conclusively that Aze makes its way into MBP, substituting for up to three of the eleven possible proline sites. Molecular modeling of a proline-rich region of the recombinant MBP illustrated that misincorporation of Aze at any site would cause a severe bend in the polypeptide chain, and that multiple Aze substitutions would completely disrupt the structure of MBP [114, 115].

A possible concern regarding Aze is that over 90% of the sugar beet grown in the USA and Canada is genetically engineered to resist glyphosate. Therefore, the crops are exposed to significant amounts of glyphosate. The electronic *Code of Federal Regulations e-CFR 180.364 Glyphosate; Tolerances for Residues*, allows up to 25 ppm residue of glyphosate in dried sugar beet pulp. In 1999, Monsanto realized that its GM sugar beet crop well exceeded the upper limit established by the EPA for glyphosate residues. They requested, and were granted, a 125-fold increase in the upper residue limit for dried beet pulp (from 0.2 to 25 ppm). At the same time, the upper limit for fresh beet was increased fiftyfold to 10 ppm.

Glyphosate has been shown to increase the risk of root rot in sugar beet, caused by fungi [116]. Aze has been demonstrated to have antifungal activity [117]. Plants tend to increase synthesis of toxins under stress conditions, and it is plausible that an increased potential for root rot would result in increased synthesis of Aze. This is especially likely given that plants increase proline synthesis under a variety of different stress conditions [118]. However, to our knowledge, whether glyphosate causes an increase in either proline or Aze synthesis in sugar beet has not been investigated.

Consumption of milk worldwide is strongly correlated with MS risk (Spearman's correlation test = 0.836; $P < 0.001$) [119]. For the past several decades, cows' feed has been supplemented with either beet

molasses or sugar beet pulp, left as a residue after the sugar has been extracted [120]. Aze has been experimentally found in three sugar beet by-products that are fed to farm animals: sugar beet molasses, and both shredded and pelleted sugar beet pulp [38]. Casein is relatively enriched in proline [121]. If cows are exposed to Aze from the sugar beet, it will likely get inserted by mistake into casein, causing it to resist proteolysis. MBP's critical proline-rich sequence is vulnerable to misincorporation of Aze. The characteristic plaques of MS show loss of MBP within lesions in axon sheaths [107]. It is unclear whether this autoimmune reaction would arise through molecular mimicry from antibodies to unmetabolized peptides from casein or as a direct result of improperly folded MBP due to Aze insertion.

Glyphosate, an analogue of glycine, can be expected to be found in all tissues, including the milk of all mammals consuming glyphosate residues in the diet. Radiolabeled glyphosate studies conducted with lactating goats found ^{13}C and ^{14}C residues of glyphosate (N-phosphonomethylglycine), N-acetylglyphosate and other radiolabeled metabolites in milk. Monsanto found daily average ^{14}C residue levels from 19 to 86 ppb, with levels falling after five days of depuration to 6 ppb prior to sacrifice for organ examination. Results disseminated by Monsanto indicate that lactating animals (goats) fed a diet containing glyphosate and AMPA can be expected to have measured residue levels in edible tissues and milk [122]. In 2007 Dupont, in a similar study, examined the metabolism of N-acetylglyphosate in lactating goats. Detectable residues of N-acetylglyphosate, glyphosate and AMPA were detected in milk and other tissues. Milk, liver and kidney each contained 0.03% of the administered dose. Individual daily radiolabeled residues in the milk ranged from 0.030 to 0.036 μg/g [123].

Lactobacillus plays an important rôle in metabolizing casein in the human gut. A detailed study of the prolyl aminopeptidase from *Lactobacillus* revealed that it is a member of the class of α/β hydrolases. Multiple sequence alignment has revealed three distinct highly conserved regions in this family and all three contain at least two highly conserved glycines [124] that would be vulnerable to displacement by glyphosate. The motif gly-x-ser-x-gly-gly characterizes the domain surrounding the catalytic serine residue of prolyl oligopeptidases in general. The glycine residues in this motif contribute to the correct positioning of the catalytic serine with respect to its substrate. A second glycine-rich domain appears essential to activity, as it likely corresponds to the oxyanion hole. The function of the third highly conserved glycine-rich domain, with the motif asp-x-x-gly-x-gly-x-ser, remains unknown. *Lactobacillus spp*. are also highly dependent on manganese to protect them from oxidative damage, hence glyphosate's preferential chelation of manganese likely harms *Lactobacillus* [125].

An examination of collagen in the jugular veins of MS patients undergoing surgical reconstruction revealed an abnormal collagen structure, characterized by thin, loosely packed type III fibres [126]. Collagen is rich in proline. If too many of the prolines in procollagen are displaced by Aze, the polypeptide does not fold into a stable triple-helical conformation, which is a prerequisite for normal secretion of procollagen [127]. This reduces the release of procollagen and the misfolded molecules are subjected to proteolysis for recycling. resulting in the useless expenditure of energy for building and degrading procollagen molecules. Those that are released can be expected to produce defective collagen matrices. Collagen is even more highly enriched in glycine than in proline, as its core structure consists of a triple peptide repeat, where glycine is always the third residue of the triplet, and proline and hydroxproline often occupy the other two positions [128]. Glyphosate substitution for glycine in structural proteins; i.e., collagen, elastin, fibronectin and laminin; would contribute to disrupted folding as well as defective strength and elasticity.

Conserved prolines also play a crucial rôle in ion channel gating, the regulation of hypoxia-inducible factor (HIF) and embroygenesis; in fact, substituting Aze for proline is a technique used to test whether a particular proline residue is critical to the protein's proper functioning [37].

8.2 Rôle of *Acinetobacter* and *Pseudomonas aeruginosa* in MS

A series of papers by Ebringer et al. have suggested an important rôle for the Gram-negative bacteria *Acinetobacter* and *Pseudomonas aeruginosa* in MS [129–131] as well as a proposed link to prion diseases. Their most recent paper in *Medical Hypotheses* presents the evidence to support this idea from multiple dimensions [130]. First, MS patients were shown to have elevated levels of antibodies to these two microbes but not to the common gut microbe *E. coli* [132, 116]. They have autoantibodies to MBP and myelin oligodendrocyte glycoprotein (MOG) [131]. MS patients are also prone to sinusitis and *Acinetobacter* is one of the most common microbes found in nasal sinuses. Ebringer et al. also proposed that the increased prevalence of sinusitis in colder climates may explain the geographical distribution of MS in more northerly latitudes [130]. *P. aeruginosa* causes upper respiratory infections and it is among the microbes that have developed multiple antibiotic

resistance in recent years, presenting a huge problem in hospital infection [133]. *Acinetobacter* has also become resistant to multiple antibiotics [134].

The number of microbial species that can metabolize glyphosate is quite small. A 1996 study showed that *Acinetobacter* is able to fully metabolize both glyphosate and AMPA and utilize these molecules as a source of phosphorus [135]. A study of agricultural soil heavily polluted with glyphosate identified only three species capable of degrading glyphosate when exposed at a level of 1000 ppm: *Pseudomonas putida*, *P. aeruginosa* and *Acetobacter faecalis* [136]. Another study on marine species identified *Pseudomonas* as being among the rare microbial species that can utilize the phosphonate in glyphosate as a source of phosphorus [137]. It can be predicted that *Pseudomonas* and *Acinetobacter* species in the nasal or digestive tracts would have a substantial advantage over other microbes if they can degrade glyphosate. On the other hand, they would also be heavily exposed if they actively take it up, and it would not be unreasonable to assume that some of the glyphosate might end up in their synthesized proteins by mistake in place of glycine. Both *Pseudomonas aeruginosa* and *Acinetobacter* strains have recently become a serious problem in hospitals, and a public health issue, due to their multiple-antibiotic resistance [138]. Glyphosate has been shown to induce generic antibiotic resistance in other microbial species, including *E. coli* and *Salmonella*, through the induction of a generic capability to export toxic chemicals through efflux pumps [139].

A PEP transferase enzyme synthesized by *Acinetobacter calcaceticus* has sequence homology with a bovine prion sequence, and antibodies against synthetic peptides containing the structurally related sequences were found to be significantly elevated in cattle with bovine spongiform encephalopathy (BSE) compared to negative controls [140]. Ebringer et al. (2005) [129] link MS to BSE, also known as "mad cow disease", and to the related human disease, Creutzfeldt–Jakob disease (CJD). Cows suffering from BSE manifest hindquarters paralysis early after onset, similar to the mobility issues afflicting MS patients at onset. Ebringer et al. found elevated levels of antibodies to both *Acinetobacter* and *Pseudomonas*, along with autoantibodies to both white and grey matter components, in BSE-affected animals, as is also the case for MS [129].

Of particular note are the molecular similarities they identified between certain peptides found in these two microbes and peptides in MOG and MBP that are known to be allergenic. Strikingly, all three of the microbial sequences they identified and all three of their human protein analogues contain conserved glycines (Table 5).

Table 5. Amino acid sequences of three peptides from *Acinetobacter* and *Pseudomonas* and the corresponding human peptides from MBP that they mimic.[a]

Microbe	Acinetobacter	Acinetobacter	Pseudomonas
Protein	3-OACT-A	4-CMLD	Gamma-CMLD
Peptide	Leu-Tyr-Arg-Ala-Gly-Lys	Ser-Arg-Phe-Ala-Tyr-Gly	Thr-Arg-His-Ala-Tyr-Gly
MBP	Leu-Tyr-Arg-Asp-Gly-Lys	Ser-Arg-Phe-Ser-Tyr-Gly	Ser-Arg-Phe-Ser-Tyr-Gly

[a] Note that all six peptides have a glycine residue.

MOG is strongly implicated in the disease pathology of MS; autoantibodies recognizing MOG have been found in the CNS of MS patients [141]. One of the major encephalitogenic peptides in MOG is the sequence from residue 92 to residue 106, which contains a highly conserved glycine near its centre [142].

Both diabetes and MS are associated with abnormal T-cell immunity to proteins found in cow's milk [143]. In a study conducted in dairy cows by Monsanto in 1973, ^{14}C-radiolabeled glyphosate was studied in the distribution of residues in milk, urine, faeces and other tissues of the lactating cow. Glyphosate contamination of milk ranged from 9 to 15 ppb with the highest accumulation in the kidney and rumen fluid (201 ppb and 109 ppb, respectively) [201]. An epitope of bovine serine albumin found in milk that is linked to MS but not to diabetes is BSA193. It shows structural homology with exon 2 of MBP through the peptide sequence GLCHMYK. Note that the first peptide in this sequence is glycine. Exon 2 is a target peptide in both MS autoimmunity and in experimental autoimmune encephalitis (EAE), an animal model of MS [144–146]. Exon 2 of MBP is implicated in remyelination [144]. Its expression is largely restricted to the developing brain and to areas of myelin reconstruction, notably MS lesions [147].

The gly-ser-gly-lys tetrapeptide is highly conserved among MBPs from multiple species [148]. The serine in this sequence is the site of attachment of polyphosphoinositide. The highly conserved nature of this sequence suggests that the phospholipidation of MBP is important biologically. Substitution of glyphosate for either of the glycines would likely disrupt this modification.

9. MMR VACCINE AND AUTISM

In this section, we make a case for a direct link between the measles, mumps, and rubella (MMR) vaccine and autism, via autoantibody induction through molecular mimicry. In a paper provocatively titled, "Peptide cross-reactivity: the original sin of vaccines", Kanduc makes the point that massive cross-reactivity between antigens in vaccines and similar sequences in human proteins makes it almost inevitable that vaccines lead to autoimmune disease through molecular mimicry [149]. Reported post-vaccination autoimmune diseases include systemic lupus erythematosus, rheumatoid arthritis, inflammatory myopathies, multiple sclerosis, Guillain–Barré syndrome and vasculitis [150].

It is becoming increasingly acknowledged that autism may be an autoimmune disease. Family members of autistic children have a significant increased risk to other known autoimmune diseases such as hypothyroidism, rheumatic fever and multiple sclerosis [151]. Several studies on both humans and monkeys have revealed a potential link between maternal antibodies directed against specific foetal brain proteins and a future autism diagnosis in the foetus [152–155]. Furthermore, it has already been demonstrated that vaccines are capable of inducing autoimmune antibodies against proteins in the brain. The narcolepsy epidemic in Europe following an aggressive immunization campaign against the H1N1 'flu virus was eventually conclusively resolved as being attributed to autoimmune reactions to the hypocretin receptor through molecular mimicry from a peptide in the surface-exposed region of the influenza nucleoprotein A that was present in the H1N1 vaccine [156] (hypocretin is an important regulator of sleep).

Much controversy surrounds the concept that the MMR vaccine may be contributing to the autism epidemic in the USA and elsewhere. In an immune-compromised child, the live measles virus from the vaccine is capable of infecting the brain and sustaining a chronic measles infection, resulting in loss of neurons, eosinophilic intranuclear inclusions and gliosis, a condition termed "subacute measles encephalitis". This can result in a seizure disorder and developmental delay in language and motor skills (as was clearly observed in a case study involving an HIV-positive 2-year-old boy [157]).

Singh et al. have published a series of papers over the past two decades [14, 158–160] proposing that there is a subpopulation among the autism community who can be characterized as suffering from "autoimmune autistic disorder" [14]. The 1998 study by Singh et al. found that 90% of measles-IgG-positive autistic sera were also positive for anti-MBP antibodies, supporting the hypothesis that a virus-induced autoimmune response may be causal in autism [158]. A follow-on serologic study of antibodies to viruses associated with autism published in 2003 revealed a statistically significantly elevated level of measles antibody in children with autism compared to their siblings ($P = 0.0001$) or to unrelated children ($P = 0.003$), but not with antibodies to mumps or rubella [159]. In a later study, 60% of 125 autistic children had significantly elevated levels of antibodies to measles haemagglutinin unique to the MMR strain of the virus, compared to the 92 control children [160]. Over 90% of the children who had elevated antibody levels also tested positive for MBP autoantibodies. It was suggested that this could be linked to virus-induced autoimmunity through mimicry.

In fact, there is a sequence homology of 78% between a peptide sequence from MBP (EISFKLGQEGRDSRSGTP) and one found in a measles virus protein, MP3 (EISDNLGQEGRASTSGTP) [161, Table 2, p. 7]. Three of the matches between these two sequences are glycines. Measles virus-neutralizing antibodies are mainly directed to haemagglutinin, implying that it is essential for acquired immunity from the vaccine [162]; yet over-production, particularly if the virus penetrates the blood–brain barrier, runs the risk of inducing an autoimmune response to the myelin sheath. In fact, high measles antibody titres have been previously linked to MS [163].

Gonzalez-Granow et al. found high titres of autoantibodies in both the IgG and IgA classes specific to MBP in the serum of patients with autism [15]. The IgA antibodies in particular were shown to act as serine proteinases to degrade MBP *in vitro*. They also induced a decrease in long-term potentiation in perfused rat hippocampi. Reduced long-term potentiation in the hippocampus is a feature of autism, as has been clearly demonstrated in studies using mouse models of autism [164].

Dr Andrew Wakefield was the first to reveal a possible connexion between MMR and autism. His controversial *Lancet* paper, published in 1998 and then later retracted, proposed that this vaccine caused an acute reaction in children with gut dysbiosis (abdominal pain, diarrhoea, food intolerances, bloating etc.) [9]. The paper reported on a group of 12 children who had experienced developmental delay following an MMR vaccine and who were diagnosed with autism. These children suffered from rash, fever, delirium and seizures following the vaccination with MMR. He and several colleagues later published additional papers elaborating the hypothesis that dysbiosis in the gut, combined with impaired protein hydrolysis, leads to autoimmune lesions in the duodenum that are associated with extensive colonic lymphoid hyperplasia. The release of undigested peptides

into the vasculature across a leaky gut barrier and, ultimately, from the vasculature across a leaky blood–brain barrier, could induce encephalopathy [165–167].

In an epidemiological study from 1998, encephalopathy was clearly demonstrated as an acute reaction to measles vaccine, where 48 cases were found following vaccination, with no cases identified after administration of either monovalent mumps or rubella [168]. Among these 48 children, eight died, and the remainder experienced mental regression, chronic seizures, movement disorders and sensory deficits in the subsequent months.

The FDA's vaccine adverse event reporting system (VAERS) database is a valuable tool for uncovering trends in vaccine adverse reactions. Our earlier studies on VAERS comparing MMR with an age-matched, equal-sized distribution of all other vaccines showed a significant association of MMR with autism ($P < 0.007$) [169]. This was puzzling, because MMR has never contained either aluminium or mercury, the two prime candidates for the kind of neurological damage that might lead to autism [170–174]. Strong associations also appeared with fever and rash. In that paper, we proposed that the adverse reaction might be caused by the acetaminophen administered to the child to try to curb the seizures.

Since glyphosate usage on crops has gone up dramatically since the GM Roundup Ready crops were first introduced in 1996, we decided it would be worthwhile to compare the early data on MMR in VAERS with the later data. We defined a cutoff date on 1 January 2003, such that the events where MMR was included as an administered vaccine could be separated into "early" and "late", based on whether they were before or after that date. Each dataset represented a 13-year interval. We found 10 639 events in the early set and 19 447 events in the late set; thus, the raw number of events nearly doubled in the later years.

We also tabulated the frequency of different adverse reactions in the two sets, and used a standard statistical analysis to compute the significance of any differences observed: we randomly down-sampled both sets as needed such that there was an identical total count and an identical distribution over age in the two datasets. Results were surprising: many symptoms associated with atopy or with an allergic reaction were significantly higher in the later set, and "hospitalization" was highly significantly overrepresented in the later set [Table 6]. Other overrepresented symptoms included seizures, dyspnea, hyperventilation, asthma, eczema, autism, hives, anaphylatic [shock], and irregular heart rate. Interestingly, the early set had more frequent occurrences of joint pain and arthritis, suggesting that the toxic elements in the vaccine impacted the joints rather than the brain.

Table 6. Frequency of various adverse reactions to MMR before and after January 2003 [US FDA, VAERS]. The *P*-values were computed according to a χ^2 goodness-of-fit test.

More common before 2003			
Reaction	Count < 2003	Count ≥ 2003	*P*-value
Arthritis	52	18	0.045
Joint pain	175	75	0.012

More common after 2002			
Reaction	Count < 2003	Count ≥ 2003	*P*-value
Hospital	132	423	0.00041
Seizures	314	534	0.0055
Dyspnea	139	279	0.0086
Hives	444	654	0.011
Anaphylactic	28	91	0.017
Eczema	10	47	0.028
Autism	105	184	0.031
Hyperventilation	18	57	0.035
General infection	77	136	0.044
Asthma	22	58	0.046
Immunoglobulin G	0	17	0.048
Ear infection	32	72	0.048
Heart rate irregular	11	39	0.049

To our knowledge, there have been no significant changes to the formulation of MMR since its introduction. The explanation for the significant changes in adverse reactions must, therefore, lie in external factors, one of which is likely to be glyphosate. We suggest that both chronic exposure to glyphosate from food, water and air and direct exposure to glyphosate residues in the vaccine are relevant factors. A child with a disrupted gut microbiome due to chronic glyphosate exposure will also suffer from a leaky blood–brain barrier, and this will lead to a much greater possibility of measles antigenic proteins entering the brain and causing anaphylaxis and seizures.

The measles virus is a member of the family of paramyxoviruses, which have two highly-conserved glycine residues at positions 3 and 7 in the hydrophobic fusion peptide (FP) region of the viral fusion-mediating glycoproteins [175]. This FP region is the most highly conserved region of the glycoproteins, and it plays a critical rôle in destabilizing the membrane of the host cell to gain entry. Substitutions of other amino acids for either the G3A or G7A glycines caused increases in both cell–cell fusion and the reactivity of the protein to antibodies, leading to both a higher infection rate and increased chances for an autoimmune reaction. Glyphosate substitution is likely to do the same, as well as leading to a form of the protein that would resist proteolysis.

The FPs of both the influenza virus and human immunodeficiency virus (HIV) gp41 contain numerous glycine residues at regular intervals, with glycine overall making up 29 and 26%, respectively, of the total peptide sequence [175]. Optic neuritis, an immune-mediated demyelinating injury of the optic nerve, has been recognized as a side effect of the influenza vaccine that can lead to blindness [176].

10. OTHER AUTOIMMUNE DISEASES

10.1 Neuromyelitis optica and aquiporin

Neuromyelitis optica is a rare severe inflammatory demyelinating disorder of the central nervous system, which is related to multiple sclerosis but distinctly different and manifested mainly by paralysis and optic nerve damage [177, 178]. It has been conclusively demonstrated that this condition is caused by an autoimmune reaction to aquaporin-4, which is highly expressed in the astrocyte membrane [177, 178].

Aquaporins are important membrane proteins, which can transport water molecules through pores into the cell while excluding protons [179]. They are highly expressed by astrocytes, one of whose rôles is to mediate water flow among the vasculature, the cerebrospinal fluid and the lymph system [178]. Thus, aquaporins are implicated in brain oedema [180]. Plants produce aquaporins as well, and mimicry between plant and human aquaporins has been proposed as a mechanism for the development of an autoimmune sensitivity to this protein [181]. Plants considered to show aquaporin mimicry notably include corn and soy as well as tomato, tobacco and spinach [182].

Autoimmune sensitivity to aquaporin has also been found in association with MS [182]. Vojdani et al. found significant elevations in antibodies against both human and plant aquaporin 4, in addition to antibodies against MB, MOG and S100 calcium-binding protein B (S100B) in patients suffering from MS.

Among the aquaporins, aquaporin-6 is unique in that it operates as an anion channel instead of as a water channel. Analysis of the peptide sequence in comparison to other aquaporins reveals that aquaporin-6 has an asparagine substituted in place of a glycine at residue 60. This one small difference completely changes the way the molecule behaves in the membrane. A glycine at this position is conserved among all the other aquaporins. Furthermore, aquaporins are constructed of α-helices, and there are three sites where the helices cross. Highly conserved glycine residues are found at all three sites [57, 183].

Aquaporin is also found in bacteria, although homology with human aquaporin is only about 20%. The bacterial aquaporin is a 27 kDa trypsin-resistant protein called aquaporin-Z, which was originally described in *E. coli* [184]. Sequence analysis conducted by Ren et al. [185] revealed four regions where homology was considerably stronger (90%, 60%, 50% and 45% respectively). They convincingly showed cross immunoreactivity between the human and bacterial versions of the protein. Antibodies to aquaporin Z bind to astrocytes, activate complement, and cause death.

Ren et al. [185] identified all the residues where the bacterial and human peptides were identical (Fig. 1 in [185]). A tally of counts reveals that glycine was by far the most common among these matched residues, representing 14 of the total 66 matches. The second most common amino acid was lysine with 8 matches. Alanine, isoleucine and valine had 7, 5 and 4 matches respectively, and all other amino acids had less than four.

Thus, it appears that glyphosate-substituted trypsin-resistant aquaporin from both gut microbes and from GM glyphosate-resistant corn and soy foods are plausible sources of antigens that could induce neuromyelitis optica and contribute to the disease process in MS through misincorporation.

10.2 Type 1 diabetes

Type 1 diabetes is considered a genetic disease, but its incidence has been increasing by 3–4% worldwide every year in the recent past [186, 168]. Although an environmental component is highly suspected, environmental factors have not yet been identified. An increased incidence of type 1 diabetes is associated with both MS [187] and autism [188]. The disease is characterized by an autoimmune reaction to various proteins expressed in the pancreatic islet cells. Specifically, antibodies against glutamic acid decarboxylase (GAD65) are often found [189]. Cross-reactivity with proteins from foods and microbes in the gut are both possibilities.

One microbe that may be inducing antibody production through mimicry is *Mycobacterium avium paratuberculosis* (MAP). Blast analysis revealed 75% homology between a previously identified antigenic region of GAD65 [190] and a MAP heat-shock protein (HSP65) [189]. The specific 16-residue matched sequence in HSP65 centrally contains a pair of glycines which could be substituted by glyphosate to cause resistance to proteolysis. This microbe has been linked to numerous other human diseases including ulcerative colitis, irritable bowel syndrome, sarcoidosis, Hashimoto's thyroiditis, MS and autism [188]. With respect to MS and autism, cross-reactivity between HSP65 and MBP through mimicry may provide the link.

Patients with type-1 diabetes commonly have an antibody reaction to bovine serum albumin, a component of cows' milk [191]. The hypothesized explanation is an autoimmune reaction to a beta-cell specific surface protein through mimicry.

Insulin-derived amyloidosis is a condition that can develop following long-term insulin therapy, whereby an "insulin ball" develops at the site of injection. This hard mass has been analysed and found to contain accumulations of insulin fibrils reminiscent of amyloid β-plaque in the Alzheimer's brain. Insulin amyloidosis is more common for animal (cows and pigs)-derived than human-derived insulin products. Nowadays, cows and pigs are chronically exposed to glyphosate in their feed. The rôle of glycine residues in proteins may indeed be to protect from aggregation into amyloid fibrils [192]. Substitution of glyphosate for any of these conserved glycines would therefore tend to promote amyloidosis.

Glutamic acid and glycine are by far the largest component amino acids of bovine proinsulin and make up 25% of the amino acid residues in the molecule [193]. The same is true for human insulin, which differs very little from the animal versions. The herbicide glufosinate is a natural noncoding amino acid analogue of glutamic acid (Fig. 2). Substitution of either glufosinate for glutamic acid or glyphosate for glycine in insulin is likely to impair its function, and may also lead to amyloidosis.

The widespread appearance of glyphosate-resistant weeds among the glyphosate-resistant crops has forced some farmers to turn to glufosinate as the herbicide of choice [194]. Glufosinate-tolerant corn and soybean have been available on the US market since their approval by the USDA in 1995 and 1996, respectively. A tri-resistant form of soybean tolerant of glyphosate, glufosinate, and 2,4-D was approved by the FDA in September 2014. Dual resistance to glufosinate and glyphosate in corn was approved in November 2015.

10.3 Coeliac disease

Coeliac disease and, more generally, gluten intolerance, have reached epidemic proportions in the USA in the past decade [195]. Wheat grown there is being routinely sprayed with glyphosate for staging and desiccation just before harvest. This practice clears the field of weeds prior to harvest and planting of the next crop, but increases the amount of residual glyphosate in the grain. The practice has been increasing in popularity in step with the increase in gluten intolerance. Glyphosate is systemic in the plant and enters the seed as the plant dies, hence eventually ending up in wheat-based foods.

Proline residues make up 20% of the first 100 amino acids of both α- and γ-gliadins [54]. Related proteins from rye and barley are also unusually proline-rich [56]. As we implied earlier, proline is inaccessible to most digestive proteases because the bond between the peptide nitrogen atom and the side group complicates hydrolytic attack. As a consequence, specialized prolyl aminopeptidases detach the amino-terminal proline from a peptide. These enzymes depend on manganese as a catalyst, and manganese is one of the metals most dramatically affected by glyphosate chelation [125]. Unhydrolysed gliadin peptides bind to HLA-DQ molecules (receptors on antigen-presenting cells) and trigger pathogenic T-cell responses [196]. Genetic variants of HLA-DQ are linked to both coeliac disease and type 1 diabetes [197, 198].

Analysis of the X-ray crystal structure of a human cytosolic prolyl aminopeptidase worked out in 2008 revealed that it is a dimer with a dependency on two manganese ions as the catalytic centres [199]. The full sequence of the catalytic domains of six prolyl peptidases from both human and microbial species is shown in Fig. 6 in ref. 199. Six of the twenty sites of fully conserved residues across all species were glycine residues, three were histidine, two were tyrosine and two were proline. The remaining seven were seven different amino acids.

11. CONCLUSION

In this paper, we have shown that widespread misincorporation of glyphosate for glycine during protein synthesis could explain the aetiology of multiple autoimmune diseases that are currently increasing in incidence in the USA. Misincorporation is plausible by analogy with multiple known toxins produced by organisms in defence against pathogens, including Aze, BMAA, L-canavanine and glufosinate, which work in a similar manner. We have shown that proteins from foods such as milk, wheat and sugar beet, as well as peptides derived from microbes resident in the gut or nasal tract or introduced iatrogenically through vaccination, are all potential causes of autoimmune disease induced through molecular mimicry. It is highly significant that two microbes linked to MS through molecular mimicry are among the very few microbes that can fully metabolize glyphosate. Using the VAERS database, we have shown that severe adverse reactions to the MMR vaccine have increased significantly over the past decade in step with the increased use of glyphosate. Glyphosate in MMR may originate from growth of the live virus on culture materials derived from glyphosate-exposed animals and/or from gelatin used as an excipient stabilizer. We have confirmed the presence of glyphosate contamination in MMR and in many other vaccines where the live virus is cultured in eggs, bovine protein or gelatin, or where animal products are used as an excipient component. Notably, some vaccines prepared without live culture on gelatin were free of glyphosate contamination. Substitution of glyphosate for glycine during protein synthesis could yield a peptide that resists proteolysis, making it more likely to induce an immune response. Furthermore, enzymes involved in proteolysis are likely to be disrupted due to their confirmed contamination with glyphosate. A non-exhaustive list of possible diseases that can be attributed to this mechanism include autism, multiple sclerosis, type 1 diabetes, coeliac disease, inflammatory bowel disease and neuromyelitis optica.

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OPINION
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PEG: Will It Come Back to You? Polyethelyne Glycol Immunogenicity, COVID Vaccines, and the Case for New PEG Derivatives and Alternatives

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INTRODUCTION

Polymer-drug conjugation (Harris, 1992; Harris and Chess, 2003; Haag and Kratz, 2006; Pelegri-O'Day et al., 2014; Hoffman, 2016; Ekladious et al., 2019) was first described in the 1954 by German chemist, Horst Jatzkewitz, who demonstrated that covalent attachment of poly (vinyl pyrrolidone) to the psychoactive compound, mescaline, could be used to prolong its circulation and duration of action (**Figure 1A**) (Jatzkewitz, 1954; Jatzkewitz, 1955; Luxenhofer, 2020). Yet despite its novelty and utility, Jatzkewitz's innovation went largely unnoticed until the mid 1970s when it was revived by Ringsdorf, Kopecek, and Duncan, among others, who championed the notion that these novel macromolecules could enhance the suboptimal activity of various pharmaceuticals (Ringsdorf, 1975). It wouldn't be until 1990—nearly 36 years from the publication of Jatzkewitz's initial work—that the first polymer-drug conjugate would receive market approval in the form of Adagen, adenosine deaminase protein conjugated with 5 kDa poly (ethylene glycol), or PEG, used to treat a rare and hereditary, pediatric metabolic disorder called adenosine deaminase severe combined immunodeficiency (Hershfield et al., 1987).

Polymer-drug conjugates have since gradually increased in their clinical application, now with more than 29 marketed products that vary widely in polymer architecture (linear and branched), molecular weight (0.3–60 kDa per polymer), and degree of conjugation (1–69-82 per drug) and nearly all of which employ the synthetic polymer, PEG, a polyether typically produced by the ring-opening polymerization of ethylene glycol (Alconcel et al., 2011; Ekladious et al., 2019; Xu et al., 2022). In addition to the diversity of their appended polymers, these therapeutics also vary widely in their drug partner, ranging from: 1) peptides (e.g. Somavert HGH receptor antagonist) to 2) small molecules (e.g. SMANCS neocarzinostatin chemotherapy and Movanik naloxone laxative) and 3) nucleic acids (e.g. Macugen anti-VEGF aptamer antiangiogenic) (Perdue et al., 2020). More recently, polymer conjugation has demonstrated further clinical utility in stabilizing lipid nanoparticles used to deliver small interfering RNA (siRNA, Onpattro) for the treatment of hereditary transthyretin-mediated (hATTR) amyloidosis (Zhang et al., 2020), as well as both current mRNA-based vaccines for SARS-CoV-2 (COVID-19), BNT162b2/Comirnaty and mRNA-1273/Spikevax (Schoenmaker et al., 2021). Interestingly, all three nanoparticle formulations share in their use of lipids tethered with 2 kDa linear, methoxy-terminal PEG (mPEG). While Phase III clinical trials for both mRNA vaccines demonstrated overwhelming safety and efficacy (e.g. 4.7 and 2.8 anaphylactic reactions cases per million registered during the first months both vaccination campaigns, respectively (CDC COVID-19 Response Team and Food and

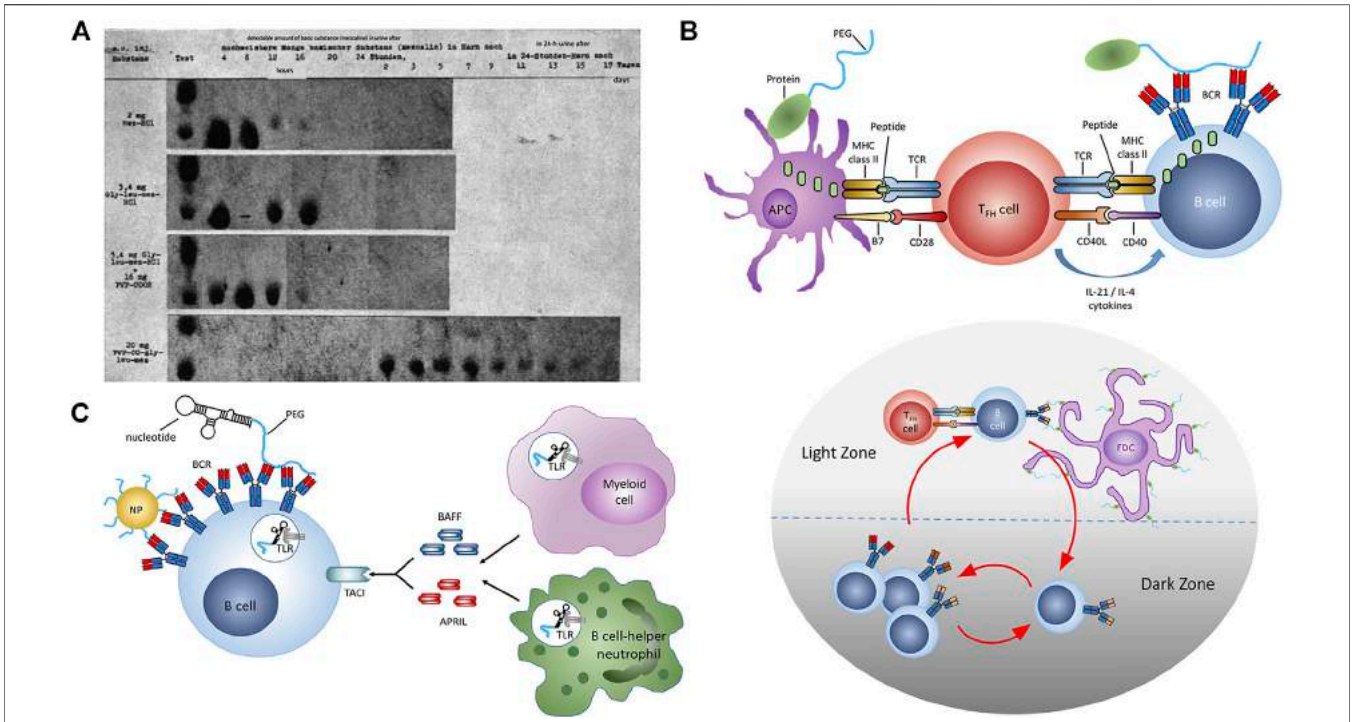

FIGURE 1 | Polymer-Drug Conjugates: Inception to Immunology. **(A)** Renal excretion of mescaline and equimolar dosages of mescaline-PVP conjugate as measured by chromatography of urinary extracts obtained following s.c. adminisration in white mice circa 1955. **(B)** Thymus-dependent immune response against PEG. (upper panel) TFH activation following antigen presentation by APCs. Somatic hypermutation and class switching in B cells following antigen encounter and interaction with activated TFH cells. (lower panel) Affinity maturation of PEG-specific B cells in the spleen. **(C)** Thymus-independent immune response against PEG. Crosslinking of BCRs by PEG and coactivation of TLRs. Reproduced with permission from Reproduced with permission from **(A)** Luxenhofer, 2020 and **(B,C)** Chen et al., 2021. Copyright **(A)** 2020 de Gruyter GmbH and **(B,C)** 2021 American Chemical Society.

Drug Administration, 2021)), their widespread use has led to concerns from some that pre-existing anti-PEG antibodies may induce hypersensitivity reactions (de Vrieze, 2021) or that drug-induced PEG immunity may impact the efficacy or safety of subsequently administered PEGylated drugs or vaccines.

PEG's remarkable hydrophilicity, flexibility, inertness, and relative biocompatibility have found the polymer numerous uses beyond modulating drug circulation or activity and today it can be found near ubiquitously in both consumer products such as detergents, cosmetics, and car wax, as well as in industrial applications including electroplating, historical artifact preservation, and molded product production (Harris, 1992; Prime and Whitesides, 1993; Harris and Chess, 2003; Li et al., 2005; Jokerst et al., 2011). PEGylating has also been used to improve stability of contrast agents for *in vivo* fluorescence imaging, photodynamic therapy, and sonodynamic therapy (Ding et al., 2018; Chen et al., 2021; Xu et al., 2022). Given PEG's near exclusive utilization in polymer-drug conjugates, our rapidly increasing consumer use of the compound, and recent, prevalent, and systemic exposure to PEG in the form of mRNA vaccines and boosters for SARS-CoV-2 (currently >0.5 bn doses (C ovid-data-tracker, 2022) in the United States) (Polack et al., 2020; Baden et al., 2021), several obvious questions arise with relevance to both public awareness and public health: *Is PEG immunogenetic? Does prior environmental exposure or PEG-drug conjugate therapy impact immune responses to PEG? Will PEG immunogenicity affect future vaccine efficacy? How can we minimize and hedge-against PEG immunogenicity in future polymer-drug formulations?*

Immunity Towards PEG Is Pre-existing and Drug Exposure-inducible

PEG was classified as a GRAS (Generally Recognized as Safe) food ingredient by the FDA in 1973 and has a long history of safe use in humans. It is the most widely used stealth polymer in drug delivery and is typically regarded as a non-immunogenic polymer. Early studies by Richter and Akerblom in 1984 found that 0.2% of treatment-naïve individuals (individuals who have never received PEGylated biopharmaceuticals), had antibodies specific to PEG in their plasma (Richter and Åkerblom, 1984). Since then, the presence of pre-existing anti-PEG antibodies has been reported to range from 4.5 to 43.1% in treatment-naïve donors (Shpetner and Vallee, 1991; Garratty, 2004; Chen et al., 2016; Lubich et al., 2016; Yang et al., 2016), leading to the hypothesis that the frequency of pre-existing anti-PEG antibodies is increasing over time (Yang et al., 2016). Recent analysis of 79 historical (samples collected from the 1970s–1990s) and 377 contemporary human serum samples, indicate the presence of anti-PEG antibodies (IgG and IgM) in

approximately 56 and 72% of samples respectively (Yang et al., 2016) with no significant difference in the measured concentrations of anti-PEG IgG and IgM, strongly suggesting that an apparent increase in pre-existing anti-PEG antibodies with time may in fact be a consequence of increased sensitivity in anti-PEG immunoassays developed in recent years (Yang et al., 2016; Chen et al., 2021). For example, direct binding assays using beads or ELISA plates are generally more sensitive compared to traditional bridging assays. Although these studies found that the prevalence of pre-existing anti-PEG antibodies was higher than was previously appreciated, the absolute concentrations of anti-PEG remain low in most positive individuals (Chen et al., 2021) and, as discussed later, drugs administered at different levels may be differentially impacted by pre-existing PEG immunity.

In addition to treatment-naïve immunity, anti-PEG antibodies are also drug-inducible and associated with systemic administration of PEGylated proteins (Chen et al., 2021), nucleic acids, liposomes, and nanoparticles (Judge and MacLachlan, 2008; Mima et al., 2015; Avci-Adali et al., 2013; Ishida et al., 2006a; Ishida et al., 2006b; Ishida et al., 2006c; Kozma et al., 2019). Drug-induced anti-PEG antibody responses occur via two principal mechanisms: T cell-dependent (TD) and T cell-independent (TI) pathways (**Figures 1B,C**). TD is typically associated with PEGylated proteins and peptides (Mima et al., 2015; Elsadek et al., 2020), while TI has been associated with systemic exposure to PEGylated nanoparticles (Freire Haddad et al., 2022). Anti-PEG antibodies induced by TD occur when peptides are presented by B cells to helper T cells, and is characterized by an initial peak of IgM, followed by class switching, and a larger peak in IgG (Freire Haddad et al., 2022). TI occurs when the antigen crosslinks receptors on IgM memory B cells and is characterized by high concentrations of IgM and low concentrations of IgG. Antibodies produced via the TI pathway have a weaker affinity for PEG compared to TD (Freire Haddad et al., 2022). While the basic underpinnings of anti-PEG immunity such as these are clear, 1) our understanding of how these processes vary with health or disease status, age, sex, or ethnicity and 2) our ability to predict the magnitude and functional impact of these responses on patients collectively remain unclear.

PEG Immunity can Induce Hypersensitivity Reactions and Alter Drug Transport/Efficacy but these Effects Vary Across Formulation Type and Mode of Administration

Hypersensitivity reactions, including anaphylaxis has been reported in association with many PEG-containing formulations including PEG-protein conjugates (pegloticase (Lipsky et al., 2014), pegvaliase (Gupta et al., 2018), pegaspargase (Hasan et al., 2017; Browne et al., 2018; Liu et al., 2019), pegcrisantaspase (Rau et al., 2018)), PEG excipients (polysorbate 80 (Pérez-Pérez et al., 2011)), contrast agents (SonoVue (de Groot et al., 2004; Geleijnse et al., 2009)), liposomes encapsulating oligonucleotides or plasmid DNA (Semple et al., 2005; Judge et al., 2006), and liposomal doxorubicin (Chanan-Khan et al., 2003; Szebeni, 2014). Pre-existing PEG antibodies, in contrast, have been implicated in hypersensitivity reactions to PEGylated medicines including pegaspargase (Liu et al., 2019) and the RNA aptamer, pegnivacogin (Povsic et al., 2013). Acute severe allergic reactions to pegnivacogin were observed only in patients with pre-existing anti-PEG antibodies, and the level of anti-PEG IgG antibodies correlated with adverse event severity (Povsic et al., 2016). In addition, 2 of 25 phenylketonuria patients treated with pegvaliase developed anaphylactic and hypersensitivity reactions to a PEGylated contraceptive (Longo et al., 2014) and 3 patients who developed allergies to pegaspargase also experienced hypersensitivity reactions when treated with pegcrisantaspase (Rau et al., 2018), indicating that anti-PEG antibodies induced by one PEGylated medicines can cross-react to other subsequently administered PEGylated medicines. The mechanism(s) by which anti-PEG antibodies induce hypersensitivity reactions is poorly understood; however, some possible mechanisms by which pegylated nanoparticles and pegylated nucleotides could induce hypersensitivity reactions include: 1) complement activation-related pseudoallergy (CARPA) (Szebeni et al., 2011; Dézsi et al., 2014; Mohamed et al., 2019), whereby anti-PEG antibodies bound to PEG on a nanoparticle or liposome surface can activate the complement cascade, liberating the anaphylatoxins C3a and C5a (Neun et al., 2018; Mohamed et al., 2019; Chen et al., 2020) and 2) Fc receptor activation of innate immune cells either by anti-PEG IgE antibodies (Shah et al., 2013; Stone et al., 2019; Zhou et al., 2021) or allergen-specific IgG that binds to Fc gamma receptors (FcγRs) expressed on platelets, macrophages, basophils, or neutrophils to release various mediators such as platelet-activating factor (PAF), cysteinyl leukotrienes (CysLTs), histamine, and serotonin (Finkelman, 2007; Reber et al., 2017; Beutier et al., 2018).

Accelerated blood clearance (ABC) of PEGylated compounds was identified in mice in 1999, and in patients treated with pegaspargase in 2007 (Cheng et al., 1999; Cheng et al., 2000; Armstrong et al., 2007) and is caused by an immune reaction associated with repeat exposure to PEG. The first injection of PEGylated drugs induces anti-PEG antibodies, which then bind and form an immune complex with the second dose of the PEGylated compound to activate the complement system. This results in the opsonization of PEG with C3 fragments and enhanced uptake by Kupffer cells in the liver and can result in altered drug pharmacokinetics and biodistribution (PK, BD) and reduced drug efficacy in subsequent doses (Dams et al., 2000; Ishida et al., 2006a; Ishida et al., 2008; Ishida and Kiwada, 2008; Hashimoto et al., 2014). Rapid drug clearance and loss of drug efficacy have been reported following treatment with PEG-uricase, pegvaliase (Gupta et al., 2018), PEGylated liposomes (Dams et al., 2000; Laverman et al., 2001; Ishida et al., 2003), and PEGylated liposomal doxorubicin. ABC has also been observed in animal models treated with empty PEGylated liposomes (Dams et al., 2000; Semple et al., 2005; Ishida et al., 2006a; Ishida et al., 2006b), poly(lactic acid) (PLA) nanoparticles, microbubbles, and lipoplexes (Ishihara et al., 2009; Fix et al., 2018). In addition, anti-PEG antibodies can hinder the

distribution of PEGylated nanoparticles to target tissues. For example, N-linked glycans present on anti-PEG antibodies bound to PEGylated nanoparticles can interact with mucin in the mucosal layer and prevent passage to epithelial surfaces (Henry et al., 2016).

Some PEGylated nanomaterials and proteins do not display ABC in animal models (Koide et al., 2008; Kaminskas et al., 2011; Koide et al., 2012; Grenier et al., 2018) and one explanation for this phenomenon is that in order for ABC to occur, a threshold molar ratio of anti-PEG antibodies to PEG compound is required for efficient clearance (Shiraishi et al., 2016; McSweeney et al., 2018). For example, the molar concentration of PEG-proteins in circulation is typically lower than that of PEG-liposomes (Grenier et al., 2018) at therapeutic dosing levels; thus, nanoparticles are thought to be less vulnerable to anti-PEG antibody-associated clearance than proteins. Indeed, prior studies show that strong ABC is observed when the number of antibodies in circulation exceeds the number of PEGylated compounds (Xu et al., 2022). This trend holds across most PEGylated compounds including proteins, liposomes, micelles, and polymeric nanoparticles and agrees with previous studies showing that three anti-PEG antibodies per PEGylated protein or about 10 anti-PEG antibodies per pegylated liposome are required for ABC (Shiraishi et al., 2016; McSweeney et al., 2018; Chang et al., 2019). These findings suggest that only compounds dosed at very low molar concentrations (*e.g.* PEG-IFNα) may be susceptible to polymer-specific ABC whereas the estimated threshold concentration of anti-PEG antibodies needed to accelerate the clearance of nucleic acid drug carriers (*e.g.* Patrisan) overwhelmingly exceed those observed in patient blood (Xu et al., 2022).

In addition to formulation-dependent susceptibility to polymer immunogenicity, mode of administration can also modulate the impact of antibody recognition. Most clinically approved polymer-drug conjugates are intravenously administered and thus their interaction with plasma IgG and IgM is higher than may be expected following intramuscular or intratumoral injection, as is common among many mRNA indications including both BNT162b2/Comirnaty and mRNA-1273/Spikevax (Schoenmaker et al., 2021). Thus, the strikingly low rates of anaphylaxis observed following SARS-CoV-2 mRNA vaccination (CDC COVID-19 Response Team and Food and Drug Administration, 2021) may be attributable in part to its intramuscular administration. Future studies focusing on the impact of polymer type/architecture/density and corresponding immunogenicity on drug efficacy and transport (*e.g.* lymphatic) following local administration are therefore warranted.

PEG Immunogenicity can be Minimized but Alternative Polymers in Clinical Use are Lacking

Having established that PEG immunogenicity can limit the clinical utility of PEG-drug conjugates and that nanoparticle-based formulations may be less vulnerable to some of these effects relative to polymer-protein drug conjugates, how can one minimize the impact and risk of immunogenicity-diminished efficacy from future polymer-conjugated drugs and vaccines? As discussed above, PEG immunogenicity can arise through a variety of mechanisms (Xu et al., 2022) and includes antibody recognition associated with hypersensitivity reactions (e.g. anaphylaxis), accelerated blood clearance, premature drug release, or cross-reaction to other PEGylated therapies, among others. While limited in number, prior studies suggest that PEG antibody recognition is strongly dependent on polymer molecular weight (Xu et al., 2022), architecture, and end-functional group (Saifer et al., 2014). For example, antibodies with affinity towards backbone ethylene oxide units recognize immobilized PEG that is 2 kDa and larger with a minimum epitope subunit of approx. 16 repeats (700 Da) (Lee et al., 2020). Given that nearly all systemically administered polymer-drug conjugates are 2 kDa and above—per linear chain—the utilization of higher densities of lower molecular weight PEG may diminish the therapeutic impact of these backbone-specific antibodies. Such an approach is conceptually illustrated by branched PEG-drug conjugates (e.g. peginterferon alfa-2a, certolizumabpegol, and pegaptanib); however, those in clinical use (and which are systemically administrable) are limited to single site-modified, di-branched PEGs with per-arm molecular weight of approx. 10–30 kDa and with methoxy terminal groups; thus, the use of increasingly branched PEGs (i.e. hyperbranched, star, dendritic, bottlebrush) of lower per-branch molecular weight may diminish recognition by backbone-specific antibodies while maintaining favorable drug circulation, solubility, stability, activity profiles.

Polymer end-terminal groups can also play an important role in engineering future, less immunologically vulnerable PEG-drug conjugates as antibodies that recognize end-groups represent the other primary class of PEG-specific antibodies detected *in vivo*. While all clinical PEG-drug conjugates are chain-terminated by methoxy groups, recent preclinical studies suggest that hydroxy-terminal PEG conjugates generate lower amounts of backbone-specific anti-PEG IgM (Shimizu et al., 2018) and, while this improved immunogenicity comes with the tradeoff of higher complement activation and second-dose ABC (and typically, slightly shorter circulation half-life (Arvizo et al., 2011)), these findings may lead to the development of future polymer-drug conjugates with less propensity for immune activation. Other polymer end-group engineering strategies include the utilization of zwitterionic (Arvizo et al., 2011), ethoxy, and n-butyl ether (Saifer et al., 2014) moieties.

In addition to direct modifications of the polymer, corresponding drugs themselves can also modulate PEG immunogenicity. The introduction of 2'-fluro-modified pyrimidines and 2′-O-methyl-modifed purines has been shown to reduce the immunogenicity of PEGylated nucleic acids (Judge et al., 2005; Wang et al., 2009; Yu et al., 2009; Lee et al., 2016) while chemotherapeutics cytotoxic to B cells such as doxorubicin, mitoxantrone or oxaliplatin (Laverman et al., 2001; Ishida et al., 2006c; Cui et al., 2008; Abu Lila et al., 2012; Nagao et al., 2013) have been shown to mitigate anti-PEG IgM induced via PEGylated liposomal drug carriers often used to deliver these compounds *in vivo* (Cui et al., 2008; Mohamed et al., 2019).

Pharmacologic approaches have been further employed to diminish the impact of polymer immunogenicity including

conjugation to or pre-treatment with immunosupressants, as well as the pre-treatment or co-infusion of tolerogenic compounds. Khanna et al. for example recently reported that pretreatment with the B/T cell immunosuppressant, mycophenolate mofetil, significantly improved treatment outcomes in a Phase I trial of patients with gout receiving pegloticase (Khanna et al., 2021). Other immunosuppressives under investigation to mitigate pegloticase immunogenicity include methotrexate, azathioprine, and leflunomide, while those used in conjunction with other ADA-prone therapies include rapamycin and anti-CD20. Likewise, pre-treatment or co-treatment with polymer, in particular high molecular weight (*i.e.* 40 kDa) PEG, has also been shown to reduce liposome-induced anti-PEG antibodies in preclinical studies (McSweeney et al., 2021). Taken together, these pharmacologic approaches are viewed by some to obviate the need PEG alternatives or derivatives; however, the deployment of immunosuppressives in combination with polymer-based vaccines and immunostimulatory therapies presents significant tradeoffs to drug efficacy, while PEG-based tolerogenics remain to be tested in patients.

Given 1) the therapeutic impact of PEG on drug immunogenicity, 2) the possible increasing prevalence of pre-existing and drug-induced PEG immunity, 3) the growing public need for safe and effective mRNA vaccines, and 4) our prevailing reliance on PEG for use in clinically approved nucleic acid and polymer-drug conjugate therapies (Schoenmaker et al., 2021), it is clear that the development and clinical validation of alternatives to (or derivatives of) PEG represents not only an unmet clinical need but also one with broad public health and national strategic interest. Indeed, the need for alternatives to PEG is a common refrain among those in the field (Harris, 1992), one as old as the first polymer-drug conjugate, Adagen; however, given the wide variety of potential candidate macromolecules such as polysaccharides, polyglycerols, and glycopolymers, (reviewed in detail elsewhere (Knop et al., 2010; Pelegri-O'Day et al., 2014; Bludau et al., 2017; Ekladious et al., 2019; Xu et al., 2022)), it begs the question as to why alternatives have yet to be approved (and studied post-approval) beyond poly(styrene co-maleic acid) (1993, Japan). Concerns over PEG immunogenicity have led some pharmaceutical companies to shy-away from or drop PEGylated products from their pipelines entirely (de Vrieze, 2020), thus the prospect of biopharma advancing clinically untested polymers through lengthy and expensive clinical trials is a difficult ask in the absence of a thoughtful incentive structure.

Given the challenging risk-reward of advancing non-PEG-based polymer-drug conjugates towards clinical translation, what can governments and funding agencies do to facilitate continued innovation in polymer-drug conjugate development and ensure the capacity for safe and effective vaccination at-scale? 1) Biosimilar-like regulatory guidelines for conjugable polymers (i.e. polysimilars) may be one approach to formalize and streamline the approval of new polymer-drug conjugates, albeit one likely requiring increased rigor given the wide structural diversity and potential health hazards of various polymer subunits relative to proteins. 2) Funding or federal lab support to perform large-scale longitudinal studies of immunogenicity towards polymers and other drug conjugates/excipients (lipids, polysaccharides, polypeptides, etc) would elucidate current (and potentially dynamic or age-, race-, and sex-specific) risks of polymer immunogenicity to human health, drug conjugate efficacy, and the strategic national need for mRNA vaccine-stabilizing polymers. 3) Federally subsidized R&D to offset the risks taken-on by companies exploring PEG- and other polymer-conjugates would greatly incentivize further innovation in this space. 4) Funding to improve our poor mechanistic understanding of polymer-induced immunogenicity and associated short- and long-term health risks would accelerate the discovery of new PEG derivatives and alternatives or propel historically utilized polymers through clinical translation. 5) Federal partnerships to ensure the financial viability of domestically manufactured, pharmaceutical-grade PEG and other polymers, as a matter of national interest, would ensure our readiness for future pandemics (and supply chain challenges) surely yet-to-come. In closing, while it is tempting to suggest a singular direction for polymer-drug conjugate development in the future, we also acknowledge that the ideal properties for a conjugation partner vary substantially with drug class, mode of administration, dosing frequency, and disease indication as discussed above; thus, with proper incentives, funding, and tools we anticipate that future conjugates will not only increase in diversity but also diverge based upon drug type and/or indication.

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CORRESPONDENCE

COVID-19 vaccine anaphylaxis: PEG or not?

To the Editor,

We read the editorial by Cabanillas et al[1] with great interest. We agree that great consideration needs to be given to the possibility that the polyethylene glycol [PEG])-2000]-N,N-ditetradecylacetamide micellar carrier system for the active mRNA spike protein component of the Pfizer–BioNTech BNT162b2 mRNA vaccine, sometimes referred to as the lipid nanoparticle (LNP) delivery system, could be evoked in the recent immediate reactions post-emergency use authorization (EUA).

In our prior work, we have described patients with immediate reactions to PEG3350. These reactions were consistent with anaphylaxis to PEG3350 bowel preparations and corticosteroids containing both PEG3350 and polysorbate (PS) 80 as an excipient (Table 1). Aside from clinical presentation consistent with anaphylaxis, the case for these being IgE-mediated was supported by positive skin tests to both PEG3350 and PS80 (which we believe to be cross-reactive when primary sensitization occurs through PEG3350) as well as the presence of specific IgE (sIgE) against PEG by two independent methods.[2,3]

We would also like to highlight a case of anaphylaxis to an intravenous medication that might be mechanistically relevant. We observed a patient with a history to suggest preexisting PEG3350 anaphylaxis who also developed anaphylaxis when later exposed to a PEGylated liposome (PEGLip) microbubble, PEGLip 5000 perflutren echocardiogram contrast (Definity®). This case was also skin test positive to PEG3350 and PS80 as well as having demonstrable anti-PEG sIgE.[4,5] In post-marketing surveillance of PEGLip perflutren, first approved by the Food and Drug Administration (FDA) in 2001, a multi-center retrospective analysis observed four cases of anaphylaxis out of 66 164 doses of PEGLip perflutren administered.[6] In this same study, an alternative formulation of perflutren conjugated to human albumin (Optison™), FDA approved in 1997, was also monitored and had no cases of anaphylaxis observed out of 12,219 doses administered.[7] Both formulations of perflutren carry a FDA black box warning for the risk of severe hypersensitivity reactions, but a hypothetical mechanism underlying these reactions had not been clearly elucidated or attributed prior to our report. While anaphylactic reactions to PEGLip perflutren appears to be rare overall, occurring in 0.006% of patients in the study by Wei et al, these reactions do occur.[6]

PEG and/or lipid complexes in vitro have been shown to cause complement activation, and given the importance of these technologies for developing new therapeutics and vaccines, there is a science behind "PEG pairing" to minimize this effect.[8] However, our clinical and laboratory observations do support that IgE-mediated reactions can occur to a PEG-containing product presumably due to previous subclinical sensitization. These patients can notably be labeled as "idiopathic anaphylaxis" or multiple drug allergy if multiple episodes to different products occur over time without knowledge of the shared excipients. An additional observation by our group and others is that for immediate reactions associated with PEG there appears to be a molecular weight (MW) threshold.[9] This was seen in our skin test-positive patients who were positive to PEG3350 but negative to PEG300 who then tolerated oral challenge with PEG300.[2,9] This MW predisposition may also vary by patient. To support this hypothesis, we have observed increasing binding avidity of anti-PEG sIgG as the molecular weight of the PEG increases.[2]

Currently, IgE-mediated reactions associated with PEG appear to both uncommon and underrecognized.[2,4] PEG2000 is crucial to the formation of micelles used as the delivery system for the mRNA vaccines. It will be important to determine whether PEG2000 is implicated in the IgE-mediated reactions in PEG allergic patients, both as a separate ingredient or as a lipid reagent as formulated in the Moderna, Pfizer–BioNTech, and future mRNA vaccines. Cases clinically compatible with anaphylaxis to the Pfizer–BioNTech mRNA vaccine have occurred on the first dose in the post-EUA phase of surveillance in healthcare workers. It is possible that these could be IgE-mediated reactions related to preexisting sensitization to a different PEG product. Until we understand more, patients with previous immediate reactions compatible with PEG anaphylaxis will be excluded from receiving the SARS-CoV-2 mRNA vaccines. Similarly, we need to understand the risk of immediate reactions to PEG products in those who have experienced anaphylaxis with the Pfizer–BioNTech SARS-CoV-2 mRNA vaccine and other mRNA vaccines if these occur. Until assessed by an allergist, it would be recommended that these individuals also avoid not only future vaccination with an mRNA SARS-CoV-2 vaccine but all components of the vaccine which would include PEG products (Table 1).

Understanding the mechanisms of immediate reactions associated with the Pfizer–BioNtech and any other mRNA vaccines that utilize different lipids in their PEG2000-micellar delivery system (Table 1), should they occur, will be crucial not only for the safety of the current COVID-19 mRNA vaccine program but for mRNA vaccines in earlier stages of development for other viruses and cancer.

TABLE 1 Selected vaccines (A) and medications (B) containing PEGs and polysorbates

Generic Name	Brand Name	Excipient
(A) Vaccines Containing PEGs or Polysorbates		
Vaccines		
Influenza	Flublok & Flublock quad	Polysorbate 20
Hepatitis A	Havrix	Polysorbate 20
Hepatitis A&B	Twinrix	Polysorbate 20
Tdap	Boostrix	Polysorbate 80
Influenza	Fluad	Polysorbate 80
Influenza	Fluarix quad	Polysorbate 80
Influenza	Flucelvax quad	Polysorbate 80
Influenza	Flulaval Quad	Polysorbate 80
HPV	Gardasil and Gardasil −9	Polysorbate 80
Hepatitis B	Heplisav-B	Polysorbate 80
DTaP	Infanrix	Polysorbate 80
Japanese encephalitis	JE-Vax	Polysorbate 80
DTaP+IPV	Kinrix	Polysorbate 80
DTaP+HepB+IPV	Pediarix	Polysorbate 80
DTaP+IPV+Hib	Pentacel	Polysorbate 80
Pneumococcal 13-valent	Prevnar 13	Polysorbate 80
DTaP+IPV	Quadracel	Polysorbate 80
Rotavirus	RotaTeq	Polysorbate 80
Zoster	Shingrix	Polysorbate 80
Meningococcal group B	Trumenba	Polysorbate 80
mRNA-1273 COVID-19	Moderna	Polyethylene glycol [PEG] 2000 dimyristoyl glycerol [DMG] (also called PEG2000-DMG)
BNT162b2 COVID-19	Pfizer and BioNTech	2 [(polyethylene glycol)-2000]-N,N-ditetradecylacetamide (also called ALC-0159)
(B) Medications Reported in Association with Anaphylaxis to PEGs or Polysorbates		
Gastrointestinal disease treatments and diagnostic aids		
PEG3350	GoLytely, Miralax	PEG3350
Aluminum hydroxide, magnesium carbonate	Gaviscon	PEG20000
Infliximab	Remicade	Polysorbate 80
Adalimumab	Humira	Polysorbate 80
Ustekinumab	Stelara	Polysorbate 80
Certilizumab pegol	Cimzia	Polysorbate 80
Rheumatologic disease treatments		
Methylprednisolone acetate (injectable)	Depo-Medrol	PEG3350
Triamcinolone acetonide (injectable)	Kenalog	Polysorbate 80
Adalimumab	Humira	Polysorbate 80
Pegloticase	Krystexxa	Polysorbate 80
Cardiovascular disease treatments and diagnostic injections		
Clopidogrel	Plavix	PEG6000
Amiodarone injection	Pacerone	Polysorbate 80
PEGylated liposomal Perflutren	Definity	N-(methoxypolyethylene glycol 5000 carbamoyl)-1,2-dipalmitoyl-sn-glycero-3-phosphatidylethanolamine, monosodium salt (also called MPEG5000 DPPE)
Radiologic Procedures		

(Continues)

TABLE 1 (Continued)

Generic Name	Brand Name	Excipient
Ultrasound gels with PEG	Multiple formulations	PEG8000
Gynecologic disease treatments		
Medroxyprogesterone acetate	Depo-Provera	PEG 3350, Polysorbate 80
Vaginal suppositories (European formulation)	Vagisan Zäpfchen, Vagisan Feuchtcreme	PEG 1500/6000/polysorbate 60
Hematologic/Oncologic disease treatments		
Etoposide	Toposar	PEG300, Polysorbate 80
Docetaxel	Taxotere	PEG300, Polysorbate 80
Erythropoietin	Retacrit	Polysorbate 20
Darbepoetin	Aranesp	Polysorbate 80
Pegaspargase	Oncaspar	PEG5000
PEGylated liposomal doxorubicin	Doxil, Caelyx	DSPE (1,2-distearoyl-sn-glycero−3-phosphoethanolamine-(PEG5000)
Biologic and monoclonal antibody medications used as chemotherapy	Various	Typically polysorbate 80
Infectious disease treatments		
Antibiotic tablets	Various formulations depending on country of origin	PEG400 most common, 1000, 4000, 6000
Phenoxymethylpenicillin injection (European formulation)	Generic	PEG6000
Bamlanivimab	Lilly	Polysorbate 80
Casirivimab/Imdevimab	Regeneron	Polysorbate 80
Allergic and Asthma disease treatments		
Omalizumab	Xolair	Polysorbate 20
Dupilumab	Dupixent	Polysorbate 80
Mepolizumab	Nucala	Polysorbate 80
Genetic disease treatments		
Pegvaliase	Palynziq	PEG20000
Miscellaneous Considerations on Other Drugs of Possible Concern		
This table does not constitute an exhaustive list. Many film coated tablets, gels, orphan drugs and injectables (especially biologics) contain PEGs and polysorbates. For FDA approved products, the NIH Daily Med Website (https://dailymed.nlm.nih.gov/dailymed/) provides a rapidly searchable database of package inserts. These formulations may vary by country and manufacturer, however		

Abbreviations: DTaP, diphtheria, tetanus, acellular pertussis; FDA, Food and Drug Administration; *HepB*, hepatitis B; *Hib*, *haemophilus influenzae* type B; *HPV*, human papillomavirus; *IPV*, inactivated polio vaccine; *NIH*, National Institutes of Health; *PEG*, polyethylene glycol; *Tdap*, tetanus, diphtheria, acellular pertussis.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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Immediate Hypersensitivity to Polyethylene Glycols and Polysorbates: More Common Than We Have Recognized

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Abstract

Background: The most common immediate hypersensitivity to macrogols is associated with PEG 3350, however the epidemiology, mechanisms and cross-reactivity are poorly understood. Thousands of medications contain either PEGs or structurally similar polysorbates.

Objective: Our objective was to better understand the mechanism, cross-reactivity and scope of PEG hypersensitivity.

Methods: Two cases with a past history of immediate hypersensitivity to PEG-containing medications were used to study potential mechanisms and cross-reactivity of immediate reactions to PEG 3350. Skin testing and oral challenges with PEG and polysorbate-containing agents were employed to determine clinical reactivity and cross-reactivity between the two allergens. Enzyme-linked immunosorbent assay (ELISA) and electrochemiluminescent immunoassay were used to detect anti-PEG specific IgG and IgE respectively, using PEGylated protein or PEG alone as antigens in two cases and six PEG 3350 tolerant controls. We searched FDA adverse event reports for immediate reactions to PEG 3350 to determine the potential scope of this problem in the United States.

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Results: Skin and provocation testing demonstrated symptomatic reactivity in both cases to PEG 3350 and polysorbate 80. Plasma samples were positive for anti-PEG specific IgE and IgG antibodies only in cases and binding increased directly proportional to the molecular weight of PEG tested. FDA adverse event reports revealed 53 additional cases of possible PEG 3350 anaphylaxis.

Conclusions: Immediate hypersensitivity to PEG 3350 with cross-reactive polysorbate 80 hypersensitivity may be under recognized in clinical practice and can be detected with clinical skin testing. Our studies raise the possibility of an IgE mediated Type I hypersensitivity mechanism in some cases.

Keywords

polyethylene glycol; PEG; immediate hypersensitivity; allergy; polysorbate

Background:

Macrogols, including polyethylene glycols (PEG) and the structurally related polysorbates (Figure 1), are compounds whose primary feature includes polyether groups. They have wide ranging use in medical and commercial settings, with molecular weights (MW) that range from 200 to 35,000g/mol.[1] PEG of MW between 3350 and 6000 are frequently used as excipients in many liquid and solid formulations of medications.[2, 3] PEG of MW 5000 is used in conjugated enzyme therapeutics, such as PEG-asparaginase and PEG-adenosine deaminase, to improve drug pharmacokinetics and lower immunogenicity. PEG of MW 3350 is the primary ingredient in commonly used oral bowel preparations for colonoscopy procedures in the United States.[1, 4] Recently, PEGs of this MW range have been receiving attention as a cause of anaphylaxis to preparations used for colonoscopies,[5] and as an immunogenic epitope in PEGylated asparaginase (Oncaspar and Pegcrisantaspase).[6, 7] There is only limited awareness of their role in reactions to medications where they are present as an excipient.[4, 8–10] Many patients report repeated cutaneous exposures[11–14] or local reactions to PEG-containing topical items[15] prior to the onset of systemic reactions to high molecular weight PEG containing medications, suggesting a cutaneous mode of sensitization. Gastrointestinal sensitization has been theorized in PEG allergic patients with an impaired epithelial barrier.[16–18] However, the scope to which macrogol hypersensitivity might be a problem in the United States and the mechanism for PEG and polysorbate reactions are not well understood.[8, 19, 20] After encountering two cases of life threatening immediate hypersensitivity to macrogols in our clinic, we sought to further understand the mechanism and scope of immediate hypersensitivity to PEG.

Methods:

Clinical Surveillance:

Cases were recruited through a dedicated drug allergy clinic at Vanderbilt University Medical Center. A detailed clinical case description was obtained from patients whose history suggested an immediate reaction to PEG 3350 containing colonoscopy preparations, laxatives, or injected corticosteroids during a 3 year period.

Skin Testing and Challenges:

To determine clinical reactivity to macrogols, including polyethylene glycols and polysorbate containing products, we used a combination of skin prick, intradermal and challenge testing with standard methodologies.[21]

Controls:

Two healthy adult volunteers served as negative controls for the skin testing protocol. Six additional healthy adult volunteers with previous exposure to PEG 3350 during colonoscopy preparation or use of laxatives during the last 5 years provided blood samples used as controls during laboratory assays.

Laboratory Methods:

To better understand the mechanism of macrogol hypersensitivity in the two cases, we next sought to detect the presence of polyethylene glycol specific antibodies. Enzyme-linked immunosorbent assay (ELISA) was used for the detection of anti-PEG antibodies. Briefly, Corning 96-well EIA/RIA assay microplates were coated with 5,000g/mol methoxy-PEG-*E.coli* asparaginase (Oncaspar) at 10 μg/ml. For anti-PEG IgG detection, plasma obtained from the aforementioned 2 cases 2~3 months after their last anaphylaxis episodes were incubated at 1:400 dilution. For anti-IgE detection, the same plasma samples were pretreated with Protein G Plus Agarose (Thermo Fisher Scientific) at 1:1 ratio to remove IgG, then incubated at 1:10 dilution. HRP-conjugated goat anti-human IgG (Sigma) or anti-human IgE (BioRad) antibodies were added at 1:1000 and 1:10,000 dilution respectively. Plates were read at dual wavelengths of 490 nm and 630 nm on an ELx808 microplate reader (BioTek). Plasma samples from 6 patients with similar exposure to colonoscopy preparations containing macrogols were used as controls.

To better determine the presence or absence of PEG specific IgE, we next used an electrochemiluminescent method with greater sensitivity for detection. Standard MULTI-ARRAY 96-well SECTOR plates were coated with Oncaspar and 5,000g/mol methoxy-PEG-bovine catalase at 10 μg/ml. Samples were processed with Protein G Plus Agarose as described above, then incubated at 1:10 dilution. Biotin-conjugated goat anti-human IgE (BioRad) antibody was added at 1:10,000 dilution. SULFO-TAG labeled Streptavidin was used as the detection reagent. Plates were read with a Sector Imager 6000 Analyzer (Meso Scale Discovery).

Furthermore, to investigate the effect of the molecular size of unconjugated PEG on anti-PEG specific IgG binding, we coated Nunc Maxisorp 96-well microplates (Thermo Fisher Scientific) with 5μg/ml HO-PEG-NH_2 of MW ranging from 1kDa to 10 kDa (Creative PEGWorks). Case and control samples were incubated at 1:100 dilution. Other steps were the same as the anti-Oncaspar IgG detection ELISA aforementioned.

Public Data Review:

To evaluate the scope to which polyethylene glycol 3350 might be associated with anaphylaxis in the United States, we next undertook a review of the publicly available FDA Adverse Event Reporting System (FAERS) database from 1989 through 2017. Using the

search terms “polyethylene glycol” and “anaphylactic shock” or “anaphylactic reaction” we reviewed the number of these complaints for polyethylene glycol containing colonoscopy preparations and laxative products. We evaluated cases associated with branded and generic colonoscopy and laxative products whose primary ingredient was PEG 3350, including colonoscopy products both with and without electrolytes.

Medication Excipient Review:

To evaluate the degree to which immediate hypersensitivity to PEG 3350 or polysorbate 80 might affect medication or vaccine safety for affected patients, we next reviewed publicly available data in the searchable “DailyMed” database provided by the National Library of Medicine,[4] which allows for search queries targeting both active and inactive ingredients of all FDA approved and over-the-counter (OTC) medications in the United States. Searches conducted on the advanced search feature of this database will return reviewable information on the first 1000 hits. Using this database, we searched with the terms “polyethylene glycol 3350” and “polysorbate 80”, selecting that these ingredients must be either an “active” or “inactive” ingredient. We then classified the first 1000 hits by route of administration and indication for the medication. We also reviewed vaccine excipient summaries provided by the CDC for vaccines containing either of the two ingredients.[22]

Results:

Description of Cases:

During our 3 year period of surveillance, we encountered two patients with a history of anaphylaxis during preparation for colonoscopy and after methylprednisolone acetate injections.

The first such patient was a 57 year old white male with an occupational history as a mechanic and electrician, who presented to our clinic for evaluation of suspected medication allergies causing anaphylaxis. 5 years prior to presentation, he noted that while preparing for a colonoscopy, taking oral Colyte® brand colonoscopy preparation (active ingredient PEG 3350[23]) he developed severe itching of his palate and throat, which was alleviated by diphenhydramine. Two years prior to presentation, he underwent injection of methylprednisolone acetate (excipient PEG 3350[24]) into his neck as treatment of radicular pain from a bulging disk. Within seconds of receiving this medication, he developed urticaria, burning all over the body, throat tightness, wheezing, and hypotension. He was immediately given epinephrine, and transferred via emergency medical services to the emergency department, where he received additional epinephrine and IV fluid therapy. One year prior to presentation, he was scheduled for routine follow up of his initial colonoscopy. During his first few sips of Moviprep® brand colonoscopy preparation (active ingredient PEG 3350[25]) he developed severe itching of his palate and throat, along with diffuse urticaria. Symptoms resolved over a couple of hours with immediate cessation of the bowel preparation and diphenhydramine. Three months prior to presentation, he attempted once again to undergo colonoscopy, using oral Gavilyte™-G generic preparation (active ingredient PEG 3350[26]). He consumed approximately 10–12 ounces and subsequently developed itching, burning urticarial rash along with the urge to defecate. He went to the

bathroom where he experienced syncope and fell, knocking a hole in the drywall with his head. Upon hearing the fall, his son, a nurse, arrived and checked his father's blood pressure, which was 60/20, and administered 0.3mg of 1:1000 concentration intramuscular epinephrine. EMS was called, and administered additional intramuscular epinephrine on arrival, taking the patient to the emergency department where he received diphenhydramine, famotidine, and intravenous fluids. He was observed overnight and discharged the next day.

The second patient was a 51 year old with an occupational history as a mechanic exposed to glycol containing hydraulic fluids, presenting for evaluation due to concern for peri-operative anaphylaxis. Four months prior to presentation, he was to receive an outpatient c-spine epidural steroid injection for cervical spine degeneration. He received lidocaine followed by omnipaque and methylprednisolone acetate. Within 5 minutes after the procedure he became itchy, red, hypotensive and a code was called. He was given ondansetron and methylprednisolone sodium succinate in addition to IV fluids. He was taken to the emergency department where he noted swelling in his hand, itching, difficulty swallowing, and hoarseness. He was given epinephrine as well as IV diphenhydramine and famotidine. He was admitted to the ICU for observation. One month prior to presentation, he began to develop a reaction just prior to a scheduled colonoscopy after use of a polyethylene glycol 3350 colonoscopy preparation. He became hypotensive and flushed and was treated with diphenhydramine, epinephrine, and IV fluids.

Skin Testing and Challenges:

The three bowel preparations and methylprednisolone acetate to which the patients had experienced immediate hypersensitivity reactions all share the ingredient PEG 3350. Both patients subsequently underwent prick and intradermal skin tests with serial dilutions of common corticosteroids, including methylprednisolone acetate (containing PEG 3350), methylprednisolone succinate (containing neither PEG nor polysorbate 80), betamethasone (containing neither PEG nor polysorbate 80), dexamethasone (containing neither PEG nor polysorbate 80), and triamcinolone acetonide (containing polysorbate 80, which shares significant structural homology to PEG) (Table I). During intradermal testing to the steroid preparations, patient 1 developed a sensation of throat and body itching, with a visible urticarial rash expanding from testing sites which was alleviated with 10 mg of cetirizine and 300 mg of ranitidine, without necessitating further treatment with epinephrine (Figure 2). Patient 1 was subsequently demonstrated to have skin test positivity to other polysorbate 80 containing products, including eye drops and conjugated pneumococcal vaccine, but was able to asymptomatically tolerate a low molecular weight PEG oral challenge with PEG 300. While Patient 2 had negative prick testing to PEG 3350 containing products and negative intradermal skin testing to methylpredisolone acetate, he did have positive testing to triamcinolone acetonide containing polysorbate 80. Upon challenge with PEG 3350 he developed diffuse urticaria, respiratory distress and hypotension requiring epinephrine and emergency department transfer. Both patients were able to tolerate challenge with parenteral steroids that did not contain macrogols.

Two healthy adult controls underwent polyethylene glycol testing on the same day as Patient 2, with negative testing and no irritation at testing sites.

Laboratory Results:

Anti-PEG specific antibody concentrations were measured as optical density (OD) from the ELISA assay using methoxy-PEG-*E.coli* asparaginase as the antigen source. Anti-PEG specific IgG (sIgG) ODs in plasma samples from the 2 cases (0.50 for Patient 1 and 0.31 for Patient 2) were significantly higher than that of the 6 PEG-exposed controls (99% CI = 0.025 ± 0.019), indicating that both cases were positive for anti-PEG sIgG in these samples obtained 2~3 months after the last reaction (Table E1, Online Only). Anti-PEG specific IgE readings for the patients were negative by this method: ODs were 0.045 and 0.020 respectively for Patient 1 and Patient 2 compared to controls of 0.019 ± 0.0037, none of which were above the uncoated well background signal (99% CI = 0.050 ± 0.011).

Using the more sensitive Meso Scale Discovery electrochemiluminescence method we were then able to detect specific IgE directed against PEG in our two cases, but not our controls. Luminescence intensity from the two cases against Oncaspar (88 for Patient 1 and 77 for Patient 2) was significantly higher than that of the controls (99% CI = 55.9 ± 4.1). Similarly, luminescence intensity from the two cases against PEG-bovine catalase (246 for Patient 1 and 194 for Patient 2) was significantly higher than that of the controls (99% CI = 54.3 ± 9.3). The increase in luminescence intensity against both PEG containing reagents, when tested with sufficient sensitivity indicates that both cases were positive for anti-PEG sIgE (Table E1, Online Only).

Using unconjugated PEG molecules of different sizes as the antigen source, samples from both cases showed strong preference towards PEGs of larger molecular weights (Figure 3). Although patients in both cases reacted clinically to PEG 3350, anti-PEG sIgG antibodies in their plasma samples displayed even higher binding for higher molecular weight PEG 5k and PEG 10k, and almost no binding towards the lowest molecular weight PEG 1k (ODs were 0.021 and 0.014 respectively) compared to controls (99% CI = 0.014 ± 0.006) who did not demonstrate binding at any molecular weight of PEG.

Public data review results:

Using the preferred search term “anaphylactic” to capture both “anaphylactic shock” or “anaphylactic reaction”, we encountered 25,905 reports to the FDA between 1989 and the end of 2017. When the additional term “polyethylene glycol” was applied, we were left with 133 reports associating polyethylene glycol with anaphylaxis. Of these, we encountered 53 reports with unique case identifiers described as either anaphylactic shock or an anaphylactic reaction in which PEG containing bowel preparations or laxatives were the primary or sole agent suspected as causal. (Table II) The average age at reaction was 48.9 years (23% missing data), and 51% of those who reacted were male (15% missing data). At the time of reaction, 51% reported the PEG containing product was the sole agent they had ingested prior to anaphylaxis and were not using any other concomitant therapies. The other 49% were taking other concomitant therapies at the time of reaction, but their reports indicated primary suspicion was on PEG containing products. In terms of the clinical context, 72% of the reactions occurred prior to colonoscopy preparation, and 28% occurred during treatment of constipation. Reported reactions were distributed across the time period from 2005–2017,

with an average of 4 cases reported per year during this time period. (Figure 4) We did not encounter any reports of PEG-related reactions prior to 2005.

Medication Excipient Review:

Using the search term “polyethylene glycol 3350” as an active or inactive ingredient returned 1155 FDA approved medications. A summary of the first 1000 hits can be found in Table E2 (Table E2, Online Only). This list demonstrates that polyethylene glycol 3350 can more commonly be found in film coated tablets, topical gels, and parenteral steroids. Using the search term “polysorbate 80” as an active or inactive ingredient returned 6821 FDA approved medications. A summary of the first 1000 hits can be found in Table E3 (Table E3, Online Only). This list demonstrates that polysorbate 80 can more commonly be found in film coated tablets, parenteral steroids, and vaccines.

Discussion:

The most commonly known clinical use of macrogols such as PEG 3350 is in colonoscopy preparation or constipation treatment.[5, 23, 25, 26] However, a review of common products and the literature demonstrates that polyethylene glycol and structurally similar polysorbate compounds can be found in vascular graft materials[10], surgical gels[27], PEGylated medications,[28–30] household and industrial compounds,[1] and as an excipient in a multitude of other medications both injectable and oral,[4, 31] In these settings, PEGs and polysorbates are not consistently described in ingredient lists.[8] The NIH DailyMed online resource through the National Library of Medicine is a useful resource for determining an individual product’s excipient content of macrogols such as PEGs and polysorbates: https://dailymed.nlm.nih.gov/.[4] Though cutaneous and systemic reactions to film coated tablets has been reported in patients with PEG hypersensitivity,[8] both of our patients were otherwise healthy and taking no daily medications that contained PEG. Neither one is known to have reacted to any products other than what we have described in this report.

A recent review of published case reports and case series in the literature by *Garvey et al.* found 37 cases of PEG hypersensitivity since 1977.[8] Our review of the FDA data adds a large number of additional cases that may not have been noticed in the medical literature. Our data suggests an average of 4 cases per year of PEG-associated anaphylaxis during colonoscopy preparation or laxative use are reported to the FDA. However, it is clear that relying on patient or physician initiated reports to the FDA will understate the true volume of the problem. Our review of FDA adverse event data focused only on drugs that contained pure polyethylene glycol 3350 at concentrations of grams per dose. Therefore we can not currently offer much additional data on whether drugs containing PEG or polysorbate 80 as an excipient at milligram or microgram concentrations can precipitate reactions in sensitized patients. We can only report that both of our patients have had anaphylaxis upon parenteral exposure to methylprednisolone acetate, formulations of which typically contain around 29 mg/ml of PEG 3350.[4]

The mechanism for macrogol hypersensitivity has been poorly understood. Anti-PEG sIgG has been detected in patients receiving PEG-conjugated protein therapeutics[6], but was not studied in unconjugated macrogol anaphylactic cases, while anti-PEG sIgE has not been

directly measured in any human studies.[32] Our findings of skin test reactivity and coexisting polyethylene glycol-directed sIgE and sIgG antibodies suggest an IgE mediated Type I hypersensitivity could be possible in clinical reactions to unconjugated macrogols. These cases may represent a separate phenotype of immediate hypersensitivity from what has been previously shown during reactions to PEG-asparaginase and other PEGylated compounds.[7, 33] Of note, the absence of binding between patient IgG antibodies and lower MW PEGs also coincided with the tolerance of PEG 300 in both skin and oral challenges *in vivo*, supporting the involvement of antibodies specific for higher MW PEGs in the clinical reactions. The stronger reactivity of the patient samples against PEGs of higher molecular weight suggests that sensitization and risk of future reactions may depend partially on the molecular weight of PEG antigen exposures, and suggest that PEG may act as the primary antigen even when not conjugated to drug molecules. Detection of sIgE directed against PEG required use of the more sensitive Meso Scale Discovery electrochemiluminescence method and polysorbate-free testing reagents. Our results suggest that development of blood testing as a modality in diagnosis of macrogol hypersensitivity may be possible.

Conclusions:

High molecular weight polyethylene glycols are common excipients in a wide variety of medications, household products and industrial products which may provide a vehicle for sensitization in a subset of susceptible individuals. Allergists should be aware that cross-reactive immediate hypersensitivity to polyether containing compounds such as macrogols/PEGs and polysorbates can occur, that they may occur via a Type I hypersensitivity mechanism, and that they may be underrecognized.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

MW	molecular weight(s)
PEG	polyethylene glycol

OTC	over-the-counter
OD	optical density
ELISA	Enzyme-linked immunosorbent assay
FDA	US Food and Drug Administration
FAERS	FDA Adverse Event Reporting System
CDC	Centers for Disease Control and Prevention

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Highlights:

What is already known about this topic?

The most common immediate hypersensitivity to macrogols is associated with PEG 3350, however the epidemiology, mechanisms and cross-reactivity are poorly understood. Thousands of medications contain either PEGs or structurally similar polysorbates.

What does this study add to our knowledge?

In vivo and *ex vivo* testing of two cases suggest an IgE mediated, Type I hypersensitivity mechanism to polyethylene glycol 3350 anaphylaxis. This hypersensitivity, while rare, may be more common than we recognize.

How does this study impact current management guidelines?

Immediate hypersensitivity to PEG 3350 with cross-reactive polysorbate 80 hypersensitivity may be under recognized in clinical practice and can be evaluated with clinical skin testing.

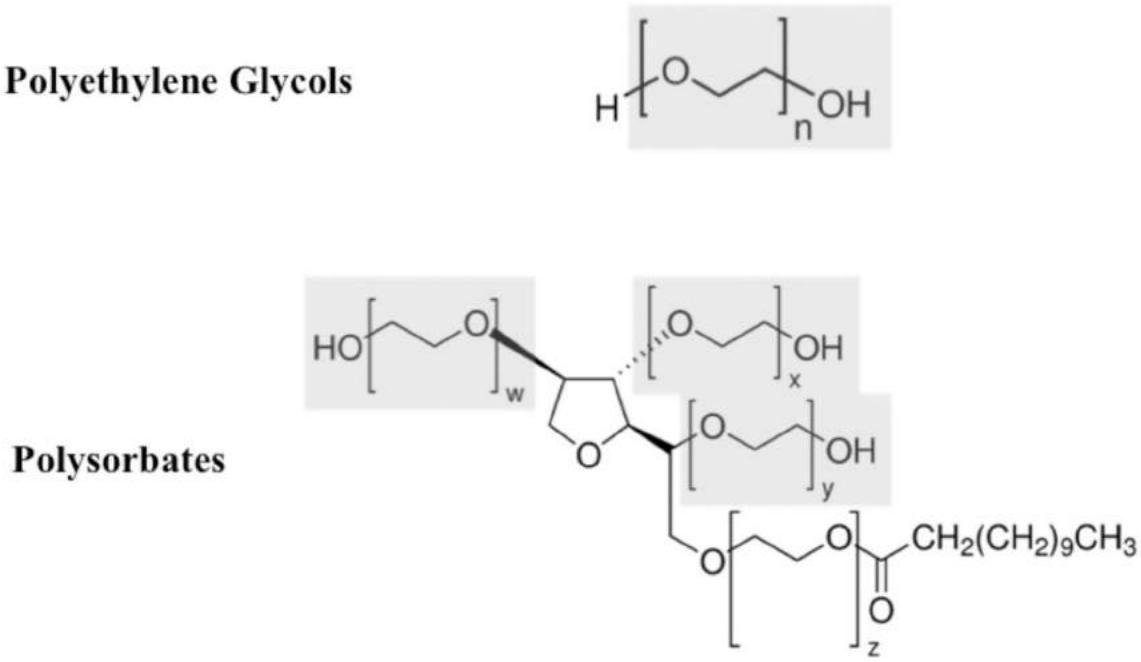

Figure 1:
Chemical structure of polyethylene glycols and polysorbates. Polysorbate 20 shown. Note the repeating polyether domains contained in both molecules, highlighted in gray. Source of chemical structure images: sigmaaldrich.com, accessed 5-15-2018. Highlights and labels added by authors to demonstrate similarity.

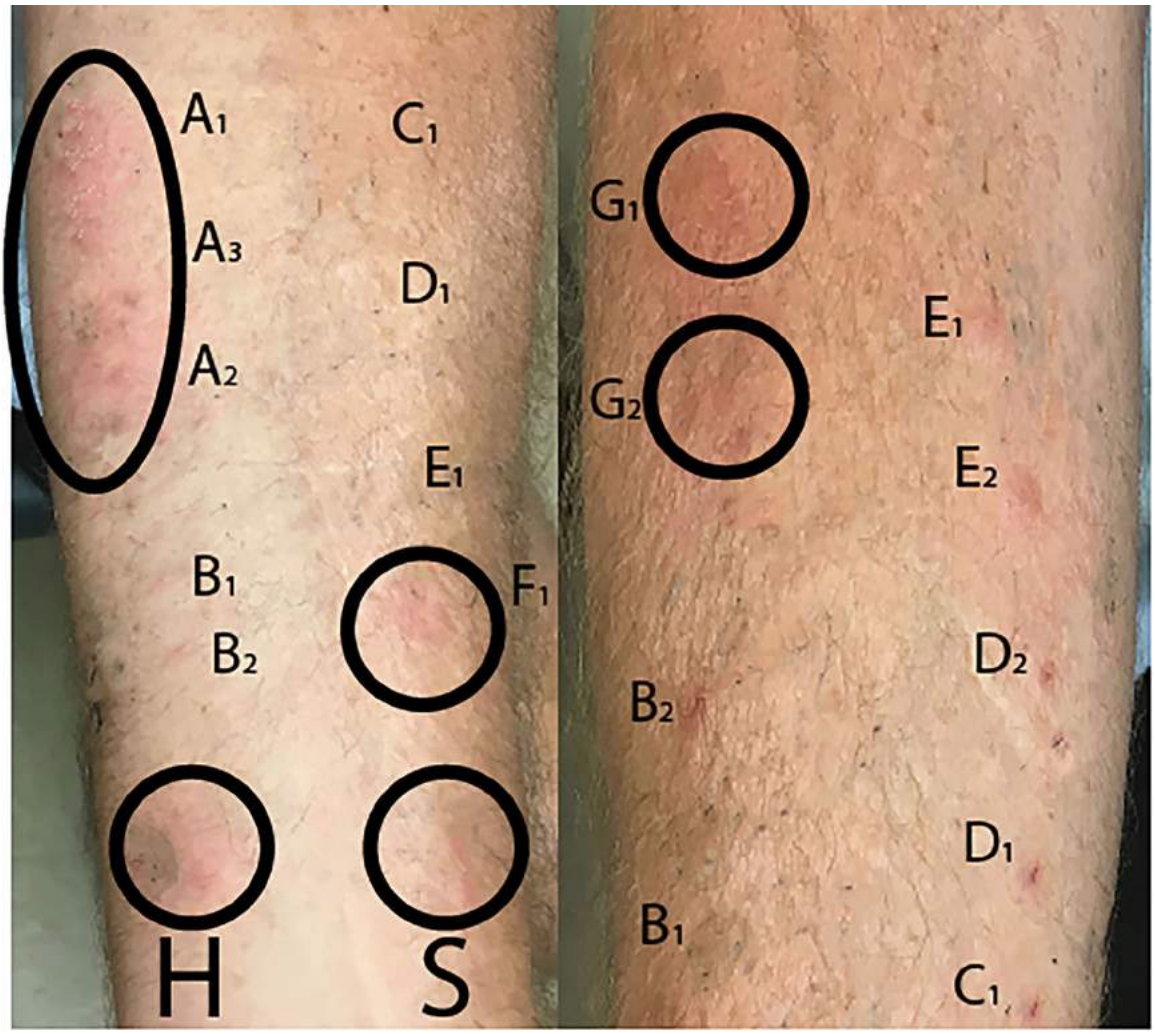

Figure 2:

Selected skin testing images for patient 1: In the left panel is skin prick testing demonstrating positive responses to methylprednisolone acetate (MP acetate), and polyethylene glycol 3350 (PEG 3350). Other tested corticosteroids were negative. In the right panel is intradermal testing, which demonstrates a positive response to triamcinolone acetate (T) at 1mg and 0.1mg. Other tested corticosteroids were interpreted as negative. (Measurements recorded in TABLE I).

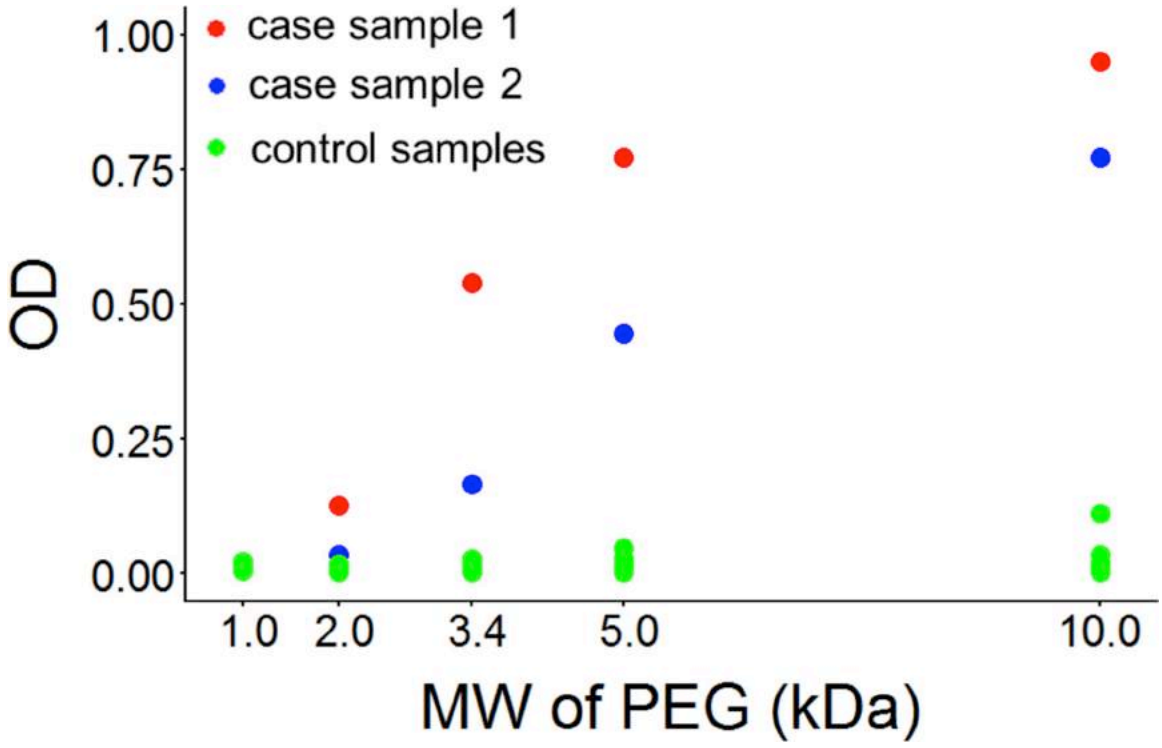

Figure 3:
IgG optical densities (ODs) of case and control plasma samples against HO-PEG-NH_2 of different molecular sizes.

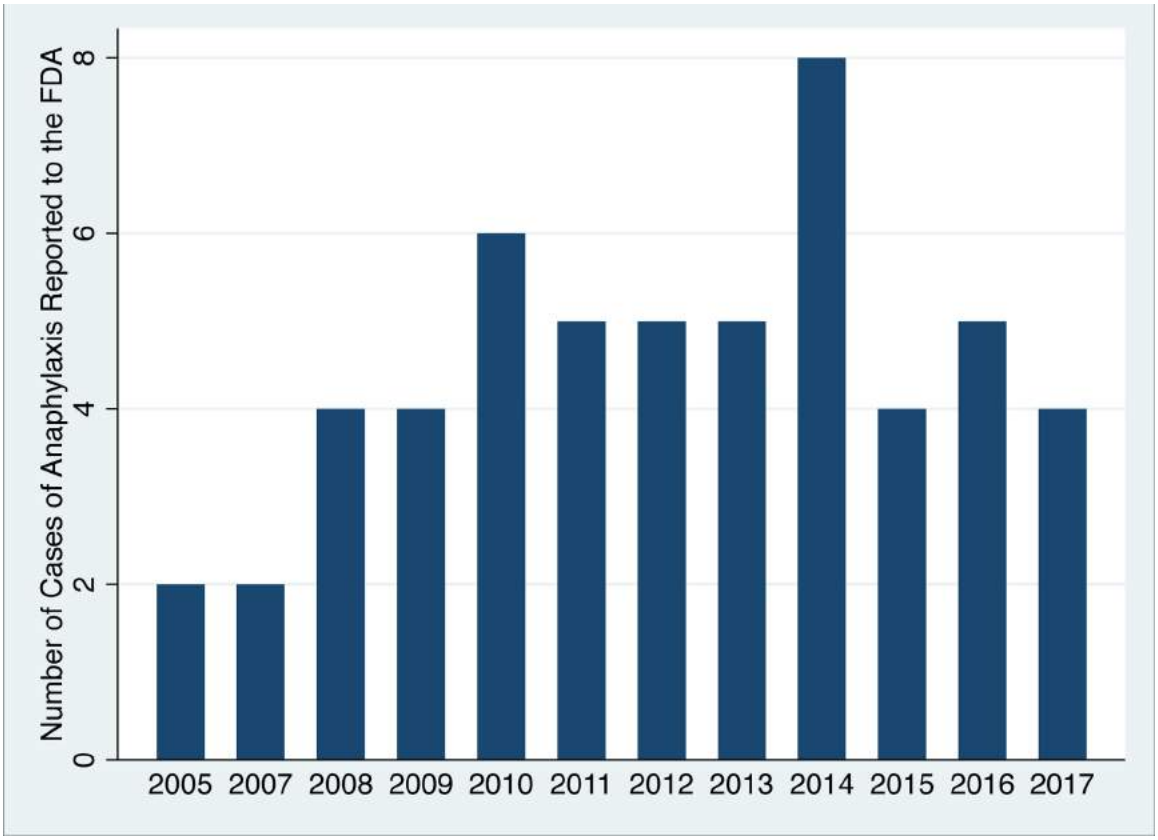

Figure 4:
Cases of anaphylaxis reported to the FDA (FAERS) implicating PEG containing bowel preparations or laxatives, by year.

Table I:

Skin Prick and Intradermal Testing with Corticosteroids and Polyethylene Glycols

Skin Prick Test Results						
	Patient 1			**Patient 2**		
Agent (Concentration)	**Wheal (mm)**	**Flare (mm)**	**Inter-pretation**	**Wheal (mm)**	**Flare (mm)**	**Interpretation**
Histamine Control (0.1mg/ml)	6	26	Positive	7	20	Positive
Saline	0	0	Negative	0	0	Negative
PEG 3350	10	26	Positive	0	0	Negative
PEG 3350 (1:10 dilution)	11	22	Positive	0	0	Negative
PEG 3350 (1:100 dilution)	11	29	Positive	0	0	Negative
PEG 300 (1:10 dilution)	0	0	Negative			
PEG 300 (1:100 dilution)	4	5	Negative			
Methylprednisolone Acetate	5	12	Positive	0	0	Negative
Methylprednisolone Sodium Succinate	3	3	Negative	0	0	Negative
Intradermal Skin Test Results						
	Patient 1			**Patient 2**		
Agent (Concentration)	**Wheal (mm)**	**Flare (mm)**	**Inter-pretation**	**Wheal (mm)**	**Flare (mm)**	**Interpretation**
Betamethasone (6 mg/ml)	6	6	Negative	0	0	Negative
Betamethasone (0.6mg/ml)	5	5	Negative	0	0	Negative
Dexamethasone (0.4mg/ml)	5	0	Negative	0	0	Negative
Dexamethasone (0.04mg/ml)	7	0	Negative	0	0	Negative
Methylprednisolone Sodium Succinate (5mg/ml)	5	6	Negative	0	0	Negative
Methylprednisolone Sodium Succinate (0.5mg/ml)	0	0	Negative	0	0	Negative
Methylprednisolone Acetate (4mg/ml)				0	0	Subacute response developed at 20 hours, with 14mm raised wheal
Methylprednisolone Acetate (0.4mg/ml)				0	0	Negative
Triamcinolone Acetonide (1mg/ml)	10	19	Positive	10	30	Positive
Triamcinolone Acetonide (0.1 mg/ml)	15	24	Positive			
Conjugated pneumococcal vaccine (w/ polysorbate 80)	20	35	Positive			
Conjugated pneumococcal vaccine (1:10 dilution)	21	30	Positive			
Polysorbate 80 containing eye drop (1:10 dilution)	15	30	Positive			

Table II:

Cases of Anaphylaxis Reported to the FDA from 2005 to 2017 Where Polyethylene Glycol 3350 Containing Formulations of Colonoscopy Preparation or Laxatives Were the Primary Drug Suspected

FAERS Report ID Number	Age	Sex	Year of Report	Formulation of PEG	Patient taking any other medications concomitantly	Indication (Colonoscopy Preparation vs. Constipation)
4852819-0	N/A	N/A	2005	Golytely	No	Preparation
4885400-8	30	Male	2005	Colyte	No	Preparation
5347102-3	42	Male	2007	Moviprep	No	Preparation
5326935-3	33	Female	2007	Polyethylene Glycol 3350-Brand not specified	No	Constipation
5792732-8	68	Male	2008	Golytely	No	Preparation
5829663-0	N/A	N/A	2008	Moviprep	No	Preparation
5909593-6	N/A	N/A	2008	Miralax	Yes	Constipation
5923262-8	64	Male	2008	Miralax	Yes	Constipation
6187140-4	52	Male	2009	Moviprep	Yes	Preparation
6262262-8	N/A	N/A	2009	Miralax	Yes	Preparation
6301790-3	52	Male	2009	Moviprep	Yes	Preparation
6446535-1	30	Female	2009	Moviprep	Yes	Preparation
6567457-1	N/A	N/A	2010	Polyethylene Glycol 3350-Brand not specified	Yes	Preparation
6583005-4	N/A	N/A	2010	Moviprep	No	Preparation
6625930-1	N/A	N/A	2010	Moviprep	No	Preparation
6649325-X	55	Female	2010	Golytely	Yes	Preparation
6681659-5	4	Male	2010	Miralax	No	Constipation
6784081-6	73	Male	2010	Miralax	No	Constipation
7610318-7	19	Male	2011	Moviprep	Yes	Preparation
7429359-8	59	Female	2011	Polyethylene Glycol 3350-Brand not specified	Yes	Preparation
7444601-5	55	Male	2011	Miralax	No	Preparation
7636123-3	64	Female	2011	Moviprep	No	Preparation
7759201-7	33	Female	2011	Polyethylene Glycol 3350-Brand not specified	No	Preparation
8274426-2	67	Female	2012	Moviprep	Yes	Preparation
8289679-4	57	Female	2012	Polyethylene Glycol 3350-Brand not specified	Yes	Constipation
8456637-6	46	Female	2012	Polyethylene Glycol 3350-Brand not specified	Yes	Constipation
8712178	N/A	Female	2012	Miralax	No	Constipation
8814458	24	Male	2012	Polyethylene Glycol 3350-Brand not specified	Yes	Constipation
9321913	16	Female	2013	Miralax	No	Preparation
9417033	56	Female	2013	Golytely	Yes	Preparation

FAERS Report ID Number	Age	Sex	Year of Report	Formulation of PEG	Patient taking any other medications concomitantly	Indication (Colonoscopy Preparation vs. Constipation)
9420162	N/A	Female	2013	Miralax	Yes	Constipation
9607762	50	Male	2013	Golytely	No	Preparation
9782506	70	Female	2013	Moviprep	No	Preparation
9828607	34	Female	2014	Miralax	Yes	Preparation
9894648	N/A	Female	2014	Miralax	Yes	Constipation
9934430	54	Male	2014	Miralax	Yes	Constipation
10235381	87	Female	2014	Moviprep	Yes	Preparation
10242352	13	Male	2014	Miralax	No	Constipation
10335513	54	Female	2014	Glycolax	No	Preparation
10428179	65	Male	2014	Moviprep	Yes	Preparation
10682474	59	Male	2014	Moviprep	No	Preparation
10710219	19	Female	2015	Moviprep	Yes	Preparation
11362693	N/A	N/A	2015	Miralax	No	Preparation
11573598	N/A	Female	2015	Moviprep	No	Preparation
11617696	74	Male	2015	Moviprep	No	Preparation
12787790	62	Male	2016	Polyethylene Glycol 3350-Brand not specified	Yes	Preparation
12849324	39	Male	2016	Colyte	Yes	Preparation
12865113	59	Male	2016	Polyethylene Glycol 3350-Brand not specified	No	Preparation
13243846	46	Male	2016	Moviprep	Yes	Preparation
13268930	64	Male	2016	Polyethylene Glycol 3350-Brand not specified	No	Preparation
13747359	68	Female	2017	Miralax	Yes	Constipation
13854981	73	Female	2017	Golytely	No	Preparation
13870252	61	Female	2017	Moviprep	No	Preparation
13896629	2	Male	2017	Golytely	Yes	Constipation

Data marked as N/A indicate that the information was not contained in the primary report to the FDA.

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Polyethylene Glycol (PEG) 3350

Polyethylene glycol is a laxative sold under the trade names MiraLAX®, ClearLax®, GaviLAX®, GlycoLax® and Purelax®. You do not need a doctor's prescription to buy PEG 3350. It is available over-the-counter.

PEG 3350 is an osmotic laxative, which means it increases the water content of stool and is effective in treating or preventing constipation. The body cannot absorb or digest this laxative.

PEG 3350 is typically taken once a day by mouth, but the dose may be adjusted higher. It comes as a powder which you will have to mix with liquid.

How do I prepare the medication?

The standard dose is 17 grams of powder mixed into 8 ounces of liquid. The bottle has a measuring cap that is marked with a line.

1. Pour the powder into the cap up to the marked line.
2. Add the powder in the cap to a full glass (8 ounces) of water, juice, soda, coffee or tea.
    - **If you are over 65, have kidney disease or have liver disease, please only use water.**
3. Mix powder well and drink the solution.

How do I take PEG 3350?

- You can take this medication on a full or empty stomach.
- PEG 3350 doesn't have any known drug interactions but you should not take other medications at the same time that you take PEG 3350. Other medications may not be digested and absorbed as well.
- Always drink plenty of **decaffeinated** liquids with this medication.

What are possible side effects?

Call your doctor immediately if you have any of the following side-effects:

- diarrhea
- difficulty breathing
- itching of the skin
- hives
- skin rash
- severe bloating
- painful swelling of the stomach
- vomiting

Other side-effects that usually do not require immediate medical attention are:

- bloating
- lower abdominal (stomach) discomfort
- cramps
- nausea
- passing extra gas

Some of these symptoms will decrease over time. Please contact your clinician if symptoms do not improve or become worse.

After starting on PEG 3350, it can take 3-5 days to have a bowel movement or see improvement in constipation. If it has been three (3) or more days since having a bowel movement, you should clean your bowels out first before starting PEG 3350.

Polysorbate 80

The Blood-Brain Barrier: Bottleneck in Brain Drug Development

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Summary: The blood-brain barrier (BBB) is formed by the brain capillary endothelium and excludes from the brain ~100% of large-molecule neurotherapeutics and more than 98% of all small-molecule drugs. Despite the importance of the BBB to the neurotherapeutics mission, the BBB receives insufficient attention in either academic neuroscience or industry programs. The combination of so little effort in developing solutions to the BBB problem, and the minimal BBB transport of the majority of all potential CNS drugs, leads predictably to the present situation in neurotherapeutics, which is that there are few effective treatments for the majority of CNS disorders. This situation can be reversed by an accelerated effort to develop a knowledge base in the fundamental transport properties of the BBB, and the molecular and cellular biology of the brain capillary endothelium. This provides the platform for CNS drug delivery programs, which should be developed in parallel with traditional CNS drug discovery efforts in the molecular neurosciences. **Key Words:** Blood-brain barrier, endothelium, drug targeting, biological transport, neurotherapeutics.

INTRODUCTION

The blood-brain barrier (BBB) is the bottleneck in brain drug development and is the single most important factor limiting the future growth of neurotherapeutics.[1] The BBB problem is illustrated in Figure 1, which is a whole body autoradiogram of a mouse sacrificed 30 min after intravenous injection of radiolabeled histamine, a small molecule of only ~100 Da in molecular mass. Histamine readily crosses the porous capillaries perfusing all peripheral tissues but is excluded from entry into the brain or spinal cord by the BBB.

The histamine example in Figure 1 refutes a common misconception that most small molecules readily cross the BBB. As discussed below, the transport of small molecules across the BBB is the exception rather than the rule, and 98% of all small molecules do not cross the BBB (FIG. 1). Moreover, all large-molecule products of biotechnology, such as monoclonal antibodies (mAbs), recombinant proteins, antisense, or gene therapeutics, do not cross the BBB (FIG. 1). Despite the large number of patients with disorders of the CNS and despite the fact that so few large- or small-molecule therapeutics cross the BBB, there are few pharmaceutical companies in the world today that have built a BBB drug targeting program (FIG. 1). However, even if a pharmaceutical company decided to develop a BBB program, there would be few BBB-trained scientists to hire because less than 1% of U.S. academic neuroscience programs emphasize BBB transport biology.

Because most drugs do not cross the BBB, and because the industry is not providing solutions to the BBB problem, it is not surprising that most disorders of the CNS could benefit from improved drug therapy (FIG. 2). For a small-molecule drug to cross the BBB in pharmacologically significant amounts, the molecule must have the dual molecular characteristics of: 1) molecular mass under a 400- to 500-Da threshold, and 2) high lipid solubility.[1] There are only four categories of CNS disorders that consistently respond to such molecules, and these include affective disorders, chronic pain, and epilepsy (FIG. 2). Migraine headache may be a CNS disorder and could also be included in this category. In contrast, most CNS disorders such as those listed in Figure 2 have few treatment options. Parkinson's disease patients are given L-dihydroxyphenylalanine (L-DOPA) for dopamine replacement therapy.[2] As discussed below in the section on BBB carrier-mediated transport, L-DOPA is an example of a BBB drug targeting strategy. However, there is no neurotherapeutic that stops the neuro-

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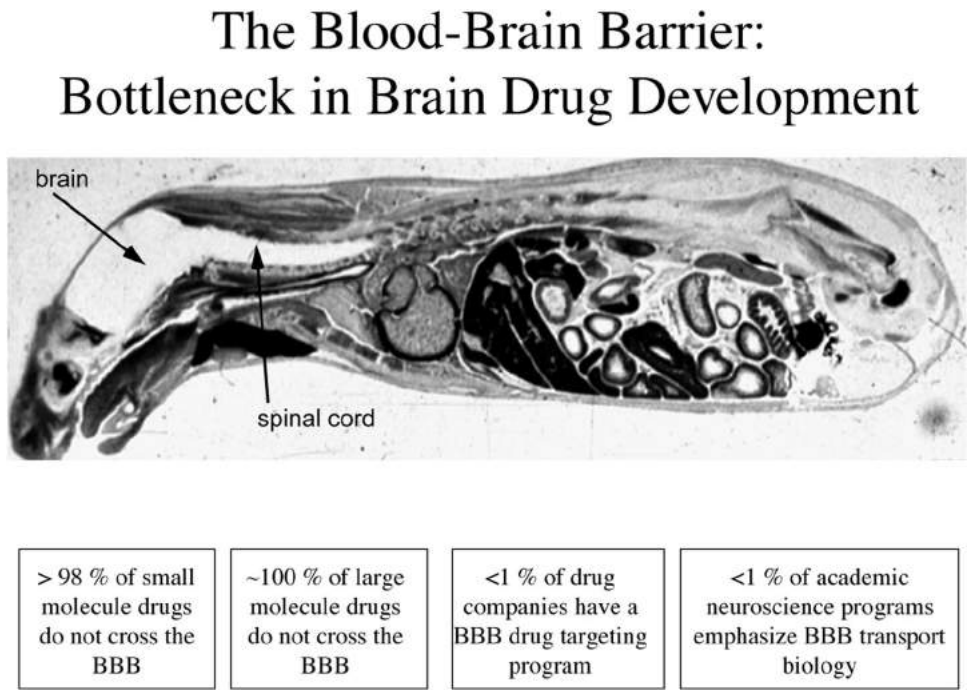

FIG. 1. Whole body autoradiogram of an adult mouse sacrificed 30 min after intravenous injection of radiolabeled histamine, a small molecule that readily enters all organs of the body, except for the brain and spinal cord.

degeneration of Parkinson's disease. Similarly, there is no therapy for other neurodegenerative diseases such as Alzheimer's disease, Huntington's disease, and amytrophic lateral sclerosis (ALS). Patients with multiple sclerosis (MS) are treated with cytokines that work on the peripheral immune system, but which do not permanently stop the progression of MS.[3] The human immunodeficiency virus (HIV) infects the brain early in the course of acquired immune deficiency syndrome (AIDS).[4] HIV in the periphery has been significantly reduced with highly active antiretroviral therapy (HAART) comprised of multiple small-molecule therapeutics. However, HAART drugs such as azidothymidine, 3TC, or protease inhibitors are substrates for BBB active efflux transporters, which are reviewed below, and HAART drugs have minimal penetration into brain parenchyma. Consequently, the brain remains a sanctuary for HIV in AIDS even with HAART.[4,5] Brain cancer, stroke, and brain or spinal cord trauma are all examples of serious CNS disorders for which there is no effective drug therapy. The childhood disorders including autism, lysosomal storage disorders, fragile X syndrome, the ataxis, and blindness, are serious disorders where there is little effective treatment. In many of these cases, the gene underlying the disease is known, but BBB delivery is the rate-limiting problem in gene therapy or enzyme replacement therapy, and no therapeutics have been developed. Many of the disorders listed in the right-hand column in Figure 2 could be treated with drugs, enzymes, or genes already discovered. However, these drugs do not cross the BBB and cannot enter into brain drug development because no BBB solutions have been developed by industry. Given the absence of effective BBB drug targeting technology, CNS drug developers are left with the traditional approaches to solving the brain drug delivery problem: small molecules, trans-cranial brain drug delivery, and BBB disruption. A review of these approaches

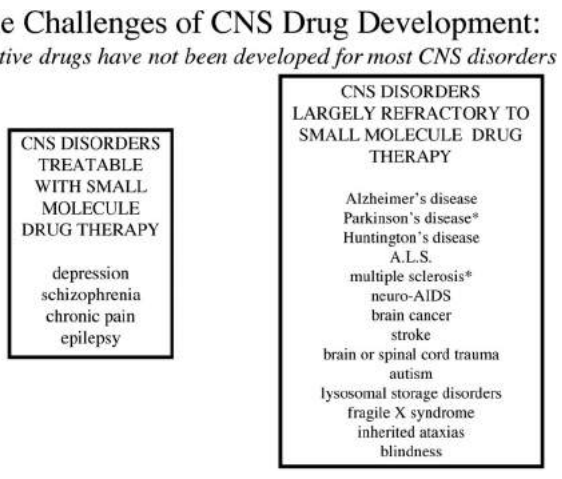

FIG. 2. A review of the Comprehensive Medicinal Chemistry database shows that, of more than 7000 small-molecule drugs, only 5% treat the CNS, and these drugs only treat four disorders: depression, schizophrenia, chronic pain, and epilepsy.[6,7] There are few effective small- or large-molecule drugs for the majority of CNS disorders, with the exception of Parkinson's disease, e.g., L-DOPA, and multiple sclerosis, e.g., cytokines.

shows that none provide solutions to the BBB problem that could be practically implemented in large numbers of patients.

SMALL MOLECULES

Most small-molecule drugs do not cross the BBB. Of over 7000 drugs in the comprehensive medicinal chemistry (CMC) database, only 5% of all drugs treat the CNS, and these CNS active drugs only treat depression, schizophrenia, and insomnia.[6] The average molecular mass of the CNS active drug is 357 Da. In another study, only 12% of drugs were active in the CNS, but only 1% of all drugs were active in the CNS for diseases other than affective disorders.[7]

BBB transport of small molecules is limited

Small molecules generally cross the BBB in pharmacologically significant amounts if 1) the molecular mass of the drug is less than 400-500 Da, and 2) the drug forms less than 8-10 hydrogen bonds with solvent water.[1]

The permeation of the drug across the BBB does not increase in proportion to lipid solubility when the molecular weight of the drug is increased. BBB permeation decreases 100-fold as the surface area of the drug is increased from 52 Angstroms^2 (e.g., a drug with molecular mass of 200 Da) to 105 Angstroms^2 (e.g., a drug of 450 Da).[8] Drug diffusion through a biological membrane is not analogous to drug diffusion through solvent water. In contrast to water, diffusion of a drug through a biological membrane is dependent on the volume of the drug. The classical Overton rules that relate membrane permeation to solute lipid solubility do not predict the molecular weight threshold effect. As noted by Leib and Stein nearly 20 years ago,[9] the molecular weight threshold effect is best predicted by the "hole-jumping" model of Trauble,[10] which posits that solutes undergo a form of molecular "hitch hiking" across a biological membrane by moving through small holes in the membrane formed by kinking of the mobile unsaturated fatty acyl side chains in the phospholipid bilayer.

Hydrogen bonding

BBB permeation decreases exponentially with the addition of each pair of hydrogen bonds added to the drug structure.[11] It does not matter whether the functional group is a hydrogen bond donor or a hydrogen bond acceptor because each hydrogen bond carries equal weight. Hydrogen bond donor groups such as hydroxyls form two hydrogen bonds because a hydroxyl group acts as both a hydrogen bond donor and hydrogen bond acceptor, whereas a carbonyl group only acts as a hydrogen bond acceptor. Once the total number of hydrogen bonds on the drug exceeds a threshold of 8-10, there is minimal transport of the drug across the BBB in pharmacologically active amounts. Both the hydrogen bonding and the molecular weight of drugs currently emanating from CNS drug discovery programs generally are higher than drugs discovered 20 years ago.[7] This is because CNS drug discovery programs now rely extensively on receptor-based high-throughput screening (HTS) programs. HTS-based drug screening invariably selects for drugs that have higher molecular weights and higher hydrogen bonding because these factors enable higher affinity drug binding to the target receptor.

HTS-based CNS drug discovery

Current CNS drug discovery programs are generally broken down into four major areas: 1) receptor target identification, 2) drug "hit" identification, 3) "lead" identification, and 4) drug lead optimization. After screening several hundred thousand small-molecule drugs with a given target, several hundred hits may be found, leading to a score of potential drug leads. The HTS drug lead compounds must then be optimized with respect to distribution, metabolism, and pharmacokinetics (DMPK).[12] However, the drugs generally require so much medicinal chemistry to block polar functional groups that the original high receptor affinity is lost in an attempt to produce a drug with acceptable DMPK properties. The difficulty in using medicinal chemistry to increase the lipid solubility of a drug is illustrated by considering that there is not a single drug currently in CNS clinical practice that is an example of a water soluble drug that was made lipid soluble with medicinal chemistry optimization such that the drug then became pharmacologically active in the brain *in vivo*.

The pharmacokinetic rule

When medicinal chemistry is used to increase the lipid solubility of the drug, this may increase penetration across the BBB, but it also increases penetration across all biological membranes *in vivo*. Therefore, the lipidized form of the drug is rapidly removed from the blood, and in pharmacokinetic terms, the plasma area under the concentration curve (AUC) is substantially decreased for the lipidized form of the drug. Drug action in brain is a function of drug uptake, expressed as percent of injected dose (ID) per gram brain, and the % ID/g is equally dependent on two factors, the BBB permeability-surface area (PS) product and the plasma AUC:

$$\%\ \mathrm{ID/g} = (\mathrm{BBB\ PS\ product}) \times (\mathrm{plasma\ AUC}) \quad \text{(Eq. 1)}$$

Although an increase in lipid solubility of the drug may increase the BBB PS product, there is a proportional decrease in the plasma AUC with lipidization. The increased BBB PS product and the decreased plasmid AUC have offsetting effects, which minimizes the increase in brain uptake caused by lipidization.[1]

Medicinal chemistry and brain drug lead optimization

The use of medicinal chemistry to increase the lipid solubility of drug to solve the BBB drug delivery problem is problematical for the reasons listed above. However, a new approach to the use of medicinal chemistry to solve the BBB drug delivery problem is discussed below. Medicinal chemistry can be used to alter the structure of a lead drug candidate to make that drug transportable on one of several carrier-mediated transport (CMT) systems within the BBB. However, redirection of the use of medicinal chemistry to increase the carrier-mediated transport of a drug, as oppose to the lipid-mediated transport of the drug, requires knowledge on the structural characteristics of a drug that enable CMT across the BBB. Therefore, a knowledge base in BBB CMT must be developed before the use of medicinal chemistry to increase drug penetration to the brain via endogenous BBB carriers.

TRANS-CRANIAL BRAIN DRUG DELIVERY

Trans-cranial brain drug delivery approaches attempt to bypass the BBB using one of three neurosurgical-based delivery approaches: intracerebral implantation, intracerebroventricular (ICV) infusion, and convection enhanced diffusion (CED). The factor limiting either the intracerebral or ICV infusion approach is that either method relies on diffusion for drug penetration into the brain from the depot site. Solute diffusion decreases with the square of the diffusion distance.[1] Therefore, the concentration of drug decreases logarithmically with each millimeter of brain tissue that is removed from the injection site, in the case of intracerebral implantation, or from the ependymal surface of the brain, in the case of ICV infusion. The concentration of a small molecule is decreased by 90% at a distance of only 0.5 mm from the intracerebral implantation site in rat brain.[13] The logarithmic decrease in drug concentration from the ependymal surface following an ICV infusion was shown in the 1970s in adult Rhesus monkeys; after ICV drug injection, the concentration of small molecules in brain parenchyma removed only 1-2 mm from the ependymal surface is only about 1-2% of the concentration in the CSF compartment.[14] The limited diffusion of drug from an intracerebral implant is shown in Figure 3, which is an autoradiogram of rat brain taken 2 days after the intracerebral implantation of a wafer embedded with radiolabeled NGF.[15] The size of the wafer is approximately equal to the magnification bar in the figure, which indicates that there has been minimal penetration of NGF into brain parenchyma from the implant site. The limited diffusion of BDNF into brain parenchyma following injection into a lateral ventricle (LV)[16] is shown in Figure 3. The BDNF is sequestered by the ependymal surface

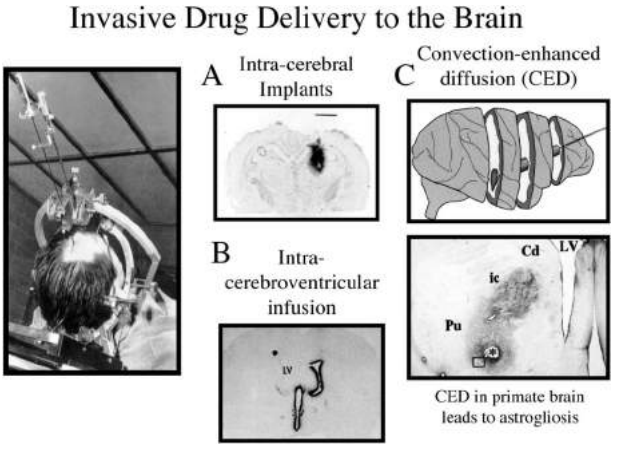

FIG. 3. Trans-cranial drug delivery to the brain. A: Autoradiogram of rat brain 48 h after an intracerebral implantation of a polymer carrying radiolabeled NGF.[15] The size of the polymer approximates the magnification bar, indicating the NGF has not significantly diffused from the implantation site. B: Autoradiogram of rat brain 24 h after an intracerebroventricular injection of BDNF into an LV.[16] The BDNF distributes to the ependymal surface of the ipsilateral LV and the third ventricle (3V), but not into brain parenchyma. C: Convection enhanced diffusion in the primate brain forces fluid through the brain tissue. The direction of fluid flow, principally via white matter tracts,[19] can be traced with immunocytochemistry using an antibody to GFAP, which shows an astrogliotic reaction in the path of fluid flow.[20] The hole in the brain left by the catheter is noted by the asterisk. The fluid moved from the catheter in the putamen (Pu) via the internal capsule (ic) white matter to the caudate (Cd).

but does not significantly diffuse into brain parenchyma. This limited diffusion of BDNF into brain parenchyma is not due to the fact that BDNF is a cationic protein, as a similar logarithmic decrease in brain penetration is found for any drug following ICV injection.[14] This slow rate of drug diffusion into brain parenchyma is to be contrasted with the rapid rate of bulk flow of CSF through the ventricular compartments. CSF is then rapidly absorped into the peripheral bloodstream at the superior sagittal sinus. The ICV injection of drug should be regarded as a slow intravenous infusion rather than a direct administration of drug into the brain.[17] The rapid rate of cytokine distribution into blood, but minimal penetration into brain, following an ICV injection has been demonstrated in adult rhesus monkeys.[18]

The effective penetration of drug into brain can be increased to a treatment radius of a few millimeters when bulk flow is used to deliver drug into brain parenchyma, and this is possible by forcing fluid through the brain with CED. However, the brain has no lymphatic system and is not designed for a significant intraparenchymal volume flow. CED in humans with glioblastoma multiform causes a preferential flow of the forced fluid along white matter tracts.[19] CED in the adult Rhesus monkey brain with glial-derived neurotrophic factor involved the infusion of relatively small volumes of ~0.1 ml/day over a 4-week period.[20] This led to diffuse white matter as-

TABLE 1. *BBB Disruption after Intracarotid Arterial Infusion of Noxious Agents*

Method	Comments (References)
Hyperosmolar	Leads to chronic neuropathologic changes and vasculopathy in the brain and seizures[21–25]
Vasoactive agents	Examples are bradykinin, histamine, and multiple other vasoactive compounds; opens BBB in brain tumor to greater extent than normal brain[72]
Solvents	BBB is solubilized with high dose ethanol, DMSO, SDS, Tween 80 (polysorbate-80)[27–30]
Alkylating agents	Examples are etoposide and melphalan; may alkylate key sulfhydryl residues similar to mercury[73,74]
Immune adjuvants	Freunds adjuvant opens BBB to IgG for weeks; enable IgG uptake into brain in rodent vaccine models, such as Alzheimer's disease[32]
Ultrasound	The combination of administration of high-dose air bubbles (2–4 μm) and high-dose ultrasound (10–1000 watt/cm^2) can induce BBB disruption[75]
Cytokines	Intracerebral interleukin-1β or CXC chemokines can attract white cells from blood and cause BBB disruption[76,77]
Miscellaneous	Intracarotid acid pH, cold temperatures, or high-dose free fatty acid all cause BBB disruption[78–80]

trogliosis, which was visualized by immunocytochemistry of the autopsy primate brain, and immunostaining with an antibody to GFAP as shown in Figure 3. In addition, there was a microglial response and demyelination around the catheter, with extension of the astrogliotic reaction from the catheter in the putamen (Pu) through the internal capsule (ic) to the caudate (cd) (FIG. 3). These findings of an intense astrogliotic reaction along white matter tracts after CED in the primate brain raise concerns about the long-term effects of this delivery approach for humans.

BLOOD-BRAIN BARRIER DISRUPTION

In parallel with trans-cranial brain drug delivery strategies, there has been a significant effort in delivering drugs to the brain with BBB disruption after the intracarotid arterial infusion of vasoactive agents such as those listed in Table 1. The intracarotid arterial infusion of 2 M concentrations of poorly diffusible solutes such as mannitol causes disruption of the BBB owing to osmotic shrinkage of the endothelial cells.[21] This is associated with severe vasculopathy[22] and chronic neuropathologic changes in rodent models[23] and is also associated with seizures in either animal models[24] or humans.[25] Plasma proteins such as albumin are toxic to brain cells,[26] and BBB disruption allows for the uptake of plasma into the brain.

Solvent/adjuvant-mediated BBB disruption

The BBB, like cell membranes in general, is subject to solvent-mediated disruption with chemicals such as ethanol, dimethylsulfoxide (DMSO), or detergents such as SDS, or Tween 80 also known as polysorbate-80.[27–30] There are numerous examples in the literature where the peripheral administration of a drug, which normally should not cross the BBB, is followed by pharmacological activity in the brain. Such an observation could arise because the drug is transported across the BBB via an endogenous transport system. However, an alternative explanation is that the drug is injected in a diluent that is membrane destabilizing, and causes BBB disruption. Often the drug is solubilized in solvents such as ethanol or DMSO, or surfactants such as SDS, a Tween detergent, or other surfactants, such as polyethyleneglycol hydroxy stearate. Doses of solvents such as ethanol or DMSO at a level of 1-4 g/kg may cause solvent-mediated disruption of the BBB.[27,28] This dose of DMSO or ethanol is given to animal models with surprising frequency, particularly small rodent models such as mice, which weigh only 20-30 g. The administration of just 50 μl of 50% DMSO to a 20-g mouse is equivalent to 1.25 g/kg DMSO, and there are examples in the literature of pharmacologic effects achieved in brain following systemic administration of drugs that normally do not cross the BBB. These drugs are administered in solvents such as ethanol or DMSO and the dose of solvent is such that BBB disruption may be caused by administration of the drug/solvent mixture. Tween 80, also known as polysorbate-80, is frequently administered in CNS drug formulations. A dose of polysorbate-80 of 3-30 mg/kg will cause BBB disruption in mice.[30] Analgesia with kyotorphin, a oligopeptide that normally does not cross the BBB, is possible following the peripheral administration of the peptide, providing Tween 80 is coadministered.[31] Low doses of another surfactant, SDS, are frequently included in CNS drug diluents. However, doses of SDS as low as 1.0 μg/kg can cause disruption of the BBB for short periods. Immune adjuvants such as Freund's complete or incomplete adjuvant cause disruption of the BBB to circulating IgG that can persist for weeks.[32] This is relevant to rodent vaccine models where active immunization is attempted as a new therapy for the treatment of brain diseases. The vaccine for Alzheimer's disease was based on the administration of the Aβ peptide mixed in

Freund's adjuvant to transgenic mice with brain amyloid.[33] The adjuvant has two effects. First, it recruits the immune system to the injection site so that antibodies are made to the target peptide, in this case the Aβ. Second, the immune adjuvant causes an inflammatory response that results in opening of the BBB. This latter property allows the circulating anti-Aβ antibodies to enter the brain. In the absence of BBB disruption, the circulating IgG cannot enter the brain. In either active or passive immunization approaches to brain disorders, the circulating IgG must be enabled to cross the BBB and enter brain to cause the intended pharmacological effect. IgG molecules do not cross the BBB, in the absence of specific transport mechanisms. It is unlikely that active or passive immunization will be effective in humans, if the BBB is not disrupted.

If a CNS drug is formulated in a vehicle other than a physiological buffer, then the amounts of any solvent, surfactant, or adjuvant, that are included in the formulation should be evaluated critically as to whether drug treatment is associated with solvent-mediated BBB disruption. In this setting, there is a high likelihood that chronic drug administration will have toxic side effects.

TRANS-NASAL DRUG DELIVERY TO THE BRAIN

The delivery of drugs after intranasal administration is based on the rationale that drugs can exit the submucous space of the nose and cross the arachnoid membrane, and enter into olfactory CSF. It is posited that drug may then enter the brain from the CSF flow tracts following intranasal administration of drug. There are two points to consider when evaluating the potential efficacy of transnasal drug delivery to the brain. First, any drug that enters into olfactory CSF will exit the CSF flow tracts and enter the peripheral bloodstream like any other ICV route of administration. The second consideration is that the arachnoid membrane, which separates olfactory CSF from the submucous spaces of the nose, has high resistance tight junctions, just like the capillary endothelium that forms the BBB.[34] Therefore, only lipid-soluble small molecules may cross the arachnoid membrane and enter into olfactory CSF in the absence of arachnoid membrane disruption. Conversely, if the arachnoid membrane and other membranes in the nose are physically or chemically disrupted, then drug may enter the CSF from the nose. The human nasal cavity can only receive about 100 μl per nostril without local injury.[35] The volume of drug administered into the nose is invariably $\gg$200 μl. Melanocyte-stimulating hormone, a seven-amino acid neuropeptide, entered CSF following intranasal instillation in humans after these subjects ingested 20 consecutive puffs of drug via an atomizer into each nares.[36] When drug is administered to the nose via volumes that are not injurious to the nose, then no distribution into CSF is found for a water-soluble drug such as vitamin B12 or a relatively lipid soluble drug such as melatonin.[35] In the absence of local injury, distribution of neuropeptides to olfactory CSF is nil, unless the protein has access to a specialized transport system that enables movement across the arachnoid membrane. This was demonstrated in the case of a conjugate of HRP and wheat germ agglutinin (WGA). The latter is a glycoprotein that crosses membranes via absorptive mediated endocytosis, based on binding to membrane lectin sites.[37] Whereas the HRP alone cannot penetrate the olfactory CSF, the HRP-WGA conjugate can cross plasma membranes via absorptive-mediated endocytosis.

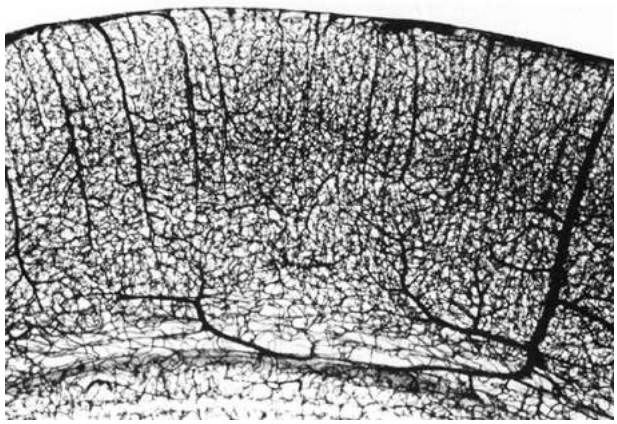

FIG. 4. India ink study shows vascular density in the cortex of adult rat brain. Reprinted with permission from Bar. The vascular system of the cerebral cortex. *Adv Anat Embryol Cell Biol* 59:I–VI, 1–62. Copyright © 1980, Springer-Verlag.[38] All rights reserved.

TRANSVASCULAR DRUG DELIVERY TO THE BRAIN VIA ENDOGENOUS BBB TRANSPORTERS

The complexity of the vascular tree in the cortex of rat brain is shown with the India ink[38] study in Figure 4. The vascular density in the human brain is even more complex. In the human brain, there are over 100 billion capillaries. The distance between capillaries is $\sim$50 μm. Therefore, the maximum diffusion distance in brain parenchyma following transvascular delivery is only 25 μm. Even a molecule as large as albumin, 68,000 Da molecular mass, will diffuse 25 μm in less than 1 s.[1] Because the intercapillary distance in brain is so small, every neuron is virtually perfused by its own blood vessel. The length of capillaries in human brain is $\sim$400 miles, and the surface area of the brain capillary endothelium in the human brain is $\sim$20 m^2. However, the volume of the intraendothelial space is only 1 μl for adult rat brain and is only 5 ml for the human brain. Therefore, the brain capillary endothelial surface, which forms the BBB *in vivo*, forms a very broad but thin

barrier system. The thickness of the endothelial cell is only ~200 nm, which is less than 5% of the thickness of most cells.

Transport across the BBB involves movement across two membranes in series: the luminal and abluminal membranes of the capillary endothelium, separated by the 200 nm of endothelial cytoplasm. The microvascular endothelium in brain is completely invested by a basement membrane, but the basement membrane constitutes no diffusion barrier. Approximately 90% of the brain side of the capillary is covered by astrocyte foot processes,[39] although these astrocyte foot processes similarly constitute no diffusion barrier. Therefore, solutes freely and instantaneously distribute throughout the entire brain extravascular volume after transport across the limiting membrane, which is the capillary endothelial membrane. The BBB has a very high resistance owing to the tight junctions, which cement adjacent endothelial cells together. Due to the presence of the tight junctions, there is no *para-cellular* pathway for solute distribution into brain interstitial fluid from blood. Circulating molecules can only gain access to brain interstitium via a *trans-cellular* route through the brain capillary endothelial membranes. If a molecule is lipid soluble and has a molecular mass less than 400 Da and is not avidly bound by plasma proteins or is a substrate for an active efflux transport system at the BBB, then the circulating molecule may gain access to brain by lipid-mediated free diffusion. In the absence of the lipid-mediated pathway, circulating molecules may gain access to brain only via transport on certain endogenous transport systems within the brain capillary endothelium. These endogenous transporters have an affinity for both small molecules and large molecules and can be broadly classified into three categories: 1) CMT; 2) active efflux transport, or AET; and 3) receptor-mediated transport, or RMT.

CMT

CMT systems for hexoses, monocarboxylic acids such as lactic acid, neutral amino acids such as phenylalanine, basic amino acids such as arginine, quaternary ammonium molecules such as choline, purine nucleosides such as adenosine, and purine bases such as adenine, are shown in Figure 5, which represents the luminal membrane of the brain capillary endothelium. The individual endogenous nutrients shown in Figure 5 are representative substrates because each carrier system transports a group of nutrients of common structure. The CMT systems shown in Figure 5 are all members of the Solute Carrier (SLC) gene family (Table 2). The BBB glucose carrier is GLUT1 (glucose transporter type 1), which is a member of the SLC2 family; the BBB monocarboxylic acid transporter is MCT1, which is a member of the SLC16 family; the BBB large neutral amino acid and cationic amino acid transporters are LAT1 and CAT1,

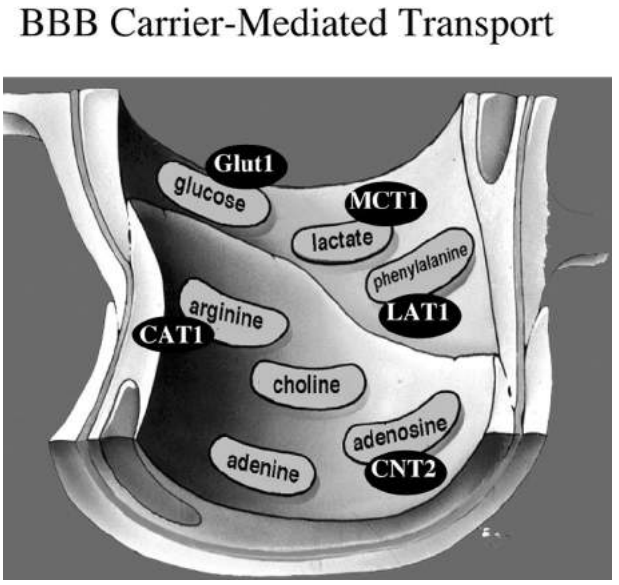

FIG. 5. BBB CMT systems are shown for seven different classes of nutrients, and the genes for five of these systems has been identified. GLUT1 = glucose transporter type 1; MCT1 = monocarboxylic acid transporter type 1; LAT1 = large neutral amino acid transporter type 1; CAT1 = cationic amino acid transporter type 1; CNT2 = concentrative nucleoside transporter type 2.

respectively, which are members of the SLC7 family; LAT1 and CAT1 are the light chains of heterodimeric proteins, and the heavy chain of the dimer is 4F2hc, which is a member of the SLC3 family; the BBB adenosine transporter is CNT2, which is a member of the SLC28 family (Table 2). Each of the SLC families shown in Table 2 represent many common genes of overlapping nucleotide identity and some of the SLC families are comprised of over 100 different genes.

BBB GLUT1 transports glucose, 2-deoxyglucose, 3-*O*-methyl-glucose, galactose, and mannose, but not L-glucose.[40] BBB MCT1 transports lactate, pyruvate, ketone bodies, and monocarboxylic acids.[41] BBB LAT1 transports the neutral amino acids with preferential affinity for the large neutral amino acids.[42] BBB CAT1 transports arginine, lysine, ornithine.[43] The BBB choline transporter transports choline, and perhaps other quaternary ammonium molecules.[44] To date, the BBB choline transporter has not been cloned. CHT1 is a sodium-dependent choline transporter member of the SLC5 family (Table 2), which corresponds to the sodium-dependent synaptosomal choline carrier. However, the BBB choline transporter is sodium independent[45] and is likely a member of a different SLC gene family. The BBB adenosine carrier transports adenosine, guanosine, and certain pyrimidine nucleosides such as uridine,[46] and is derived from the CNT2 gene,[47] where CNT = concentrative nucleoside transporter. Purine nucleosides are also transported by sodium independent or equilibrative nucleoside transporters (ENT), which are members of the SLC29 gene family (Table 2). However, BBB transport

TABLE 2. *Solute Carrier (SLC) Gene Families of Small-Molecule Transporters*

Family	Substrate Specificity	Abbreviations
SLC1	Acidic amino acid transporter	EEAT
	ASC small neutral amino acid transporter	ASCT
SLC2	Glucose transporter	GLUT
	H^+-myo-inositol transporter	HMIT
SLC3	Heavy chain of heterodimeric amino acid transporters	4F2hc
SLC4	Bicarbonate/carbonate exchangers and Na^+ coupled transporters	AE, NBC
SLC5	Sodium/substrate cotransporters (glucose, choline)	SGLT, CHT
SLC6	Neurotransmitter transporters (GABA, glycine, taurine, monoamines, creatine)	GAT, TAUT
SLC7	Cationic amino acid transporter	CAT
	Light chain of amino acid transporters	LAT
SLC8	Sodium/calcium exchanger	NCX
SLC9	Sodium/proton exchanger	NHE
SLC10	Sodium/bile salt cotransporter	NTCP, ASBT
SLC11	Natural resistance-associated macrophage protein	NRAMP
	Divalent metal-ion transporter	DMT
SLC12	Potassium/chloride cotransporter	KCC
SLC13	Sodium/sulphate cotransporter	NaS
	Sodium/dicarboxylate transporter	NaDC
SLC14	Urea transporter	UT
SLC15	Proton peptide transporter	PEPT
SLC16	Monocarboxylic acid transporter (lactate, pyruvate, ketone bodies)	MCT
SLC17	Vesicular glutamic acid transporter	VGLUT
SLC18	Vesicular amine transporter	VAT
SLC19	Vitamin transporters (folic acid, thiamine)	THTR
SLC20	Sodium-phosphate cotransporters	Pit
SLC21	Organic anion transporters	OATP
SLC22	Organic cation transporters	OCTN, OAT
SLC23	Sodium/ascorbic acid transporter	SVCT
SLC24	Sodium/calcium-potassium exchanger	NCKX
SLC25	Mitochondrial carriers	MC
SLC26	Anion exchangers	CFTR
SLC27	Fatty acid transport proteins	FATP
SLC28	Sodium dependent nucleoside transporters	CNT
SLC29	Equilibrative nucleoside transporters	ENT
SLC30	Zinc efflux transporters	ZNT
SLC31	Copper efflux transporters	CTR
SLC32	Vesicular neurotransmitter transporters	VIAAT, VGAT
SLC33	Acetyl-CoA transporters	AT
SLC34	Sodium/phosphate cotransporters	NaPi
SLC35	Nucleotide sugar transporters	UGT
SLC36	Lysosomal amino acid transporters	LYAAT
SLC37	Glucose-6-phosphate transporter	G6PT
SLC38	Sodium coupled neutral amino acid transporters	SNAT
SLC39	Metal ion transporters	ZIP
SLC40	Iron efflux transporter	MTP

in vivo on the blood side of the endothelium is sodium dependent,[48] which excludes the role of an ENT carrier in mediating uptake of circulating adenosine. Pyrimidine nucleosides are primarily transported by CNT1, and, to date, there is no evidence that the BBB expresses CNT1. Purine bases such as adenine and guanine are transported by a nucleobase transporter (NBT)[46] but, to date, no eukaryotic NBT transporter gene has been cloned.

In addition to the CMT systems shown in Figure 5, there are many other CMT genes expressed at the BBB, which enable the BBB transport of water-soluble vitamins, thyroid hormones, and other compounds. All of these CMT systems at the BBB, which may number in the dozens, are potential portals of entry of drugs to the brain. The CMT systems comprise highly stereospecific pore-based transporters, and there are significant structural requirements for transporter affinity. Therefore, it is unlikely that a drug, which is normally not transported across the BBB, would be made transportable by simply coupling to the drug to another molecule that undergoes CMT across the BBB. Rather, the structure of the pharmaceutical should be altered with medicinal chemistry so that it takes on the structure of a pseudo-nutrient and thus is able to undergo transport across the BBB via one of the

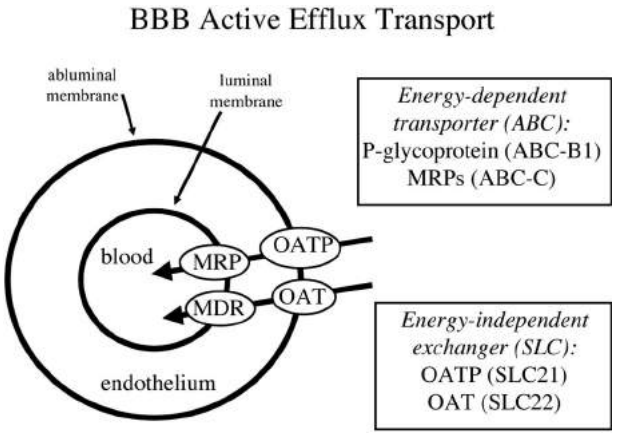

FIG. 6. BBB AET systems are comprised of an energy-dependent system at one side of the brain capillary endothelium and an energy-independent system at the opposite endothelial membrane. As a hypothetical example, members of the ABC gene family are shown at the luminal endothelial membrane, and members of the SLC gene family are shown at the abluminal endothelial membrane.

CMT systems. For example, the α-carboxylation of dopamine results in the formation of L-DOPA, and DOPA, a large neutral amino acid, is a substrate for the BBB LAT1. Once across the BBB, the L-DOPA is decarboxylated back to dopamine via aromatic amino acid decarboxylase. L-DOPA is the primary example of a pro-drug that traverses biological membranes, not via lipid mediation, but via carrier mediation.

AET

P-glycoprotein is the prototypic AET system at the BBB, and accounts for the active efflux of molecules in the brain to blood direction. P-glycoprotein, which is a product of the ABC-B1 gene (FIG. 6), is just one of many members of the ATP binding cassette (ABC) gene family of transporters. There are several multidrug resistance protein (MRP) transporters, which also belong to the ABC gene family. The excessive focus on p-glycoprotein, also called the multidrug resistance (MDR) gene product, overlooks the fact that P-glycoprotein is just one member of a large gene family, and many members of the ABC gene family may participate in BBB AET. A second consideration is that active efflux in the brain to blood direction requires the concerted actions of two different types of transporters: an energy requiring transporter at one membrane of the endothelium, and an energy-independent transporter, or exchanger, at the opposite membrane of the capillary endothelium. Examples of energy-independent exchangers are members of the solute carrier (SLC) transporter gene family and include the organic anion transporter (OAT) gene family or the organic anion transporter polypeptide (OATP) gene family (FIG. 6). OATP and OAT are members of the SLC21 and SLC22 gene families, respectively (Table 2).

Certain drugs are excluded from penetration into brain because these drugs are substrates for BBB AET systems. One strategy for increasing brain penetration of such drugs is the development of "co-drugs" that inhibit BBB AET systems and thereby allow increased brain penetration of the therapeutic drug. The development of pro-drugs to increase brain penetration of therapeutics might focus on MRP, OATP, or OAT transporters at the BBB in addition to p-glycoprotein.

RMT

Certain large-molecule peptides or proteins undergo transport from brain to blood via RMT across the BBB. There are at least three different types of BBB receptor systems as depicted in Figure 7. The transferrin receptor (TfR) is an example of a bidirectional RMT system that causes both the receptor-mediated transcytosis of holotransferrin in the blood to brain direction, and the reverse transcytosis of apo-transferrin in the brain to blood direction.[49,50] The neonatal Fc receptor (FcRn) is an example of a reverse RMT system that functions only to mediate the reverse transcytosis of IgG in the brain to blood direction, but not in the blood to brain direction.[51,52] The type 1 scavenger receptor (SR-VI) is an example of a receptor-mediated endocytosis system that mediates the uptake of modified low-density lipoprotein (LDL) from the blood compartment into the intraendothelial compartment, and this endocytosis is not followed by exocytosis into brain interstitial fluid.[53]

Molecular Trojan horses and BBB RMT

Certain endogenous ligands or peptidomimetic mAbs that bind exofacial epitopes on BBB RMT systems and that are endocytosing antibodies can act as molecular Trojan horses to ferry drugs, proteins, and nonviral gene medicines across the BBB using the endogenous RMT

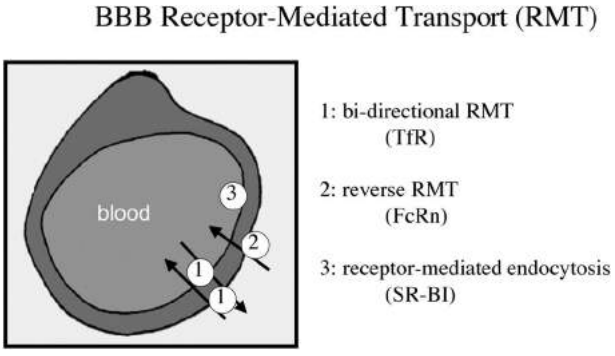

FIG. 7. BBB RMT systems are shown for three classes of systems. An example of a bidirectional RMT system is the endothelial transferrin receptor (TfR), which mediates the transport of holo-transferrin (Tf) in the blood to brain direction, and the transport of apo-Tf in the brain to blood direction. A reverse RMT system such as the neonatal Fc receptor (FcRn) transports IgG in the brain to blood direction only. An endocytosis system is illustrated by the type I scavenger receptor (SR-BI), which mediates the endocytosis of acetylated low-density lipoprotein into the endothelial compartment without transcytosis across the BBB.

systems. This BBB molecular Trojan horse technology has been reduced to practice *in vivo* in the following systems:

- Vasoactive intestinal peptide (VIP) causes a 60% increase in cerebral blood flow after intravenous injection in conscious rats.[54]
- BDNF causes 100% normalization of the pyramidal cell density in the CA1 sector of the hippocampus in adult rats subjected to transient forebrain ischemia after delayed intravenous administration.[55]
- BDNF reduces stroke volume 65-70% in adult rats with either permanent or reversible middle cerebral artery occlusion (MCAO) after delayed intravenous administration.[56,57]
- FGF-2 causes an 80% reduction in stroke volume in a permanent MCAO model in adult rats after delayed intravenous administration.[58]
- Epidermal growth factor (EGF) can be used as a peptide radiopharmaceutical to enable early detection of brain cancer that overexpresses the EGF receptor.[59]
- $A\beta^{1\text{-}40}$ can be used as a peptide radiopharmaceutical for the early detection of brain amyloid in Alzheimer's disease.[60]
- Sequence-specific peptide nucleic acids (PNA) can be used as antisense radiopharmaceuticals for the *in vivo* imaging of gene expression in brain, in either transgenic mouse models or adult rats with experimental brain cancer.[61,62]

In all of these studies, the peptide or antisense agent was ineffective in the brain *in vivo* after intravenous administration owing to the lack of transport of the molecule across the BBB. However, the intended CNS pharmacologic effect *in vivo* was achieved after intravenous administration, owing to conjugation of the peptide or antisense therapeutic to a BBB molecular Trojan horse. Molecular Trojan horses can also target liposomes[63] and nanoparticles[64] across the BBB. Nonviral plasmid DNA is encapsulated in pegylated liposomes, which are then targeted across the BBB and the brain cell membrane with peptidomimetic monoclonal antibodies that function as molecular Trojan horses.[65] The pegylated immunoliposome (PIL) nonviral gene transfer technology has enabled 100% normalization of striatal tyrosine hydroxylase activity in experimental Parkinson's,[66] and a 100% increase in survival time of adult mice with experimental brain cancer.[67] After intravenous administration of PILs carrying an exogenous reporter gene, the exogenous gene was globally expressed in all regions of the brain of the adult Rhesus monkey after intravenous injection of a nonviral formulation.[68] Plasmid DNA that produces short hairpin RNA for the purposes of silencing genes

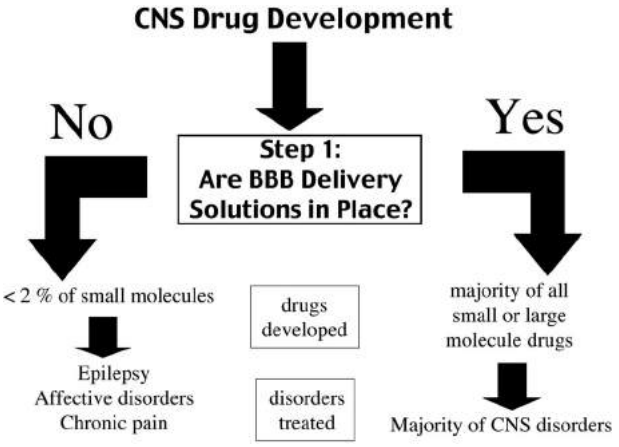

FIG. 8. Step 1 in CNS drug development is the availability of effective BBB drug or gene targeting technology. In the absence of a BBB technology, then the CNS drug developer is limited to lipid-soluble low molecular weight drugs, and only a few CNS diseases consistently respond to this class of molecule.

through a mechanism of RNA interference (RNAi) can be delivered across the BBB with the PIL gene targeting technology.[69] This resulted in an 88% increase in survival time in adult mice with experimental human brain cancer that were treated with DNA-based RNAi therapeutics directed against the human EGF receptor.[70]

CONCLUSIONS

The development of new drugs for brain disorders is a formidable challenge, and there is no effective treatment for the majority of brain diseases (FIG. 2). The inability to treat most brain diseases is incongruous with the tremendous progress made in the molecular neurosciences. The brain drug discovery sciences have, in fact, been highly successful, and many new therapeutics have been discovered, which could potentially be used to treat the brain, if the BBB problem was solved. However, if the drugs cannot be delivered across the BBB, then there is no translation from the lab to the clinic. Step number 1 in CNS drug development is providing solutions to the BBB problem (FIG. 8). If no BBB delivery solutions are in place, which is the standard in the pharmaceutical industry, then the number of drugs that can be developed as new neurotherapeutics is less than 2% of small molecules and is ~0% of large molecules. The few small molecules that do cross the BBB are those drugs that have high lipid solubility and molecular mass less than 400 Da, and these drugs generally only treat certain CNS disorders, such as epilepsy, affective disorders, and chronic pain (FIG. 8). In the absence of an effective BBB technology, the pharmaceutical industry cannot provide therapeutics for the majority of patients with brain disorders. It is estimated that the global CNS pharmaceutical market would have to grow by more than 500% just to equal the cardiovascular market,[71] and there are more

patients with CNS disorders than there are with cardiovascular disease. If BBB delivery solutions were in place for either small or large molecules, then almost any pharmaceutical could enter clinical drug development programs and therapies could be developed for most CNS disorders (FIG. 8).

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Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain

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Abstract

It was reported that nanoparticles with polysorbate 80 (Tween 80, T-80) coating represented tools used for delivering drugs to brain. Nevertheless, disputations were once aroused for some complications. Aimed to have a better understanding of the specific role of T-80 coating on nanoparticles and simplify the problem, the direct observation of brain targeting combined with in vivo experiments was carried out in this work using the model nanoparticles (MNPs). The presence of a complex composed by the model loading, T-80 and nanoparticles was found in the preparation of MNPs. The result was further supported by some surface properties of MNPs. Being bound to nanoparticles that were overcoated by T-80 later, was necessary for the loading to be delivered to brain. Partial coverage was enough for T-80 coating to play a specific role in brain targeting. It seemed that brain targeting of nanoparticles was concerned with the interaction between T-80 coating and brain micro-vessel endothelial cells. Therefore, the specific role of T-80 coating on nanoparticles in brain targeting was confirmed.

Propylene Glycol

EXPERIENCE AND REASON | SEPTEMBER 01 1983

Hyperosmolality in Small Infants Due to Propylene Glycol

ALLEN M. GLASGOW; ROGER L. BOECKX; MARILEA K. MILLER; MHAIRI G. MACDONALD; GILBERT P. AUGUST; STEPHEN I. GOODMAN

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Propylene glycol (1,2-propanediol) is used in many drug preparations. Although propylene glycol is regarded as having low toxicity in adults, in humans and animals there have been reports of CNS, renal, hematologic, and cardiac toxicity.[1-5] The absorption of propylene glycol through large burn wounds has recently been documented as a cause of serum hyperosmolality.[6,7]

Investigation of the cause of unexplained hyperosmolality in a premature infant led to the finding that several infants in our nursery were hyperosmolar due to administration of propylene glycol in a multivitamin preparation used in parenteral nutrition. This finding raises concern about the relatively large dose of propylene glycol that may be received by very small infants, especially those receiving multiple medications.

Thimerosal / Mercury

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ORIGINAL ARTICLE

Effect of thimerosal, methylmercury, and mercuric chloride in Jurkat T Cell Line

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ABSTRACT

Mercury is a ubiquitous environmental toxicant that causes a wide range of adverse health effects in humans. Three forms of mercury exist: elemental, inorganic and organic. Each of them has its own profile of toxicity. The aim of the present study was to determine the effect of thimerosal, a topical antiseptic and preservative in vaccines routinely given to children, methyl mercury, and mercuric chloride on cellular viability measured by MTT in Jurkat T cells, a human T leukemia cell line. The treatment of Jurkat T cells with thimerosal caused a significant decrease in cellular viability at 1 µM (25%, $p<0.05$; IC50: 10 µM). Methyl mercury exhibited a significant decrease in cellular viability at 50 µM (33%, $p<0.01$; IC50: 65 µM). Mercuric chloride ($HgCl_2$) did not show any significant change in cellular survival. Our findings showed that contrary to thimerosal and methyl mercury, mercuric chloride did not modify Jurkat T cell viability.

KEY WORDS: Cell Survival/drug effects; organic mercury compounds; mitochondrial membranes/drug effects; MTT; T-Lymphocytes/drug effects; Cell Death/drug effects

Introduction

Mercury, one of the most widely diffused and hazardous organ-specific environmental contaminants, exists in a wide variety of physical and chemical states, each with unique characteristics of target organ specificity (Aleo *et al.*, 2002). Mercury occurs in three forms: the elemental or metallic form, inorganic salts, and organic compounds. The toxicity of mercury is complex and depends on the form of mercury, route of entry, dosage, and age at exposure (Clarkson, 1997). The organic form of mercury, mainly methyl mercury, is known to be more toxic than the inorganic form (Shenker *et al.*, 1992). Chronic exposure to low levels of methyl mercury can modulate T- and B-cell functions (cytokine production, cell growth, and proliferation) and different cellular processes leading to apoptotic cell death (Makani *et al.*, 2002; Shenker *et al.*, 1992). Ethyl mercury is an organic mercury compound, and in the form of thimerosal has been used as a topical antiseptic and as a preservative in vaccines routinely given to children, including diphtheria-tetanus-acellular pertussis (DTP), hepatitis B, and some Haemophilus influenzae type B (Goldman & Shannon, 2001; Halsey, 1999; Pichichero *et al.*, 2002). Thimerosal (as sodium ethylmercuric thiosalicylate) contains 49.6% mercury by weight and is metabolized to ethyl mercury and thiosalicylate. The normal dose of a pediatric vaccine contains about 12.5–25 µg of mercury per 0.5 ml. (No authors listed, AAP, 1999). Massive overdoses from inappropriate use of products containing thimerosal have resulted in toxic effects (Axton, 1972; Fagan *et al.*, 1977; Lowell *et al.*, 1996; Matheson *et al.*, 1980; Pelassy *et al.*, 1994; Pfab *et al.*, 1996). Inorganic mercury (I-Hg) compounds (as mercury salts) are also a significant source of mercury overexposure in both adults and children in some countries (Clarkson, 2002). Inorganic mercury compounds have been used for many years in numerous products, including various medications, germicidal soaps, teething powders, and skin lightening cream containing mercury (Clarkson, 2002). Many of these mercury-based products are still in use today (Geier *et al.*, 2010; Goldman & Shannon, 2001). In the present study, we evaluated the effect of thimerosal, methyl mercury and mercuric chloride ($HgCl_2$) on the viability of Jurkat T cells

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by (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Methods

Cell culture

Human T leukemic Jurkat cells were purchased from American Type Culture Center (ATCC no. TIB-152) (Rockville, MD, USA) and maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 1% glutamine, and 1% antibiotics/antimicotics (pen./strep.). The cells were grown at 37 °C in a humidified atmosphere of 5% CO_2.

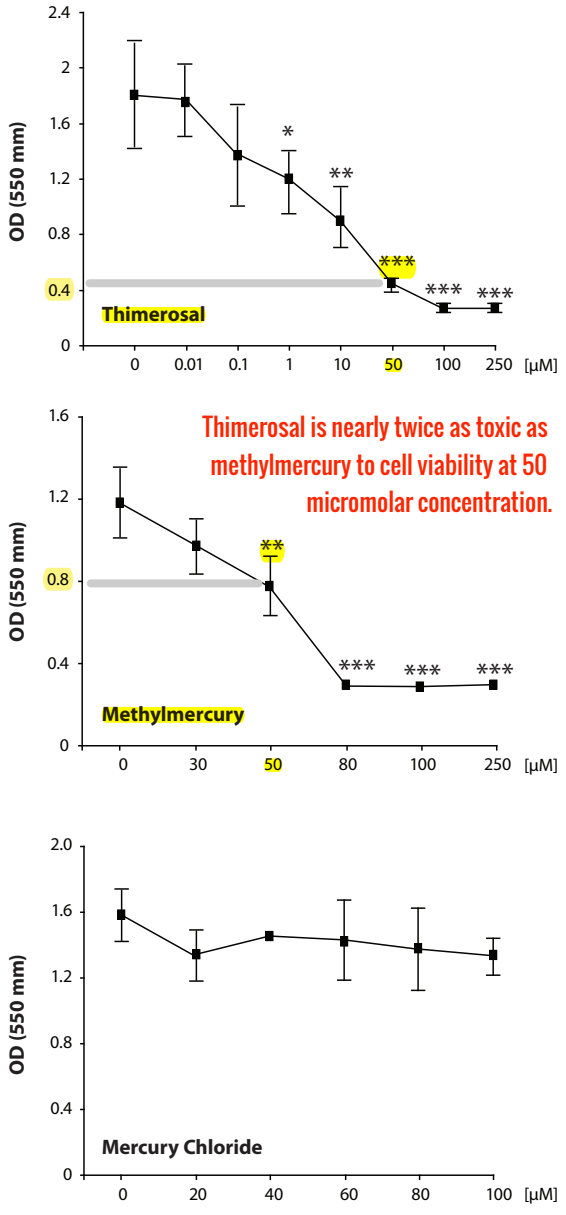

Figure 1. Viability of Jurkat cells determined by MTT-assay after exposure to thimerosal, methylmercury and mercury chloride. The data are expressed as mean ± SEM. Asterisks indicate significant differences from control cells without treatment (n=3, *p<0.05;**p<0.01;***p<0.001). Note, the x-axis is not a linear scale.

Mercury and its chemical compounds

Thimerosal (EtHg), methyl mercury (MeHg) and mercuric chloride [(mercuric (II) chloride ($HgCl_2$) also termed 'mercury two')] were purchased from Sigma. PBS and water were used to dilute mercuric chloride ($HgCl_2$) and thimerosal, respectively. Cells treated only with vehicles were used as controls.

Cytotoxicity assay (MTT)

The principle behind this technique depends on the capacity of living cells to reduce tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to a formazan crystal in their metabolizing mitochondria. The number of 1×10^4 cells/well Jurkat T cells (ATCC no. TIB-152) were seeded into 96 well plates and exposed to thimerosal, methyl mercury, and mercuric chloride ($HgCl_2$) at concentrations of thimerosal (0.01-0.1-1-10-50-100-250 µM), methyl mercury (30-50-80-100-250 µM), and mercuric chloride ($HgCl_2$ (20-40-60-80-100 µM). The plates were incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 48 hours, the medium was discarded and 20 µl/well of MTT solution (5 mg/ml) was added and incubated for 3 hours at 37 °C (5% CO_2). Finally, 20µl/well of isopropanol was added and the color intensity was read spectrophotometrically at 590 nm using a Microplate Reader (Bio-Rad Model 550, California, USA).

Statistical analysis

The ANOVA one-way test was used to determine statistical significance. A p-value of less than 0.05 was considered to be statistically significant.

Results

We exposed Jurkat T cells to thimerosal, methyl mercury and mercuric chloride in the concentrations reported in Figure 1 for 48 hours. Upon exposure to thimerosal, methyl mercury and mercuric chloride ($HgCl_2$), the viability of cells was measured with MTT assay. As shown in Figure 1, the treatment of Jurkat T cells with thimerosal caused a significant decrease in cellular viability at 1µM (25%, p<0.05; IC50: 10 µM). Methyl mercury exhibited a significant decrease in cellular viability at 50 µM (33%, p<0.01; IC50: 65 µM). Finally, at all concentrations analyzed, mercuric chloride ($HgCl_2$), did not show any significant change in cellular survival (Figure 1).

Discussion

Mercury is ubiquitous in the environment and exposure occurs from the use of mercury-containing dental

amalgam, vaccine preservatives, and ingestion of fish containing high levels of methyl mercury (Counter & Buchanan, 2004; Krantz & Dorevitch, 2004; Ratcliffe *et al.*, 1996). In the literature, however, there are few data showing the effect of organic and inorganic mercury on cell viability. Considerable concern has been expressed recently over the cumulative dose of ethyl mercury given to children through routine immunizations (Geier *et al.*, 2010; Hornig *et al.*, 2004). The source of mercury in vaccines is the antimicrobial preservative thimerosal, containing 49.9% mercury by weight. Our findings demonstrate that thimerosal at the concentration usually found in vaccines, affects significantly cellular viability. A recent paper showed that after thimerosal exposure at the same concentration as tested in the present study, a human glioblastoma cell line displayed a similar effect (James *et al.*, 2005). On the other hand, the form of mercury that accumulates in the food chain is methyl mercury. Some people may be exposed to higher levels of mercury in the form of methyl mercury if they have a diet high in fish, shellfish, or marine mammals that come from mercury-contaminated waters. Colombo *et al.* (2004) determined the sensitivity of Jurkat T cells to up to 1μM of methyl mercury after 48 hours of exposure (Colombo *et al.*, 2004). They found that cellular viability determined by MTT assay showed no toxic effects during the first 48 hours, yet exposure for up to 72 hours caused a significant decrease in cellular viability at the higher dose of mercury (1 μM) (Pelassy *et al.*, 1994). Our findings are in accordance with these data and show that organic mercury, such as methyl mercury and thimerosal, are more cytotoxic than inorganic mercury (as $HgCl_2$). Experiments are in progress to ascertain the underlying mechanisms of ethyl mercury induced cell death. It has been proposed to induce depletion of thiol reserves (*e.g.*: GSH) and ROS damage, activating death-signaling pathways (Makani *et al.*, 2002). A previous study showed that thimerosal was able to induce apoptosis and G2/M phase in human leukemia U937 cells (Woo *et al.*, 2006). Finally, according to other authors (Bahia *et al.*, 1999; Ogura *et al.*, 1996), methyl mercury showed a higher toxicity compared to mercuric chloride ($HgCl_2$). Recently, mercuric chloride ($HgCl_2$) was reported to affect the differentiative capacity instead of proliferation in neural stem cells (Cedrola *et al.*, 2003). Further studies will attempt to assess the possible effect of thimerosal as preservative in vaccines. Our data showed an effect of organic mercury on the viability of Jurkat T cells, suggesting a possible toxic effect of these compounds of mercury *in vivo*.

Conflict of interest statement

We have no conflicts of interest connected with this work.

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Low-dose mercury exposure in early life: relevance of thimerosal to fetuses, newborns and infants

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Abstract

This review explores the different aspects of constitutional factors in early life that modulate toxicokinetics and toxicodynamics of low-dose mercury resulting from acute ethylmercury (etHg) exposure in Thimerosal-containing vaccines (TCV). Major databases were searched for human and experimental studies that addressed issues related to early life exposure to TCV. It can be concluded that: a) mercury load in fetuses, neonates, and infants resulting from TCVs remains in blood of neonates and infants at sufficient concentration and for enough time to penetrate the brain and to exert a neurologic impact and a probable influence on neurodevelopment of susceptible infants; b) etHg metabolism related to neurodevelopmental delays has been demonstrated experimentally and observed in population studies; c) unlike chronic Hg exposure during pregnancy, neurodevelopmental effects caused by acute (repeated/cumulative) early life exposure to TCV-etHg remain unrecognized; and d) the uncertainty surrounding low-dose toxicity of etHg is challenging but recent evidence indicates that avoiding cumulative insults by alkyl-mercury forms (which include Thimerosal) is warranted. It is important to a) maintain trust in vaccines while reinforcing current public health policies to abate mercury exposure in infancy; b) generally support WHO policies that recommend vaccination to prevent and control existing and impending infectious diseases; and c) not confuse the 'need' to use a specific 'product' (TCV) by accepting as 'innocuous' (or without consequences) the presence of a proven 'toxic alkyl-mercury' (etHg) at levels that have not been proven to be toxicologically safe.

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Thimerosal Induces DNA Breaks, Caspase-3 Activation, Membrane Damage, and Cell Death in Cultured Human Neurons and Fibroblasts

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Abstract

Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomo-lar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced. Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250-μM concentrations of thimer-osal for 45 min to 24 h. A 4′, 6-diamidino-2-phenylindole dihy-drochloride (DAPI) dye exclusion test was used to identify non-viable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2 μM based on the manual detection of the fluorescent attached cells and at a 1-μM level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250 μM thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3– dependent apoptosis in human neurons and fibroblasts. We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.

Keywords

thimerosal; active caspase-3; apoptosis; toxicity; neurons; fibroblasts; DNA breaks; membrane damage; DAPI

Thimerosal (sodium ethylmercury-thiosalicylate) is an antibacterial and antifungal mercurial compound used as a preservative in biological products and vaccines, in concentrations ranging from 0.003 to 0.01% (30 –100 μg/ml) (Ball *et al*., 2001). Thimerosal contains 49.6 % mercury by weight and releases ethylmercury as a metabolite. In the body, ethylmer-cury can be converted to inorganic mercury, which then preferentially accumulates in the kidneys and brain (Blair *et al*., 1975). Inorganic mercury is known to induce membrane and DNA damage (Ferrat *et al*., 2002;Ben-Ozer *et al*., 2000), and in cell culture conditions it was shown to be mutagenic and generate DNA breaks in concentrations below 500 nM (Schurz *et al*., 2000). Ethylmercury

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can significantly increase the concentration of inorganic mercury in many organs (Magos *et al*., 1985). After *in vivo* administration, ethylmercury passes through cellular membranes and concentrates in cells in vital organs, including the brain, where it releases inorganic mercury, raising its concentrations higher than equimolar doses of its close and highly toxic relative methylmercury (Magos *et al*., 1985).

However, little is known about acute reactions of various types of human cells following short-time exposure to thimer-osal in micro- and nanomolar concentrations.

In this paper we used a convenient and easily reproducible combination of fluorescent techniques analyzing various markers of DNA and membrane damage, and investigated the toxicity of micromolar and nanomolar concentrations of thimerosal (125 nM–250 μM) occurring in the first 24 h of exposure in cultures of human cortical neuronal cells and in human fibroblasts.

We found that thimerosal in micromolar concentrations rapidly decreased cellular viability. Within several h after thimer-osal administration, cells lost their capability to exclude the fluorescent dye 4′,6-diamidino-2-phenylindole dihydrochlo-ride (DAPI) and developed multiple DNA breaks accompanied by caspase-3 activation and apoptotic morphology. Neuronal cell cultures demonstrated a higher sensitivity to thimerosal compared with fibroblasts.

MATERIALS AND METHODS

Cell cultures

HCN-1A Human cerebral cortical neurons (CRL-10442) were purchased from American Type Culture Collection (ATCC, Manassas, VA) and were cultured according to ATCC recommendations. The line was derived from cortical tissue removed from a patient undergoing hemispherec-tomy for intractable seizures. As recommended by ATCC, the cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with 4 mM L-glutamine, modified to contain 4.5g/l glucose and 1.5g/l sodium bicarbonate, supplemented with 10% fetal bovine serum, and the pH adjusted to 7.35 prior to filtration.

Normal neonatal human foreskin HCA 2 fibroblasts (PD32) were obtained from the laboratory of Dr. Olivia Smith-Pereira, Ph.D. The cells were grown in DMEM supplemented with 10% fetal bovine serum medium, and the pH was adjusted to 7.4 prior to filtration. For the experiments, all cells were subcultured in 24-well cell culture plates (Fisher, Pittsburgh, PA). All experiments were reproduced in triplicates. Each of the parallel series yielded identical results.

Thimerosal

Thimerosal (minimum 97% HPLC), SigmaUltra (Sigma, St. Louis, MO) was added to cell cultures in 30 μl of double-distilled water to final concentrations of 250 μM, 50 μM, 10 μM, 2 μM, 1 μM, 500 nM, 250 nM, and 125 nM. Concentrations of 1 μM–125 nM were used with neuronal cells only. Control cell cultures received 30 μl of water without thimerosal.

Dye exclusion test using DAPI

DAPI is a nonintercalating DNA-specific dye with an emission maximum in the blue spectrum (Shapiro, 1985). It is widely used for counterstaining cellular nuclei in fixed sections and has been demonstrated to be useful for the detection of nonviable cells with compromised membranes in live cell cultures (Boutonnat *et al*., 1999;McCarthy and Hale, 1988).

The DAPI exclusion test was performed as described (Boutonnat *et al*., 1999). Briefly, cells were incubated with DAPI (Sigma, St. Louis, MO) diluted in a cell culture medium at a final concentration of 100 ng/ml for 30 min at 20°C (Boutonnat *et al*., 1999). A fluorescent signal was monitored and representative images were taken at 45 min and 2, 4, 6, and 24 h after the addition of thimerosal, The DAPI incubation started 30 min before each observation was made (at 15 min, 90 min, etc). Images were acquired using an Olympus IX-70 fluorescent microscope equipped with a MicroMax digital camera system (Princeton Instruments, Inc., Trenton, NJ) containing an RTE/CCD-1300-Y/HS array cooled by a Peltier device. Image acquisition was performed using the *MetaMorph* 4.1 program (Advanced Scientific, Inc., Meraux, LA). The micrographs were taken at central parts of the wells, where cellular density was most uniform.

Terminal transferase-based nick-end labeling (TUNEL)

Cells were fixed in ice cold methanol, and TUNEL staining for detection of DNA breaks was performed using the ApoTaq Fluorescein and ApoTaq Rhodamine kits for indirect immunofluorescence (Serologicals, Gaithersburg, MD), employing the standard technique recommended by the manufacturer. Following washing, the cells were counterstained with the DNA binding dye DAPI (1 μg/ml) for visualization of all cellular nuclei and were mounted in Vectashield (Vector Laboratories, Burlingame, CA) for observation by fluorescence microscopy.

Caspase-3 detection

Detection of active caspase-3 in live cell cultures was performed using an APO LOGIX™ carboxyfluorescein (FAM) caspase detection kit (Cell Technology, Minneapolis, MN). The kit detects active caspases in living cells through the use of a FAM-labeled DEVD fluoromethyl ketone (FMK) caspase inhibitor, which irreversibly binds to active caspase-3 (Amstad *et al*., 2000;Bedner *et al*., 2000;Smolewski *et al*., 2001). The inhibitor is cell permeable and noncytotoxic. With lesser affinity, FAM-DEVD-FMK binds to the other caspases participating in apoptosis: caspase-8 >caspase-7 >caspase-10 >caspase-6 in the order of decreasing binding affinity (Carcia-Calvo *et al*., 1998).

The kit was used as recommended by the manufacturer. Briefly, 10 μl of 30X Working Dilution FAM-Peptide-FMK was added to 300 μl of cell culture medium/per well, directly in 24-well cell culture plates after 5 h or 23 h of incubation with thimerosal. Cells were incubated for 1 h at 37°C under 5% CO_2, protected from light. Then the medium was carefully removed, and the cells were washed twice with 2 ml/per well of 1X Working Dilution Wash Buffer. The fluorescent signal was observed under an Olympus IX-70 fluores-cence microscope equipped with a MicroMax digital camera system (Princeton Instruments, Inc.) containing an RTE/CCD-1300-Y/HS array cooled by a Peltier device. Caspase-positive cells appeared fluorescing green. Representative images were taken at 6 h and 24 h after the addition of thimerosal. Image acquisition was performed using the *MetaMorph* 4.1 program (Advanced Scientific, Inc.). Positive controls included cultures of cortical neurons treated with 0.5 μM staurosporin to induce caspase-3 activation. In several series of experiments, we added DAPI to cell cultures to the concentration of 100 ng/ml for 30 min immediately after 1 h of incubation with the FAM-Peptide-FMK solution. This made the co-localization of active caspase-3 and DAPI signals possible.

Fluorescence measurements using a microplate reader

In a separate set of experiments, we measured both active caspase-3 and DAPI signals in co-localization experiments using a GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence (Tecan Inc., Research Triangle Park, NC). Neuronal cells were incubated with 1–250 μM concentrations of thimerosal for 6 h and processed as described for simultaneous DAPI and active caspase-3 detection. Both FITC and DAPI fluorescence were

measured directly in 24-well plates using a Chroma Technology bandpass filter set: FITC excitation D490/40, emission 520/10; DAPI excitation D360/40, emission 460/20. The reactions were repeated twice and yielded the same dose-dependent increase in thimerosal toxicity. Background fluorescence was subtracted from the experimental series, and the results were represented as graphs of average values using Microsoft Excel.

RESULTS

Thimerosal-Induced Changes in Membrane Permeability and Cell Viability

Changes in cell viability rapidly occurred after administration of thimerosal in all cell cultures and were detected by the loss of ability to exclude the fluorescent dye DAPI. DAPI is classified as a semipermeant dye, which requires a relatively short (30-min) exposure time of cell cultures to the dye prior to the signal observation in a DAPI exclusion test (Boutonnat *et al*., 1999). Under these conditions, the dye has been shown to be useful for the detection of nonviable cells and can be utilized as a selective marker of membrane integrity. Indeed, it is a less toxic alternative to propidium iodide (PI) (Boutonnat *et al*., 1999).

The results of the experiments show a dose- and time-dependent increase of membrane permeability to DAPI, first detected after 2 h of incubation with thimerosal and resulting in the penetration of the dye into the nuclei and DNA staining (Figs. 1 and 2). Figure 1 presents experiments performed on human cultured cortical neurons (HCN-1A) and shows that, after 2 h of incubation with thimerosal at a concentration of 250 μM, the DAPI penetrated through cellular membranes and stained the cellular nuclei. The inability to exclude the dye indicates the loss of cellular membrane integrity and cell death (Boutonnat *et al*., 1999;McCarthy and Hale, 1988). After 4 h of incubation, thimerosal-induced membrane permeability and DNA staining were observed at a concentration of 10 μM. After 6 h of incubation with thimerosal, changes in membrane permeability were detected at concentrations as low as 2 μM, based on the appearance of DAPI-stained cells attached to the bottom of the wells. In control cell cultures, which were treated with DAPI alone, only sporadic dead cells were detected, and their numbers stayed the same 2, 4, and 6 h after the addition of DAPI (Fig. 1). There was no change in cell membrane permeability for DAPI for up to 24 h if no thimerosal was added.

We performed direct counts of DAPI-positive cells for the initial quantitative assessment of our results. We counted all DAPI-positive cells in two ×40 fields of view for each of the thimerosal concentrations after 6 h of incubation. All counts were taken in central parts of the wells, where the cellular density was most uniform. The comparison with the average density of cells in these areas revealed that, at 2-μM thimer-osal, 11% were DAPI-positive; at 10-μM thimerosal, 58% were DAPI-positive; at 50-μM thimerosal, 61% of the cells were DAPI-positive; and at 250-μM thimerosal, 100% of the neurons had compromised cellular membranes. In controls, less than 1% of the cells were DAPI positive, due to cell death naturally occurring in the cell cultures.

No changes in membrane permeability and DAPI staining were observed with thimerosal concentrations lower than 2 μM at times of incubation up to 24 h.

Since dying cells disattach from the bottom shortly after death and float in the media, they cannot be counted. This explains the similar numbers of DAPI-positive cells counted after 10- and 50-μM thimerosal treatments, and it could have some affect on the sensitivity of the lower limit of toxicity measurements. To address this issue and to take into consideration all DAPI-stained cells, we used a fluorescent microplate reader, which detects the fluorescence of both attached and floating dead cells (see Fig. 3). Using a GENios Plus Microplate Reader, we detected the lower limit of thimerosal toxicity for neuronal cells after 6 h of incubation to be at 1-μM concentration of thimerosal.

Experiments with cultured human fibroblasts produced similar results, although, when compared with neuronal cells, the fibroblasts demonstrated a slightly lower sensitivity to thimer-osal toxicity by the DAPI exclusion test in terms of the number of DAPI-stained cells (Fig. 2).

Similar to neuronal cells, significant numbers of DAPI-stained nuclei were first observed after 2 h of incubation with thimerosal at 250 μM concentration in the fibroblast culture experiments (Fig. 2). After 4 h of incubation, nuclear staining was detected at 10-μM concentration of thimerosal. However, unlike the neuronal cells, the human fibroblasts did not show toxicity at 2-μM concentration of thimerosal after 6 h of incubation.

Detection of Thimerosal-Induced DNA Damage

We used TUNEL to detect DNA breaks generated in neurons and fibroblasts after 6 h of incubation with thimerosal. Following incubation, the cells were fixed, labeled by TUNEL, and counterstained by DAPI, which in these experiments was employed as a fluorescent DNA marker to visualize all cell nuclei in fixed cell cultures.

The results of these experiments are presented in Figure 4. The figure demonstrates that TUNEL-positive cells were detected in all cell cultures after 6 h of incubation, up to the concentration of 2 μM of thimerosal.

To determine if extending the time of incubation with thimerosal at concentrations below 2μM would result in the generation of DNA breaks, we extended the time of incubation to 24 h in a separate series of experiments. After 24 h, a TUNEL signal was detected in neuronal cells at 1-μM concentration of thimerosal (versus 2 μM at 6 h) (not shown). Incubation of neuronal cells for 24 h with concentrations of thimerosal below 1 μM (125, 250, and 500 nM) did not produce a TUNEL signal.

Detection of Apoptotic Morphology in Thimerosal-Treated Cells

We performed a morphological evaluation of the fixed and fluorescently stained cell cultures after thimerosal treatment for the purpose of identifying apoptotic cells. To identify apoptotic morphology, the cells were fixed and then stained by DAPI. In this experiment, DAPI was employed not as a vital dye, as in our previous study, but rather as a fluorescent histological nuclear stain. Although DAPI is an important marker used in live cell cultures to selectively label nonviable cells (Boutonnat *et al*., 1999;McCarthy and Hale, 1988), it is also frequently used in fixed cells to visualize nuclear morphology and apo-ptotic bodies. We used it for this purpose in these tests.

Apoptotic morphology was detected in thimerosal-treated cells. Figure 5 demonstrates that, after 6 h of incubation, both fibroblasts and neurons showed morphological signs of apo-ptosis, which included chromatin condensation on the nuclear membrane, the appearance of characteristic doughnut-shaped nuclei, different stages of apoptotic body formation, and freely positioned apoptotic bodies. After 6 h of incubation, apoptotic morphology was observed at concentrations as low as 2 μM of thimerosal (Fig. 5), whereas, at 24 h after incubation, similar apoptotic morphology was observed at concentrations as low as 1 μM.

To further confirm the apoptotic nature of cell death induced by thimerosal, we performed detection of active caspase-3, which is a sensitive and specific indicator of apoptosis.

Active Caspase-3 in Thimerosal-Treated Cells

Caspase-3 activation serves as a sensitive marker of ap-optosis, developing through caspase-3–dependent mechanisms, which constitutes one of the most frequent apoptotic pathways. We

employed visualization of active caspase-3 directly in living cells through the use of a FAM-labeled peptide caspase inhibitor (FAM-Peptide-FMK) (see Materials and Methods).

We detected caspase-3–positive neuronal cells after 6 h of incubation with thimerosal at concentrations ranging from 250 to 2 μM. The intensity of the signal was dose-dependent and much lower at the 2-μM concentration, compared to higher concentrations, probably due to an earlier stage of caspase-3 activation (Fig. 6).

Assessment of 200 cells per well randomly, using the fluo-rescent microscope, revealed that active caspase-3 was expressed in 20% of the cells at 2-μM thimerosal, 26% at 10-μM thimerosal, 83% at 50-μM thimerosal, and 97% of the neurons at 250-μM thimerosal concentration. In the controls, less than 1% of the cells was caspase-3–positive, due to cell death naturally occurring in the cell cultures.

At 2-μM thimerosal, the active caspase-3 signal was predominantly observed in the cytoplasm, which represents the early stage of its activation, whereas, at higher concentrations of thimerosal, the signal was detected in both the cytoplasm and the nuclei (Fig. 6). (Nuclear localization of active caspase-3 is characteristic for later stages of the apoptotic process.)

When we used a fluorescent microplate reader, which detects signals from the detached cells, we detected active caspase-3 activation at 1-μM concentration of thimerosal after 6-h incubation, probably due to the added contribution from floating dead cells (Fig. 3).

When we extended the incubation time with thimerosal from 6 to 24 h, detectable numbers of attached cells with active caspase-3 were observed at 1-μM concentration of thimerosal (Fig. 7). An active caspase-3 signal at 1-μM concentration was cytoplasmic, demonstrating an earlier stage of caspase-3 activation. Interestingly, after 24 h of incubation, the neurons treated with 2-μM thimerosal showed the migration of caspase-3 from the cytoplasm to the nuclei (Fig. 7). The majority of caspase-3–positive cells were also DAPI-positive, which indicates membrane damage occurring simultaneously with apoptotic response. However, at the higher 250-μM concentration of thimerosal, a number of cells were only DAPI-positive without caspase-3 activation, demonstrating necrotic death (Fig. 7). We did not detect active caspase-3 at 24 h of incubation in untreated neurons, or in neuronal cultures treated with lower concentrations of thimerosal (500, 250, and 125 nM).

DISCUSSION

Our data indicate that thimerosal is toxic to human neurons and fibroblasts if applied in micromolar concentrations (1–250 μM). An early sign of thimerosal toxicity is a change in cellular membrane permeability to the vital dye DAPI, which is associated with the loss of cell viability (Boutonnat *et al*., 1999;McCarthy and Hale, 1988). This can be detected as early as 2 h after incubation.

DAPI proved to be useful for analyzing thimerosal toxicity, because it is a sensitive marker of membrane integrity. It is employed as a propidium iodide substitute in cell viability assays and labels nuclei of dying cells, which lack an intact plasma membrane (Boutonnat *et al*., 1999;Castro-Hermida *et al*., 2000;McCarthy and Hale, 1988;Robertson *et al.*, 1998). Dual staining experiments using propidium iodide and DAPI co-staining with FACS analysis demonstrated that DAPI stains only dead cells (McCarthy and Hale, 1988). Viable cells that are not stained by PI also exclude DAPI (McCarthy and Hale, 1988).

The nature of cell death labeled by DAPI in the case of thimerosal treatment deserves additional discussion. The DAPI exclusion method relies on the fact that this dye is largely impermeable to cells with an intact plasma membrane. However, when cell membrane integrity becomes

compromised, DAPI gains access to the nucleus, where it complexes with DNA and renders the nucleus highly fluorescent. Early compromised integrity of plasma membranes is a characteristic feature of necrotic cell death, whereas, in apoptosis, cellular membranes are compromised at later times. This is why intra-cellular staining by DAPI (and also by its more toxic substitute propidium iodide) is regularly interpreted as a sign of necrosis (Boutonnat *et al.*, 1999). However, in the case of thimerosal, the changes in membrane permeability coincided with the activation of apoptosis-specific caspase-3 (Fig. 3). In our opinion, this indicates a separate direct membrane damaging effect of thimerosal that developed simultaneously with apoptotic changes, such as caspase-3 activation.

In many cases, the importance of caspase-3 activation is related to its connection to specific and extensive apoptotic DNA cleavage (Porter and Janicke, 1999). This DNA fragmentation can be labeled by the TUNEL technique and is widely used for the visualization of apoptotic cells. A caspase-activated deoxyribonuclease (CAD, or DFF 40) is implicated as a direct executioner of the cleavage (Liu *et al*., 1997;Mukae *et al.*, 1998). Most of the time, the enzyme is kept inactive by the binding of an inhibitor (ICAD, or DFF 45). Activation of the nuclease occurs when the inhibitor is cleaved by activated caspase-3 (Enari *et al*., 1998;Sakahira *et al*., 1998). However, the exact sequence of events in case of a human brain is likely different from this scheme. In human CNS neurons, other caspase-3–related pathways and possibly the other DNA cleaving enzymes are more important, and the role of the CAD-mediated mechanism is likely limited, because no expression of CAD mRNA was detected in human brain cells (Mukae *et al*., 1998).

Similar to our results, high cellular toxicity of thimerosal in low micromolar concentrations was recently reported using another cell culture model (Makani *et al*., 2002). The effects of different concentrations of thimerosal were examined in Jurkat cells. The cells were incubated with 5- to 0.5-μM concentrations of thimerosal for 24 h. Concentration-dependent apopto-sis was detected and measured by TUNEL. Caspase-3 activation was also detected after 4 and 6 h of incubation with thimerosal. The study concluded that thimerosal induced caspase-3–dependent apoptosis in Jurkat cells. This apoptosis was associated with the depolarization of the mitochondrial membrane and release of cytochrome c. In this same study, a significantly enhanced generation of reactive oxygen species was also detected, as a result of incubation with thimerosal (Makani *et al*., 2002). We hypothesize that these elevated levels of free radicals and the subsequent oxidation may play role in apoptosis induction and might also be involved in the direct membrane-damaging effects of thimerosal identified in our study.

We showed that the concentrations of thimerosal that induced toxic effects in human cortical neurons ranged from 1 to 250 μM. However, comparisons of the nuclear morphology of dying cells after incubation with higher versus lower concentrations of thimerosal demonstrate important differences. Although caspase-3 activation was detected in both high and low concentrations of thimerosal, the morphology of dying cells was different in these two situations. The cell bodies of neurons treated with higher concentrations of thimerosal (50 – 250 μM) were swollen, which is more characteristic of necrotic cell death, whereas cells treated with low concentrations (2–10 μM) were shrunken, as is typical for apoptosis (Fig. 7). Similarly, the nuclei of dying neurons treated with 250-μM thimer-osal were larger in size and swollen, in contrast to the shrunken nuclei of cells treated with 2-μM thimerosal (Fig. 7). Thus, cell death occurring after incubation of neuronal cells with higher concentrations of thimerosal has features of both apoptosis (caspase-3 activation) and necrosis (cell edema and nuclei swelling). This can be explained by a direct membrane-damaging effect of thimerosal, which rapidly leads to the loss of membrane integrity and cell swelling. This process likely occurs simultaneously with apoptosis induction, the initiation of the caspase cascade, and the activation of caspase-3. At lower concentrations of thimerosal, direct membrane-damaging effects are weaker, and no swelling is observed.

Investigation of thimerosal toxicity is especially important at the present time, because this compound is used in biological products and can be administered in toxic doses either accidentally or intentionally (Ball *et al*., 2001).

In our study, the concentrations of thimerosal that induced toxic effects ranged from 1 μM (405 μg/l) to 250 μM (101 mg/l), which is equivalent to the levels of inorganic mercury from 201 μg/l to 50 mg/l. In clinical cases of accidental or intentional usage in high concentrations, thimerosal was administered in doses from 3 mg/kg to several hundred mg/kg (Ball *et al*., 2001). Such doses resulted in local necrosis at the application site and severe central nervous system and kidney injury.

Much lower concentrations are reached during normal vaccination, when thimerosal-containing vaccines are used. In the case of a full series of vaccinations containing thimerosal, up to 403 μg of thimerosal (equivalent to 200 μg of mercury) are received by 6 months of age (calculated from Ball *et al*., 2001). This results in the administration of 200/3.81 = 52 μg/kg, 200/5.22 = 38 μg/kg, and 200/6.27 = 32 μg/kg of mercury. These calculations utilize averages of the 5th, 50th, and 95th% weight for females at birth (2.36 kg, 3.23 kg, 3.81 kg) and at 6 months (5.25 kg, 7.21 kg, 8.73 kg) = 3.81 kg, 5.22 kg, 6.27 kg, reported by (Ball *et al*., 2001) when used in calculating exposure limits for mercury in comparisons of various agencies guidelines.

The lowest toxic concentration of mercury contained in the thimerosal doses in our present study (201 μg/l) is less than four times higher than some of these estimated concentrations. The rapidly developing toxicity of thimerosal in low micro-molar concentrations over short time frames is of concern and suggests that additional research is necessary to estimate the effects of prolonged exposure to thimerosal in lower doses.

In this paper we demonstrated that extending the time of incubation with thimerosal from 2 to 6 h is associated with toxicity that was not seen after a shorter time of exposure. For this reason, further studies of lower concentrations and longer exposure times appear to be warranted. These results indicate that additional research is needed to more fully delineate the dose- and time-dependent toxicity of thimerosal in sub-micro-molar concentrations and suggests that toxicity may occur at even lower doses than those utilized in these experiments, with longer times of exposure. Because mercury can be retained in body organs for months to years, the study of longer incubation times is warranted. We also conclude that a proposed combination of fluorescent techniques combining the assessment of DNA, membrane damage, and active caspase-3 is useful in studying thimerosal toxicity.

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Integrating experimental (in vitro and in vivo) neurotoxicity studies of low-dose thimerosal relevant to vaccines

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Abstract

There is a need to interpret neurotoxic studies to help deal with uncertainties surrounding pregnant mothers, newborns and young children who must receive repeated doses of Thimerosal-containing vaccines (TCVs). This review integrates information derived from emerging experimental studies (in vitro and in vivo) of low-dose Thimerosal (sodium ethyl mercury thiosalicylate). Major databases (PubMed and Web-of-science) were searched for in vitro and in vivo experimental studies that addressed the effects of low-dose Thimerosal (or ethylmercury) on neural tissues and animal behaviour. Information extracted from studies indicates that: (a) activity of low doses of Thimerosal against isolated human and animal brain cells was found in all studies and is consistent with Hg neurotoxicity; (b) the neurotoxic effect of ethylmercury has not been studied with co-occurring adjuvant-Al in TCVs; (c) animal studies have shown that exposure to Thimerosal-Hg can lead to accumulation of inorganic Hg in brain, and that (d) doses relevant to TCV exposure possess the potential to affect human neuro-development. Thimerosal at concentrations relevant for infants' exposure (in vaccines) is toxic to cultured human-brain cells and to laboratory animals. The persisting use of TCV (in developing countries) is counterintuitive to global efforts to lower Hg exposure and to ban Hg in medical products; its continued use in TCV requires evaluation of a sufficiently nontoxic level of ethylmercury compatible with repeated exposure (co-occurring with adjuvant-Al) during early life.

ASYMPTOMATIC TRANSMISSION & SHEDDING

Live Virus Vaccines

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Horizontal transmission of live vaccines

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Dear Editor,

Horizontal transmission has been rarely reported with of many live attenuated vaccines. Different mumps vaccines have shown rarely such transmission.[1–5] A study in US reported evidence of the transmission of rubella vaccine virus from vaccinees to two susceptible contacts.[6]

With live varicella vaccines, there are at least three reports. The brother of a 3-y-old vaccinated girl developed fever and a rash; horizontal transmission of vaccine virus was later confirmed.[7] A pregnant mother contracted the vaccine virus after her 12-mo-old boy received varicella vaccine.[8] Horizontal transmission was reported in 15 (17%) susceptible healthy siblings after varicella vaccination of 156 children with leukemia.[9] The package insert of live varicella vaccine (Varivax, Merck) states that "Post-marketing experience suggests that transmission of vaccine virus may occur rarely between healthy vaccinees who develop a varicella-like rash and healthy susceptible contacts. Transmission of vaccine virus from vaccinees who do not develop a varicella-like rash has also been reported."[10]

There are two reports with rotavirus vaccines. A randomized, double-blind study on human rotavirus vaccine (Rotarix™, Glaxo) in 100 pairs of healthy twins found that the transmission rate among placebo recipients was 18.8%.[11] In another case, rotavirus vaccine (RotaTeq, Merck) transmission was reported from a vaccinated infant to an older, unvaccinated sibling, resulting in symptomatic rotavirus gastroenteritis.[12]

A study on live attenuated influenza vaccine (FluMist, MedImmune) in a Finnish day care showed that one child in the placebo group had transiently detectable vaccine virus, indicating transmission from a vaccinated child; the child remained asymptomatic.[13]

Despite these reports, these live vaccines are used in millions of doses across the world. Clearly, the benefit of vaccination outweighs the very low risk of vaccine virus transmission.

Conflict of Interest

All three authors are employed by Serum Institute of India Ltd.

Footnotes

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Influenza Vaccine

Infectious virus in exhaled breath of symptomatic seasonal influenza cases from a college community

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Significance

Lack of human data on influenza virus aerosol shedding fuels debate over the importance of airborne transmission. We provide overwhelming evidence that humans generate infectious aerosols and quantitative data to improve mathematical models of transmission and public health interventions. We show that sneezing is rare and not important for—and that coughing is not required for—influenza virus aerosolization. Our findings, that upper and lower airway infection are independent and that fine-particle exhaled aerosols reflect infection in the lung, opened a pathway for a deeper understanding of the human biology of influenza infection and transmission. Our observation of an association between repeated vaccination and increased viral aerosol generation demonstrated the power of our method, but needs confirmation.

Abstract

Little is known about the amount and infectiousness of influenza virus shed into exhaled breath. This contributes to uncertainty about the importance of airborne influenza transmission. We screened 355 symptomatic volunteers with acute respiratory illness and report 142 cases with confirmed influenza infection who provided 218 paired nasopharyngeal (NP) and 30-minute breath samples (coarse >5-μm and fine ≤5-μm fractions) on days 1–3 after symptom onset. We assessed viral RNA copy number for all samples and cultured NP swabs and fine aerosols. We recovered infectious virus from 52 (39%) of the fine aerosols and 150 (89%) of the NP swabs with valid cultures. The geometric mean RNA copy numbers were 3.8×10^4/30-minutes fine-, 1.2×10^4/30-minutes coarse-aerosol sample, and 8.2×10^8 per NP swab. Fine- and coarse-aerosol viral RNA were positively associated with body mass index and number of coughs and negatively associated with increasing days since symptom onset in adjusted models. Fine-aerosol viral RNA was also positively associated with having influenza vaccination for both the current and prior season. NP swab viral RNA was positively associated with upper respiratory symptoms and negatively associated with age but was not significantly associated with fine- or coarse-aerosol viral RNA or their predictors. Sneezing was rare, and sneezing and coughing were not necessary for infectious aerosol generation. Our observations suggest that influenza infection in the upper and lower airways are compartmentalized and independent.

influenza virus aerosol airborne infection vaccination effects viral shedding

The nature of infectious contacts and the relative importance of contact, large-droplet spray, and aerosol (droplet nuclei) transmission remain controversial (**1**⇓⇓⇓⇓⇓–**6**). Nonpharmaceutical interventions have been employed to control and reduce the impact of influenza epidemics and pandemics (**7**). However, to design

effective nonpharmaceutical interventions, it is necessary to accurately define the relative and absolute contribution of each route of transmission (**8**) and implement interventions that impede those of principal importance.

Mathematical models that have been used to understand and estimate the contribution of each mode are very sensitive to estimates of unmeasured parameters (**9**, **10**), such as the viral load in exhaled breath and coughs and the frequency of sneezing by influenza cases (**8**). However, due to limitations inherent to sampling virus shedding via various routes from infected individuals, and the difficulty of distinguishing routes of transmission in observational studies, the quantitative dynamics and relative contributions of each route remain elusive (**4**, **8**). Recent reports have shown that infectious influenza virus can be recovered from exhaled aerosols (**11**⇓–**13**). These studies, based on small numbers of cases or artificial breathing maneuvers, do not provide sufficient data to quantify the extent of aerosol shedding during natural breathing, nor do they identify the contributions of spontaneous coughs and sneezes commonly thought to be the most important mechanism for viral shedding, or identify other factors that may impact viral aerosol shedding. We address these key knowledge gaps by characterizing influenza virus in exhaled breath from community-acquired influenza cases during natural breathing, prompted speech, coughing, and sneezing, and assess the infectivity of naturally occurring influenza aerosols.

Results

We screened 355 volunteers with acute respiratory illness; the 178 volunteers who met enrollment criteria provided 278 visits for sample collection. We confirmed influenza infection in 156 (88%) of the enrolled participants using qRT-PCR; 152 had at least one positive nasopharyngeal (NP) swab and 4 (3%) were confirmed based on positive aerosol samples alone. NP swab analysis was positive for 8 (33%) of 24 randomly selected volunteers from among the 177 screened who did not meet enrollment criteria; thus, sensitivity and specificity of our enrollment criteria, during the 2012–2013 season, were ~73% [95% confidence interval (CI) 62–84%] and 84% (95% CI 80–88%), respectively. In the reported analyses, we excluded 8 visits made on the day of symptom onset, 10 made >3 d after onset, 7 with missing data for cough, and 3 with incomplete qRT-PCR data (Fig. S1 and Table S1). The resulting dataset for confirmed cases with complete data on RNA copies, cough, and symptoms included 218 visits by 142 cases: 89 influenza A (83 H3, 3 pdmH1, 3 unsubtypable), 50 influenza B, and 3 dual influenza infection cases.

Our study population (**Table 1**) consisted mostly of young adults (19–21 y) with a high asthma prevalence (21%), normal body mass index (BMI, median = 22.7; 7% underweight, 20% overweight, and 8% obese) (Table S2), and a low self-reported influenza vaccination rate (22%). We observed at least one cough during 195 (89%) and one or more sneezes during 11 (5%) of the 218 visits. Cough frequency varied considerably, from 5 per 30 min at the 25th percentile to 39 per 30 min at the 75th. Most volunteers rated their upper respiratory symptoms as mild to moderate, systemic symptoms as moderate to severe, and lower respiratory symptoms as mild (**Fig. 1**).

Table 1.
Characteristics of study population

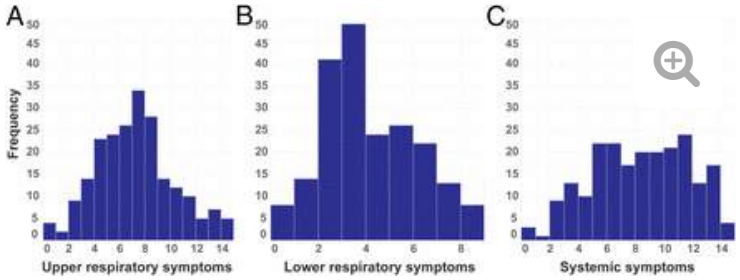

Fig. 1.
Histograms of symptom scores. (**A**) Upper respiratory symptoms (runny nose, stuffy nose, sneezing, sore throat, and earache, score range 0–15). (**B**) Lower respiratory symptoms (chest tightness, shortness of breath, and cough, score range 0–9). (**C**) Systemic symptoms (malaise, headache, muscle/joint ache, fever/sweats/chills, and swollen lymph nodes, score range 0–15).

Infectious virus was recovered from 52 (39%) fine-aerosol samples and 150 (89%) NP swabs (**Table 2**). Quantitative cultures were positive for 30% of the fine-aerosol samples, with a geometric mean (GM) for positive samples of 37 fluorescent focus units (FFU) per 30-min sample (**Fig. 2A**) and for 62% of NP swabs with GM for positive samples of 2,500. Using Tobit analysis to adjust the estimate of the GM for the presence of samples below the limit of detection, we obtained a GM 1.6 (95% CI 0.7–3.5) for fine aerosols and a GM 60.6 (95% CI 22.7–1.6 × 10^2) for NP swabs.

Table 2.
Viral shedding

VIEW INLINE **VIEW POPUP**

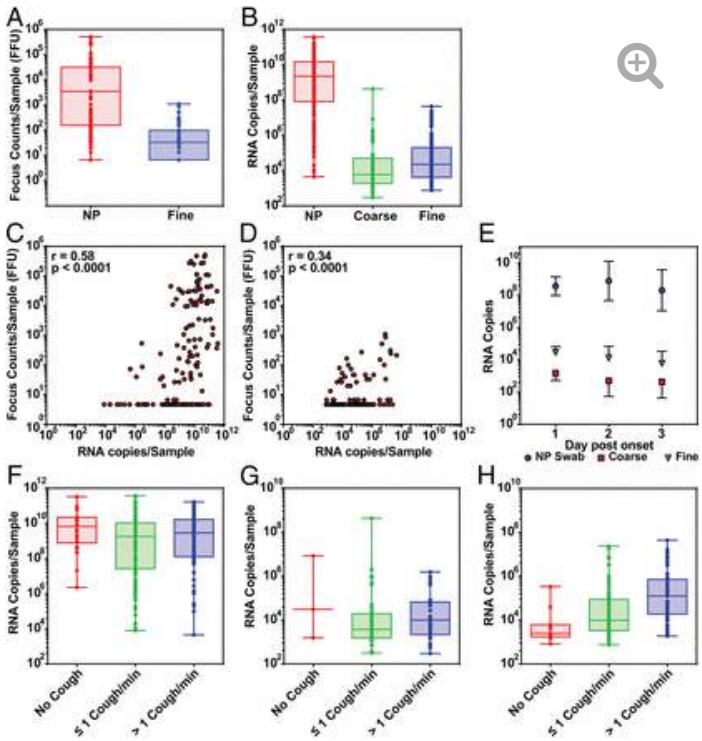

Fig. 2.
Viral shedding: (**A**) infectious influenza virus (fluorescent focus counts) in NP swabs and fine aerosols and (**B**) RNA copies in NP swabs, coarse, and fine aerosols. (**C** and **D**) Scatter plots and Spearman correlation coefficients of infectious virus plotted against RNA copies for (**C**) NP swabs and for (**D**) fine-aerosol samples. (**E**) The effect of day after symptom onset on RNA copies observed in NP swabs, coarse, and fine aerosols plotted as GM adjusted for missing data using Tobit analysis with error bars denoting 95% CIs. (**F–H**) The effect of cough frequency on RNA copies observed in (**F**) NP swabs, (**G**) coarse aerosols, and (**H**) in fine aerosols. Coarse: aerosol droplets >5 μm; Fine: aerosol droplets ≤5 μm in aerodynamic diameter.

Influenza virus RNA was detected in 76% of the fine-aerosol samples, 40% of the coarse-aerosol samples, and 97% of the NP swabs of enrolled volunteers. For the positive samples, the GM viral RNA content of fine-aerosol samples was 3.8×10^4, for coarse aerosols was 1.2×10^4, and for NP swabs was 8.2×10^8 (**Fig. 2B**). The adjusted GMs were 1.2×10^4 (95% CI 7.0×10^3 to 1.9×10^4) for fine aerosols and 6.0×10^2 (95% CI 3.0×10^2 to 1.2×10^3) for coarse aerosols. Quantitative culture was correlated with RNA copies in both NP swabs (**Fig. 2C**) (**r** = 0.58) and fine aerosols (**Fig. 2D**) (**r** = 0.34). The time course of shedding is shown in **Fig. 2E**.

Viral RNA in NP swabs was not correlated with cough frequency (number of coughs per 30 min) (**Fig. 2F** and Fig. S2**A**) (**r** = 0.02). Viral RNA in coarse aerosols was weakly correlated with cough frequency (**Fig. 2G** and Fig. S2**B**) (**r** = 0.24). However, viral RNA copy number in fine aerosols was moderately well correlated with cough frequency (**Fig. 2H** and Fig. S2**C**) (**r** = 0.45). Only 3 (13%) of 23 coarse-aerosol samples where no coughs were observed had detectable viral RNA, while 11 (48%) of the corresponding 23 fine-aerosol samples had detectable viral RNA and 8 were positive by culture. RNA copies in the fine-aerosol, no-cough samples ranged up to 3.7×10^5 (adjusted GM 1.5×10^3, 95% CI 4.2×10^2 to 5.3×10^3) and infectious virus to 1.4×10^2 FFU per 30-min sample. The few sneezes observed were not associated with greater RNA copy numbers in either coarse or fine aerosols (Fig. S3).

Results of regression analyses to identify predictors of viral RNA shedding are shown in **Table 3**, controlled for random effects of subject and repeated observations on individuals.

Table 3.
Predictors of viral RNA shedding

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The day after symptom onset (comparing day 1 postonset with days 2 and 3) was associated with a significant decline in viral RNA shed into fine aerosols (**P** < 0.05 for day 2 and **P** < 0.01 for day 3 in adjusted models), a borderline significant decline in coarse-aerosol shedding (**P** < 0.10), and was not associated with a significant change in shedding detected in NP swabs (**P** > 0.10).

In regression analyses, cough frequency was significantly associated with increased fine- (**P** < 0.001 to <0.0001) and coarse- (**P** < 0.01) aerosol shedding, but was not associated with NP shedding. Fine-aerosol shedding was significantly greater for males. Analysis of an interaction of cough with sex indicated that males produced, on average, 3.2 times more virus than did females per cough. However, females also coughed significantly (**P** = 0.005) more frequently than males: 33 (SD 39) per 30-min observation and 21 (SD 21), respectively (Fig. S4).

BMI was positively associated with shedding in fine and coarse aerosols in unadjusted models (**P** < 0.10). BMI was retained in the best-fitting adjusted models for both fine and coarse aerosols, where it was significantly associated with fine-aerosol shedding (**P** < 0.05). However, BMI was not associated with shedding detected in NP swabs (**P** > 0.10). Standard categories of BMI were not as good a fit as the continuous BMI and were not significantly associated with shedding (Table S3), although a positive trend is evident for overweight and obese individuals in the adjusted model.

Self-reported vaccination for the current season was associated with a trend (**P** < 0.10) toward higher viral shedding in fine-aerosol samples; vaccination with both the current and previous year's seasonal vaccines, however, was significantly associated with greater fine-aerosol shedding in unadjusted and adjusted models (**P** < 0.01). In adjusted models, we observed 6.3 (95% CI 1.9–21.5) times more aerosol shedding among cases with

vaccination in the current and previous season compared with having no vaccination in those two seasons. Vaccination was not associated with coarse-aerosol or NP shedding (**P** > 0.10). The association of vaccination and shedding was significant for influenza A (**P** = 0.03) but not for influenza B (**P** = 0.83) infections (Table S4).

Viral load in NP swabs was not a significant predictor of aerosol shedding (**P** = 0.16 for fine and **P** = 0.48 for coarse aerosols). Temperature measured at the time of sampling, asthma history, smoking, and influenza type were not significantly associated with the extent of measured shedding. While self-reported symptoms were not associated with aerosol shedding, they were significantly associated with shedding measured by the NP swab; only upper respiratory symptoms remained significant when adjusted for other symptoms and age. Increasing age was associated with a significant decrease in shedding in the NP swab; however, age was not associated with aerosol shedding.

Discussion

We recovered infectious influenza virus from 52 samples of fine aerosols collected from exhaled breath and spontaneous coughs produced by 142 cases of symptomatic influenza infection during 218 clinic visits. Finding infectious virus in 39% of fine-aerosol samples collected during 30 min of normal tidal breathing in a large community-based study of confirmed influenza infection clearly establishes that a significant fraction of influenza cases routinely shed infectious virus, not merely detectable RNA, into aerosol particles small enough to remain suspended in air and present a risk for airborne transmission. Because these data were collected without volunteers having to breathe through a mouthpiece or perform forced coughs, they allow us to provide estimates of average shedding rates, variability, and time course of and risk factors for shedding that can be used to provide well-grounded parameter estimates in future models of the risk of airborne influenza transmission from people with symptomatic illness.

The first published estimates of the numbers of influenza virus variants transmitted from donor to recipient host indicated that the bottleneck for transmission between humans is fairly wide and highly variable (mean 192 with 95% confidence 66–392) (**14**, **15**). Our observation that cases shed considerable quantities of virus into aerosols, GM $>10^4$ RNA copies per 30 min, and up to 10^3 infectious virus particles per 30 min, suggests that large numbers of variants could be transmitted via aerosols, especially via the short-range mode (**16**). However, longer-range aerosol transmission, as might be observed in less-crowded environments than in the initial report from Hong Kong, would be expected to usually result in lower exposures and transmission of fewer variants, consistent with the narrower bottleneck described in ferret models (**17**, **18**).

Sobel Leonard et al. (**14**) suggested that the width of the bottleneck increased with severity of illness, as indicated by a borderline significant positive association between temperature and number of variants transmitted. We did not see a significant association between measured temperature and shedding by any route. In contrast, symptoms were not a significant predictor of bottleneck size, and in our data, symptoms were not significant predictors for shedding into aerosols. Symptoms were, however, significant predictors for nasal shedding as measured in NP swabs. Thus, if aerosols were the more important route of transmission, our observations would be consistent with the currently available bottleneck analysis.

We observed that influenza cases rarely sneezed, despite having just undergone two NP swab collections (a procedure that generally makes one feel an urge to sneeze). Sneezing was not observed in the absence of cough and was not associated with greater aerosol shedding than we observed with cough alone (Fig. S3). Thus, sneezing does not appear to make an important contribution to influenza virus shedding in aerosols.

Sneezing might make a contribution to surface contamination. Because sneezes generate considerable amounts of large-droplet spray composed of many ballistic droplets not collected by our sampler, we cannot assess that possibility with our data.

Cough was prevalent and was a strong predictor of virus shedding into both coarse and fine aerosols. However, cough was not necessary for infectious aerosol generation in the ≤5-μm (fine) aerosol fraction; we detected culturable virus in fine aerosols during 48% of sampling sessions when no coughs were observed. This suggests that exhaled droplets, generated by mechanisms other than cough, are responsible for a portion of the viral load observed in the fine-aerosol fraction. Several researchers have recently shown that exhaled aerosol particles are frequently generated from normal healthy lungs by small airway closure and reopening (**19**–**21**). It has been hypothesized that during respiratory infections, airway closure and reopening frequency would be increased due to inflammation with a commensurate increase in aerosol generation and contagiousness (**22**).

Cough is thought to produce aerosols from large airways by shear forces that produce relatively coarse-aerosol droplets (**23**). Our finding that only 13% of cases not observed to have coughed during sample collection produced detectable viral RNA in their coarse aerosols is consistent with that hypothesis. The remaining aerosols may have resulted from speaking; each subject was required to recite the alphabet three times. One might expect that viral replication in the large airways combined with cough-generated coarse-aerosol droplets would produce the majority of viral aerosols. However, we observed a weak correlation of coarse-aerosol RNA copy number with cough frequency and a much stronger association of fine-aerosol copy number with cough frequency, even though cough would be expected to be the primary source of coarse aerosols. These observations suggest that cough is, at least in part, an epiphenomenon, more of a response to irritation associated with high viral loads in distal airways than a direct source of infectious aerosols.

A striking finding was the association of gender with shedding into fine aerosols. This relationship appears to have resulted from a threefold greater impact of coughing on shedding in males. We observed these gender and gender-by-cough interaction effects only for the fine-aerosol fraction. Absence of a gender effect in the coarse-aerosol fraction suggests that this is not an effect of cough on aerosol generation by shear forces in the upper airway. We did not measure lung volumes and therefore cannot control for a lung size effect. An equally plausible explanation may be that women tend to have more sensitive cough reflexes (**24**). Thus, women may have tended to cough in response to lower viral loads and coughed more frequently at a given viral load, which could have produced the observed steeper slope of viral load regressed on cough frequency in males compared with females. Consistent with this suggestion, we did observe a significantly greater cough frequency in females (**P** = 0.005) and a steeper slope of fine-aerosol viral RNA with cough in males (Fig. S4).

BMI was a borderline significant predictor of aerosol shedding in most models, was retained as an important predictor of both coarse and fine aerosols in adjusted models, and reached statistical significance for fine aerosols when adjusted for other factors; it was not a significant predictor of nasal shedding. This observation might be consistent with reports of increased inflammation in models of obesity and influenza and severity of influenza-like illness in obese persons (**25**–**30**). Alternatively, increasing BMI is associated with increased frequency of small airways closure, and the resulting increased aerosol generation during airway reopening as described above may explain the stronger association of BMI with fine than coarse aerosols and lack of association with NP swabs (**31**).

Our analysis found a clear separation of factors associated with shedding from the nose and those with shedding into aerosols, especially fine-particle aerosols. Upper airway symptoms, as would be expected, were strongly associated with shedding detected in NP swabs, and greatly reduced the size and significance of lower

respiratory and systemic symptoms in the fully adjusted model. Age was negatively associated with nasal shedding but not a predictor of aerosol shedding. More surprisingly, no symptoms, including lower respiratory and systemic systems, were strongly associated with shedding into aerosols, in this population with relatively mild lower respiratory symptoms (**Fig. 1**). Furthermore, nasal shedding was not a significant predictor of aerosol shedding and none of the strong predictors of aerosol shedding were associated with nasal shedding. Thus, we can conclude that the head airways made a negligible contribution to viral aerosol generation and that viral aerosols represent infection in the lung. Moreover, upper and lower airway infection appear to behave as though infection is compartmentalized and independent. In this context, it is notable that Varble et al. (**18**) observed that intrahost viral variants differ in the nasopharynx and lung of ferrets.

We did not observe a significant decline over time of viral load detected in NP swabs. If day 1 after onset of symptoms (used as baseline for these analyses) in our cases was equivalent to a mixture of day 1 and day 2 after experimental influenza virus inoculation in the report by Hayden et al. (**32**), then our lack of finding a clear drop in nasal shedding over the next 2 d is reasonably consistent with the pattern reported for experimental infection. There is no available data for comparison of aerosol shedding from published experimental infections. That we saw a much clearer pattern of rapid decline over time in aerosol shedding again suggests a separation of infection into upper and lower airway compartments in humans.

The association of current and prior year vaccination with increased shedding of influenza A might lead one to speculate that certain types of prior immunity promote lung inflammation, airway closure, and aerosol generation. This first observation of the phenomenon needs confirmation. If confirmed, this observation, together with recent literature suggesting reduced protection with annual vaccination, would have implications for influenza vaccination recommendations and policies.

Materials and Methods

Study Population and Sample Collection Procedures.
We recruited volunteers with acute respiratory illness on the University of Maryland–College Park campus and surrounding community from December 2012 through March 2013. The University of Maryland Institutional Review Board approved the study, and we obtained a signed consent (or assent and parental verbal assent) from volunteers who reported fever with a cough or sore throat (Fig. S5).

During the initial visit, we administered a brief screening questionnaire, measured oral temperature, height, weight, and collected two NP swabs (Copan) for each volunteer screened. One swab was used to perform QuickVue A/B rapid tests for influenza (except when results of a rapid test performed by medical provider were available). The second NP swab was used for viral culture and PCR for those meeting enrollment criteria and for PCR in a random sample of 24 of those not enrolled.

Participants were asked about sex, age, antipyretic use, vaccination status, use of steroid medications, medical and smoking history, to rate current symptoms on a four-level scale (none = 0, mild = 1, moderate = 2, severe = 3), and to rate the worst symptoms during the illness thus far. We defined symptoms as upper respiratory (runny nose, stuffy nose, sneezing, sore throat, and earache), lower respiratory (chest tightness, shortness of breath, and cough), and systemic (malaise, headache, muscle/joint ache, fever/sweats/chills, and swollen lymph nodes).

Volunteers were enrolled in exhaled breath collection if they met the following criteria: (**i**) positive QuickVue rapid test, or oral temperature >37.8 °C plus cough or sore throat, and (**ii**) presented within the first 3 d of symptom onset. Exhaled breath samples were collected using the Gesundheit-II (G-II) human source bioaerosol sampler,

as previously described (**12**, **33**). We collected exhaled breath for 30 min while the participant was seated with their face inside of the large open end of a cone-shaped inlet for the G-II. The inlet cone draws in 130 L of air per minute and allowed participants to breathe, talk, cough, and sneeze naturally throughout sample collection while maintaining >90% collection efficiency for exhaled and coughed droplets ≤100 μm. Subjects were asked to breathe normally and to recite the alphabet once at 5, 15, and 25 min. We collected "coarse" (>5 μm) aerosol droplets by impaction on a Teflon surface and "fine" droplets (≤5 μm and >0.05 μm) by condensation growth and impaction on a steel surface constantly rinsed into a buffer containing (PBS with 0.1% BSA) liquid reservoir. Audible spontaneous coughs and sneezes during breath collection were counted by direct observation in real-time (**n** = 59) or by playback of digital recordings (**n** = 159).

Participants enrolled before the third day after symptom onset were asked to come in for up to two consecutive daily follow-up visits (Fig. S5) with repeat questionnaire, NP swab, and exhaled breath collections. Final analyses included only visits for enrolled cases occurring on days 1–3 after symptom onset with complete data on cough and sneeze, symptoms, PCR results for swab and aerosol samples.

Laboratory Methods.

Detailed methods are described in the **SI Materials and Methods**. Briefly, NP swabs were eluted in 1 mL of PBS with 0.1% BSA (PBS/0.1% BSA) or universal transport medium (Copan), and Teflon impactors were scrubbed with a nylon swab saturated with PBS/0.1% BSA. The swab was eluted in 1 mL PBS/0.1% BSA. Fine-aerosol samples were concentrated to 1 mL using centrifugal ultrafiltration.

RNA was extracted from NP swab, fine- and course-aerosol samples, and whole-virion standards using an automated Qiagen system and viral RNA was quantified by one-step real-time RT-PCR using Taqman primer probe sets designed by the US Centers for Disease Control and Prevention and made available through our cooperative agreement. Standard curves were calibrated for virus copy number using plasmids containing a cDNA copy of the qRT-PCR target amplicon. Experimentally determined limits of detection and quantification for each of the qRT-PCR reactions are shown in Table S5.

Virus culture on Madin–Darby canine kidney (MDCK) cells was used to detect infectious virus in NP swab and fine-aerosol samples. Coarse-aerosol samples were not cultured for infectious virus because impaction on a dry Teflon surface was expected to reduce infectivity of those samples. Infectious influenza virus was quantified using an immunofluorescence assay for influenza nucleoprotein, and positive cells were counted as FFU by fluorescence microscopy. Details of laboratory methods can be found in **SI Materials and Methods**.

Statistical Analysis.

We entered and cleaned data using locally hosted REDCap data-capture tools (**34**) and performed data management and analyses in R (v3.2.3 R Development Core Team, Vienna, Austria) and SAS (v9.4, Cary, NC), and produced graphics with Prism Software (PRISM software v7.0; GraphPad). We used the delta method to estimate confidence limits for sensitivity and specificity. We used Spearman correlation, generalized linear models (SAS Proc GENMOD), and Tobit regression (**35**) with nested random effects of sample within subject in (SAS Proc NLMIXED) to analyze infectious virus counts, RNA copy numbers, and compute GM virus concentrations. Tobit regression accounted for uncertainty and censoring of the observations by the limit of quantification. We included all independent variables with unadjusted $\mathbf{P} < 0.10$ in initial adjusted models and selected final models using the Akaike information criterion while retaining adjustment for age and sex. Regression model results are presented as the ratio of shedding at the 75th percentile to shedding at the 25th percentile of the distribution of the independent variable, so that clinical and epidemiological meaning of the relationship can be more easily interpreted.

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View Abstract

Measles (MMR) Vaccine

Post-vaccine measles in a child with concomitant influenza, Sicily, Italy, March 2015

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We describe the occurrence of measles in an 18 month-old patient in Sicily, Italy, in March 2015, who received the first dose of a measles-containing vaccine seven days before onset of prodromal symptoms. Measles virus infection was confirmed by PCR and detection of specific immunoglobulin; viral genotyping permitted the confirmation of a vaccine-associated illness. The patient had a concurrent influenza virus infection, during a seasonal epidemic outbreak of influenza.

Case description

In early March 2015, measles-mumps-rubella-varicella zoster (MMRV) vaccine was administered to an apparently healthy 18-month-old child living in Sicily, Italy. Seven days later, the child presented to the family paediatrician with fever (40.1 °C), catarrhal cough, runny nose and eyelid oedema. Macular rash appeared over the body two days later, starting on the trunk and then spreading to the neck and face. By day 13, the rash was fading, but due to the persistence of symptoms, the child was admitted to a children's hospital and reported as a possible case of vaccine-related measles to the Epidemiology Department of the Regional Public Health.

The local health authority carried out an epidemiological investigation: a standard measles notification form was sent to the regional health authorities and immediately forwarded to the Ministry of Health and to the Infectious Diseases Epidemiology Unit of the National Institute of Health. No direct link was identified with other measles cases in the community and the family had no history of travel outside Sicily. Moreover, contact investigation revealed no household members or pre-school contacts with symptoms consistent with measles. One of the child's parents developed influenza-like illness (ILI) symptoms (fever (›38 °C) and cough, which lasted for three consecutive days) one day after administration of MMRV vaccine to the patient.

Urine and throat swab specimens were collected from the child and submitted to the Regional Reference Laboratory in Palermo for nucleic acid-based testing for measles, mumps, rubella and varicella zoster viruses and genotyping of any detected viruses. Given that this patient with suspected vaccine-associated measles developed symptoms during a seasonal epidemic outbreak of influenza viruses, and taking into account reports of morbilliform rash associated in patients with influenza B who tested negative for measles virus infection [1,2], testing was also requested for influenza and other respiratory viruses.

While no viruses could be detected in the urine specimen, measles, influenza A(H3N2) and respiratory syncytial viruses were detected in the throat swab.

On day 17, the patient's symptoms resolved without complications and the patient was discharged from hospital (Figure).

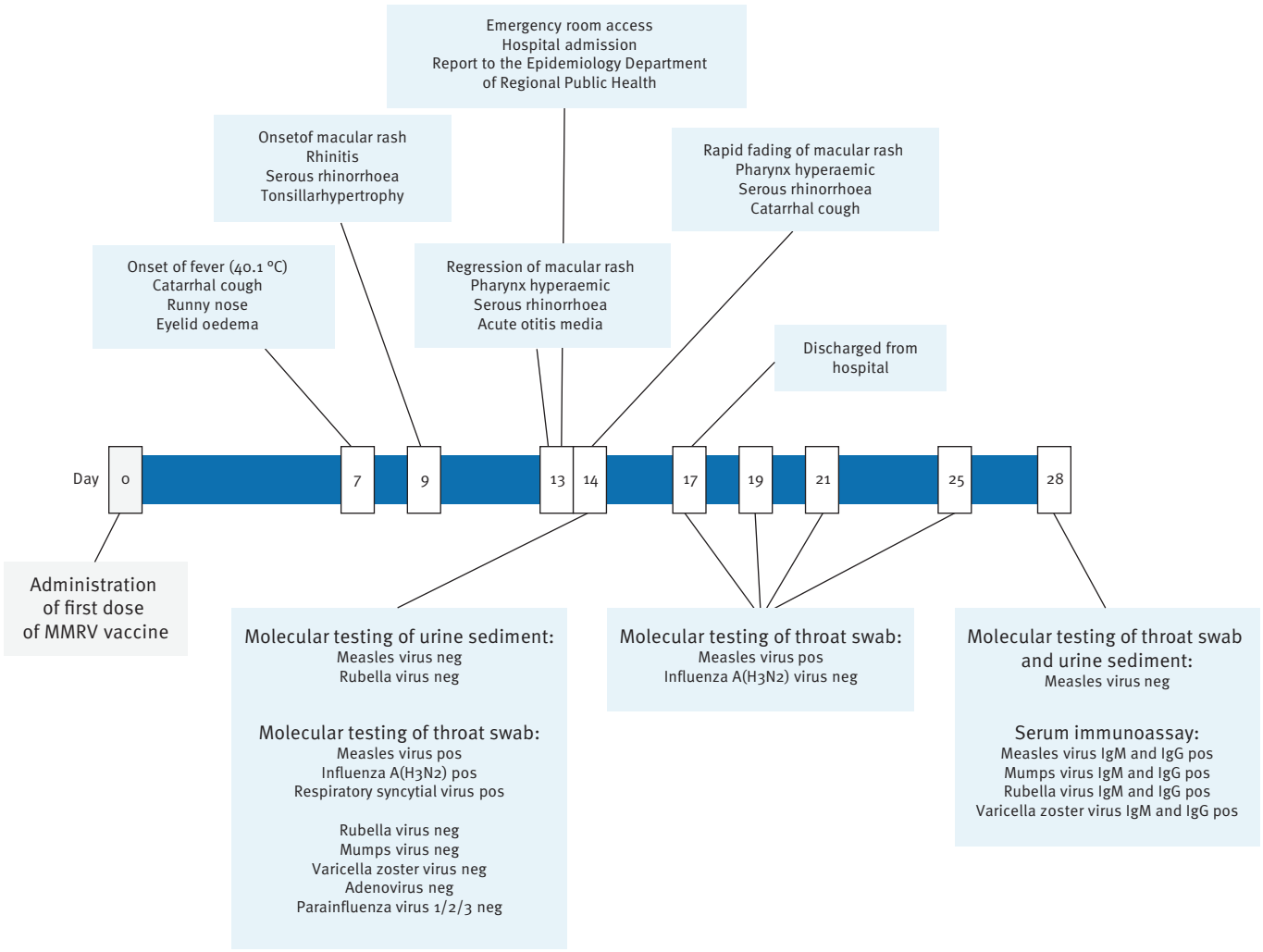
Measles virus was detected in throat swabs taken on days 17, 19, 21 and 25, but no influenza or other respiratory viruses were detectable in these specimens.

Measles virus was not detected on day 28 from a throat swab and urine specimen. A blood sample was taken at this time for serological testing for measles, mumps, rubella and varicella zoster viruses. A time line of events is shown in the Figure.

Seroconversion following MMRV immunisation was evaluated through the detection of specific measles, rubella, mumps and varicella zoster IgM and IgG antibodies by chemiluminescent immunoassay (CLIA) (measles virus: IgM = 3.1 arbitrary units (AU)/mL, IgG ›300

Figure

Time line of symptoms and physical signs in a child with post-vaccine measles and concomitant influenza, case management, specimen collection and laboratory results, Sicily, Italy, March 2015



MMRV: measles-mumps-rubella-varicella zoster; neg: negative; pos: positive.

AU/mL; mumps virus: IgM = 1.3 AU/mL, IgG = 78.9 AU/mL; rubella virus: IgM = 1.97 AU/mL, IgG = 18.0 international units (IU)/mL; varicella zoster: IgM = 0.71 AU/mL, IgG = 271.8 mIU/mL).

The measles virus was determined to be the Schwarz vaccine strain, genotype A, MVs/Palermo.ITA/12.15 [A] (VAC) [3] by sequence analysis of the genome.

Laboratory investigations

Serological and nucleic acid-based tests were performed for surveillance of measles and rubella, and genotype determination at the Regional Reference Laboratory of Palermo, formerly a member of the national network for influenza surveillance and genotyping (INFLUNET).

For the detection of specific measles, rubella, mumps and varicella zoster IgM and IgG antibodies, commercial CLIA tests were used (LIAISON (DiaSorin) and VITROS (Ortho Clinical Diagnostics)), which have the following cut-off values: measles IgM ≥ 1.0; measles IgG ≥ 13.5; mumps IgM ≥ 1.0; mumps IgG ≥ 10.0; rubella IgM ≥ 1.2; rubella IgG ≥ 15.0; varicella zoster IgM ≥ 1.0; varicella zoster IgG ≥ 100.0.

Throat swabs and the sediment of urine samples were tested using a real-time PCR instrument (QuantStudio 7 Flex Real-Time PCR system, Applied Biosystems), using specific primer/TaqMan probe sets for measles [4], mumps [5], rubella [4] and varicella zoster [6,7] viruses after extraction of total RNA using QIAmp Viral RNA Mini Kit (Qiagen).

Measles genotyping was conducted to distinguish wild-type from vaccine-associated measles viral strains. PCR products, targeting either the N gene or the H gene [8], were obtained from throat swab and sequenced using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems).

SuperScript One-Step RT-PCR kit with Platinum Taq (Invitrogen) were used for both endpoint reverse transcription RT-PCR and real-time RT-PCR reactions.

Sequences were confirmed as measles virus following comparison with the BLAST algorithm and they were phylogenetically analysed to assign genotype and cluster. The sequences were identified as Schwarz vaccine strain (genotype A) and were submitted to GenBank (accession numbers KR262162 (gene N) and KR262161 (gene H)).

Background

In Italy, vaccination against measles is included in the national vaccination schedule. Two doses of measles-mumps-rubella (MMR) vaccine have been recommended in all regions since the early 1990s [9], sometimes in association with varicella vaccination. The first dose is given at 13–15 months-old and the second at the age of 5–6 years [10].

In accordance with the national measles elimination plan [11], an enhanced surveillance system was introduced in 2007 [12] with the aim of improving timeliness, completeness of case reporting and case investigation, including laboratory confirmation of diagnosis and viral genotyping.

As the incidence of wild-type measles decreases in countries with high levels of vaccination coverage, vaccine-associated cases could be misreported [13,14], suggesting that there is a need to improve the ability to distinguish between vaccine-associated measles and 'true' wild-type measles virus infection [15].

Post-marketing surveillance of vaccines is mandatory in Italy and adverse reactions observed after the administration of vaccines are reported through the national pharmacovigilance network. According to the latest data available [16], these are mainly represented by fever, skin rash and febrile seizures, while post-vaccination viral shedding is a very uncommon event, which has been rarely documented so far [17,18].

Discussion

With an estimated more than 500 million doses administered in over 60 countries since the 1970s, the benefit of measles vaccination in preventing illness, disability and death appear unchallengeable [19,20].

Moreover, vaccine safety is annually validated by accurate post-marketing surveillance of adverse reactions conducted by the Italian Medicines Agency (AIFA). As for other live attenuated vaccines, adverse reactions following MMR or MMRV immunisation rarely present with clinically significant illness [16]: such illness is indistinguishable from wild-type measles. In this context, the reference laboratory for molecular surveillance plays a fundamental role in measles virus characterisation, through viral sequencing and genotyping, in order to promptly differentiate between wild-type and vaccine-related strains [14,18].

In this report, we documented the pharyngeal excretion of the Schwarz measles vaccine virus in an apparently healthy child with a febrile rash after measles vaccination and with laboratory-confirmed influenza A(H3N2) coinfection.

On the basis of our data, some points can be noted.

Firstly, although unlikely, measles after MMRV vaccination is possible, and this can mimic wild-type infection, leading to potential measles case misclassification. The application of molecular techniques for viral genotyping is helpful to correctly classify a case and to drive the decisions of public health authorities at the local level.

Secondly, this is the first report of a measles case with concurrent influenza and respiratory syncytial virus detection: we cannot exclude the possibility that the co-presence of other viral natural infections in a very young child, showing a slight hypogammaglobulinaemia in serum protein electrophoresis, may have favoured, or even determined, the occurrence of vaccine-related measles virus in pharyngeal secretions. Unfortunately, the parent showing ILI symptoms was not tested for influenza virus, making us unable to assess, although very likely, an intrafamilial transmission of influenza virus infection.

Notably, virus excretion was demonstrated over a 25-day period after vaccination, which is longer than previously reported [17,21,22]. Interference with other coinfecting viruses or a defective host immune response could play a role in this unexpected persistence of measles virus, although this hypothesis will require further investigation.
Thirdly, virus excretion was repeatedly detected in the throat, but not in urine sediment. This finding partially contrasts with World Health Organization (WHO) guidance for laboratory diagnosis for measles virus infection, which suggests to test preferentially for the virus in the sediment of urine samples that have been collected within at least five days after the onset of rash [23]. In the case presented here, in accordance with WHO guidance, matched urine and throat specimens were collected on the fifth day after the onset of macular rash.

Detection of measles virus in respiratory samples up to 16 days after the onset of rash suggests that other host cell pathways or viral mechanisms, potentially related to other concomitant viral infections, might be responsible for such an event. However, also in this case, further studies are necessary to better explain such an anomaly.

In conclusion, development of measles in individuals who have received MMR or MMRV vaccine is a possible,

although extremely rare, event. Therefore, especially in geographical areas with a low incidence of measles, maintenance of efficient molecular surveillance systems and the improvement of the timeliness of both case reporting and virus genotyping is of paramount importance, to ensure correct differentiation between vaccine-related illness and natural measles infection [24].

Conflict of interest

None declared.

Authors' contributions

Conceived and designed the study: FT, FV. Collected clinical and epidemiological data: PD, CD, NC. Analysed data: FT. Wrote the paper: FT, FV.

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Case of vaccine-associated measles five weeks post-immunisation, British Columbia, Canada, October 2013

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We describe a case of vaccine-associated measles in a two-year-old patient from British Columbia, Canada, in October 2013, who received her first dose of measles-containing vaccine 37 days prior to onset of prodromal symptoms. Identification of this delayed vaccine-associated case occurred in the context of an outbreak investigation of a measles cluster.

In this report we describe a case of measles-mumps-rubella (MMR) vaccine-associated measles illness that was positive by both PCR and IgM, five weeks after administration of the MMR vaccine. Based on our literature review, we believe this is the first such case report which has implications for both public health follow-up of measles cases and vaccine safety surveillance.

Between 29 August and 2 September 2013, three unlinked persons from across the Fraser Valley, British Columbia, Canada, presented with rash illness consistent with clinical measles [1]. Based on the outbreak investigation by the local health authority, none of the three cases had an identified exposure to a measles case or travel history outside of Canada during the incubation period, and a source case was never identified. All three cases had the same measles genotype B3 sequence type (MVs/British Columbia.CAN/34.13, MeaNS id 39928, GenBank accession numbers KF704002 and KF704001). Measles genotype B3 is endemic in the World Health Organization's African and Eastern Mediterranean regions [2]. Two additional cases of measles due to secondary transmission from one of the above cases were identified in British Columbia in the third week of September.

Case report

In early October 2013, a two-year-old child living in the Fraser Valley presented to the family physician with fever, rash, conjunctivitis and coryza. Symptoms had begun two days before, with a runny nose, followed by fever on the day hereafter. A macular rash appeared on the day of visiting the physician, starting on the face and progressing to the rest of the body; fever measured by the parents was at 39 °C.

Clinical examination of the child by the family physician found a fever of 39.5 °C, marked bilateral conjunctivitis, and macular rash over the body. Three days later, fever had dissipated, rash was fading and symptoms resolved without complications.

Public health alerts had been issued to community physicians regarding the recent cluster of measles in September, which may have raised suspicion for measles in this case. Additionally, the child's family was aware of measles cases in the community from a relative who attended the same church as one of the original cases, but no direct link was identified and they had no travel history outside of Canada. Contact investigation revealed no ill household members or preschool contacts. The child's past medical history indicated anaphylaxis to peanuts and eggs. Primary series of immunisations were not up-to-date, as she had just received her first dose of MMR vaccine 37 days prior to the onset of illness. At the same visit, the child had received meningococcal C and pneumococcal conjugate vaccines.

Laboratory investigations

Laboratory testing for measles was performed on specimens collected on the day of rash onset. Measles RNA was detected in the nasopharyngeal swab by the RT-PCR assay [3]. Acute and convalescent measles specific IgM and IgG antibodies were detected in the blood by ELISA (Enzygnost Anti-Measles Virus IgM and IgG (Dade Behring, Marburg, Germany): IgM detectable (0.213), IgG 1294 mIU/mL, and IgM detectable (0.246), IgG 2,413 mIU/mL, respectively. Virus genotype was determined by the National Microbiology Laboratory in Winnipeg, Canada as vaccine strain, genotype A, MVs/British Columbia/39.13 [A] (VAC) [4]. Other virology testing found no detectable Parvovirus B19 specific IgG or IgM antibody, and detectable human herpesvirus

(HHV)-6 specific IgG antibody but no detectable HHV-6 DNA.

Public health measures

While genotyping results were pending, case management proceeded as for a wild-type measles infection. Public health follow-up lead to the identification of 87 contacts. As per guidelines, post-exposure prophylaxis was provided within six days of exposure to 45 susceptible contacts (41 contacts with a history of one dose of MMR vaccine received an additional MMR dose, and four contacts with no history of MMR vaccine or with contraindications to MMR vaccination, received immunoglobulin) [1]. All contacts received education on signs and symptoms of measles, and those who received immunoglobulin were recommended to subsequently receive MMR vaccine, if this was not contraindicated.

Discussion

The incubation period of measles is typically eight to 12 days from exposure to rash onset, with a range from seven to 21 days. Public health interventions are based on this established incubation period for determining the epidemiological links between cases and for estimating periods of exclusion for contacts in high risk settings [5,6]. Based on our review of the literature, this report documents the first case of MMR vaccine-associated measles, 37 days post-immunisation, well beyond 21 days and the routine 30 days post-MMR immunisation period used by the Canadian adverse event following immunization (AEFI) surveillance system.

Measles-containing vaccines are used globally, have been part of the British Columbia immunisation schedule since 1969, and have an impressive record of safety validated by careful, ongoing AEFI surveillance. Rash and/or mild clinical illness following MMR vaccine are not uncommon [7]. Clinically significant vaccine-associated illness is rare, but when it occurs it is indistinguishable from wild-type measles, except by genotyping [8]. Detection of vaccine virus has been documented up to 14 days post-immunisation by RT-PCR, and up to 16 days by immunofluorescence microscopy of urine sediment [9-12]. Complications from vaccine-associated measles have been documented in both immune-competent and compromised individuals [13,14]. Of note, only one case report of transmission from vaccine-associated measles has been identified [15,16].

Possible explanations for this prolonged shedding of measles vaccine virus include interference with the immune response by host or vaccine factors. Immunoglobulin administration early in the incubation period has been reported to extend the time to onset of symptoms, but in this child there was no such history and no known immunosuppressive illness [5]. The two-fold rise between acute and convalescent measles-specific IgG suggests the vaccine-mediated immune response had been underway prior to the onset of symptoms. Investigations clarified that there were no shipping, handling or cold-chain deviations for the specific vaccine used, and that it was administered by a public health nurse trained in immunisations. The potential immunological impact of the older age of the child at the time of receiving the first dose of MMR vaccine, 33 months versus the typical 12-15 months of age, and the co-administration of meningococcal C and pneumococcal conjugate vaccines are areas for future investigation.

It is possible that the case's symptoms were not measles-vaccine-related but an inter-current illness confounding the presentation. However, symptoms of marked conjunctivitis, continued fever with rash, and progression of macular rash from face to the whole body, are all more suggestive of measles versus other exanthems caused by viral diseases. Parvovirus and HHV-6 results were negative, and the absence of intake of medications excludes a drug reaction. Rubella serology was not done as it was expected to be positive given the recent MMR vaccine administration. Therefore, the combination of classic measles symptoms, detection of measles vaccine virus and reactive measles IgM, and lack of evidence of an alternative illness explanation, were highly suggestive of measles vaccine-associated illness.

Heightened surveillance and awareness of measles because of the ongoing outbreak likely contributed to the identification of this case. Although this is the first such reported case, it likely represents the existence of additional, but unidentified, exceptions to the typical timeframe for measles vaccine virus shedding and illness. Such cases have important public health implications for the investigation of measles clusters because while there is uncertainty about case classification (wild-type vs vaccine-type), case and contact management should proceed as if for wild-type to prevent secondary transmission. In this case, uncertainty from the presence of a measles outbreak, symptom onset on day 37 after MMR vaccine administration, and a two-week period between the RT-PCR findings and genotype determination, resulted in the initially reasonable presumption that this was a wild-type measles case and subsequent resource-intense follow-up of contacts. Awareness of the frequency of such exceptions to the typical measles timeframe and improving the timeliness of measles vaccine virus genotyping could help focus public health resources on cases of wild-type measles. Further investigation is needed on the upper limit of measles vaccine virus shedding based on increased sensitivity of the RT-PCR-based detection technologies and the immunological factors associated with vaccine-associated measles illness and virus shedding.

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Conflict of interest

None declared.

Authors' contributions

BH, FH, MM and PVB contributed to the clinical and public health management of the case. MK, MP and JH provided laboratory testing. MM drafted the manuscript; all authors critically revised and approved the final version of the manuscript.

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VIROLOGY

Rapid Identification of Measles Virus Vaccine Genotype by Real-Time PCR

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ABSTRACT During measles outbreaks, it is important to be able to rapidly distinguish between measles cases and vaccine reactions to avoid unnecessary outbreak response measures such as case isolation and contact investigations. We have developed a real-time reverse transcription-PCR (RT-PCR) method specific for genotype A measles virus (MeV) (MeVA RT-quantitative PCR [RT-qPCR]) that can identify measles vaccine strains rapidly, with high throughput, and without the need for sequencing to determine the genotype. We have evaluated the method independently in three measles reference laboratories using two platforms, the Roche LightCycler 480 system and the Applied Biosystems (ABI) 7500 real-time PCR system. In comparison to the standard real-time RT-PCR method, the MeVA RT-qPCR showed 99.5% specificity for genotype A and 94% sensitivity for both platforms. The new assay was able to detect RNA from five currently used vaccine strains, AIK-C, CAM-70, Edmonston-Zagreb, Moraten, and Shanghai-191. The MeVA RT-qPCR assay has been used successfully for measles surveillance in reference laboratories, and it could be readily deployed to national and subnational laboratories on a wide scale.

KEYWORDS measles, PCR, genotyping, measles vaccine, molecular methods

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Endemic transmission of measles virus (MeV) was interrupted in the Americas in 2002 (1), but since then, importations of measles from areas of endemicity have caused frequent and sometimes large outbreaks (2–6) and a recent transitory suspension of the elimination status (7). An important component of the public health response to a measles outbreak is vaccination of unimmunized contacts (8). Since approximately 5% of recipients of measles virus-containing vaccine experience rash and fever which may be indistinguishable from measles (9), it is very important to identify vaccine reactions to avoid unnecessary isolation of the patient, as well as the need for contact tracing and other labor-intensive public health interventions. Recent measles outbreaks in the Canadian provinces of Alberta and British Columbia have emphasized the need for rapid differentiation of vaccine reactions (18, 19) from reactions to infection with the wild-type virus. During the measles outbreak in California in 2015, a large number of suspected cases occurred in recent vaccinees (3). Of the 194 measles virus sequences obtained in the United States in 2015, 73 were identified as vaccine sequences (R. J. McNall, unpublished data). In contrast, only 11 of 542 cases genotyped in the National Reference Center for Measles, Mumps, and Rubella in Germany were associated with the vaccine virus.

Genotyping is used to confirm the origin of an outbreak and to exclude endemic circulation, but it is also the only way to distinguish vaccine strains from wild-type viruses. Genetic characterization of MeV is accomplished by sequencing of the 450

TABLE 1 The lower limit of detection of MeVA RT-qPCR compared to MeV RT-qPCR was determined by testing serial dilutions of synthetic MeV RNA with a known copy number

Assay	Copy no.	No. of samples with positive results/total no. of samples tested	% positive results
MeVA RT-qPCR	10^3	18/18	100
	10^2	18/18	100
	10^1	13/20	65
	10^0	1/18	6
	10^{-1}[a]	0/3	0
MeV RT-qPCR	10^3	18/18	100
	10^2	18/18	100
	10^1	18/18	100
	10^0	4/18	22
	10^{-1}[a]	0/3	0

[a]This concentration was tested only 3 times since it is undetectable by both assays and therefore was not informative in the determination of the lower limit of detection.

nucleotides (nt) coding for the COOH terminal 150 amino acids of the nucleoprotein (N-450) (10). The WHO currently recognizes 24 genotypes of measles virus, and all of the vaccine strains are in a single genotype, genotype A. Wild-type viruses of genotype A are no longer circulating (11).

It is difficult, especially during outbreaks, to perform rapid confirmation of vaccine reactions by sequencing, and there is interest in developing rapid molecular tests to detect vaccine strains (12). Here, we describe a real-time reverse transcription-PCR (RT-PCR) method that detects the vaccine genotype (MeVA RT-quantitative PCR [RT-qPCR]) and that can provide rapid discrimination between wild-type-virus infections and vaccine reactions. The method was developed initially on the Roche LightCycler 480 platform at the Canadian National Microbiology Laboratory (NML) and then independently evaluated at the Robert Koch-Institute (RKI) in Germany using the same platform and at the US Centers for Disease Control (CDC) using the Applied Biosystems 7500 platform.

RESULTS

Assay development and evaluation at the NML. The analytical sensitivity of the MeVA RT-qPCR on the Roche LightCycler 480 platform was established using the synthetic RNA standard, which was serially diluted from 10^3 to 10^{-1} copies per reaction and tested in triplicate in at least 6 separate assays in parallel with the MeV RT-qPCR. The lower limit of detection of the MeVA RT-qPCR was 10 to 100 copies per reaction, compared to a sensitivity of 1 to 10 copies per reaction for the MeV RT-qPCR (Table 1).

Eighty-eight surveillance specimens that were previously genotyped as genotype A, 96 specimens of nonvaccine measles virus genotypes (B3, C2, D3, D4, D6, D7, D8, D9, E, H1, and H2), and isolates for genotypes B2, C1, D2, D5, D6, D7, D10, G1, G2, and H2 (WHO Measles Strain Bank, US Centers for Disease Control, Atlanta, GA, USA) were tested with MeVA RT-qPCR and produced no false-positive results. The amplification curves of 33 wild-type measles virus samples, including all the genotypes listed above, did not rise significantly in comparison to the curves of samples containing vaccine strain RNA (Fig. 1). However, 3 of 88 genotype A specimens were not detected by the MeVA RT-qPCR (Table 2). These three specimens were near the lower limit of detection (crossing-point [Cp] value, >35) for the MeV RT-qPCR. The sensitivity of the MeVA RT-qPCR in relation to the MeV RT-qPCR was 97% (90% to 99%, 95% confidence interval [CI]), and the specificity was 100% (95% to 100%, 95% CI) (Table 3). Specificity was further evaluated by testing a panel of other viral agents from cell culture-derived material or clinical specimens (parvovirus B19, dengue virus serotypes 1 to 4, influenza virus H3N2, poliovirus Sabin 1 species C, enterovirus D68-2 [EV-D68-2] species D, Coxsackie virus, EV71, parechovirus, echovirus 18, herpes simplex virus 1 [HSV1], HSV2, Epstein-Barr virus [EBV], cytomegalovirus [CMV], human herpesvirus 6 [HHV-6], HHV7,

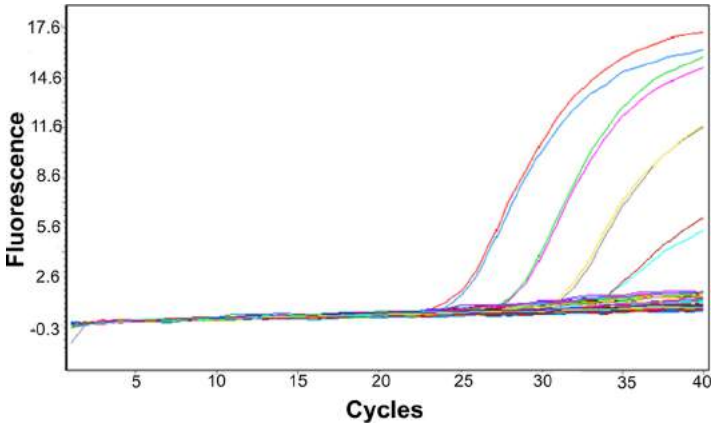

FIG 1 Amplification curve from the MeVA RT-qPCR on the Roche LightCycler 480 system. The bundle of the flat curves includes 33 wild-type measles virus specimens comprising the following genotypes: B2, B3, C1, C2, D2, D3, D4, D5, D6, D7, D8, D9, D10, G1, G2, E, H1, and H2. The amplification curves are from MeV vaccine RNA from 10^2 to 10^5 copy numbers, assessed in duplicate. The QuantiTect Probe RT-PCR kit was used for these reactions.

varicella zoster virus [VZV], rubella virus, and mumps virus). All specimens were negative by MeVA RT-qPCR.

Fifty specimens that were positive for vaccine strain A were tested in parallel by MeVA RT-qPCR and MeV RT-qPCR, and there was a good correlation of the Cp values between the two methods, with a slope of 0.88 (0.82 to 0.94, 95% CI). The slope was significantly different from 1.00, and a *y* intercept of 4.1 (2.2 to 6.0, 95% CI) confirmed that the sensitivity and limit of detection of the MeVA RT-qPCR method were lower than those of the MeV RT-qPCR (Fig. 2).

Assay evaluation at RKI. The MeVA qPCR was also independently evaluated at RKI by testing 46 archival measles virus specimens of genotype A and 112 samples containing wild-type MeV, including genotypes B3, D4, D5, D6, D8, D9, D10, G2, and H1. The same LightCycler 480 platform was used. The MeV RT-qPCR (16) includes the SuperScript III Platinum One-Step qRT-PCR kit (Invitrogen), so an evaluation was performed comparing the SuperScript III and QuantiTect reagent kits. The SuperScript III PCR kit produced suboptimal results, with significant increases of the amplification baseline of nonvaccine measles virus genotypes D10, D8, and B3 (Fig. 3A).

When the QuantiTect Probe RT-PCR kit was used for the MeVA RT-qPCRs, the test was 89% sensitive and 99.5% specific for genotype A measles virus (Table 3), with amplification curves comparable to those shown in Fig. 1. There was a single false-positive result from a genotype D5 wild-type strain, which produced amplification with

TABLE 2 Comparison of MeVA RT-PCR and MeV RT-qPCR in three reference laboratories

Reference laboratory and MeVA RT-qPCR result	No. of MeV RT-qPCR samples		Total no. of samples
	Genotype A	Not genotype A	
NML			
Positive	85	0	85
Negative	3	96	99
Total	88	96	184
CDC			
Positive	12	0	15
Negative	1	12	13
Total	13	12	28
RKI			
Positive	41	1	42
Negative	5	111	116
Total	46	112	158

TABLE 3 Summary of sensitivity and specificity of MeVA RT-qPCR for the detection of MeV genotype A

Center	No. of samples	% sensitivity (95% CI)	% specificity (95% CI)	Genotypes tested
NML	184	97 (90–99)	100 (95–100)	B3, B2, C1, D2, C2, D3, D4, D5 D6, D7, D8, D9, D10, G1, G2, E, H1, H2
RKI	158	89 (0.76–0.96)	99 (94–100)	B3, D4, D5, D6, D8, D9, D10, G2, H1
CDC	28	92 (66–100)	100 (70–100)	B3, D4, D8, D9, G3, H1, AIK, CAM-70, Edmonston-Zagreb, Moraten, Shanghai-191[a]
Overall	370	94 (88–97)	99 (97–100)	

[a]Genotypes D4 and G3 and the non-Edmonston vaccine strains, tested using synthetic RNAs and culture lysate, respectively, were not included in the sensitivity and specificity calculations.

the MeVA RT-qPCR. The region targeted by the MeVA assay was sequenced, and the sequence differed from the vaccine strain sequence by a G at position 517 in the probe region (conserved in all wild-type genotypes listed in Fig. 4), by a C at position 538 in the reverse primer region (similar to genotypes D4, D7, and D8), and by a T at position 548, at the 5′ terminus of the reverse primer (similar to genotypes B3 and D6). These genotypes did not produce any cross-reactivity with the MeVA-specific assay, and the reason for the false-positive result for this D5 specimen is unclear.

Assay evaluation at CDC on the ABI 7500 platform. The MeVA RT-qPCR method was independently evaluated at the CDC on the ABI 7500 instrument, which is a commonly used instrument in state public health laboratories and is available in many laboratories in the WHO Measles Rubella Laboratory Network (14). Similarly to the results seen with the LightCycler 480 platform, the MeVA RT-qPCR assay performed suboptimally with the SuperScript III kit with respect to the resulting amplification curves for wild-type measles virus genotypes (Fig. 3B).

The MeVA RT-qPCR and MeV qPCR were compared using the ABI 7500 platform and the QuantiTect kit, and the samples included synthetic MeV RNAs serially diluted from 10^5 to 10^1 copies per reaction. The dilutions were tested in duplicate on at least four separate assays. The results were similar to those obtained from the Roche LightCycler 480 system (Table 1) in that the lower limit of detection of the MeVA RT-qPCR assay was approximately 1 Log_{10} higher than for the MeV RT-qPCR assay.

To assess the specificity of the MeVA RT-qPCR assay on the ABI 7500 platform and to compare it to the performance of the MeV RT-qPCR assay, three sets of samples were used, i.e., synthetic RNAs containing the entire N gene open reading frames from six currently circulating wild-type genotypes (B3, D4, D8, D9, G3, and H1) (B. Bankamp, unpublished data), RNA from cell culture lysates from five vaccine strains (AIK-C, CAM-70, Edmonston-Zagreb, Moraten, and Shanghai-191), and RNA extracted from 28

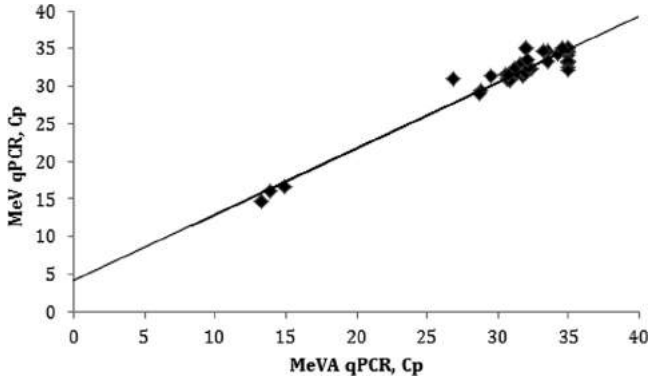

FIG 2 Correlation between Cp values of 50 genotype A measles virus specimens tested by MeVA RT-qPCR and the standard MeV RT-qPCR method. The regression line has a slope of 0.88 (0.82 to 0.94, 95% CI), a *y* intercept of 4.1 (2.2 to 6.0, 95% CI) and an R^2 value of 0.949 ($P < 0.0001$). The QuantiTect Probe RT-PCR kit was used for these reactions.

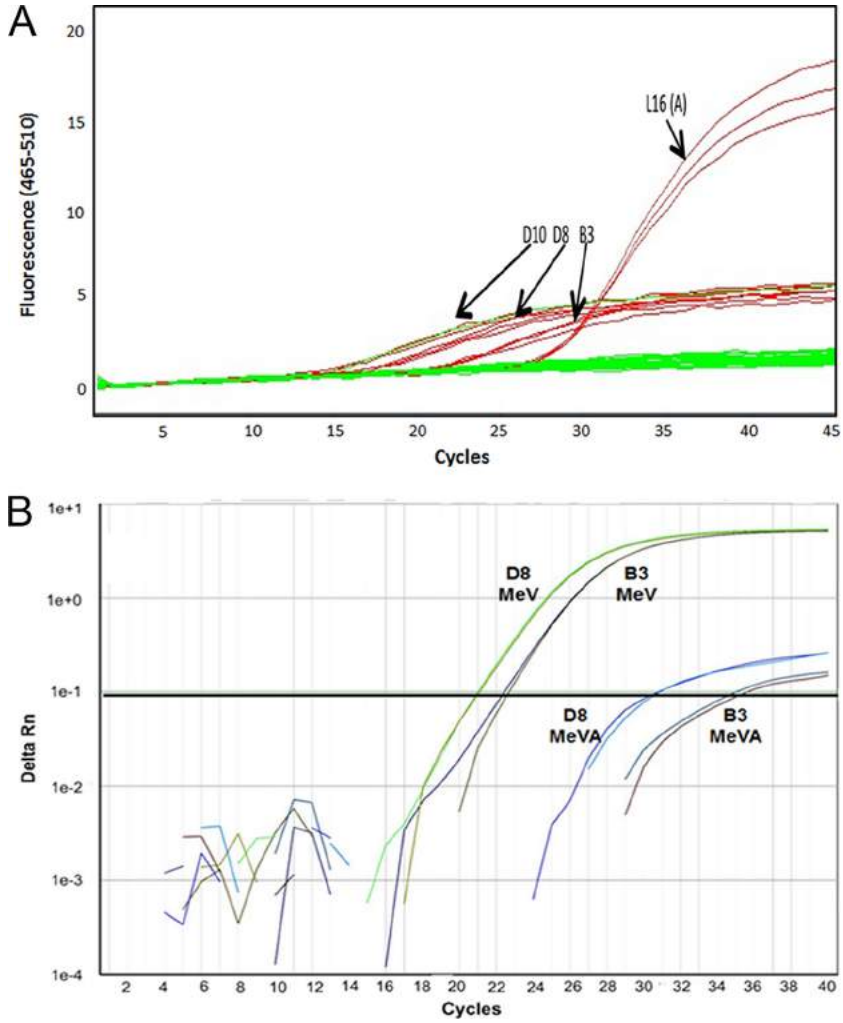

FIG 3 Negative effect of the use of the SuperScript III Platinum One-Step quantitative RT-PCR kit on the specificity of the MeVA RT-qPCR for the vaccine genotype. (A) The use of SuperScript III on the Roche LightCycler 480 platform caused a significant rise in the baseline of the amplification curves for genotype D10, D8, and B3. (B) Results of the use of SuperScript III on an ABI 7500 platform in amplification curves from wild-type measles virus RNA.

archival respiratory swabs and urine specimens that were submitted to the CDC for routine surveillance. Three archival specimens were negative by MeV RT-qPCR, and the other 25 were positive by MeV RT-qPCR and included clinical specimens from measles cases and vaccine reactions (with threshold cycle [C_T] values ranging from 14 to 36).

Of the positive archival specimens, all specimens with wild-type genotypes ($n = 12$) were negative in the MeVA RT-qPCR assay but positive in the MeV RT-qPCR assay and 12 of 13 specimens from vaccine reactions were positive in both assays (Table 2). Three of the specimens from the vaccine reactions had C_T values ranging from 38 to 40 in the MeV RT-qPCR assay and from 38 to 40 in the MeVA RT-qPCR.

The RNA from all five vaccine strains was detected in both assays with slightly lower sensitivity (C_T value, 2 to 3) in the MeVA RT-qPCR assay than in the MeV RT-qPCR assay (data not shown). In addition, the MeVA RT-qPCR assay did not produce a positive signal in samples containing high copy numbers of synthetic RNA from the six commonly circulating wild-type genotypes (Fig. 5).

If we consider all samples that were amplified within 40 PCR cycles, as was done at NML and RKI for the LightCycler platform, the sensitivity of the MeVA test on the ABI 7500 platform was 94% and the specificity was 100% (Table 3).

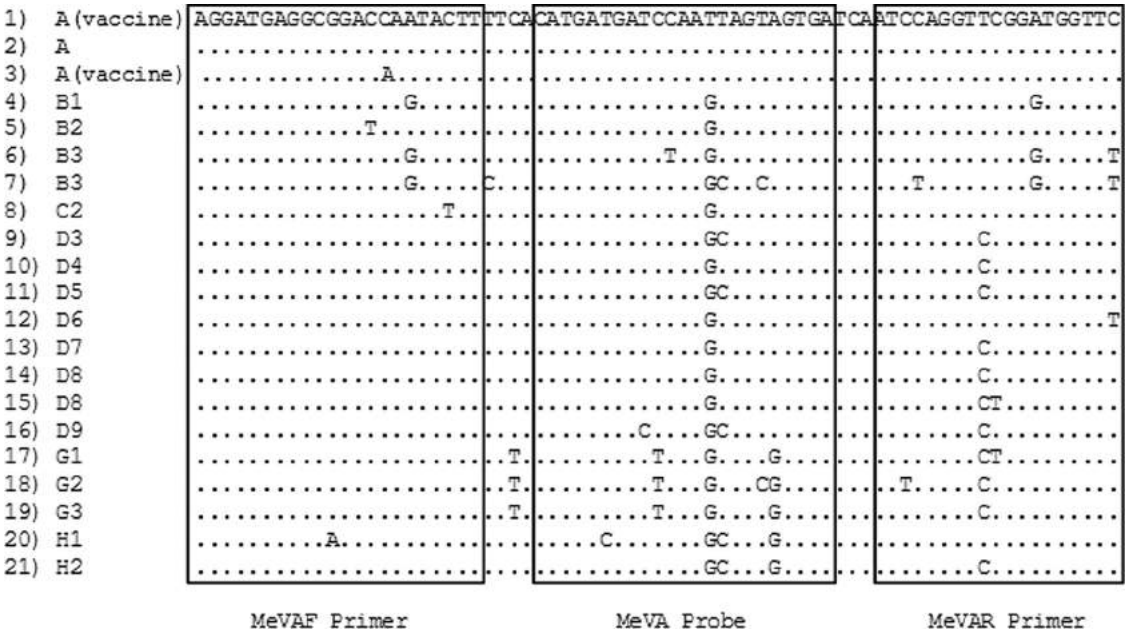

FIG 4 Alignment of the N gene region (positions 478 to 548) amplified by the MeVA RT-qPCR. The alignment includes examples of each genotype available on GenBank for this region, except for genotype D9, which was sequenced from one of our archival specimens. Row 1, 31 identical sequences from various vaccine strains; row 2, MVi/Maryland.USA/54 (A); row 3, two vaccine strains showing a 1-nt difference in the forward primer region; row 4, MVi/Yaounde.CMR/12.83 (B1); row 5, MVi/Libreville.GAB/84 (B2); row 6, MVi/Ibadan.NIE/971 (B3); row 7, MVi/New_York.USA/94 (B3); row 8, MVi/Maryland.USA/77 (C2); row 9, MVi/Illinois.USA/89/1 (D3); row 10, MVi/Montreal.CAN/89 (D4); row 11, Bankok.THA/12.93 (D5); row 12, MVi/New_Jersey.USA/94/1 (D6); row 13, MVs/Dundee.UNK/82 (D7); row 14, MVi/BritishColumbia.CAN/13.10/1 (D8); row 15, MVi/Manchester.GBR/30.94 (D8); row 16, MVs/Ontario.CAN/14.14 (D9); row 17, MVi/Berkeley.USA/83 (G1); row 18, MVi/Amsterdam.NLD/49.97 (G2); row 19, MVi/Gresik.IDN/17.02 (G3); row 20, MVi/Hunan.CHN/93/7 (H1); row 21, MVi/Beijing.CHN/94/1 (H2).

DISCUSSION

In response to the need for prompt differentiation between vaccine reactions and wild-type measles virus infection cases, laboratories have been developing methods that do not require sequencing of N-450. A method targeting a region on the hemagglutinin gene has been described and tested with a small number of vaccine and wild-type specimens or isolates (15). Here, we describe the development and validation of a measles virus genotype A-specific RT-qPCR, MeVA RT-qPCR, that targets the N gene of MeV. This assay produces rapid results and is capable of high throughput. The MeVA RT-qPCR was thoroughly tested at three global reference laboratories. Two RT-qPCR platforms and over 300 samples were included in the evaluation. Overall, our data show very high (99.5%) specificity for the A genotype, albeit with lower (94%) sensitivity than the standard MeV RT-qPCR (16). Because of the lower sensitivity, the MeVA RT-qPCR is intended to be used as a tool for rapid detection of genotype A sequences and not as a primary diagnostic test. The MeVA RT-qPCR should be performed in parallel with the MeV RT-qPCR method. Multiplexing of the two tests is in progress to increase the efficiency of this method.

We have shown that the MeVA RT-qPCR can be used on both the Roche LightCycler 480 and the ABI 7500 platforms, which are available in a large number of laboratories around the world. We also demonstrated that the QuantiTect kit gave optimal performance on both platforms.

An alignment of the nt 478 to 548 region used for the MeVA RT-qPCR (Fig. 4) shows that some wild-type strains differ only by a single nucleotide in the probe region, a G at position 517, although other mismatches that may favor specificity are present in the primer regions. This point mutation may be stable, since it results in an amino acid change (serine in wild-type strains to isoleucine in vaccine strains), but it is conceivable that wild-type strains may arise with a mutation in this position that cross-reacts with the MeVA assay. Therefore, we currently still confirm every MeVA RT-qPCR result by WHO-recommended sequencing of the N-450 region.

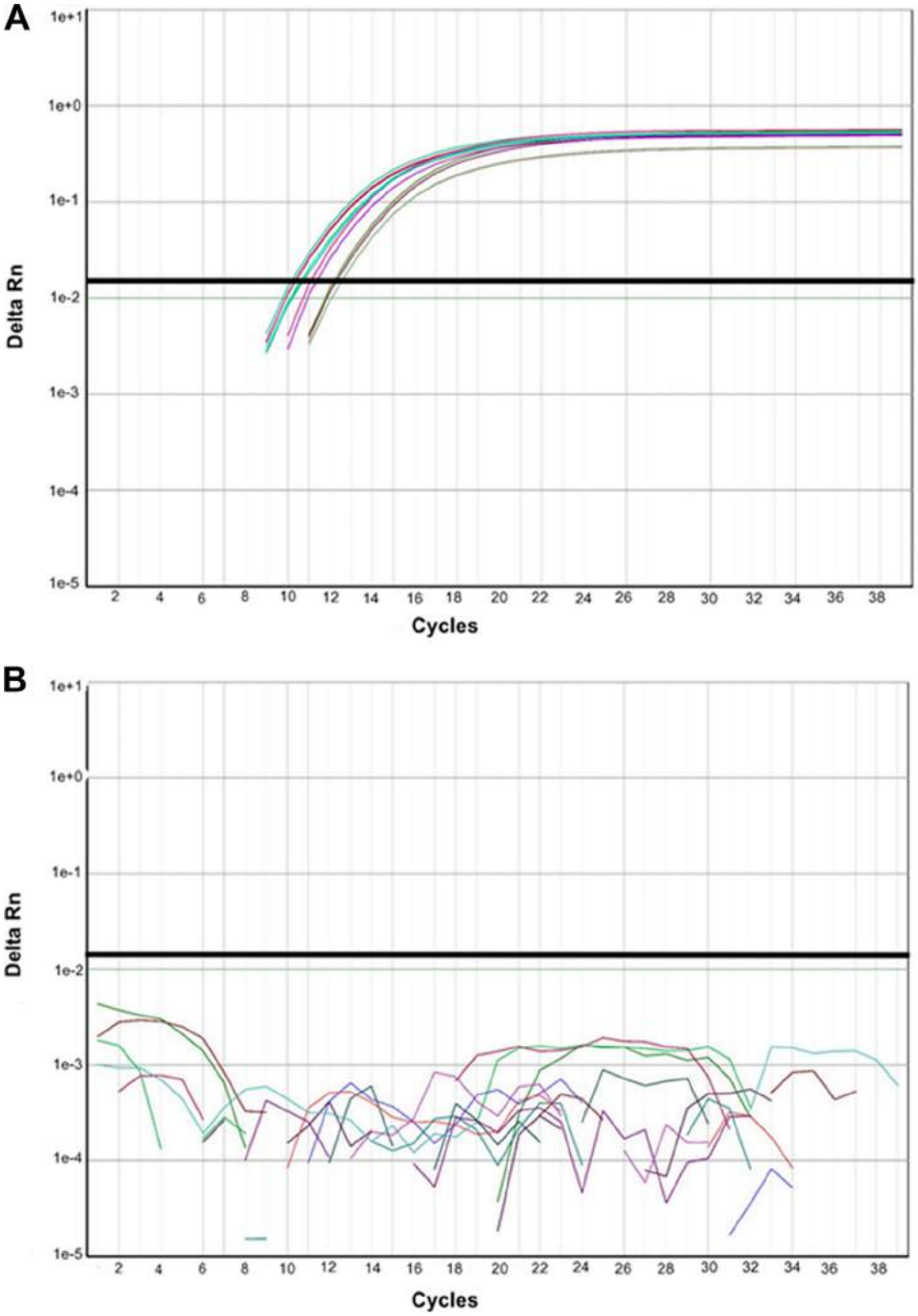

FIG 5 Specificity of the MeVA RT-qPCR assay, using an Applied Biosystems 7500 platform. Synthetic RNA from the six active wild-type measles virus genotypes (B3, D4, D8, D9, G3, and H1) was tested. Panel A shows detection of 10^7 copies of RNA/reaction in the MeV qPCR assay, and panel B shows the lack of amplification of 10^7 copies of RNA/reaction in the MeVA RT-qPCR assay. The QuantiTect Probe RT-PCR kit was used for these reactions.

The specificity of the test has been assessed on most of the measles virus genotypes currently circulating in the world (except D11 and G3) (11, 14), and there was only one genotype D5 specimen that gave a false-positive result. The sequence of the MeVA RT-qPCR target region of this genotype D5 virus differed from that of the vaccine strains by the G at position 517, conserved in all measles virus wild-type strains, and by two additional nucleotides in the reverse primer. Genotypes D4, D6, and D8 have the same sequence as this D5 strain in the probe region and one fewer difference in the reverse

primer region (Fig. 4), but they did not cross-react with the MeVA RT-qPCR. Another genotype D5 strain, tested at the NML, and 12 additional D5 strains, tested at the RKI, were not amplified by the MeVA RT-qPCR. Therefore, the reasons for this false-positive result remain unclear.

During measles outbreak investigations, rapid detection of measles vaccine reactions is necessary to avoid unnecessary public health interventions. In Canada, the NML has been using the MeVA and MeV RT-qPCRs with a turnaround time of 2 days. Therefore, local health authorities can initiate appropriate public health responses without waiting for sequencing results, which often take several days to obtain. The MeVA RT-qPCR is especially useful during large measles outbreaks, when it is difficult for laboratories to perform sequencing on a large number of specimens in a timely manner. Similarly, recent measles outbreaks in the United States have reinforced the need for rapid confirmation of vaccine reactions. In countries such as Germany, which is still experiencing frequent measles outbreaks, this RT-PCR-based method has already proven to be a valuable tool for guiding the public health responses. The MeVA RTqPCR assay is a straightforward application of real-time RT-PCR methodology, and the two platforms evaluated here are available in many laboratories. This assay could be readily deployed to national and subnational laboratories on a wide scale.

MATERIALS AND METHODS

Primers, probes, and control RNA. The primers and probe for the vaccine-specific assays were designed following analysis of 31 sequences available on GenBank from Edmonston-derived and non-Edmonston-derived vaccine strains. These sequences are identical in the target region of MeVA RT-qPCR (the 3′ region of the MeV N gene between nt 478 and nt 548 of the Edmonston strain [GenBank accession no. AF266288.2]), including the more divergent non-Edmonston-derived strains Shanghai-191 and CAM-70 (Fig. 4) (15). Two vaccine strains, Schwarz FF-8 (GenBank AB591381.1) and Edmonston AIK-C (GenBank S58435.1), have a 1-nt difference in the sequence of the forward primer, but they are identical to the other vaccine strains in the probe region (Fig. 4). The primers (Invitrogen) for reverse transcription and cDNA amplification were 5′-AGGATGAGGCGGACCAATACTT-3′ (MeVAF) and 5′-GAACCATCCGAACC TGGAT-3′ (MeVAR). Both primers were used at a concentration of 0.9 μM. Amplification was detected by a TaqMan probe (TIB Molbiol) with 6-carboxyfluorescein (FAM) as a fluorophore, at a concentration of 0.25 μM. The probe had the sequence 5′-FAM-CATGATGATCCAATTAGTAGTGA-BBQ-3′ (MeVA probe [BBQ, black berry quencher]), where the underlined characters indicate locked nucleic acid bases containing a 2′O,4-C methylene bridge which has the effect of increasing the melting temperature (T_m) and potentiating the destabilizing effect of a nucleotide mismatch (17).

As a standard for the measurement of MeV copy numbers, synthetic measles virus RNA was prepared by *in vitro* transcription, using a MEGAscript T7 transcription kit (Invitrogen, Life Technologies Inc.), either from a plasmid containing the open reading frame of the N gene of genotype A (16) or from PCR amplicons that included the T7 promoter in the forward primer (Bankamp, unpublished). DNase-treated RNA was purified with a MEGAclear transcription cleanup kit (Ambion, Life Technologies Inc.) and quantitated fluorometrically (Qubit, Life Technologies Inc.). The absence of residual DNA was verified by real-time RT-PCR (MeV RT-qPCR) (16) in the presence or absence of the reverse transcriptase.

Samples tested. For this study, 370 samples were tested to evaluate the sensitivity and specificity of the MeVA RT-PCR. The majority of these were clinical samples that were submitted to NML, CDC, or RKI as part of routine surveillance activities for measles.

Roche LightCycler 480 platform. Archival nasopharyngeal swabs and urine specimens sent to the NML for molecular surveillance were used. These specimens tested positive for measles virus by MeV RT-qPCR using a previously described method (16) and were genotyped using the N-450 target (10, 11). RNA was extracted using the QIAamp viral RNA minikit (Qiagen; catalog no. 52904) or the MagNA Pure liquid chromatograph (LC) total nucleic acid isolation kit—high performance (Roche Diagnostics; catalog no. 05323738001) on the MagNA Pure LC 2.0 instrument (Roche Diagnostics). For RT-PCR, 2 μl of extracted RNA was subjected to one-step reverse transcription and qPCR using the QuantiTect Probe RT-PCR kit (Qiagen; catalog no. 204443) according the instructions of the manufacturer. The RT-qPCR mixtures (total volume, 20 μl) were incubated at 50°C for 20 min (RT step) and 95°C for 15 min (activation of the polymerase) and subjected to 40 cycles of amplification (95°C for 5 s and 60°C for 1 min) on the Roche LightCycler 480 instrument. The RT-qPCR result was considered positive if there was amplification within 40 cycles, but crossing-point (Cp) values were recorded for only the first 35 cycles.

At the National Reference Center for Measles, Mumps, and Rubella at the RKI, archival surveillance specimens were extracted using the QIAamp viral RNA minikit (Qiagen; catalog no. 52906) and amplified by using the SuperScript III Platinum One-Step quantitative RT-PCR kit (Invitrogen; catalog no. 11732-088) or the QuantiTect Probe RT-PCR kit (Qiagen; catalog no. 20443). MeVA RT-qPCR, MeV RT-qPCR, and genotyping at the N-450 region were performed as described above. The RT-PCR result was considered positive if amplification was detected within 40 cycles.

Applied Biosystems 7500 platform. At the CDC, RNA was extracted with the QIAamp viral RNA minikit as described above. The MeV RT-qPCR was performed using the same reaction conditions and primers and probes (16). As for the Roche LightCycler 480, the SuperScript III and QuantiTect reagent kits were evaluated as described in Results. For the comparisons described in this report, the RT-qPCR result was considered positive if there was amplification within 40 cycles; however, during routine use of this assay at the CDC, specimens with threshold cycle (C_T) values between 38 and 40 are considered to represent equivocal results.

Statistical analyses. Sensitivity and specificity of MeVA RT-qPCR were calculated using the VassarStats website (13). Linear regression and related statistics were calculated using an online calculator developed by GraphPad Software, Inc. (http://www.graphpad.com/quickcalcs/linear1) and graphed using Microsoft Excel.

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The findings and conclusions in this report are ours and do not necessarily represent the official position of the Centers for Disease Control and Prevention. Names of specific vendors, manufacturers, or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers, or products by the Centers for Disease Control and Prevention or the US Department of Health and Human Services.

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Whooping Cough (Pertussis) Vaccine

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FDA Study Helps Provide an Understanding of Rising Rates of Whooping Cough and Response to Vaccination

November 27, 2013

A new study is helping to provide a better understanding of vaccines for whooping cough, the common name for the disease pertussis. Based on an animal model, the study conducted by the U.S. Food and Drug Administration (FDA) and published November 25, 2013, in *The Proceedings of the National Academy of Sciences*, shows that acellular pertussis vaccines licensed by the FDA are effective in preventing the disease among those vaccinated, but suggests that they may not prevent infection from the bacteria that causes whooping cough in those vaccinated or its spread to other people, including those who may not be vaccinated.

Whooping cough rates in the United States have been increasing since the 1980s and reached a 50-year high in 2012. Whooping cough is a contagious respiratory disease caused by *Bordetella pertussis* bacteria. Initial symptoms include runny nose, sneezing, and a mild cough, which may seem like a typical cold. Usually, the cough slowly becomes more severe, and eventually the patient may experience bouts of rapid, violent coughing followed by the "whooping" sound that gives the disease its common name, when trying to take a breath. Whooping cough can cause serious and sometimes life-threatening complications, permanent disability, and even death, especially in infants and young children.

There are two types of pertussis vaccines, whole-cell and acellular. Whole-cell pertussis vaccines contain a whole-cell preparation, which means they contain killed, but complete, *B. pertussis* bacteria. The acellular pertussis vaccine is more purified and uses only selected portions of the pertussis bacteria to stimulate an immune response in an individual. In response to concerns about the side effects of the whole cell pertussis vaccine, acellular vaccines were developed and replaced the use of whole-cell pertussis vaccines in the U.S. and other countries in the 1990s; however, whole-cell pertussis vaccines are still used in many other countries.

"This study is critically important to understanding some of the reasons for the rising rates of pertussis and informing potential strategies to address this public health concern," said Karen Midthun, M.D., director of the FDA's Center for Biologics Evaluation and Research, where the study was conducted. "This research is a valuable contribution and brings us one step closer to understanding the problem. We are optimistic that more research on pertussis will lead to the identification of new and improved methods for preventing the disease."

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NIAID At a Glance

- Director, Anthony S. Fauci, M.D.
- Overview of NIAID Research
- History
- Budget & Planning
- Congressional Testimony

While the reasons for the increase in cases of whooping cough are not fully understood, multiple factors are likely involved, including diminished immunity from childhood pertussis vaccines, improved diagnostic testing, and increased reporting. With its own funds plus support from the National Institutes of Health (NIH), the FDA conducted the study to explore the possibility that acellular pertussis vaccines, while protecting against disease, might not prevent infection.

"There were 48,000 cases reported last year despite high rates of vaccination," said Anthony S. Fauci, M.D., director of the NIH's National Institute of Allergy and Infectious Diseases. "This resurgence suggests a need for research into the causes behind the increase in infections and improved ways to prevent the disease from spreading."

The FDA conducted the study in baboons, an animal model that closely reproduces the way whooping cough affects people. The scientists vaccinated two groups of baboons-one group with a whole-cell pertussis vaccine and the other group with an acellular pertussis vaccine currently used in the U.S. The animals were vaccinated at ages two, four, and six months, simulating the infant immunization schedule. The results of the FDA study found that both types of vaccines generated robust antibody responses in the animals, and none of the vaccinated animals developed outward signs of pertussis disease after being exposed to *B. pertussis*. However, there were differences in other aspects of the immune response. Animals that received an acellular pertussis vaccine had the bacteria in their airways for up to six weeks and were able to spread the infection to unvaccinated animals. In contrast, animals that received whole-cell vaccine cleared the bacteria within three weeks.

This research suggests that although individuals immunized with an acellular pertussis vaccine may be protected from disease, they may still become infected with the bacteria without always getting sick and are able to spread infection to others, including young infants who are susceptible to pertussis disease.

For more information, see FDA: Vaccines.

Content last reviewed on November 27, 2013

Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model

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Edited by Rino Rappuoli, Novartis Vaccines and Diagnostics Srl, Siena, Italy, and approved October 22, 2013 (received for review August 5, 2013)

Pertussis is a highly contagious respiratory illness caused by the bacterial pathogen *Bordetella pertussis*. Pertussis rates in the United States have been rising and reached a 50-y high of 42,000 cases in 2012. Although pertussis resurgence is not completely understood, we hypothesize that current acellular pertussis (aP) vaccines fail to prevent colonization and transmission. To test our hypothesis, infant baboons were vaccinated at 2, 4, and 6 mo of age with aP or whole-cell pertussis (wP) vaccines and challenged with *B. pertussis* at 7 mo. Infection was followed by quantifying colonization in nasopharyngeal washes and monitoring leukocytosis and symptoms. Baboons vaccinated with aP were protected from severe pertussis-associated symptoms but not from colonization, did not clear the infection faster than naïve animals, and readily transmitted *B. pertussis* to unvaccinated contacts. Vaccination with wP induced a more rapid clearance compared with naïve and aP-vaccinated animals. By comparison, previously infected animals were not colonized upon secondary infection. Although all vaccinated and previously infected animals had robust serum antibody responses, we found key differences in T-cell immunity. Previously infected animals and wP-vaccinated animals possess strong *B. pertussis*-specific T helper 17 (Th17) memory and Th1 memory, whereas aP vaccination induced a Th1/Th2 response instead. The observation that aP, which induces an immune response mismatched to that induced by natural infection, fails to prevent colonization or transmission provides a plausible explanation for the resurgence of pertussis and suggests that optimal control of pertussis will require the development of improved vaccines.

whooping cough | T-cell memory | animal models | adaptive immunity | IL-17

Pertussis is a highly contagious, acute respiratory illness caused by the bacterial pathogen *Bordetella pertussis* (1, 2). Infection results in a wide spectrum of clinical manifestations ranging from mild respiratory symptoms to a severe cough illness accompanied by marked leukocytosis and the hallmark inspiratory whoop and posttussive emesis (3). Because acellular pertussis vaccines replaced whole-cell vaccines in the 1990s, pertussis has reemerged at a startling rate in the United States despite nationwide vaccine coverage in excess of 95% (4). With a 50-y high of 42,000 reported cases in the United States in 2012, pertussis is the most common of the vaccine-preventable diseases (5). This resurgence is mirrored throughout the industrial world despite similar high rates of vaccination (6–9). Two common hypotheses for the resurgence have been proposed: *i*) current acellular pertussis vaccines (aP) vaccines are less effective than the whole-cell pertussis (wP) vaccines they replaced and *ii*) aP-induced immunity wanes more quickly than anticipated (10–13). However, pertussis resurgence is not completely understood (14, 15).

Hampering our ability to counteract this resurgence is the fact that pertussis pathogenesis and immunity to natural infection have not been well studied in humans because typical pertussis is sporadic given high rates of vaccination in developed countries. Human challenge studies have been proposed but never conducted due to a variety of logistical and ethical problems including the potential for severe disease, the lack of an effective therapeutic for established disease, and the highly contagious nature of pertussis. Although a variety of small-animal models have been used to study pertussis, none of them adequately reproduce the human disease (16). To address this gap, we recently developed a nonhuman primate model of pertussis using baboons (*Papio anubis*) and found the disease is very similar to severe clinical pertussis. Upon challenge, baboons experience 2 wk of heavy respiratory colonization and leukocytosis peaking between 30,000–80,000 cells/mL, similar to the range in pertussis-infected infants (1, 17). In addition, baboons experience a paroxysmal cough illness characterized by repeated fits of 5–10 coughs. The coughing fits last on average >2 wk in the baboon, although this is less than some severely infected children, where the cough can last up to 12 wk (1, 17). We also characterized airborne transmission of *B. pertussis* from infected to naïve animals, which is the route of transmission postulated to occur between humans (18). Because this is the only model of pertussis to reproduce the cough illness and transmission of the human disease, we believe it provides the unique opportunity to test our hypothesis that aP vaccines fail to prevent *B. pertussis* colonization, thus enabling transmission among vaccinated individuals.

Using this model we have confirmed that, as in humans, aP vaccines provide excellent protection against severe disease in baboons. However, aP vaccines do not prevent colonization following direct challenge or infection by transmission. In addition, aP-vaccinated animals are capable of transmitting disease to naïve contacts. By comparison, wP-vaccinated animals cleared infection significantly more quickly than aP-vaccinated or naïve

Significance

Pertussis has reemerged as an important public health concern since current acellular pertussis vaccines (aP) replaced older whole-cell vaccines (wP). In this study, we show nonhuman primates vaccinated with aP were protected from severe symptoms but not infection and readily transmitted *Bordetella pertussis* to contacts. Vaccination with wP and previous infection induced a more rapid clearance compared with naïve and aP-vaccinated animals. While all groups possessed robust antibody responses, key differences in T-cell memory suggest that aP vaccination induces a suboptimal immune response that is unable to prevent infection. These data provide a plausible explanation for pertussis resurgence and suggest that attaining herd immunity will require the development of improved vaccination strategies that prevent *B. pertussis* colonization and transmission.

Author contributions: J.M.W. and T.J.M. designed research; J.M.W., L.I.Z., and T.J.M. performed research; J.M.W. and T.J.M. analyzed data; and J.M.W. and T.J.M. wrote the paper.

The authors declare no conflict of interest.

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See Commentary on page 575.

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animals. We also found that aP vaccination induces T helper 2 (Th2) and T helper 1 (Th1) immune memory responses, whereas infection and—to a lesser extent—wP vaccination induce Th17 and Th1 memory. Our results suggest that in addition to the potential contribution of reduced efficacy and waning immunity of aP, the inability of aP to prevent colonization and transmission provides a plausible explanation for pertussis resurgence.

Results

Acellular Pertussis Vaccines Protect Against Disease but Fail to Prevent Infection. Several observational studies recently concluded that children primed with aP vaccine are at greater risk for pertussis diagnosis compared with wP-primed children (19–22). Although these data suggest aP vaccine is less effective than wP vaccine at preventing colonization, the rate of undiagnosed *B. pertussis* carriage in vaccinated individuals is unknown. To assess the ability of each vaccine to prevent colonization and clinical pertussis symptoms, baboons were vaccinated according to the US schedule at 2, 4, and 6 mo of age with human doses of combination diphtheria, tetanus, and pertussis vaccines containing aP or inactivated wP (Table 1 provides a list of the components of each vaccine). At 7 mo of age, vaccinated, naïve, and previously infected (convalescent) animals were challenged with D420, a *B. pertussis* clinical isolate that causes severe infection in humans and baboons (17). Naïve animals were heavily colonized with peak levels between 10^7–10^8 cfu/mL in nasopharyngeal washes (Fig. 1*A*). After 2 wk, colonization gradually decreased, and the infection cleared after 30 d. Consistent with our previous finding, none of the convalescent animals were colonized (17). Compared with naïve animals, aP-vaccinated animals had slightly reduced colonization for the first 10 d but remained consistently colonized before clearing after 35 d. In wP-vaccinated animals the initial colonization was similar to aP-vaccinated animals but the infection cleared after 18 d, significantly faster than naïve and aP-vaccinated animals (Fig. 1*B*).

To assess the efficacy of the vaccines in preventing the symptoms of severe pertussis, peripheral blood was drawn serially, and complete blood counts were performed to monitor leukocytosis, a significant marker of morbidity in pertussis-infected infants (23). Compared with preinfection levels, naïve animals had a significant increase in circulating white blood cells at each time point, peaking at over 40,000 cells per μL, an eightfold increase over preinfection levels (Fig. 1*C*). In contrast to the colonization data, aP vaccination, wP vaccination, and convalescence all prevented leukocytosis (Fig. 1*C*). In addition, wP-vaccinated, aP-vaccinated, and convalescent animals did not cough and showed no reduction of activity, loss of appetite, or other outward signs of disease.

Acellular Vaccines Fail to Prevent Infection Following Natural Transmission. To assess the ability of vaccination to prevent pertussis infection by transmission, two aP-vaccinated animals and one unvaccinated animal were cohoused with a directly challenged, unvaccinated animal. Similar to our previous findings (18), all animals became colonized 7–10 d after cohousing with the infected animal (Fig. 2). The peak levels and kinetics of colonization were indistinguishable between the naïve and aP-vaccinated animals.

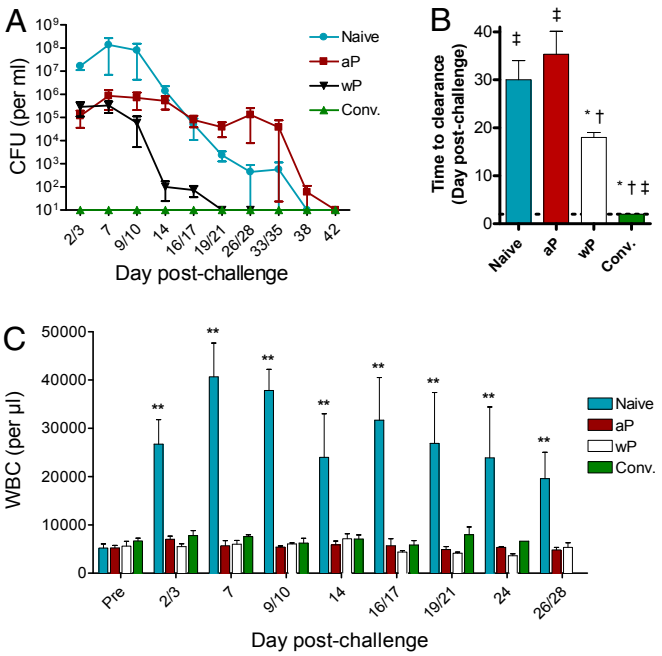

Fig. 1. The effect of vaccination or convalescence on colonization and leukocytosis. Naïve animals, aP-vaccinated animals, wP-vaccinated animals, and previously infected [convalescent conv.)] animals were directly challenged with *B. pertussis* (n = 3–4 per group). (*A*) Colonization was monitored by quantifying *B. pertussis* cfu per mL in biweekly nasopharyngeal washes with a limit of detection of 10 cfu per mL. For each animal the time to clearance is defined as the first day that no *B. pertussis* cfu were recovered from nasopharyngeal washes. (*B*) The mean time to clearance is shown for each group (n = 3 per group). Because no *B. pertussis* organisms were recovered from the conv. animals, the mean time to clearance was defined as the first day of sampling (day 2, indicated by the dashed line). *P < 0.05 vs. Naïve, †P < 0.05 vs. aP, ‡P < 0.05 vs. wP. (*C*) The mean circulating white blood cell counts before and after challenge are shown for each group of animals (n = 3–4 per group). **P < 0.01 vs. preinfection from same group.

Acellular-Vaccinated Animals Are Capable of Transmitting *B. pertussis* to Naïve Contacts. Because aP fails to prevent colonization we hypothesized that aP-vaccinated animals can transmit *B. pertussis* infection to contacts. To test this hypothesis, two aP-vaccinated animals were challenged with *B. pertussis* and placed in separate cages. After 24 h, a naïve animal was added to each cage, and all animals were followed for colonization. Both of the naïve animals were infected by transmission from their aP-vaccinated cage mates (Fig. 3).

Vaccination and Previous Infection Induce Robust Antibody Responses. Sera collected before vaccination or primary infection and again at 1 wk before challenge were analyzed for IgG antibodies against heat-killed *B. pertussis* and the vaccine antigens

Table 1. Components of aP and wP vaccines used in this study

Vaccine component	Daptacel	Infanrix	Triple antigen
Diphtheria toxoid	15 Lf	25 Lf	20–30 Lf
Tetanus toxoid	5 Lf	10 Lf	5–25 Lf
Whole-cell *Bordetella pertussis*	—	—	≥4 IU
Inactivated pertussis toxin	10 μg	25 μg	—
Filamentous hemagglutinin	5 μg	25 μg	—
Pertactin	3 μg	8 μg	—
Fimbriae types 2 and 3	5 μg	—	—
Aluminum (from aluminum phosphate)	0.33 mg	≤0.625 mg	≤1.25 mg

IU, international units; Lf, limit of flocculation units.

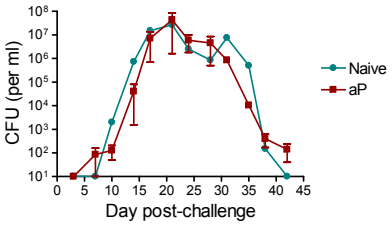

Fig. 2. aP does not protect against colonization following natural transmission. A naïve animal was directly challenged. After 24 h, a naïve animal and two aP-vaccinated animals were placed in the same cage as the directly challenged animal and followed for colonization as in Fig. 1.

pertussis toxin (PT), filamentous hemagglutinin (FHA), pertactin (PRN), and fimbriae types 2 and 3 (FIM). We show that wP, aP, and natural infection induce high-antibody titers to all antigens, and the aP group generally possessed equivalent or greater pre-challenge titers, suggesting that the differences in colonization between the groups do not correlate with levels of circulating antipertussis antibodies (Fig. 4). Following challenge, the titers for vaccinated animals were essentially unchanged, whereas boosting was observed for some antigens in convalescent animals (Fig. S1).

T-Cell Memory Response Elicited by Acellular Pertussis Vaccination Is Mismatched Compared with Natural Infection. Although a large number of clinical studies have characterized the antibody response to pertussis infection and vaccination, key deficiencies remain in our understanding of pertussis-induced helper T-cell immune responses in humans and primates. Importantly, no clinical studies have investigated whether the primary series of pertussis vaccines induce Th17 memory, a recently identified T cell that specializes in controlling extracellular bacterial infections at mucosal surfaces through stimulating neutrophil recruitment (24). To assess *B. pertussis*-specific T-cell memory responses in naïve, aP-vaccinated, wP-vaccinated, and convalescent animals, peripheral blood mononucleated cells (PBMCs) were collected 1 wk before infection. Total PBMC were incubated either with medium alone or with heat-killed *B. pertussis* as an ex vivo simulation of the memory responses recalled during the ensuing challenge. Following an overnight incubation, nonadherent PBMC, including T cells, were collected and separated using magnetic beads into the following fractions: CD4−, CD4+, CD95−CD4+, or left unseparated (total nonadherent cells). Memory helper T cells in primates are characterized by surface expression of CD4 and CD95 (25, 26). After further culture of all fractions, the supernatants were analyzed for secretion of IL-17, IFN-γ, and IL-5; cytokines that are characteristic of Th17, Th1, and Th2 cells, respectively. Very low background cytokine secretion was observed from nonstimulated cells isolated from naïve, vaccinated, or convalescent animals or from stimulated cells from naïve animals (Figs. S2 and S3). When stimulated with heat-killed *B. pertussis*, both total nonadherent cells and CD4+ cells from convalescent animals secreted high levels of IL-17, some IFN-γ, and no IL-5. When the CD95+ memory cells were depleted, the CD95−CD4+ cells did not secrete IL-17 or IFN-γ, consistent with induction of *B. pertussis*-specific Th17 and Th1 memory cells (Fig. 5). Stimulated total nonadherent cells and CD4+ cells from aP-vaccinated animals secreted significant IFN-γ, but the response was weaker than convalescent cells ($P = 0.01$), and there was no significant increase in IL-17 secretion. However, there was a significant IL-5 response, consistent with skewing toward Th2 and Th1 memory (Fig. 5). Total nonadherent cells and CD4+ cells from wP-vaccinated animals secreted similar IFN-γ compared with aP cells, but no IL-5. IL-17 secretion was between levels for naïve and convalescent cells, suggesting that T-cell memory induced by wP vaccination is similar to natural infection, but the Th17 and Th1 memory responses were weaker.

Discussion

The introduction of whole-cell vaccines consisting of inactivated *Bordetella pertussis* organisms in the United States in the 1940s caused a precipitous decrease in pertussis incidence (27). However, over the past 30 y, pertussis has resurged in the United States. The resurgence began during the wP vaccine era, but the pace has quickened since aP vaccines were recommended for all primary and booster doses (11). This correlation has led many to hypothesize that aP vaccines are less effective on a population scale than the wP vaccines they replaced (10, 12, 13). Consistent with this notion, several recent observational studies concluded that children primed with aP vaccine had a twofold to fivefold greater risk of pertussis diagnosis compared with wP-primed children (19–22). Our results in nonhuman primates add to these findings by showing that animals vaccinated with wP cleared infection by a direct challenge twice as fast as animals vaccinated with aP. However, neither vaccine was able to prevent colonization as well as immunity from a previous infection.

Another hypothesis as to why pertussis is reemerging is that the duration of immunity in aP-vaccinated children is shorter than anticipated. Although some first-generation acellular vaccines had poor immunity and efficacy, double-blinded clinical trials and field-efficacy studies for the US-licensed acellular vaccines estimated the short-term efficacy to be excellent: ∼85% after three doses and 98% after five doses (28–30). However, recent cohort and case-control studies concluded that 5 y following the fifth aP dose, children are fourfold to 15-fold more likely to acquire pertussis compared with within the first year, consistent with waning aP immunity (30–33).

We hypothesized an additional explanation for pertussis resurgence is that aP-vaccinated individuals can act as asymptomatic or mildly symptomatic carriers and contribute significantly to transmission in the population. Observational studies suggest that asymptomatic pertussis can occur in vaccinated children and adults based on PCR or serological data (34, 35). However, during the aP vaccine trials, participants were not screened for *B. pertussis* infection unless they presented with pertussis-like symptoms and at least 7–21 d cough (12). Therefore, no experimental data exist on whether vaccination prevents *B. pertussis* colonization or transmission in humans. In the present study we show that aP-vaccinated primates were heavily infected following direct challenge, and the time to clearance was not different compared with naïve animals. Similarly, there was no difference in the kinetics or peak level of colonization between aP-vaccinated and naïve animals that were infected by natural transmission. Importantly, we also show in two experiments that aP-vaccinated animals transmitted *B. pertussis* to naïve cage mates. Together these data form the key finding of this study: aP vaccines do not prevent infection or

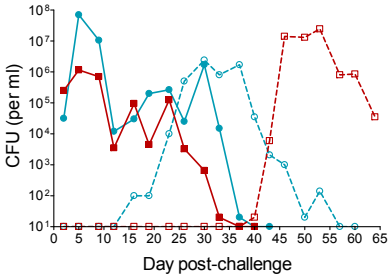

Fig. 3. Infected aP vaccinees can transmit pertussis to naïve contacts. Two animals vaccinated with aP were housed in separate cages, and each was directly challenged. Twenty four hours after challenge, an unchallenged naïve animal was placed in each cage. All animals were followed for colonization as in Fig. 1. One cage pairing is shown with turquoise lines with circles, and the other is shown with maroon lines with squares. Solid lines with closed symbols indicate the aP-vaccinated, directly challenged animals, and open symbols with dashed lines are used for the unchallenged, naïve contacts.

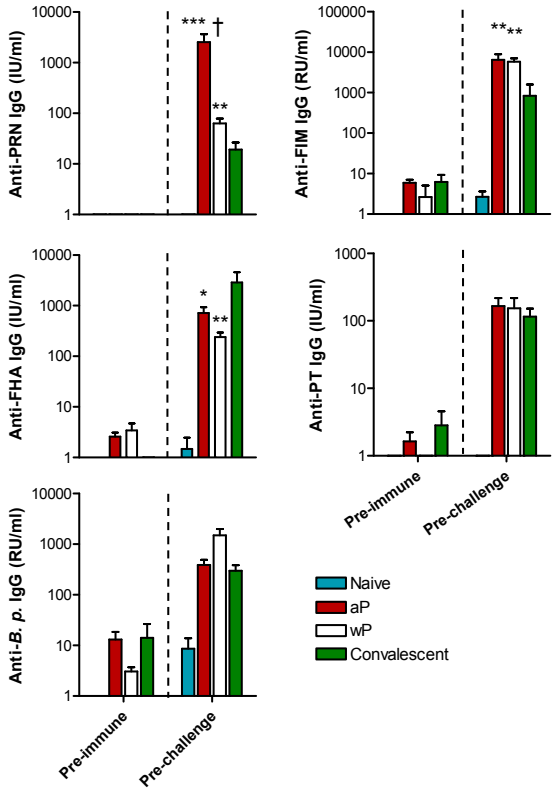

Fig. 4. Vaccination and previous infection induce robust serum antibody responses. Antibody responses to the four vaccine antigens—PRN, FIM, FHA, and PT—and to heat-killed *B. pertussis* (*B. p.*) were measured by ELISA. Preimmune sera were collected from vaccinated animals before immunization and from conv. animals before initial infection (n = 3–4 per group). Because Infanrix does not contain FIM, four Daptacel-vaccinated animals were included in the anti-FIM ELISA. Prechallenge sera were collected from all animals 1 wk before challenge. International Units (IU) or relative units (RU) in each sample were determined by comparing the responses to the WHO international standard pertussis antiserum on each plate. ***P < 0.001, **P < 0.01, *P < 0.05 vs. Convalescent. †P < 0.001 vs. wP.

transmission of *Bordetella pertussis* even 1 mo after completing the primary vaccination series.

We show that wP, aP, and natural infection all induce high-antibody titers. The prechallenge titers in aP-vaccinated animals were generally equivalent or higher than those observed in convalescent and wP-vaccinated animals, suggesting that aP is immunogenic in baboons and that the inability to prevent infection was not due to low-antibody titers. Compared with the large number of clinical studies that have characterized the antibody response to pertussis infection and vaccination, very few have investigated pertussis-induced helper T-cell immune responses in humans. Taken as a whole, these limited data suggest that aP vaccination induces Th2 or mixed Th2/Th1 responses, whereas wP vaccination and natural infection induce a Th1 response (13). However, none of these studies tested for Th17 memory, a recently identified T cell that specializes in controlling extracellular bacterial infections at mucosal surfaces (24). Our data show that natural infection induced robust Th17 and Th1 immunity. Animals vaccinated with wP, which cleared infection faster than naïve and aP-vaccinated animals, showed similar but weaker T-cell responses. wP vaccination is generally believed to induce strong Th1 responses, but what we observed here was relatively weak. This observation might be explained by heterogeneity in the manufacturing of different wP vaccines. Future studies will compare the immune response induced by wP vaccines produced by three different manufacturers. In comparison with natural infection and wP, aP-induced immunity was mismatched, showing a Th2 response with a weaker Th1 response and no significant Th17 response.

Together, the cytokine and T-cell immunological data observed in baboons are generally consistent with those observed in mice (13). We previously showed that pertussis infection in baboons induces a mucosal immune response characterized by production of IL-17 and a variety of chemokines and cytokines associated with IL-17 signaling, including IL-6 and IL-8. This primary immune response correlated with long-lived Th17 and Th1 memory responses that lasted >2 y (36). Mice infected with *B. pertussis* also express mucosal IL-17, IL-6, and IL-8 homologs and induce Th17 and Th1 memory (37–40). Mice vaccinated with wP also develop Th17 and Th1 memory that results in partial protective immunity, similar to what we observed in the baboon model (41, 42). A recent report by Ross et al. (42) concluded that an aP containing PT, FHA, and PRN induces Th1, Th2, and Th17 immune responses in C57BL/6 mice (42). However, a previous study from the same group found Th1 and Th2 but no

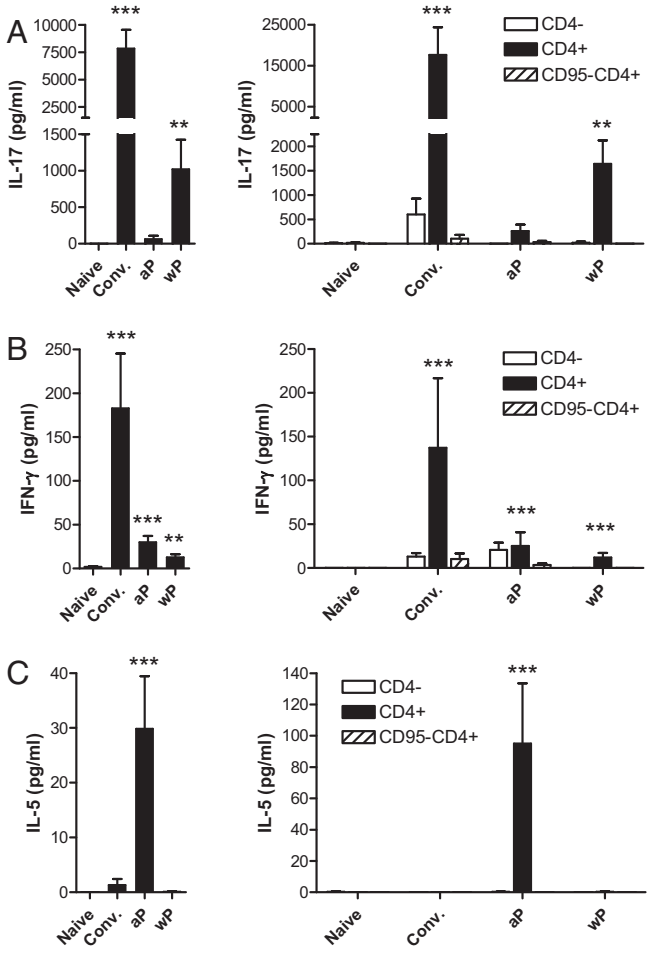

Fig. 5. Helper T-cell responses induced by vaccination and infection. PBMC collected from naïve, aP-vaccinated, wP-vaccinated, and conv. animals 1 wk before infection were incubated overnight with either medium alone or medium containing heat-killed *B. pertussis* (n = 3–4 per group). For each growth condition, nonadherent cells were collected and either left unseparated (total nonadherent cells) or separated using anti-CD4 and anti-CD95 magnetic particles. Total nonadherent, CD4–, CD4+, and CD95–CD4+ cells were then cultured under the same conditions as before (with medium alone or stimulated with heat-killed *B. pertussis*). After 36 h, supernatants were collected and analyzed for IL-17 (*A*), IFN-γ (*B*), and IL-5 (*C*). Cytokine secretion in response to *B. pertussis* stimulation is presented for total nonadherent cells (*Left*) and separated cells (*Right*). ***P < 0.001, **P < 0.01, *P < 0.05 vs. same fraction from naïve animals.

significant Th17 responses in C3H/HeJ and C3H/HeN mouse strains vaccinated with an aP containing PT and FHA (41). Nevertheless, data from two clinical studies recently showed negligible Th17 recall responses (~10 pg/mL) in PBMC isolated from aP-vaccinated 4-y-old children before and after booster, suggesting aP does not induce Th17 memory in humans (43, 44).

Taken as a whole, the data presented in this study suggest that antibodies induced by aP vaccination are sufficient for preventing severe pertussis symptoms but do not mitigate colonization. Inhibition of leukocytosis likely occurs through antibody-mediated neutralization of PT, a toxin which interferes with leukocyte extravasation by blocking chemokine receptor signaling (1). The mechanism by which aP prevents coughing despite heavy bacterial colonization is not known but deserves further attention. On the other hand, induction of Th17/Th1 memory responses correlated with the ability to clear infection: convalescent and wP-vaccinated animals possessed strong Th17 responses and Th1 responses and cleared infection more quickly than aP-vaccinated animals which lacked Th17 responses but possessed Th1/ Th2 memory. Although we have not definitively shown that Th17 cells are required for *B. pertussis* clearance, this correlation is consistent with the role these cells play in fighting extracellular bacterial infections at mucosal surfaces by inducing neutrophil chemotaxis. The current studies were not designed to look at immune cell recruitment to the respiratory tract, but additional experiments are underway to determine the role of neutrophils in the immune response to pertussis infection and vaccination in baboons. We are also investigating other possible mechanisms that could prevent mucosal colonization; for example, a possible role for IgA and IgD which are secreted in primate lower and upper respiratory tracts, respectively (45, 46).

The baboon model offers many advantages, chiefly the ability to investigate pertussis pathogenesis, transmission, and host immune responses to infection and vaccination in a primate species that is >96% genetically similar to humans (47). However, there are also several limitations associated with this model. There are far fewer animals available for research compared with smaller-animal models. In addition, there is a paucity of immunological reagents that are validated for baboons compared with mice and humans. Although antibodies against cell surface markers are generally cross-reactive, anti-cytokine antibodies tend to be much more species-specific. For this reason we have so far been unable to assess T-cell responses using intracellular cytokine staining and flow cytometry. This led us to develop the cell separation assay as an alternative method for phenotyping the memory T-cell responses induced by pertussis infection and vaccination (36). One limitation of our assay is that during the CD4+ cell purification, antigen-presenting cells such as macrophages and dendritic cells are removed after an overnight incubation. This likely explains the low IFN-γ secretion observed in all groups because antigen-presenting cells increase IFN-γ secretion by antigen-specific CD4+ T cells through a positive feedback loop (48). In line with this hypothesis, our previous data showed that restimulated whole PBMC from convalescent animals secreted much higher levels of IFN-γ. In addition, restimulation assays using human PBMC or murine splenocytes after infection or vaccination also show higher levels of secreted IFN-γ (42, 49). Together these observations suggest that although our assay is valuable for phenotyping T-cell memory, it likely underrepresents the magnitude of Th1 memory responses. We used heat-killed *B. pertussis* as an antigen for our restimulation assays because we believe this is the most relevant method for ex vivo simulation of T-cell memory recalled during infection. However, it is possible that this assay underdetects immune responses that would be observed had we used purified vaccine antigens. Another disadvantage of primate models is that it is not feasible to directly link an immune response to protection. Although protection from pertussis has been shown to be mediated by IFN-γ and, to a lesser extent, IL-17 signaling using knockout mouse strains lacking specific gene products (13), the relative protection afforded by Th17 or Th1 responses in vaccinated or convalescent baboons or humans is not known.

Currently, a major focus of public health agencies is the prevention of pertussis infection in young infants who have not completed their primary aP series and have considerable morbidity and mortality to pertussis infection (1). One recommendation to reduce transmission of pertussis to infants is by "cocooning," or vaccinating people who have contact with infants (11). Our data show that aP-vaccinated animals are infected and transmit pertussis to naïve contacts. Consistent with these findings, seroepidemiological studies have concluded that *B. pertussis* circulation is still high in countries with excellent aP uptake (27, 50), and a cross-sectional study showed that postpartum aP vaccination of mothers did not reduce pertussis illness in young infants (51). These data suggest that cocooning is unlikely to be an effective strategy to reduce the burden of pertussis in infants. However, it is important to note that our data in combination with human data show that vaccination with aP provides excellent protection from severe pertussis (52). Therefore, any short-term plan for addressing the resurgence of pertussis should include continued efforts to enhance aP immunization. However, to protect the most vulnerable members of the population and achieve optimal herd immunity, it will be necessary to develop a vaccination strategy that effectively blocks pertussis infection and transmission.

Materials and Methods

Ethics Statement. All animal procedures were performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International in accordance with protocols approved by the Center for Biologics Evaluation and Research Animal Care and Use Committee and the principles outlined in the *Guide for the Care and Use of Laboratory Animals* by the Institute for Laboratory Animal Resources, National Research Council (53).

Bacterial Strains and Media. *B. pertussis* strain D420 was grown on Bordet–Gengou and Regan–Lowe plates prepared as described previously (17). Heat-killed *B. pertussis* was prepared by resuspending to an OD_{600} of 0.90 (5×10^9 cfu/mL) in PBS and heating at 65 °C for 30 min.

Vaccination, Infection, and Evaluation of Baboons. Baboons obtained from the Oklahoma Baboon Research Resource at the University of Oklahoma Health Sciences Center were inoculated with human doses of aP or wP administered intramuscularly at 2, 4, and 6 mo of age. For studies using aP, equal numbers of animals were vaccinated with Daptacel (Sanofi Pasteur Ltd.) and Infanrix (GlaxoSmithKline). For wP, animals were vaccinated with Triple Antigen (Serum Institute of India Ltd.), which meets the World Health Organization (WHO) recommendations for potency. Naïve animals were age-matched but not vaccinated. Previously infected animals were clear of *B. pertussis* infection for 1 to 2 mo before reinfection. Direct challenge and transmission studies were performed as described previously (17, 18). The inoculum for each direct challenge was between 10^9–10^{10} cfu as determined by measurement of optical density and confirmed by serial dilution and plating to determine the number of cfu per mL of inoculum. Baboons were evaluated twice weekly as described previously for enumeration of circulating white blood cells and serum separation (17). Nasopharyngeal washes were diluted and plated on Regan–Lowe plates to quantify bacterial cell counts.

Isolation of PBMC and Cell Separation. Baboons were anesthetized, and PBMC were isolated from peripheral blood as described previously (36) and cryopreserved in RPMI-1640 medium supplemented with 10% (vol/vol) DMSO and 12.5% (wt/vol) BSA using Mr. Frosty containers (Nalgene). After thawing, cells were washed twice and nonadherent cells were collected as described previously. For each growth condition, cells were incubated overnight with either medium alone or medium containing heat-killed *B. pertussis* (50 bacteria:1 PBMC). Nonadherent cells were collected, and 2×10^6 cells were left unseparated (total nonadherent cells). Using the method previously described, 4×10^6 cells were separated using anti-CD4 magnetic particles, and another 4×10^6 cells were depleted of CD95+ cells and then separated with anti-CD4 magnetic particles (36). The following fractions were collected: Total nonadherent, CD4–, CD4+, and CD95–CD4+. After incubation with or without heat-killed *B. pertussis*, cells were pelleted and supernatants were collected for IL-17A quantitation by ELISA (Aniara) and quantitation of IFN-γ and IL-5 using the Milliplex MAP nonhuman primate kit according to the manufacturer's instructions (Millipore). Data are presented as

the cytokine concentration secreted by *B. pertussis*-stimulated cells minus the basal concentration secreted by cells incubated with medium alone.

Detection of Serum Antibodies to Pertussis Antigens. Nunc Maxisorp 96-well plates were coated overnight with 0.2 μg/mL PT, 0.5 μg/mL FHA, 2 μg/mL PRN, or 0.2 μg/mL FIM (List Biologicals) as described previously (17, 54). For whole-bacteria ELISA, plates were coated overnight at 37 °C with heat-killed *B. pertussis* prepared as described above. Serum IgG for each antigen was measured as described previously (17). Each plate contained a standard curve from the WHO international standard pertussis antiserum (National Institute for Biological Standards and Control) used to assign international units for PT, FHA, and PRN and relative units for FIM and heat-killed *B. pertussis* by comparison with the linear portion of the standard curve. Because Infanrix does not contain FIM, only Daptacel-vaccinated animals were included in the anti-FIM ELISA.

Statistics. All data are reported as mean ± SEM. Statistical analyses were performed by ANOVA with post hoc *t* test using JMP (version 9) software (SAS Institute, Inc.). Antibody and cytokine data were normalized by log transformation before analysis.

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Acellular pertussis vaccination facilitates *Bordetella parapertussis* infection in a rodent model of bordetellosis

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Despite over 50 years of population-wide vaccination, whooping cough incidence is on the rise. Although *Bordetella pertussis* is considered the main causative agent of whooping cough in humans, *Bordetella parapertussis* infections are not uncommon. The widely used acellular whooping cough vaccines (aP) are comprised solely of *B. pertussis* antigens that hold little or no efficacy against *B. parapertussis*. Here, we ask how aP vaccination affects competitive interactions between *Bordetella* species within co-infected rodent hosts and thus the aP-driven strength and direction of in-host selection. We show that aP vaccination helped clear *B. pertussis* but resulted in an approximately 40-fold increase in *B. parapertussis* lung colony-forming units (CFUs). Such vaccine-mediated facilitation of *B. parapertussis* did not arise as a result of competitive release; *B. parapertussis* CFUs were higher in aP-relative to sham-vaccinated hosts regardless of whether infections were single or mixed. Further, we show that aP vaccination impedes host immunity against *B. parapertussis*—measured as reduced lung inflammatory and neutrophil responses. Thus, we conclude that aP vaccination interferes with the optimal clearance of *B. parapertussis* and enhances the performance of this pathogen. Our data raise the possibility that widespread aP vaccination can create hosts more susceptible to *B. parapertussis* infection.

Keywords: pathogen evolution; *Bordetella parapertussis*; disease; acellular vaccination; epidemiology; co-infection

1. INTRODUCTION

Despite decades of worldwide pertussis vaccination, whooping cough is re-emerging in highly vaccinated countries (CDC 2002; Celentano *et al*. 2005). A rise in non-vaccine alleles coincident with widespread vaccination has been documented for *Bordetella pertussis* (Elomaa *et al*. 2005; Van Amersfoorth *et al*. 2005; Van Gent *et al*. 2009) leading some authors to propose that vaccine-driven epitope-evolution in *B. pertussis* is one factor—among several others (Berbers *et al*. 2009)—that may contribute to whooping cough re-emergence in humans (Mooi *et al*. 2001). However, it is not clear how *Bordetella parapertussis*—the other major aetiological agent of human whooping cough—might respond to the selective pressure exerted by large-scale pertussis vaccination. Here, we postulate that the widespread and long-term use of acellular subunit pertussis vaccines creates hosts that are more favourable for *B. parapertussis*.

All commercial whooping cough vaccines currently contain either killed whole cells or purified antigens of *B. pertussis*—herein referred to as whole cell (wP) and acellular vaccines (aP), respectively. Currently, aP vaccines are largely favoured over their wP predecessors owing to their reduced reactogenicity (Anderson *et al*. 1988). Although aP vaccines are very effective at reducing the incidence of *B. pertussis* infection (Mattoo & Cherry 2005), they hold little or no efficacy against *B. parapertussis* (Stehr *et al*. 1998; Willems *et al*. 1998; Liese *et al*. 2003; David *et al*. 2004). In fact, *B. parapertussis* prevalence is predicted to increase slightly in response to vaccines that are less protective against *B. parapertussis* than natural *B. pertussis* infection (Restif *et al*. 2008). Thus, analogous to the serotype specificity observed for conjugate vaccines against other infectious diseases and the serotype replacement associated with their use (Obaro *et al*. 1996; Lipsitch 1997), we hypothesize that the prolonged and widespread use of *B. pertussis*-specific aP vaccines has the potential to increase carriage of species not included in the vaccine, namely *B. parapertussis*.

The rationale to design and employ vaccines that target only *B. pertussis* stems from the assumption that *B. parapertussis* infections are not widely prevalent. Indeed, the vast majority of whooping cough studies do not attempt to identify *B. parapertussis* because differential diagnosis does not affect clinical management and this probably leads to under-reporting. However, when differential diagnosis has been carried out, *B. parapertussis* was found to comprise between 2 and 36 per cent of cases (Watanabe & Nagai 2004) and, in one study, to constitute the major aetiological agent (Borska & Simkovicova 1972). Both mixed and sequential infections of *B. pertussis* and *B. parapertussis* have been reported in epidemiological studies (Mertsola

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1985; Iwata *et al.* 1991; He *et al.* 1998; Mastrantonio *et al.* 1998; Stehr *et al.* 1998; Bergfors *et al.* 1999), showing that *B. pertussis* and *B. parapertussis* co-circulate in the same populations and sometimes the same hosts.

Some aP vaccine efficacy studies report a significantly higher proportion of *B. parapertussis* relative to *B. pertussis* in aP-vaccinated compared with unvaccinated individuals (Bergfors *et al.* 1999; Liese *et al.* 2003). These data are consistent with the hypothesis that *B. parapertussis* gains a selective advantage under aP vaccination. We can envisage at least three possible mechanisms by which aP vaccination could generate this selective advantage, all of which are based on the observation that aP vaccination confers less protection against *B. parapertussis* than the immunity induced by natural *B. pertussis* infection or wP vaccination.

First, aP vaccination could drive competitive release within individual hosts (Grech *et al.* 2008; Read & Mackinnon 2008). The transmission success of a given pathogen genotype depends on its intrinsic fitness and competitive ability (Read & Taylor 2001). Theory has predicted that *B. pertussis* must have a competitive advantage over *B. parapertussis* in unvaccinated co-infected hosts (Restif *et al.* 2008). However, aP vaccination can give *B. parapertussis* two potential fitness advantages; first, it can better survive aP vaccination than *B. pertussis* (Stehr *et al.* 1998; Willems *et al.* 1998; Liese *et al.* 2003; David *et al.* 2004) and second, by removing *B. pertussis* competitors, it could open up ecological space for *B. parapertussis*, which can greatly enhance the rate of spread of non-vaccine *B. parapertussis* (competitive release hypothesis; Lipsitch 1997; Hastings & D'Alessandro 2000). A second possibility is that by focusing immune responses on *B. pertussis*, aP vaccination interferes with an optimal immune response against *B. parapertussis*, resulting in slower clearance or enhanced establishment of *B. parapertussis* (enemy release hypothesis (ERH)). ERH is a term used widely in plant ecology when a plant species experiences a decrease in regulation by 'natural enemies' and rapidly increases in distribution and abundance (Mitchell & Power 2003). Such natural enemies might constitute herbivores in the case of plant ecology and, in pathogen biology, host immunity. Results from one aP vaccine efficacy study examining *B. parapertussis* in mice are consistent with an aP-driven enhancement of *B. parapertussis* infection (David *et al.* 2004), but is unclear whether a lack of immune regulation was driving this enhancement. A third possibility—but one not easily testable empirically for ethical reasons—is that aP vaccination could increase the number of humans susceptible to *B. parapertussis* by reducing levels of cross-immunity that would have otherwise been generated by natural *B. pertussis* infections or wP vaccination. Under this scenario, vaccination is in effect creating new ecological opportunities for *B. parapertussis* (the vacant niche filling hypothesis).

Here, we used a rodent model of *B. pertussis* and *B. parapertussis* infection to investigate the competitive ERH. By vaccinating laboratory mice with a commercial aP vaccine (which selectively targets *B. pertussis* and not *B. parapertussis*) and challenging them with single- or mixed-species infections (table S1, electronic supplementary material), the level of protection and immune stimulation was estimated over time in terms of changes in lung colony-forming units (CFUs), cytokine milieu, neutrophil recruitment and pathogen-specific antibody responses. If *B. parapertussis* is competitively suppressed by *B. pertussis* infection, *B. parapertussis* lung CFU will be lower in mixed relative to single infections (tested with the term 'infection type', a two-level factor describing the number of *Bordetella* species present in an infection; single or mixed). Following from this, competitive release of *B. parapertussis* would present as a significant interaction between infection type and 'vaccination'—a two-level factor describing the vaccination regime administered, sham or aP, and the infection type. If enemy release is occurring, we expect *B. parapertussis* CFUs to be higher in aP-vaccinated relative to sham-vaccinated hosts (tested with the term vaccination), regardless of whether infections were alone or in a mixture (which would present as a significant main effect of vaccination and a non-significant interaction between vaccination and infection type). Evidence that aP vaccination interferes with an optimal host immune response against *B. parapertussis* would further support the ERH. Our results support the enemy release model: aP vaccination interferes with the optimal clearance of *B. parapertussis* and enhances the performance of this pathogen.

2. MATERIAL AND METHODS

(a) *Bacteria strains and growth conditions*

Bordetella pertussis 1740 is a derivative of Tohama I (Kasuga *et al.* 1954), rendered kanamycin resistant by the chromosomal insertion of pSS4266 (Goebel *et al.* 2008) and was a kind gift from Dr Scott Stibitz (USDA). *Bordetella parapertussis* 12822 was isolated from German clinical trials (Heininger *et al.* 2002) and 12822G is a gentamicin-resistant derivative of the parent strain (Wolfe *et al.* 2005). Bordetellae were maintained on Bordet-Gengou (BG) agar (Difco) containing 10 per cent defibrinated sheep blood (Hema Resources) at 37°C for approximately 72 h. Supplementing BG plates with kanamycin or gentamicin (50 and 20 $\mu g\,ml^{-1}$, respectively; Sigma Aldrich) allowed differentiation between bacteria in mixed infections. For experimental inocula, liquid culture bacteria were grown overnight at 37°C and shaken to mid-log phase (optical density at 600 nm of approx. 0.3) in Stainer-Scholte broth.

(b) *Hosts, vaccination and inoculation*

Four- to six-week-old female C57BL/6 mice (Jackson Laboratories) were maintained in specific pathogen-free rooms at Pennsylvania State University and were handled in accordance with Institutional Animal Care and Use Committee guidelines. In two experiments, a total of 200 mice were divided into eight treatment groups. Half of all mice received two 50 µl subcutaneous injections (on days 0 and 14) of the commercial Adacel vaccine (referred to as aP; Sanofi Pasteur) at one-fifth the human dose, whereas the other half were sham vaccinated sterile phosphate buffered saline (PBS) and both treatments were administered with Imject Alum adjuvant (Thermo Scientific). Using this vaccination protocol, vaccine efficacy in human clinical trials was shown to correlate with bacterial clearance in a murine model of *B. pertussis* (Mills *et al.* 1998; Guiso *et al.* 1999). Adacel vaccines are provided as combined tetanus–diphtheria–pertussis formulation adsorbed to alum and contain the following five *B. pertussis* antigens: 5 $\mu g\,ml^{-1}$ of

detoxified pertussis toxin, 10 μg ml^{-1} filamentous haemagglutinin, 6 μg of pertactin and 10 μg of fimbriae types 2 and 3.

In both vaccinated and sham-vaccinated groups, 27 mice were each infected with *B. pertussis* alone, *B. parapertussis* alone or a mixture of both, and 21 were sham infected with sterile PBS (table S1, electronic supplementary material). Mice were challenged intranasally with 5×10^6 CFU three weeks after the second vaccination (day 35), as described (Harvill *et al.* 1999). For mixed infections, the 50 μl inocula contained 5×10^6 CFU of each of *B. pertussis* and *B. parapertussis*. The same dose of each bacterium in mixed and single infections was used as we wanted to compare the dynamics of each bacterium on its own versus in mixed infections. On each day of sacrifice (table S1, electronic supplementary material; experiment 1: days 0, 3, 7, 14 and 35 post-infection (p.i.) and experiment 2: days 0, 3 and 7 p.i.), three to four mice per group were sacrificed in experiments 1 and 2, respectively. In both experiments, lungs were aseptically removed and homogenized in 1 ml of sterile PBS. Serial dilutions of organ homogenate were plated on BG agar plates containing the relevant antibiotics and cells were incubated for 3–5 days at 37°C to quantify the number of viable bacteria. In experiment 1, blood was collected on each day of sacrifice for the assessment of *Bordetella*-specific serum antibodies.

(c) *Lung cytokines, neutrophil numbers and antibody enzyme linked immunosorbent assays (ELISAs)*

In experiment 1, levels of the lung cytokines interleukin (IL)-4, IL-5, interferon gamma (IFN-γ) and granulocyte macrophage-colony stimulating factor (GM-CSF) were quantified using a flow cytometric cytokine assay, according to the manufacturer's instructions (Bio-Plex Mouse Cytokine T_H1/T_H2 Panel Cytokine Assay and a Bio-Plex cytokine reagent kit, Bio-Rad).

In experiment 2, lung leukocyte numbers were quantified by performing lung perfusions on days 0, 3 and 7 p.i. Briefly, lungs were perfused with sterile PBS on day of sacrifice to remove red blood cells before homogenizing through sterile cell strainers (BD Biosciences). Homogenates were laid on a Histopaque gradient 1119 (Sigma Aldrich), centrifuged at 1500 g for 30 min and the leukocyte layer collected and counted on a haemocytometer at ×40 magnification. Aliquots of cells were stained with fluorescein labelled antibodies (FITC)-labelled anti-Ly-6G to detect neutrophils (*e*Bioscience). The percentage of FITC-positive cells was multiplied by the total number of leukocytes to calculate neutrophil numbers.

Bacteria were grown overnight (optical density of 0.7 at 600 nm), diluted in carbonate buffer and 200 μl added to each well of a 96-well plate. Serum from experiment 1 was added to the first row of coated 96-well plates at a 1 : 50 dilution and serially diluted across the plates to a final dilution of 1 : 102 400. Incubation, wash and development steps were carried out as detailed (Wolfe *et al.* 2005). Total immunoglobulin (Ig) titre was quantified using biotin-conjugated anti-mouse Ig (Southern Biotechnology Associates) and peroxidase-conjugated streptavidin (BD Pharmingen). Results were reported as endpoint titres.

(d) *Statistical analyses*

All analyses were performed in R v. 2.7.0 (http://www.R-project.org) using generalized linear models (GLM; Crawley 2007; R 2008). Analyses focused on 200 mice, with experimental groups as detailed above and in table S1, electronic supplementary material. We assumed lognormal errors in CFU, cytokine and antibody titres and carried out the analysis on the $\log_{10}$ transformed data, using least squares with normal errors and the identity link. Data from duplicate bioassay and triplicate antibody enzyme linked immunosorbent assay (ELISA) plate wells were averaged and the respective titres induced by naive animals were subtracted from experimental animals before being $\log_{10}(n+1)$ transformed to satisfy homogeneity-of-variance and normality-of-error assumptions of models used.

CFU, lung cytokine and antibody data were analysed from days 3 to 35 p.i. inclusive in order to capture the full post-peak dynamics of infection. Main effects were vaccination (aP versus sham vaccinated), infection type (single versus mixed infection) and day p.i. (fitted as a categorical variable). The main effects of infection, vaccination and the infection by vaccination interaction terms explicitly test the main hypothesis of this study. To control for the dynamic kinetics of *Bordetella* infection, the main effect of day—as well as all two-way interactions between day and vaccination or infection type—was included in all analyses. In no cases were any of the three-way interactions significant and so they are not reported. Qualitative differences owing to infection type and vaccination were strong and consistent across experimental blocks and quantitative differences were controlled for by including experimental block as a factor in all analyses. Maximal models were first fit to the data and minimal models reached by removing non-significant terms ($p > 0.05$), beginning with the highest level interaction. Reported parameter estimates were taken from the relevant minimal models.

3. RESULTS

(a) *Vaccine-mediated interactions and mixed infection*

As expected, aP vaccination significantly reduced the CFU of *B. pertussis* (figure 1*a*,*b*; CFU days 3–35 inclusive, vaccination (acellular or sham): $F_{1,72} = 145.9$, $p < 0.0001$; vaccination × day: $F_{3,72} = 6.5$, $p = 0.001$). The average bacterial abundance produced throughout the infection was approximately 700-fold lower in aP-vaccinated relative to sham-vaccinated hosts. By contrast, aP vaccination significantly increased *B. parapertussis* CFU (figure 1*c*,*d*; CFU days 3–35 inclusive, vaccination: $F_{1,72} = 16.9$, $p < 0.0001$; vaccination × day: $F_{3,72} = 5.5$, $p = 0.002$). The average bacterial abundance produced throughout infection was approximately 40-fold higher in aP-vaccinated relative to sham-vaccinated hosts.

We found no evidence to support within-host competitive suppression of either pathogen species by the other. For *B. pertussis*, the vaccine-driven decrease in bacterial abundance was observed independent of whether infections were alone or in a mixture with *B. parapertussis* (figure 1*a*,*b*; CFU days 3–35 inclusive, infection type (mixed versus single) and infection type × vaccination, infection type × day, all $p > 0.05$) and was of similar magnitude in both experimental blocks (block × vaccination: $p > 0.05$). Likewise, the increase in *B. parapertussis* CFU was unaffected by the presence of *B. pertussis* (figure 1*c*,*d*; CFU days 3–35 inclusive, infection type, infection type × vaccination, infection

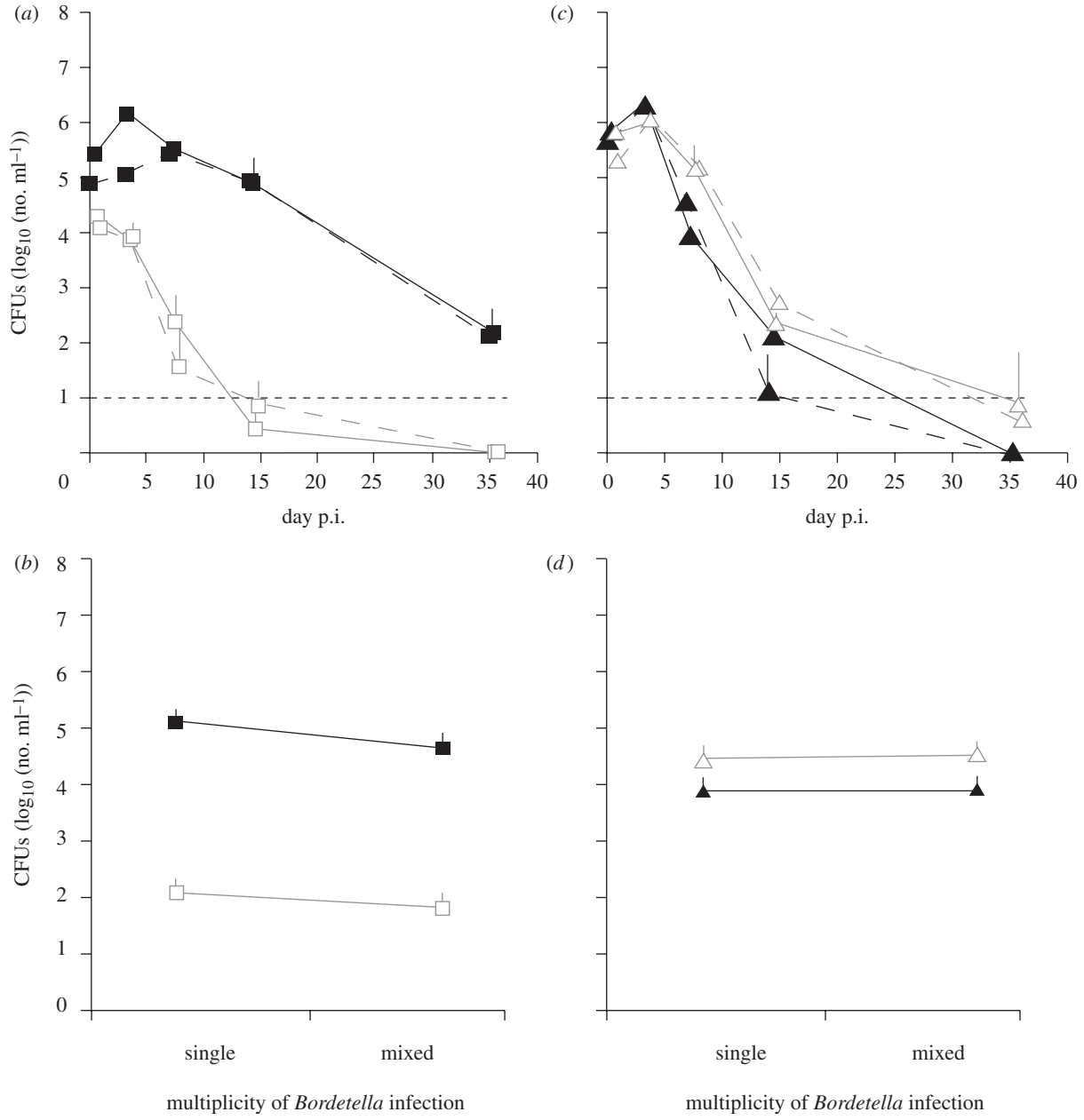

Figure 1. Bacterial lung CFUs. (*a*) Timeline of *B. pertussis* and (*c*) *B. parapertussis* in single and mixed infections of aP- or sham-vaccinated hosts. Average CFU between days 3–35 p.i. (least-squares mean ± s.e.m.) from (*b*) GLMs of *B. pertussis* CFU and (*d*) *B. parapertussis* CFU. Shown is the $\log_{10}$ transformed mean CFU from 200 independent infections produced in two replicate experiments. The *x*-axis is jittered for clarity and dotted grey lines indicate limit of detection. (*a*) Filled (sham) and open (aP) squares represent *B. pertussis* infections alone (sold lines) or in a mixed infection (dashed lines). (*c*) Filled (sham) and open (aP) triangles represent *B. parapertussis* infections alone (solid lines) or in a mixed infection (dashed lines).

type × day and block × vaccination, all $p > 0.05$). Thus, we found no support for the competitive release hypothesis: there was no competition and hence no expansion of *B. parapertussis* when *B. pertussis* was selectively suppressed by aP vaccination.

Consistent with this absence of competition, mixed infections had an approximately twofold higher average CFU relative to single infections (CFU days 3–35 inclusive, infection type: *B. pertussis*, $F_{1,75} = 67.9$, $p < 0.0001$; *B. parapertussis*, $F_{1,75} = 39.4$, $p < 0.0001$, respectively), implying that there is no constrained 'niche space' over which the two species were competing. Thus, aP vaccination enhanced *B. parapertussis* CFUs in the lung, reversing the dominance from *B. pertussis* to *B. parapertussis* independent of the multiplicity of infection, consistent with the ERH.

(b) *Lung cytokines and neutrophil recruitment*

The lung immune response was skewed from a T_H1 towards a predominantly T_H2 response by aP vaccination. Specifically, aP-vaccinated mice produced significantly lower IFN-γ and higher lung IL-5 and IL-4 levels—a cytokine profile characteristic of T_H2 cells—relative to sham-vaccinated mice (figure 2*a–c*; (cytokine) between days 3 and 35, inclusive: IFN-γ vaccination, $F_{1,64}=23.75$, $p < 0.0001$ and vaccination × day, $F_{3,64}=8.9$, $p < 0.0001$; IL-5 vaccination, $F_{1,67} = 14.5$,

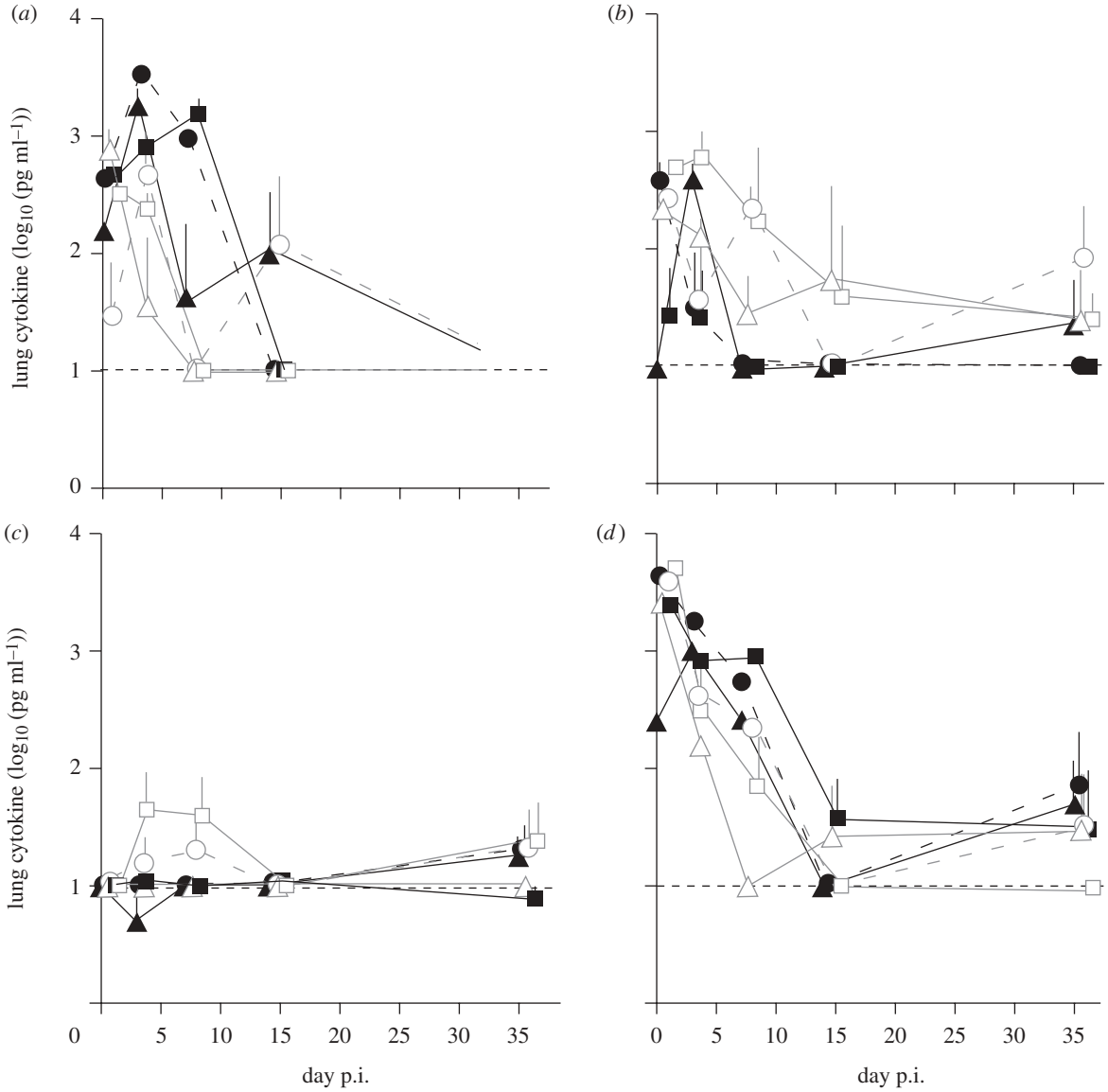

Figure 2. Lung cytokine profiles. Plots show (*a*) timeline of IFN-γ, (*b*) IL-5, (*c*) IL-4 and (*d*) GM-CSF levels induced in lung homogenate during single- and mixed-*B. pertussis* and *B. parapertussis* infections in aP- or sham-vaccinated hosts. The values from 90 independent infections are presented as mean lung cytokine titres ± s.e.m. The *x*-axis is jittered for clarity and dotted grey lines indicate lower limit of detection of assay used. Filled (sham) and open (aP) triangles represent *B. parapertussis*; filled (sham) and open (aP) squares represent *B. pertussis*; and filled (sham) and open (aP) circle represent mixed infections.

$p < 0.0001$ and vaccination × day, $p > 0.05$; IL-4 vaccination, $F_{1,70} = 6.0$, $p = 0.02$ and vaccination × day, $p > 0.05$) and this was true for both *Bordetella* species (infection type, infection type × vaccination and infection type × day terms, all $p > 0.05$). In addition, lung GM-CSF levels were significantly reduced from day 3 p.i. onwards in aP-vaccinated hosts, regardless of the *Bordetella* species or multiplicity of infection (figure 2*d*; (cytokine) between days 3 and 35 inclusive: vaccination, $F_{1,64} = 20.39$, $p < 0.0001$; vaccination × day, $F_{3,64}=3.0$, $p = 0.03$; infection type, infection type × vaccination and infection type × day terms, all $p > 0.05$).

Although the number of neutrophils recruited to the lungs early in infection was significantly lower in aP- relative to sham-vaccinated hosts, the extent of this aP-driven reduction in neutrophils depended on *Bordetella* species; aP-vaccinated hosts infected with *B. parapertussis* (either as a single or mixed infection) had significantly lower neutrophil numbers compared with *B. pertussis*-infected individuals (figure 3; lung neutrophil numbers on days 3–7: vaccination, $F_{1,58} = 4.2$, $p = 0.04$; vaccination × day, $p > 0.05$; infection type, $F_{2,58} = 0.46$, $p = 0.6$; infection type × day, $F_{2,58} = 3.1$, $p = 0.02$; infection type × vaccination, $F_{2,58} = 3.8$, $p = 0.03$).

(c) *Pathogen-specific antibody response*

Acellular vaccination enabled both *B. pertussis*- and *B. parapertussis*-infected hosts to mount more rapid anti-*B. pertussis*- and anti-*B. parapertussis*-specific Ig responses, respectively, relative to their sham-vaccinated counterparts (figure 4*a*,*b*; *B. pertussis*: vaccination, $F_{1,37} = 23.3$, $p < 0.001$; vaccination × day, $F_{3,37} = 4.9$, $p = 0.006$; figure 4*c*,*d*; *B. parapertussis*: vaccination, $F_{1,37} = 24.2$, $p < 0.0001$; vaccination × day, $F_{3,37} = 3.9$, $p = 0.02$, respectively). The extent to which aP vaccination affected the anamnestic responses depended on whether the infection was single or a mixture (figure 4*a*–*d*; *B. pertussis*:

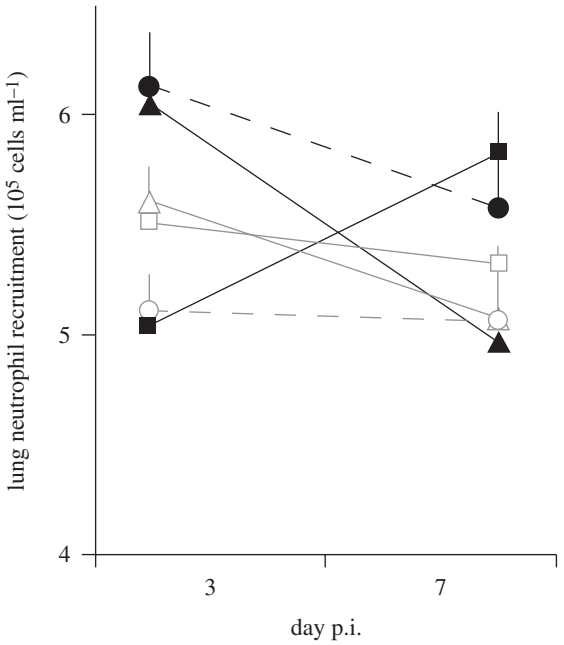

Figure 3. Lung neutrophil recruitment. Plots show neutrophil numbers in the lung on days 3 and 7 p.i. broken down by infection. Each plotted point represents the mean of 12 mice ± s.e.m. Filled (sham) and open (aP) triangles represent *B. parapertussis*; filled (sham) and open (aP) squares represent *B. pertussis*; and filled (sham) and open (aP) circle represent mixed infections.

infection type, $F_{1,37} = 0.8$, $p = 0.4$; infection type × vaccination, $F_{1,37} = 4.6$, $p = 0.03$; *B. parapertussis*, infection type, $F_{1,37} = 0.08$, $p = 0.8$; infection type × vaccination, $F_{1,37} = 4.7$, $p = 0.04$).

4. DISCUSSION

Here we show that aP vaccination accelerated the clearance of *B. pertussis* from the lower respiratory tract (LRT) of mice (figure 1*a*,*b*), but delayed *B. parapertussis* clearance, resulting in approximately 40-fold higher total *B. parapertussis* lung CFUs (figure 1*c*,*d*). Importantly, no evidence to support competitive interactions between *B. pertussis* and *B. parapertussis* was found in either sham- or aP-vaccinated co-infected hosts (figure 1*a*–*d*). An aP vaccine-driven reduction in inflammatory cytokine responses (figure 2) as well as neutrophil recruitment to the lung in response to *B. parapertussis* infection (figure 3)—two key players in the clearance of this pathogen (Kirimanjeswara *et al.* 2005; Mann *et al.* 2005; Wolfe *et al.* 2009)—correlated with delayed *B. parapertussis* clearance. In addition, antibody responses in vaccinated *B. parapertussis*-infected hosts, although robust, were likely to have reduced efficacy relative to non-vaccinated hosts owing to species differences in prominent surface molecules preventing immune cross-protection (Wolfe *et al.* 2007; Zhang *et al.* 2009*a*,*b*). Thus aP vaccination, by priming the host response against *B. pertussis* clearance, confers an advantage to *B. parapertussis* by interfering with optimal immune clearance and resulting in increased lung CFUs, consistent with the ERH outlined in §1 (Mitchell & Power 2003).

Bordetella parapertussis and *B. pertussis* have been thought to compete directly with one another as they exploit the same respiratory tract niche (Bjørnstad & Harvill 2005). However, we found no evidence of within-host competition between *B. parapertussis* and *B. pertussis* in our study: CFUs of each species appeared to be unaffected by the presence of the other (figure 1*a*–*d*). The lack of competition probably arose as total infection densities were not constrained in the lung: infection with two species resulted in total pathogen densities twice that of single-species infections. Indeed, by colonizing discrete areas in the LRT, these distinct infections may avoid direct interaction. Another possibility is that by focusing solely on the LRT, we failed to capture within-host competition between *B. parapertussis* and *B. pertussis* in the upper respiratory tract (URT). *Bordetella* infection is initiated by the attachment of organisms to epithelial cell cilia of the URT, a respiratory area that is thought to act as an important reservoir of *Bordetella* infection (Mattoo & Cherry 2005). Experiments examining the localization of distinct bacterial populations in both the URT and LRT, as well as transmission of bacteria from the respiratory tract (which can be carried out experimentally in rat or rabbit models of bordetellosis), would increase our understanding of colonization and shedding processes respectively, and how these may vary with vaccination status or *Bordetella* species.

What mechanisms are behind the 'enemy release' of *B. parapertussis* under aP vaccination and why does wP vaccination or prior exposure to *B. pertussis* not drive similar increases in *B. parapertussis* CFU (Wolfe *et al.* 2007; Zhang *et al.* 2009*b*)? First, robust T_H1 inflammatory responses and neutrophil recruitment to the LRT are required for optimal anamnestic responses against *B. parapertussis* (Kirimanjeswara *et al.* 2005; Mann *et al.* 2005; Wolfe *et al.* 2005, 2009). Here, we show that aP vaccination skews the host immune response towards a T_H2 response (Barnard *et al.* 1996; Ryan *et al.* 1997) and it is likely that this lack of inflammatory help—reduced lung inflammatory responses and neutrophil recruitment—enables *B. parapertussis* to evade rapid antibody-mediated clearance in our study (Wolfe *et al.* 2009). Second, omission of the critical protective O-antigen from aP vaccine preparations is also likely to reduce aP vaccine efficacy against *B. parapertussis* (Zhang *et al.* 2009*a*) and could contribute towards enhanced infection. Third, aP vaccination may have the potential to provoke immune interference in the form of original antigenic sin. Of those *B. pertussis* antigens contained in the aP vaccine expressed by *B. parapertussis*, antigenic differences exist between the *Bordetella* species and so individuals exposed to a *B. parapertussis* antigen similar, but not identical to one encountered previously, may induce an immune response to the latter antigen directed against the first (Francis 1953; Webster 1966; Klenerman & Zinkernagel 1998). Thus, subunit vaccines with limited epitopes—such as the aP vaccine—may have the potential to prevent appropriate immune responses against challenging *B. parapertussis* bacteria whose epitopes are divergent from those of the vaccine variant and lead to sub-optimal clearance and perhaps enhanced infection.

Importantly, following the effects of aP vaccination on infection dynamics over time allowed us to resolve previously conflicting results concerning the effect of aP on *B. parapertussis* (David *et al.* 2004; Zhang *et al.* 2009*b*).

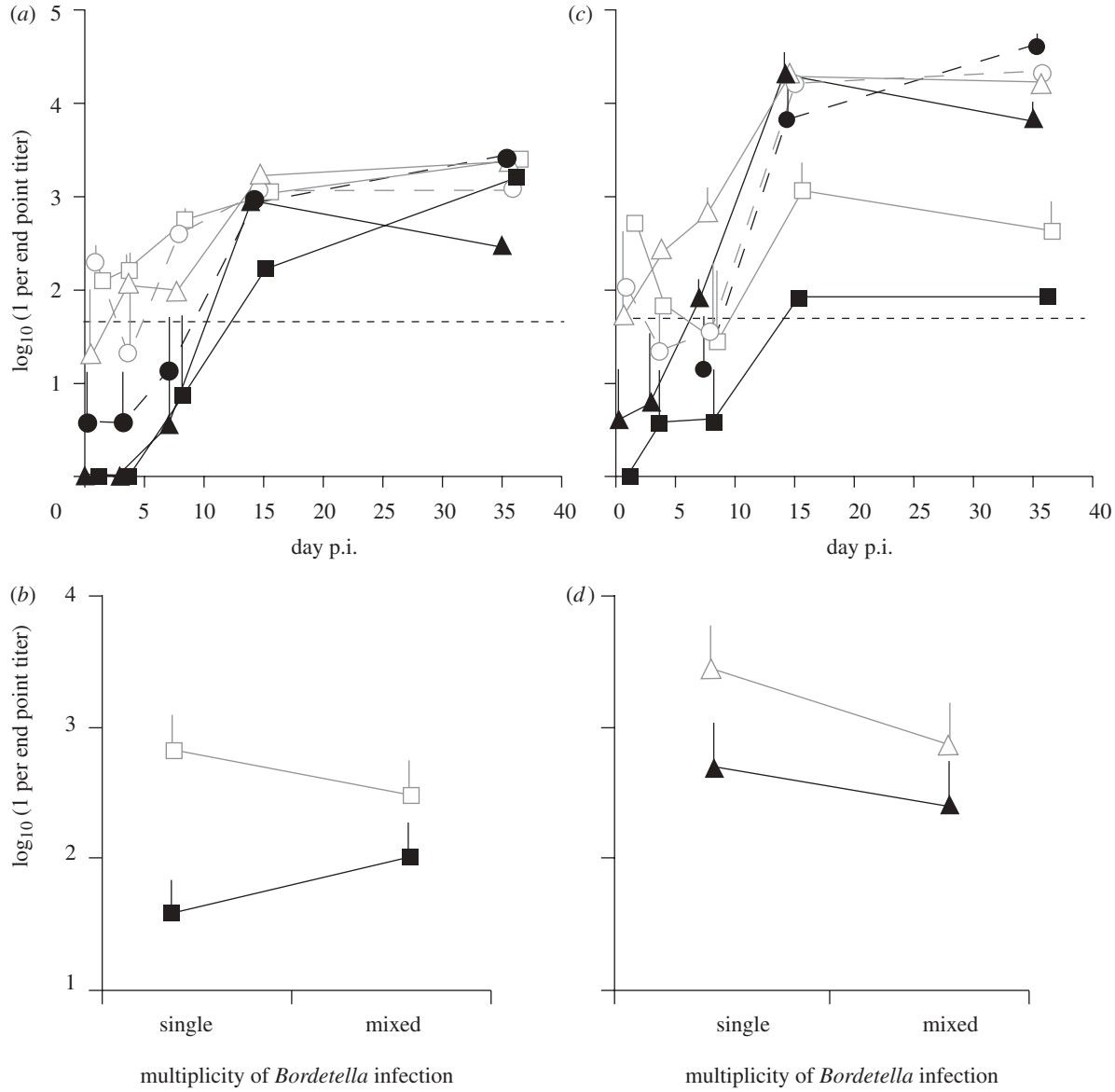

Figure 4. *Bordetella* species-specific serum Ig titres. Timeline showing titres of (*a*) anti-*B. pertussis* total Ig and (*c*) anti-*B. parapertussis* total Ig during single and mixed *B. pertussis* and *B. parapertussis* infections of sham or vaccinated hosts. (*a* and *c*) Filled (sham) and open (aP) triangles represent *B. parapertussis*, filled (sham) and open (aP) squares represent *B. pertussis*, and filled (sham) and open (aP) circles represent mixed infections. Shown is the $\log_{10}$ transformed mean endpoint titre $\pm$ s.e.m. Average Ig titre between days 3–35 p.i. (least-squares mean $\pm$ s.e.m.) from (*b*) GLMs of anti-*B. pertussis* Ig and (*d*) anti-*B. parapertussis* Ig. The means from 120 independent infections are presented. The *x*-axis is jittered for clarity and dotted grey lines indicate limit of detection.

Specifically, we show that the effect of aP vaccination on *B. parapertussis* infection varied temporally—aP vaccination did not affect *B. parapertussis* lung CFU on day 3 p.i. consistent with Zhang *et al.* (2009*b*), but enhanced CFU on day 7 p.i. consistent with David *et al.* (2004) (figure 1*a–d*)—which resolves these previously conflicting studies and highlights the importance of tracking dynamics throughout infection in order to capture full effects of the treatment of interest. It is possible that these findings may be relevant only to the specific strains we have examined and further studies should be carried out to determine if our results hold across *B. pertussis* and *B. parapertussis* strains.

As always, it is important to be cautious about extrapolating from animal models to humans. The dynamics of *B. pertussis* and *B. parapertussis* infection in rodent hosts shares many similarities with human infection, but like all experimental models, differs from the human situation in a number of key ways (Elahi *et al.* 2007). However, the relative efficacies of pertussis vaccines in the rodent model correspond to those obtained in clinical trials (Mills *et al.* 1998; Guiso *et al.* 1999), and we note that epidemiological evidence in human whooping cough infections is consistent with an enhancement effect for *B. parapertussis* (Bergfors *et al.* 1999; Liese *et al.* 2003). Directly proving aP vaccination puts treated people at risk of acquiring *B. parapertussis* is very difficult, but we hope our study highlights the need for more thorough *B. parapertussis* epidemiological data and encourages further work in this neglected area. If our experiments are capturing the phenomenology of what is happening under aP vaccination in humans, it may be important to consider the introduction of vaccines that better

protect against both bordetellae; for example, live attenuated *B. pertussis* nasal vaccines (Mielcarek *et al.* 2006), wP vaccines containing both *B. pertussis* and *B. parapertussis* (Burianova-Vysoka *et al.* 1970), or supplementation of aP vaccines with *B. parapertussis* protective antigens (Zhang *et al.* 2009*a*). An enhanced understanding of the evolutionary consequences of widespread aP vaccination is needed in order to optimize the next generation of vaccination strategies and fully reap the benefits of this powerful medical intervention.

All procedures were carried out in accordance with Institutional Animal Care and Use Committee guidelines.

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The impact of parental postpartum pertussis vaccination on infection in infants: A population-based study of cocooning in Western Australia

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Abstract

During a pertussis epidemic in 2011-2012 the Western Australian (WA) Department of Health implemented a 'cocooning' programme, offering free pertussis-containing vaccine (dTpa) to new parents. We assessed the impact of vaccinating parents with dTpa on the incidence of pertussis infection in newborns. Births in WA during 2011-2012 were linked to a register of parental pertussis vaccinations and to notified reports of laboratory-proven pertussis in children <6 months of age. Parents who received dTpa during the four weeks after their child's birth were defined as 'vaccinated postpartum.' Cox proportional-hazards methods were used to calculate hazard ratios (HRs) and 95% confidence intervals (CIs) for the risk of pertussis infection among infants born to parents vaccinated postpartum vs. unvaccinated parents, adjusted for maternal age, geographic region, timing of birth, and number of siblings. Of 64,364 live-births, 43,480 (68%) infants had at least one vaccinated parent (60% of mothers and 36% of fathers). After excluding records where parent(s) were either vaccinated prior to the birth, vaccinated >28 days after the birth, the vaccination date was uncertain, or the child died at birth (n=42), the final cohort contained 53,149 children, 118 of whom developed pertussis. There was no difference in the incidence of pertussis among infants whose parents were both vaccinated postpartum compared to those with unvaccinated parents (1.9 vs 2.2 infections per 1000 infants; adjusted HR 0.91; 95%CI 0.55-1.53). Similarly, when assessed independently, maternal postpartum vaccination was not protective (adjusted HR 1.19; 95%CI 0.82-1.72). Supplemental sensitivity analyses which varied the time period for parental vaccination and accounted for under-reporting of vaccination status did not significantly alter these findings. In our setting, vaccinating parents with dTpa during the four weeks following delivery did not reduce pertussis diagnoses in infants. WA now provides dTpa vaccine to pregnant women during the third trimester.

Keywords: Bordetella pertussis; Cocooning; Immunisation; Pertussis vaccine; Public health; Whooping cough.

Finding the 'who' in whooping cough: vaccinated siblings are important pertussis sources in infants 6 months of age and under

Christina Bertilone, Tania Wallace, Linda A Selvey

Abstract

Objectives: To describe the epidemiology of pertussis, and to identify changes in the source of pertussis in infants 6 months of age and under, during the 2008–2012 epidemic in south metropolitan Perth.

Design and setting: Analysis of all pertussis cases notified to the South Metropolitan Population Health Unit and recorded on the Western Australian Notifiable Infectious Disease Database over the study period. Information on the source of pertussis was obtained from enhanced surveillance data.

Results: Notification rates were highest in the 5–9 years age group, followed by the 0–4 years and 10–14 years age groups. There was a significant increase in the proportion of known sources who were siblings from the early epidemic period of 2008–2010, compared with the peak epidemic period of 2011–2012 (14.3% versus 51.4%, $p = 0.002$). The majority of sibling sources were fully vaccinated children aged 2 and 3 years.

Conclusions: The incidence of pertussis was highest in children aged 12 years and under in this epidemic. At its peak, siblings were the most important sources of pertussis in infants 6 months and younger, particularly fully vaccinated children aged 2 and 3 years. Waning immunity before the booster at 4 years may leave this age group susceptible to infection. Even if cocooning programs could achieve full vaccination coverage of parents and ensure all siblings were fully vaccinated according to national schedules, waning immunity in siblings could provide a means for ongoing transmission to infants. Recent evidence suggests that maternal antenatal vaccination would significantly reduce the risk of pertussis in infants 3 months of age and under. *Commun Dis Intell* 2014;38(3):E195–E200.

Keywords: pertussis, whooping cough, infants, source, vaccination, immunisation

Introduction

The incidence of pertussis (whooping cough) has risen both in Australia and internationally over recent years, and large epidemics have occurred.[1,2] Increased clinician awareness and laboratory testing are likely to be partially responsible for the apparent increase in disease incidence.[3] However, the epidemiology of pertussis in Australia and the United States of America has also changed in recent times, with an increasing proportion of disease occurring in children.[4–7] Possible reasons for this include the increasing use of less effective acellular vaccines[8–10] and increasing circulation of *Bordetella pertussis* strains deficient of vaccine antigen.[11,12] Within vaccinated populations, the fewer whole cell vaccines received, the greater the risk of pertussis.[8,10] Additionally, immunity from acellular pertussis vaccination wanes more rapidly than that from whole cell vaccination.[13–15] Pertussis morbidity and mortality are greatest in infants under the age of 6 months, who are too young to have completed a primary vaccination course. The implications of these changes for the source of infant pertussis remain unclear.

Household contacts are the most likely sources of infant pertussis, but there is variation in the proportion of sources reported to be parents as opposed to siblings. A recently published Australian review on infant pertussis sources reported the source as a parent in 55% (range 39%–57%) and a sibling in 16%–43%.[16] The proportion of sources that were siblings varied widely between studies, in comparison to the proportion that were parents, which were more consistent. The conclusion was that siblings may be more important sources of infant pertussis than previously realised.[16]

A prolonged outbreak of pertussis occurred in Australia, including south metropolitan Perth, between 2008 and 2012. A cocooning strategy involving the vaccination of caregivers of newborns was implemented in Western Australia and ran for 2011 and 2012 in attempts to protect newborns during the outbreak. This strategy can only be effective if caregivers are the main source of pertussis in infants.

Over the study period, the South Metropolitan Population Health Unit (SMPHU) collected enhanced surveillance data for pertussis cases in

children under 5 years of age. These data are not collected or reported at the national level so provide valuable additional information, particularly regarding source of infection, to that routinely collected for the National Notifiable Diseases Surveillance System. This study aimed to describe the epidemiology of the epidemic in south metropolitan Perth in relation to the source of infant pertussis, as well as any changes in the epidemiology and the source that occurred over the 5-year period.

Methods

The SMPHU is responsible for the follow up of notifiable diseases for the area covered by the South Metropolitan Health Service, which spans all of metropolitan Perth south of the Swan River and services approximately 37% of the Western Australian population.[17] Over the study period, the SMPHU collected enhanced surveillance data for pertussis cases in children under 5 years of age. The process involves a trained public health nurse interviewing the treating doctor and caregiver of the notified case, in order to obtain further information such as the likely source of infection and any high risk contacts. Enhanced surveillance defines a source of pertussis as a contact of the notified case who had either prolonged coughing illness or known pertussis infection, who was in contact with the notified case during the latter's incubation period (from 6 to 21 days prior to symptom onset). In the case of multiple possible sources, the source was assumed to be the individual who first became symptomatic, provided that the source's infectious period coincided with the notified case's incubation period.

Enhanced surveillance data for notified cases in infants 6 months of age and under were examined retrospectively, as well as pertussis notification data recorded on the Western Australian Notifiable Infectious Disease Database (WANIDD) for all age groups. All confirmed and probable cases meeting the case definition for pertussis were included if the optimal date of onset of pertussis occurred any time from 1 January 2008 to 31 December 2012, and residential postcode was within the SMPHU catchment area. The optimal date of onset refers to the earliest date recorded on WANIDD reflecting disease onset. In some situations, such as those where the caregiver of the notified case could not be contacted by telephone, enhanced surveillance data were not available. Notified cases and sources were defined as being fully vaccinated for age if on the optimal date of onset of illness they had received all pertussis vaccinations recommended by the Western Australian immunisation schedule for their age. This would potentially include vaccinations given within the 14 days preceding disease onset. The dates of vaccination for the source were not available so any such cases would be misclassified as being fully vaccinated for age at disease onset. Notified cases from the 2008–2010 and 2011–2012 periods were compared because this distinction allowed comparison of the pre-cocooning period with the cocooning period, and the early epidemic period with the peak epidemic period. Differences in age specific risk of infection as well as source of infant pertussis in the 2 periods were assessed.

Denominator data for notification rates were obtained from the Epidemiology Branch of the WA Department of Health. All analyses were performed in SPSS version 21. All comparisons were performed using chi-squared analyses or Fisher's exact test for categorical variables, and Mann-Whitney U testing for continuous variables. The study was approved by the Curtin University Human Research Ethics Committee (protocol approval SPH-16-2013). Ethics approval was not sought elsewhere, as this study formed part of the core business of the SMPHU.

Results

There were 3,611 cases of pertussis notified to the SMPHU from 2008 to 2012, with this period demonstrating a dramatic increase in notifications in comparison with previous years (Figure 1). Of these cases, 37.3% (n = 1348) occurred in children 12 years of age or under. At the peak of the epidemic in the December 2011 quarter, notification rates were markedly higher in children in age categories 14 years of age and under in comparison with the remainder of the population (Figure 1, Figure 2). The notification rate for the 5–9 years age group in the December 2011 quarter was 341.4 per 100,000, and 243.0 per 100,000 for the 10–14 years age group. Notification rates peaked in adults in this quarter also, but the amplitude of the peak was much less marked (56.0 per 100,000). Notification rates in children 4 years of age and under did not peak until the following quarter, at 206.8 per 100,000.

Of the 115 cases of pertussis in infants 6 months of age and under, enhanced surveillance data were available for 106 (92.2%). The optimal date of onset was the date of symptom onset for 111 of 115 cases, and the laboratory specimen date for the remaining four. There were no significant differences between those who had undergone enhanced surveillance and those who had not, comparing gender ($p = 0.74$), age ($p = 0.56$), ethnicity ($p = 1.00$) and hospitalisation status ($p = 0.48$).

The source was identified in 65 of 106 cases (61.3%). Two potential sources were identified for two of these cases, and one for the remaining 104 cases.

Figure 1: Notification rates of pertussis, south metropolitan Perth, 2008 to 2012, by quarter and age group

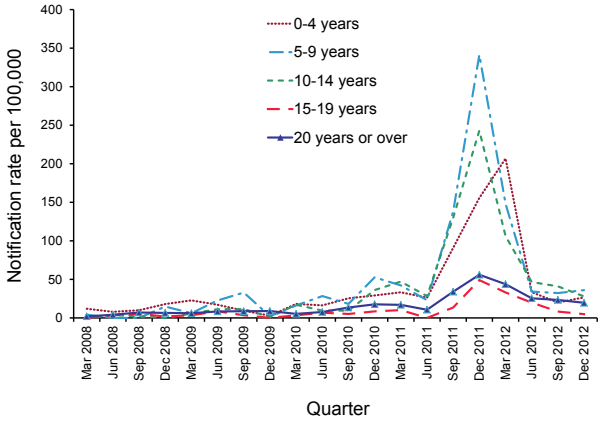
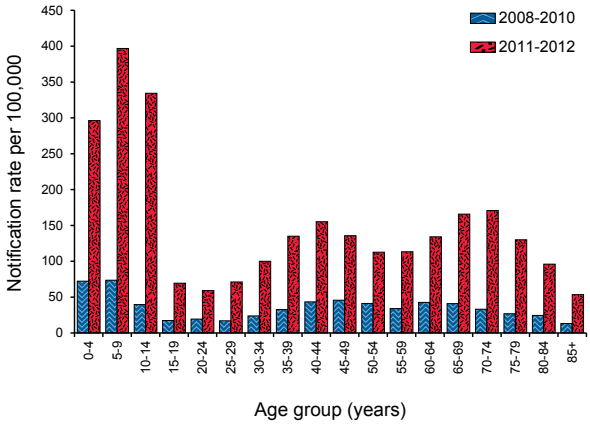


Figure 2: Notification rate of pertussis, south metropolitan Perth, 2008 to 2010 compared with 2011 to 2012, by age group



The proportion of sources whose diagnosis was confirmed with laboratory testing was unknown. Over the 5-year period, the source was a parent in 38.5% (n = 25) of cases and a sibling in 35.4% (n = 23) of cases. The most likely source of pertussis differed in the 2008–2010 period compared with the 2011–2012 period (Table). The proportion of parents as a source was lower in the 2011–2012 period (32.4%, n = 12 versus 46.4%, n = 13). However this difference was not statistically significant (p = 0.25). In contrast, the proportion of sources that were siblings was significantly higher in the 2011–2012 period (51.4%, n = 19 versus 14.3%, n = 4; p = 0.002).

During the 2011–2012 peak epidemic period, the ages of 14 of 19 sibling sources were known. Eight of these sources were aged from 2 to 4 years with five being fully vaccinated, one partially vaccinated, one unvaccinated, and one of unknown vaccination status. The true number of children in the 2–4 years age group may have been higher as the ages of 5 children were not recorded. Three sources were aged 6–11 years, and three were aged 12–19 years. Of all children in south metropolitan Perth diagnosed with pertussis in 2008–2012 and aged from 7 months to 4 years, 78.1% (n = 267) were fully vaccinated for age.

Discussion

Recent studies have shown an increasing incidence of pertussis in children but the implications of this for the source of infant pertussis have not been fully described. Identifying the source of pertussis in infants 6 months of age and under is crucial for the development of effective preventive strategies in this age group. However, the most likely source of infection will reflect local epidemiology, and if

Table: Source of pertussis in infants 6 months of age and under, south metropolitan Perth, 2008 to 2010 compared with 2011 to 2012

	2008-2010			2011-2012			Total		
	n	Known source %	Notified cases %	n	Known source %	Notified cases %	n	Known source %	Notified cases %
Parent	13	46.4	24.5	12	32.4	19.4	25	38.5	21.7
Sibling	4	14.3	7.5	19	51.4	30.6	23	35.4	20
Other household contact	3	10.7	5.7	2	5.4	3.2	5	7.6	4.3
Grand parent	3	10.7	5.7	3	8.1	4.8	6	9.2	5.2
Cousin	3	10.7	5.7	0	0	0	3	4.6	2.6
Other household contact	2	7.1	3.8	1	2.7	1.6	3	4.6	2.6
Total known source	28			37			65		
Notified cases with available enhanced surveillance data	45			61			106		
Notified cases 6 months of age and under	53			62			115		

the age specific risk of infection changes during epidemics, the source of pertussis in infants could vary at different points in the epidemic cycle. This study demonstrates changes in the source of infant pertussis corresponding with changing age specific risk of infection during an epidemic period.

Notification rates were highest in children in this epidemic, particularly at its peak in the 2011–2012 period. This correlated with a dramatic rise in the proportion of sibling sources. There are several possible explanations for the high notification rates in children. Recent studies suggest that acellular pertussis vaccine immunity wanes more rapidly than that of the whole cell pertussis vaccine.[8,10,13–15] The vaccine effectiveness of the whole cell pertussis vaccine previously administered in Australia was estimated at 91% (95% CI 85.5%–94.4%) in infants aged 8–23 months, and 84.5% (95% CI 78.3%–88.9%) in the 2–4 years age group.[19] In contrast, a recent Australian study reported the vaccine effectiveness of acellular vaccine to be 83.5% (95% CI 79.1%–87.8%) in infants aged 6–11 months, falling to 70.7% (95% CI 64.5%–75.8%) in children aged 2 years, and 59.2% (95% CI 51.0%–66.0%) in children aged 3 years.[20] In the whole cell pertussis vaccine effectiveness study, children had received 5 doses of pertussis vaccine by age 5 (2, 4, 6, 18 months and 4 years). In contrast, the acellular pertussis vaccine effectiveness for the children aged 2 and 3 years was calculated for children receiving 3 doses of vaccine, reflecting the current pertussis vaccination schedule of 2, 4, 6 months and 4 years.[20]

The high notification rates in children and the higher percentage of sibling sources could also be epidemic specific features, given the timing of this study. This is feasible as studies of contact patterns have shown high levels of assortative mixing in children.[21] Age specific infection risk and infant pertussis source types may be different in the inter-epidemic period. This would be congruent with the findings of this study, given that proportions of sources that were parents and siblings in the 2008–2010 period were comparable with those reported in previous literature.[16] Even if high incidence of pertussis in children and high proportions of siblings as sources are purely epidemic specific features, there are still implications for infant pertussis control measures during epidemics.

Cocooning programs are challenging to implement and there is no definitive evidence that they are successful in reducing the incidence of infant pertussis.[22,23] Parents remain susceptible to pertussis for 14 days following immunisation, due to the time taken to mount an immune response.[24] The earlier parental immunisation is performed post-natally, the better protected infants will be, making hospital-based vaccination ideal. Barriers to this have been identified, including legal issues related to vaccinating fathers (who are not hospital patients), and the need to provide after-hours services.[25] In Western Australia in 2011, an estimated 60% of mothers and 41% of fathers of newborns had been administered government funded pertussis vaccine, although the timing of this vaccination post-natally is unknown (2012 data not available at the time of publication).[26] These rates were similar to coverage rates reported in Victoria for the duration of their state wide cocooning program, where it was found that of those eligible, 68% of mothers and 49% of fathers were vaccinated.[22] In metropolitan areas of Victoria, 6% of mothers and 10% of fathers were vaccinated in the maternity hospital, compared with 70% of mothers and 42% of fathers in rural areas, suggesting that (particularly in metropolitan areas) vaccination may not have been given early enough in the neonatal period.[22] In this study, although the proportion of sources that were parents was lower in the cocooning period (2011–2012) compared with the pre-cocooning period (2008–2010), this observation did not reach statistical significance. While this may be a real finding, there were insufficient numbers in this study to determine that. If the difference in the proportion of source cases that were parents in the 2 periods were real, cocooning may explain this reduction, but it is likely to be insufficient to explain the observed increase in the proportion of sibling sources.

The increasing proportion of sibling sources over time reflected the increasing proportion of pertussis notifications in children 12 years of age and under over the 2008–2012 epidemic. In the peak epidemic period, sibling sources of infection were most likely to be aged 2 or 3 years. This suggests that the impact of high notification rates was greatest in the youngest siblings, despite the greatest numbers of cases occurring in children aged 7–11 years. Possible reasons for this include that siblings tend to be close in age, and that younger children are generally less able to control respiratory secretions. The only other recent Australian study of infant pertussis sources had similar findings, demonstrating that siblings aged 3 and 4 years were particularly important sources of infant pertussis during the 2009 epidemic in New South Wales.[27] Dutch research published in 2010 speculated that the high proportion of infant pertussis sources that were siblings (41%) in their study may have been related to the introduction of acellular pertussis vaccine in the Netherlands, as well as prior use of a less effective whole cell vaccine.[24] In that study, the source was a sibling aged 1–4 years in 18% of cases (95% CI 12%–25%), a sibling aged 5–8 years in 15% of cases (95% CI 9%–21%), and a sibling aged 9–13 years in

8% of cases (95% CI 4%–13%). The vaccination schedule for that population involved vaccination at 2, 3, 4 and 11 months, with a booster at 4 years introduced 5 years prior to the commencement of the study. There is a possibility that with the introduction of acellular pertussis vaccine, the interval between primary vaccination and booster doses in both the Dutch and Australian populations is now too long, resulting in waning immunity before the booster at 4 years. Even if all household contacts of newborns (including siblings) could be routinely fully vaccinated, the issue of breakthrough disease prior to the booster at 4 years would leave a certain proportion of siblings as possible infant pertussis sources, limiting the effectiveness of cocooning.

Vaccination in the 3rd trimester of pregnancy is an alternative measure for prevention of infant pertussis, with the benefit of placental transfer of maternal IgG to the infant. The vaccine effectiveness of the maternal antenatal vaccination program in the United Kingdom was estimated at 91% (84%–95% CI) for infants aged 3 months or less.[28] Following the introduction of the program, significant reductions in infant pertussis mortality, numbers of confirmed cases and numbers of hospitalisations were reported.[28] Adverse event surveillance has not detected any significant complications of maternal vaccination to date,[29] but further investigation is required into the possibility of infant immune response blunting.[28] Neonatal vaccination is an alternative possible means of infant pertussis control but similar concerns exist regarding immune blunting, requiring further study.[30] More research is also required to determine whether these observed antibody responses translate into lower incidence of pertussis in infants.

This study is a retrospective review of the data collected as part of the routine surveillance of pertussis, meaning there are several limitations. The source of pertussis was unable to be identified in 38.7% (n = 41) of cases who underwent enhanced surveillance. Previously published Australian studies on the source of infant pertussis have been unable to identify a source in 31%[27] and 49%[31] respectively. This could be due to the source being an asymptomatic or mildly unwell household contact, or a contact from outside the household unknown to the notified case or caregiver undergoing interview. If previously vaccinated adults are more likely to experience mild or asymptomatic illness, the proportion of infant pertussis sources that were parents could be underestimated in studies relying on the recall of the notified case and epidemiologic linkage rather than laboratory testing. However, siblings were the most common source of infant pertussis in a recently published study, which performed laboratory testing on all household contacts in order to identify the source.[24]

Another reason for the higher proportion of siblings noted in the 2011–2012 period could be that as the epidemic progressed, clinician awareness of pertussis in younger children increased, with a concurrent increase in laboratory testing. If this were the case, previous reports of sibling sources of infant pertussis may have underestimated the true proportion of sources attributable to siblings. Regardless, there are still implications for infant pertussis prevention and control measures.

This study has shown that a rapid increase in notification rates in children at the peak of the 2008–2012 epidemic in south metropolitan Perth was accompanied by a significant increase in siblings as sources of pertussis in young infants. In the face of widespread vaccination with a less effective acellular pertussis vaccine, it seems likely that notification rates will remain high in children. Fully vaccinated siblings aged 2 and 3 years were the most important infant pertussis sources in the peak epidemic period of this study, suggesting that immunity may wane in this age group before the vaccine booster at 4 years . Even if it were possible to fully cocoon infants through a combination of parental vaccination and ensuring siblings were fully vaccinated, the possibility of transmission via breakthrough disease in siblings would persist. The risk of sibling transmission to infants would be significantly reduced through the addition of a pertussis vaccine booster at 18 months and maternal antenatal vaccination, for which evidence of effectiveness at preventing pertussis in infants 3 months of age or less is mounting.

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Polio Vaccine

IPV

Inactivated poliovirus vaccine

Inactivated polio vaccine (IPV) was developed in 1955 by Dr Jonas Salk. Also called the Salk vaccine IPV consists of inactivated (killed) poliovirus strains of all three poliovirus types. IPV is given by intramuscular or intradermal injection and needs to be administered by a trained health worker. IVP produces antibodies in the blood to all three types of poliovirus. In the event of infection, these antibodies prevent the spread of the virus to the central nervous system and protect against paralysis.

Advantages

- As IPV is not a 'live' vaccine, it carries no risk of VAPP.
- IPV triggers an excellent protective immune response in most people.

Disadvantages

- IPV induces very low levels of immunity in the intestine. As a result, when a person immunized with IPV is infected with wild poliovirus, the virus can still multiply inside the intestines and be shed in the faeces, risking continued circulation.
- IPV is over five times more expensive than OPV. Administering the vaccine requires trained health workers, as well as sterile injection equipment and procedures.

Safety

IPV is one of the safest vaccines in use. No serious systemic adverse reactions have been shown to follow vaccination.

Efficacy

IPV is highly effective in preventing paralytic disease caused by all three types of poliovirus.

Recommended use

An increasing number of industrialized, polio-free countries are using IPV as the vaccine of choice. This is because the risk of paralytic polio associated with continued routine use of OPV is deemed greater than the risk of imported wild virus.

However, as IPV does not stop transmission of the virus, OPV is used wherever a polio outbreak needs to be contained, even in countries which rely exclusively on IPV for their routine immunization programme.
Once polio has been eradicated, use of all OPV will need to be stopped to prevent re-establishment of transmission due to VDPVs.

World Health Organization

Poliomyelitis: Vaccine derived polio

19 April 2017 | Q&A

What is vaccine-derived polio?

Oral polio vaccine (OPV) contains an attenuated (weakened) vaccine-virus, activating an immune response in the body. When a child is immunized with OPV, the weakened vaccine-virus replicates in the intestine for a limited period, thereby developing immunity by building up antibodies. During this time, the vaccine-virus is also excreted. In areas of inadequate sanitation, this excreted vaccine-virus can spread in the immediate community (and this can offer protection to other children through 'passive' immunization), before eventually dying out.

On rare occasions, if a population is seriously under-immunized, an excreted vaccine-virus can continue to circulate for an extended period of time. The longer it is allowed to survive, the more genetic changes it undergoes. In very rare instances, the vaccine-virus can genetically change into a form that can paralyse – this is what is known as a circulating vaccine-derived poliovirus (cVDPV).

It takes a long time for a cVDPV to occur. Generally, the strain will have been allowed to circulate in an un- or under-immunized population for a period of at least 12 months. Circulating VDPVs occur when routine or supplementary immunization activities (SIAs) are poorly conducted and a population is left susceptible to poliovirus, whether from vaccine-derived or wild poliovirus. Hence, the problem is not with the vaccine itself, but low vaccination coverage. If a population is fully immunized, they will be protected against both vaccine-derived and wild polioviruses.

Since 2000, more than 10 billion doses of OPV have been administered to nearly 3 billion children worldwide. As a result, more than 13 million cases of polio have been prevented, and the disease has been reduced by more than 99%. During that time, 24 cVDPV outbreaks occurred in 21 countries, resulting in fewer than 760 VDPV cases.

Until 2015, over 90% of cVDPV cases were due to the type 2 component in OPV. With the transmission of wild poliovirus type 2 already successfully interrupted since 1999, in April 2016 a switch was implemented from trivalent OPV to bivalent OPV in routine immunization programmes. The removal of the type 2 component of OPV is associated with significant public health benefits, including a reduction of the risk of cases of cVDPV2.

The small risk of cVDPVs pales in significance to the tremendous public health benefits associated with OPV. Every year, hundreds of thousands of cases due to wild polio virus are prevented. Well over 10 million cases have been averted since large-scale administration of OPV began 20 years ago.

Circulating VDPVs in the past have been rapidly stopped with 2–3 rounds of high-quality immunization campaigns. The solution is the same for all polio outbreaks: immunize every child several times with the oral vaccine to stop polio transmission, regardless of the origin of the virus.

Rotavirus Vaccine

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Comparison of virus shedding after lived attenuated and pentavalent reassortant rotavirus vaccine

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Abstract

Transmission of rotavirus vaccine or vaccine-reassortant strains to unvaccinated contacts has been reported. Therefore, it is essential to evaluate and characterize the nature of vaccine-virus shedding among rotavirus vaccine recipients. Two groups of healthy infants who received a complete course of RotaTeq (RV5) or Rotarix (RV2) were enrolled (between March 2010 and June 2011) to compare fecal shedding for one month after each vaccine dose. Shedding was assessed using both enzyme immunoassay (EIA) and real-time reverse transcription-polymerase chain reaction (RT-PCR). Eighty-seven infants (34 girls and 53 boys) were enrolled in the study. After the first vaccine dose, the peak time of virus shedding occurred between day 4 and day 7, with positive detection rates of 80-90% by real-time RT-PCR and 20-30% by EIA. In both groups, vaccine shedding occurred as early as one day and as late as 25-28 days. Mixed effects logistic regression analysis of real-time RT-PCR data showed no significant differences between two groups when shedding rates were compared after the first vaccine dose (odds ratio [OR] 1.26; P=0.71) or after the second vaccine dose (odds ratio [OR] 1.26; P=0.99). However, infants receiving RV2 shed significantly higher viral loads than those receiving RV5 when compared after the first vaccine dose (P=0.001) and after the second dose (P=0.039). In terms of shedding rates detected by real-time RT-PCR, vaccine uptake of RV5 or RV2 among infants in Taiwan was comparable. Clinical significance of higher shedding viral loads in RV2 should be further observed.

Keywords: Rotavirus vaccine; Shedding.

Chickenpox (Varicella) Vaccine

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Transmission of varicella-vaccine virus from a healthy 12-month-old child to his pregnant mother

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PMID: 9255208 DOI: 10.1016/s0022-3476(97)70140-9

Free article

Abstract

A 12-month-old healthy boy had approximately 30 vesicular skin lesions 24 days after receiving varicella vaccine. Sixteen days later his pregnant mother had 100 lesions. Varicella-vaccine virus was identified by polymerase chain reaction in the vesicular lesions of the mother. After an elective abortion, no virus was detected in the fetal tissue. This case documents transmission of varicella-vaccine virus from a healthy 12-month-old infant to his pregnant mother.

Comment in

Transmission of varicella-vaccine virus: what is the risk?

Wald ER.

J Pediatr. 1998 Aug;133(2):310-1. doi: 10.1016/s0022-3476(98)70250-1.

PMID: 9709733 No abstract available.

EFFECTIVENESS / OUTBREAKS / HERD IMMUNITY

Antibodies

Vaccine. 2001 Oct 15;20 Suppl 1:S38-41. doi: 10.1016/s0264-410x(01)00288-2.

What are the limits of adjuvanticity?

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PMID: 11587808 DOI: 10.1016/s0264-410x(01)00288-2

Abstract

Vaccines developed traditionally following empirical approaches have often limited problems of immunogenicity, probably due to the low level of purity of the active component(s) they contain. The application of new technologies to vaccine development is leading to the production of purer (e.g. recombinant) antigens which, however, tend to have a poorer immunogenicity as compared to vaccines of the previous generation. The search for new vaccine adjuvants involves issues related to their potential limits. Since the introduction of aluminium salts as vaccine adjuvants more than 70 years ago, only one adjuvant has been licensed for human use. The development of some of these new vaccine adjuvants has been hampered by their inacceptable reactogenicity. In addition, some adjuvants work strongly with some antigens but not with others, thus, limiting their potentially widespread use. The need to deliver vaccines via alternative routes of administration (e.g. the mucosal routes) in order to enhance their efficacy and compliance has set new requirements in basic and applied research to evaluate their efficacy and safety. Cholera toxin (CT) and labile enterotoxin (LT) mutants given along with intranasal or oral vaccines are strong candidates as mucosal adjuvants. Their potential reactogenicity is still matter of discussions, although available data support the notion that the effects due to their binding to the cells and those due to the enzymatic activity can be kept separated. Finally, adjuvanticity is more often evaluated in terms of antigen-specific antibody titers induced after parenteral immunization. It is known that, in many instances, antigen-specific antibody titers do not correlate with protection. In addition, very little is known on parameters of cell-mediated immunity which could be considered as surrogates of protection. Tailoring of new adjuvants for the development of vaccines with improved immunogenicity/efficacy and reduced reactogenicity will represent one of the major challenges of the ongoing vaccine-oriented research.

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Science News

from research organizations

Antibodies are not required for immunity against some viruses

Date: March 1, 2012

Source: Cell Press

Summary: A new study turns the well established theory that antibodies are required for antiviral immunity upside down and reveals that an unexpected partnership between the specific and non-specific divisions of the immune system is critical for fighting some types of viral infections. The research may lead to a new understanding of the best way to help protect those exposed to potentially lethal viruses, such as the rabies virus.

FULL STORY

A new study turns the well established theory that antibodies are required for antiviral immunity upside down and reveals that an unexpected partnership between the specific and non-specific divisions of the immune system is critical for fighting some types of viral infections. The research, published online on March 1st in the journal *Immunity* by Cell Press, may lead to a new understanding of the best way to help protect those exposed to potentially lethal viruses, such as the rabies virus.

The immune system has two main branches, innate immunity and adaptive immunity. Innate immunity is a first line of defense that relies on cells and mechanisms that provide non-specific immunity. The more sophisticated adaptive immunity, which counts antibody-producing B cells as part of its arsenal, is thought to play a major role in the specific response to viral infections in mammals. However, adaptive immune responses require time to become fully mobilized.

"Mice infected with vesicular stomatitis virus (VSV) can suffer fatal invasion of the central nervous system even when they have a high concentration of anti-VSV antibodies in their system," explains senior study author, Dr. Ulrich H. von Andrian, from Harvard Medical School. "This observation led us to revisit the contribution of adaptive immune responses to survival following VSV infection."

The research team studied VSV infection in mice that had B cells but did not produce antibodies. Unexpectedly, although the B cells themselves were essential, survival after VSV exposure did not require antibodies or other aspects of traditional adaptive immunity."We determined that the B cells produced a chemical needed to maintain innate immune cells called macrophages. The macrophages produced type I interferons, which were required to prevent fatal VSV invasion," says co-author Dr. Matteo Iannacone.

Taken together, the results show that the essential role of B cells against VSV does not require adaptive mechanisms, but is instead directly linked with the innate immune system. "Our findings contradict the current view that antibodies are absolutely required to survive infection with viruses like VSV, and establish an unexpected function for B cells as custodians of macrophages in antiviral immunity," concludes Dr. von Andrian. "It will be important to further dissect the role of antibodies and interferons in immunity against similar viruses that attack the nervous system, such as rabies, West Nile virus, and Encephalitis."

Journal reference: Moseman et al.: "B Cell Maintenance of Subcapsular Sinus Macrophages Protects against a Fatal Viral Infection Independent of Adaptive Immunity."

Story Source:

Materials provided by **Cell Press**. *Note: Content may be edited for style and length.*

Immunity. 2012 Mar 23;36(3):415-26. doi: 10.1016/j.immuni.2012.01.013. Epub 2012 Mar 1.

B cell maintenance of subcapsular sinus macrophages protects against a fatal viral infection independent of adaptive immunity

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Affiliations

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Free PMC article

Abstract

Neutralizing antibodies have been thought to be required for protection against acutely cytopathic viruses, such as the neurotropic vesicular stomatitis virus (VSV). Utilizing mice that possess B cells but lack antibodies, we show here that survival upon subcutaneous (s.c.) VSV challenge was independent of neutralizing antibody production or cell-mediated adaptive immunity. However, B cells were absolutely required to provide lymphotoxin (LT) α1β2, which maintained a protective subcapsular sinus (SCS) macrophage phenotype within virus draining lymph nodes (LNs). Macrophages within the SCS of B cell-deficient LNs, or of mice that lack LTα1β2 selectively in B cells, displayed an aberrant phenotype, failed to replicate VSV, and therefore did not produce type I interferons, which were required to prevent fatal VSV invasion of intranodal nerves. Thus, although B cells are essential for survival during VSV infection, their contribution involves the provision of innate differentiation and maintenance signals to macrophages, rather than adaptive immune mechanisms.

Case Reports Neurology. 1992 Apr;42(4):761-4. doi: 10.1212/wnl.42.4.761.

Severe tetanus in immunized patients with high anti-tetanus titers

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Abstract

Severe (grade III) tetanus occurred in three immunized patients who had high serum levels of anti-tetanus antibody. The disease was fatal in one patient. One patient had been hyperimmunized to produce commercial tetanus immune globulin. Two patients had received immunizations 1 year before presentation. Anti-tetanus antibody titers on admission were 25 IU/ml to 0.15 IU/ml by hemagglutination and ELISA assays; greater than 0.01 IU/ml is considered protective. Even though one patient had seemingly adequate anti-tetanus titers by in vitro measurement (0.20 IU), in vivo mouse protection bioassays showed a titer less than 0.01 IU/ml, implying that there may have been a hole in her immune repertoire to tetanus neurotoxin but not to toxoid. This is the first report of grade III tetanus with protective levels of antibody in the United States. The diagnosis of tetanus, nevertheless, should not be discarded solely on the basis of seemingly protective anti-tetanus titers.

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Letter to the Editor

SARS-CoV-2 infection despite high levels of vaccine-induced anti-Receptor-Binding-Domain antibodies: a study on 1110 health-care professionals from a northern Italian university hospital

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To the Editor,

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccination campaigns are at an advanced stage in many countries, but few concerns have been raised about cases of post-vaccination infection [1]. Real-world data are needed to guide health-policy-makers, especially now that the implementation of a third-dose administration protocol is being discussed.

Thanks to a longitudinal study (Covidiagnostix), funded by the Italian Ministry of Health, we investigated the antibody response, over a 6-month period, in 1110 health-care professionals (HCPs) injected with both doses of the BNT162b2 vaccine (January–February 2021) at the San Raffaele Hospital in Milan, Italy.

Health-care professionals previously infected by SARS-CoV-2 were identified by testing their sera, a few minutes before the first vaccination dose (T_0), for the presence of antibodies against the viral nucleocapsid-protein (N) (Roche Anti-SARS-CoV-2 electrochemiluminescence immunoassay (ECLIA); Roche, Basel, Switzerland). They were further tested after 21 (T_1) (immediately before the second vaccination dose), 42 (T_2) and 180 (T_3) days for the presence of antibodies against the receptor-binding domain (RBD) of the viral spike (S) protein (Roche Anti-SARS-CoV-2-S ECLIA). As part of a follow-up programme, HCPs were also sporadically subjected to RT-PCR amplification tests of nasopharyngeal swabs as well as to serological tests at time-points different from those of the Covidiagnostix.

At T_0, 90 HCPs (8.2%) were anti-N seropositive and showed the previously observed exceptional anti-RBD titre increase at T_1 upon receiving the first dose [2]. The remaining 1020 seronegative HCPs showed the production of anti-RBD antibodies upon receiving the first dose (T_1), which was boosted by administration of the second dose (T_2) [2] and was followed by a decrease of approximately 70%, at T_3, in the majority of the HCPs ($n = 929$, 91.1%). The remaining group ($n = 91$, 8.9%) showing T_3 minus T_2 anti-RBD titres ≥ 0 was tested (T_3) for the presence of anti-N antibodies. Ten of them resulted positive, indicating post-vaccination infections. As a control group, 410 HCPs showing T_3 minus T_2 titres ≤ 0 were also tested for the presence of anti-N antibodies; all of them were negative and none showed a positive RT-PCR swab test. Two more HCPs who were infected after vaccination, showing T_3 minus T_2 titres <0 (Table 1, participants 11 and 12), were identified through post-vaccination RT-PCR swab tests. Their infections were confirmed by the detection of anti-N serum antibodies at T_3.

Eight HCPs infected after vaccination were female, aged 49.8 ± 6.8 years, and four were male, aged 55.5 ± 15.3 years (Table 1). One individual was infected between the first and second vaccine doses, nine were infected between 7 and 99 days after the second dose and two were oblivious to having being infected (Table 1). All the individuals were asymptomatic, except for four

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Table 1
Demographic characteristics, serological results and COVID-19 related information of the 12 HCPs post-vaccination infected by SARS-CoV-2

Subject	Sex	Age (years)	Anti-RBD (BAU/mL)			PCR cycles[a]		Type of variant	2nd dose to infection interval (days)[b]	Symptoms	Close contacts[f]	Time length of negativization[c] (days)	HCP position
			T_1	T_2	T_3	RdRp gene	E Gene						
1	M	76	2.93	2122	>2500	22.9	20.9	B.1.1.7	64	Asymptomatic	Yes	21	Institutional Review Board
2	F	42	142	>2500	>2500	34.1	N/A	N/A	59	Asymptomatic	Yes	13	Psychologist
3	M	54	<0.4	196	897	25.6	24.2	N/A	69	Partial anosmia/ageusia	Yes	13	Nurse (Pediatric)
4	M	39	1019	1866	>2500	N/A	N/A	N/A	N/A [d]	Asymptomatic	No	?	Administrative
5	F	57	<0.4	2047	>2500	N/A	N/A	N/A	N/A [d]	Asymptomatic	No	?	Administrative
6	F	55	208	>2500	>2500	N/A	N/A	N/A	<0[e]	Asymptomatic	No	?	Nurse (Infectious Diseases)
7	F	49	77.5	>2500	>2500	22.3	22.1	B.1.1.7	67	Partial anosmia/ageusia, cold, generalized pain	Yes	13	Nurse (Psychiatry)
8	M	53	0.79	339	>2500	223.1	23.5	B.1.1.7	84	Asymptomatic	No	16	Technician (Echography)
9	F	42	18.5	1131	>2500	30.8	31.8	N/A	42	Asymptomatic	Yes	14	Nurse (Psychiatry)
10	F	55	76.3	2046	>2500	30.1	30.3	N/A	99	Partial anosmia/ageusia, cold	Yes	22	Technician (Pathological Anatomy)
11	F	42	50.1	>2500	2495	21.4	20.8	N/A	14	Partial anosmia/ageusia, cold	Yes	14	Nurse (General medicine)
12	F	56	5.7	1066	714	28.1	27.8	N/A	7	Asymptomatic	Yes	17	Nurse (Cardiology Department)

[a] Values refers to the first positive swab test. Values were considered: positive (between 14 and 34) slightly positive (between 34 and 40), negative (>40).
[b] Intervals are calculated from the day of the 2nd dose to the day of the first positive RT-PCR test.
[c] Time length of negativization was calculated from the day of the first positive RT-PCR test to the day of the first negative RT-PCR test.
[d] COVID-19 was asymptomatic and the HCPs found out about the infection only through the serological test at T_3.
[e] Positivity was discovered by an occasional anti-N test performed at T_1.
[f] "Close contacts" refers to the presence of a SARS-CoV-2 positive unvaccinated household at the time of infection.

who reported partial anosmia and ageusia accompanied, in three cases, by a mild cold and, in one of those three cases, by a generalized pain (Table 1). The possibility of an in-hospital outbreak was ruled out because the 12 HCPs perform, within the hospital, different tasks (Table 1) with the exception of two nurses from the Psychiatric Department, but they were infected 1 month apart. Notably, 8 out of 12 HCPs infected after vaccination reported the presence of a SARS-CoV-2-positive family member (not vaccinated) as the potential source of infection (Table 1).

Reduced vaccination efficiency has been observed in older individuals (>60 years old) [2], but the 12 HCPs were between 39 and 57 years old, except for one who was 76 years old. Seven of the individuals had anti-RBD titres at T_2 above 2000 binding antibody units (BAU)/mL, three were between 1000 and 2000 BAU/mL and only two had titres below 400 BAU/mL (Table 1). An anti-RBD titre threshold of approximately 1300 BAU/mL was associated with neutralizing activity as previously described by Ferrari et al. [3]. Although the latter is not the only correlate for vaccine efficacy, with memory B and T cells possibly playing a key role in protection, we would have expected a better consistency between high anti-RBD antibody serum levels and protection from infection. These data further highlight the difficulty of finding a reliable and unique correlate of protection by assessing only the serum neutralizing antibody titres. It must be noted that two HCPs (individuals 3 and 5) did not respond to the first vaccine dose and showed T_1 anti-RBD titres <0.4 U/mL (Table 1).

In conclusion, 6 months after the vaccination of 1110 HCPs, 12 of them were infected despite receiving the proper BNT162b2 administration protocol (except for one, who was infected between the two doses). However, because some of the HCPs did not undergo anti-N serological testing, the number of infections might be underestimated. Post-vaccination infections, distributed throughout the whole observation period, were often associated with the presence of unvaccinated SARS-CoV-2-infected households. Importantly, no in-hospital (or related public areas) secondary cases were observed among colleagues (>95% of the San Raffaele Hospital employees were vaccinated). Our study showed that, in the observed cohort of HCPs, no severe clinical manifestations of coronavirus disease 2019 occurred. We might speculate that the latter is the consequence of the efficacy of the BNT162b2 vaccine, but infection and symptomatology were not related to a low anti-RBD antibody response. In the light of these data, we think that implementation/modification of current vaccine protocols should focus on further studies evaluating clinical outcomes in individuals who are infected after vaccination, their anti-RBD antibody titre and, importantly, the possible key role of memory immunity in the protection from severe coronavirus disease 2019.

Transparency declaration

The authors declare that they have no conflicts of interest.

Author contributions

DF designed the study, performed the data analysis and interpretation and wrote the article; NC, NM and ML wrote the article and supervised the study.

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Statement on research ethics

The COVIDIAGNOSTIX study was approved by the San Raffaele Hospital Institutional Ethical Review Board (CE:199/INT/2020).ht.

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Measles virus neutralizing antibody response, cell-mediated immunity, and IgG antibody avidity before and after a third dose of measles-mumps-rubella vaccine in young adults

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Abstract

Background—Two doses of measles-mumps-rubella (MMR) vaccine are 97% effective against measles, but waning antibody immunity and two-dose vaccine failures occur. We administered a third MMR dose (MMR3) to young adults and assessed immunogenicity over 1 year.

Methods—Measles virus (MeV) neutralizing antibody concentrations, cell-mediated immunity (CMI), and IgG antibody avidity were assessed at baseline, 1-month, and 1-year after MMR3.

Results—Of 662 subjects at baseline, 1 (0.2%) was seronegative (<8 mIU/mL) and 23 (3.5%) had low (8-120 mIU/mL) MeV neutralizing antibodies. At 1-month post-MMR3, 1 (0.2%) subject was seronegative and 6 (0.9%) had low neutralizing antibodies with only 21/662 (3.2%) showing a ≥4-fold rise in neutralizing antibodies. At 1-year post-MMR3, none were negative and 10 (1.6%) of 617 subjects had low neutralizing antibodies. CMI results showed low-levels of spot-forming

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Conflict of Interest: Laura A. Coleman worked for Marshfield Clinic Research Foundation at the time of the study, but she currently works for Abbott Nutrition, Columbus, OH. All other coauthors do not report any conflict of interest.

Meetings: The MeV neutralizing antibody results were presented at the Infectious Disease Week Conference, October 8-12, 2014, Philadelphia, PA.

Publisher's Disclaimer: Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Food and Drug Administration.

cells after stimulation, suggesting T-cell memory, but the response was minimal post-MMR3. MeV IgG avidity results did not correlate with neutralization results.

Conclusions—Most subjects were seropositive pre-MMR3 and very few had a secondary immune response post-MMR3. Similarly, CMI and avidity results showed minimal qualitative improvements in immune response post-MMR3. We did not find compelling data to support a routine third dose of MMR vaccine.

Keywords

measles; third dose measles-mumps-rubella (MMR) vaccine; measles vaccine immunogenicity; vaccine preventable disease; immunization; cell-mediated immunity; measles virus antibody avidity

Background

Measles is a contagious, viral rash illness; complications including pneumonia and encephalitis can result in death[1]. High two-dose measles-mumps-rubella (MMR) vaccination coverage and improved measles control in the World Health Organization (WHO) Region of the Americas resulted in the declaration of measles elimination in the U.S. in 2000[2].

Two doses of MMR vaccine are generally sufficient to provide long-lasting protection against measles[3]. Nonetheless, measles virus (MeV) is one of three components in the MMR vaccine, and third doses have been administered during mumps outbreaks among highly vaccinated populations[4, 5] and in non-outbreak settings among healthcare personnel, military recruits, international travelers, and college students who may have been two-dose vaccinated but lacked documentation[6-8].

The immunogenicity of the MeV component of a third MMR dose has not been studied. We assessed the magnitude and duration of an aggregate MeV neutralizing antibody response, cell-mediated immune response, and IgG antibody avidity before and after a third MMR dose (MMR3) in a healthy, young adult population.

Methods

Setting

The study population comprised patients of the Marshfield Clinic, a private, multispecialty group practice with regional centers throughout central and northern Wisconsin. The Clinic maintains an electronic vaccination registry (www.recin.org) for immunizations administered by Marshfield Clinic providers, local public health agencies, and immunization providers. No measles cases were reported in the area during the study period.

Subjects

Two cohorts comprising 685 subjects were enrolled during 2009-2010. Cohort 1 (N=113 subjects) participated in a 10-year longitudinal study at the Marshfield Clinic examining immunogenicity and adverse events following the second MMR vaccine dose, hereafter

called the longitudinal study[9, 10]. To achieve adequate sample size, Marshfield's vaccination registry was used to recruit subjects from Cohort 2 who had two documented MMR doses but did not participate in the longitudinal study (N=572 subjects). Invitation letters were mailed to both cohorts and follow-up phone calls were made. Additionally, Cohort 1 subjects who participated in the measles cell-mediated immunity (CMI) sub-study during the longitudinal study were asked to participate in the current CMI sub-study.

Although only 16 (14.2%) Cohort 1 subjects had low or negative MeV antibody concentrations during the longitudinal study, 93/113 Cohort 1 subjects with ≥1 low or negative antibody concentration to measles, mumps, or rubella during the longitudinal study (defined previously[10-12]) and all Cohort 2 subjects were offered a third dose of MMR vaccine (M-M-R II; Merck & Co.). Serum was collected from all subjects immediately before (baseline), and one month and one year after MMR3.

Study design

At each visit, subjects were questioned about measles disease, exposures, vaccinations, and other health events. MMR vaccine was administered during the initial visit. Informed consent was obtained from all subjects. Institutional Review Boards of the Marshfield Clinic Research Foundation and the Centers for Disease Control and Prevention approved the study. Sample size determination and exclusion criteria were previously described[13].

Cell-mediated immunity sub-study

The 60 participants of the longitudinal measles CMI sub-study or subjects with a low or negative MeV antibody concentration on ≥1 serum specimen draw during the longitudinal study were asked to participate in the current CMI sub-study. However, only 34 (56.7%) subjects meeting these criteria were re-enrolled. A convenience sample from Cohort 2 was used to reach the recruitment goal of 60 subjects.

Laboratory Methods

Laboratory testing was performed at the end of the study. Other than each subject's unique identifier code and serum collection dates, laboratories were blinded to study information.

Plaque reduction neutralization—Plaque reduction neutralization (PRN) testing was performed using low-passage Edmonston MeV on Vero cell monolayers, as previously described[14]. Endpoints were determined for all serum samples tested and ND_{50} titers calculated using the Kärber method. Serial four-fold dilutions of serum were tested in duplicate starting at 1:8 and ending at 1:8192 against virus diluted to give 25-35 plaques/well and run in parallel with the Second WHO International Standard Reference Serum (66/202). After incubating the virus-serum mixtures at 37° C with 5% CO_2, the mixtures were transferred onto corresponding 24-well tissue culture plates containing confluent Vero monolayers; after incubating for 1 hour at 37° C, the inoculum was removed and cells overlaid with medium containing carboxymethylcellulose and returned to the incubator for 5 days prior to staining with neutral red and plaque counting. Serum samples from individual subjects were tested in the same assay run. Titers were standardized against the WHO reference serum with a titer of 1:8 corresponding to 8 mIU/mL in this assay.

Cell-mediated immunity—Cryo-preserved peripheral blood mononuclear cells (PBMCs) were thawed and cultured overnight in 5% CO_2 at 37°C with Roswell Park Memorial Institute (RPMI) media supplemented with 4% human serum type AB (Lonza), 1% penicillin/streptomycin, and 1% 200 mM L-glutamine. Following the overnight culture, IFN-γ production by T-cells was assessed using enzyme-linked immunospot assays of PBMCs (5×10^5 cells/well), as previously described[15]. PBMCs were stimulated either with a mixture of MeV hemagglutinin, fusion, and nucleoprotein proteins as 20 amino acid peptides (11 amino acids overlapping) at 1μg/mL or with a lysate from MeV-infected Vero cells (Advanced Biotechnologies) at 10μg/mL for 40 hours. RPMI media and Con A (5μg/mL) were used as negative and positive controls, respectively. After stimulation, the plates were incubated with biotin-conjugated antibodies to human IFN-γ, then developed and read, as previously described[15]. Low and positive T-cell responses were categorized as <20 and ≥20 spot-forming cells (s.f.c.)/million PBMCs, respectively.

Avidity—MeV IgG antibody avidity was evaluated to determine whether there was a correlation between neutralizing antibody concentrations and strength of antibody binding. Avidity testing occurred after neutralization results were available using the method described previously[16]. Serum samples from all 662 subjects were split into quartiles based on baseline PRN antibody concentration. Subjects with negative neutralizing antibody concentrations were negative for MeV IgG by the Captia Measles IgG enzyme immunoassay assay (Trinity Biotech, Jamestown, NY), thus avidity could not be measured. All subjects with low MeV neutralizing antibody concentrations at baseline, 1-month, or 1-year post-MMR3 were tested for MeV antibody avidity. A random number generator selected specimens from at least 10 subjects from each of the remaining 3 quartiles for avidity testing of 59 subjects. The specimen was classified as negative if at 1:21 dilution it had undetectable IgG by the Captia assay, low avidity if the end titer avidity index percentages (etAI%) were ≤30%, intermediate between 30%-70% (intermediate results were retested), or high avidity if the etAI% was ≥70%.

Data analysis

Based on previous studies[17, 18], serum samples were categorized as: (1) negative (<8 mIU/mL), susceptible to infection and disease; (2) low (8-120 mIU/mL), potentially susceptible to infection and disease; (3) medium (121-900 mIU/mL), potentially susceptible to infection but not disease; and (4) high (>900 mIU/mL), not susceptible to infection or disease. Serum samples were also dichotomized as potentially susceptible (<121 mIU/mL) and not susceptible (≥121 mIU/mL).

We combined Cohorts 1 and 2 during analysis because there were no statistically significant differences between the cohorts by sex, race/ethnicity, or age. However, Cohort 1 had significantly lower geometric mean concentrations (GMCs) of MeV neutralizing antibody at baseline (p=0.0289), so we stratified the chi-squared risk factor analysis at 1-month and 1-year post-MMR3 by baseline MeV neutralizing antibody concentrations.

Mantel-Haenszel chi-squared and Fisher's exact tests were run to assess categorical variables. Wilcoxon Rank Sum tests were used for continuous variables. Potential risk

factors for negative or low MeV neutralizing antibody levels included: sex, age at first MMR dose, time since second MMR dose (we used <15 years versus ≥15 years prior based on average age of subjects at enrollment minus the age when the second dose was recommended), and (for post-MMR3 serum samples) the binary variable of whether the subject had low or negative MeV neutralizing antibody levels at baseline. In multivariate logistic regression, a backwards selection approach that used p-values <0.4 for inclusion and <0.05 for retention identified factors independently associated with negative or low MeV neutralizing antibody levels at baseline, 1-month and 1-year post-MMR3.

For the CMI analysis, the mean number of spot-forming cells resulting from PBMC stimulation with MeV peptide and MeV lysate was determined at baseline, 1-month, and 1-year post-MMR3. The MeV-specific T-cell response was calculated by subtracting the mean spontaneous response (no stimulation) from the mean peptide or lysate response. MeV T-cell responses were correlated with MeV neutralizing antibody levels at baseline, 1-month, and 1-year post-MMR3. For the avidity analysis, end titer avidity index percentages were correlated with MeV neutralizing antibody levels at all 3 time points.

GMCs of MeV neutralizing antibody were calculated from base 2 log-transformed data. Statistical significance was assigned for P-values <0.05. Data were analyzed with SAS 9.3 (Cary, NC). Reverse cumulative distribution curves were created in Excel to compare the shift in curves from baseline, 1-month, and 1-year post-MMR3.

Results

Enrollment

We contacted 194/200 persons from the longitudinal study; 113 (58.2%) were enrolled, 45 (23.2%) refused, and 36 (18.5%) were ineligible (15 had previously received MMR3 and 21 had other reasons). To achieve adequate sample size, we contacted 1379 (76.8%) of an additional 1795 persons. Of those, 572 (41.4%) were enrolled, 664 (48.2%) refused, and 143 (10.4%) were ineligible (4 had previously received MMR3 and 139 had other reasons) (Supplementary Figure 1).

Baseline serum samples were obtained from 685 enrolled subjects. We excluded 20 (2.9%) Cohort 1 subjects who had medium or high antibody concentrations for all 3 antigens throughout the longitudinal study and were, therefore, not given MMR3. An additional 3 (0.4%) were excluded because they only had baseline samples. There were 662 (96.6%) subjects who received MMR3 and completed the 1-month draw; 617 (92.6%) completed the 1-year draw. Subjects were aged 18-28 years, (mean: 20.8 years, standard deviation: +/-2.1); 294 (44.4%) were male and 649 (98.0%) were self-declared non-Hispanic, white. The mean interval between the second and third MMR doses was 15.8 years (range: 6.7–20.4 years).

MeV neutralizing antibody concentrations pre- and post-MMR3

Of 662 subjects at baseline, 1 (0.2%) was seronegative, 23 (3.5%) had low MeV neutralizing antibody concentrations, 337 (50.9%) had medium concentrations, and 301 (45.5%) had high concentrations (Figure 1). The seronegative subject was a female aged 20 years who received her last MMR dose 18 years prior. At 1-month and 1-year post-MMR3, she had

medium MeV neutralizing antibody concentrations. Of 23 subjects with low baseline antibody concentrations, 1 was negative, 5 were low, 14 were medium, and 3 were high 1-month post-MMR3. One year post-MMR3, 19 of 23 had sera drawn; 5 had low, 14 had medium, and 0 had high MeV neutralizing antibody concentrations.

Overall, at 1-month post-MMR3, 1/662 (0.2%) subjects had no detectable MeV neutralizing antibodies, 6 (0.9%) had low, 256 (38.7%) had medium, and 399 (60.3%) had high neutralizing antibody concentrations. One year post-MMR3, all 617 subjects who returned were positive for MeV neutralizing antibodies: 10 (1.6%) had low, 299 (48.5%) had medium, and 308 (49.9%) had high neutralizing antibody concentrations.

When assessed as a continuous variable, subjects with low or negative baseline MeV neutralizing antibody concentrations were more likely to have low or negative antibody concentrations 1-month and 1-year post-MMR3. Whereas subjects with high baseline concentrations were more likely to have high neutralizing antibody concentrations at 1 month (R^2=0.54, P<0.0001) and 1 year (R^2=0.68, P<0.0001)(Figure 2).

GMCs were significantly different between baseline and 1-month post-MMR3 (727 vs. 1060 mIU/mL, P<0.0001), and between baseline and 1-year post-MMR3 (727 vs. 843 mIU/mL, P<0.05). However, the reverse cumulative distribution curves show the shift in MeV antibody concentrations from baseline to 1-month to 1-year post-MMR3 was minimal (Figure 3).

Four-fold rises

Twenty-one (3.2%) of 662 subjects had ≥4-fold rises from baseline to 1-month post-MMR3, of whom at baseline 1 was seronegative, 8 had low antibody concentrations, and 12 had medium PRN concentrations. Eight (1.3%) of 617 subjects had ≥4-fold rises from baseline to 1-year post-vaccination, of whom at baseline 1 was seronegative, 4 had low concentrations, and 3 had medium PRN concentrations.

Risk factors for negative or low MeV neutralizing antibody concentrations pre- and post-MMR3

The unadjusted odds ratios showed that those who had their first MMR dose at age 12-<15 months ([Odds Ratio] OR:3.47, [Confidence Interval] CI:1.24–9.72, p=0.01) had a higher odds of having lower or negative baseline antibody concentrations compared with those who had their first dose at age ≥15 months, and those who had their second MMR dose <15 years prior had a lower odds of having low or negative baseline MeV neutralizing antibody levels versus those who had their second dose ≥15 years prior (OR:0.22, CI:0.05–0.93, p=0.03) (Table 1).

Of 50 (7.6%) subjects who received their first dose at age 12-<15 months, 5 (10.0%) had negative or low baseline MeV antibody concentrations, versus 19/612 (3.1%) subjects who were vaccinated with their first dose at age ≥15 months. Of 190 (28.7%) subjects who received their second dose <15 years prior, 2 (1.1%) had negative or low baseline MeV antibody concentrations, versus 22/472 (4.7%) subjects who received their second dose ≥15 years prior. In multivariate analysis, having the first MMR dose at 12-<15 months of age

remained a significant risk factor at baseline (OR:3.94,CI:1.37–11.30, p=0.01), and those who had their second MMR dose <15 years prior continued to have a lower odds of having low or negative MeV antibody concentrations (OR:0.18,CI:0.04–0.80, p=0.02).

At 1-month post-MMR3, there were no significant risk factors for having low or negative MeV antibody concentrations when adjusting the chi-squared analysis by controlling for baseline GMCs. In multivariate analysis, a significant risk factor for negative or low MeV antibody concentrations 1-month post-MMR3 was whether a subject had low or negative baseline MeV antibody concentrations (OR:195.8,CI:21.8–>999.9, p<0.0001).

At 1-year post-MMR3, females had a lower odds of having low or negative MeV antibodies (OR:0.34, CI:0.06–1.80, p=0.04) versus males when adjusting the chi-squared analysis by controlling for baseline GMCs. In multivariate analysis at 1-year post-MMR3, being female remained protective (OR:0.19, CI:0.04–0.99, p=0.049) and low or negative baseline MeV neutralizing antibody concentrations were a risk factor (OR:54.95, CI:10.90–277.14, p<0.0001).

Cell-mediated immunity

Of 60 CMI sub-study subjects, 7 were excluded (6 did not receive MMR3 and 1 had insufficient blood drawn); 1 (1.9%) of 53 subjects missed the 1-month draw and 6 (11.3%) missed the 1-year draw. MeV lysate stimulation results were missing for an additional 2 subjects at baseline and 1 subject at 1 month. Positive controls were positive for all CMI subjects, indicating viable cells capable of spot-formation. The unstimulated T-cell mean spot-forming cells (s.f.c.)/million PBMCs was 0.1±0.1 at baseline, 0.1±0.1 at 1-month, and 0.2±0.2 at 1-year post-MMR3.

Of 53 CMI sub-study subjects, none had negative baseline MeV neutralizing antibody concentrations and 5 (9.4%) had low baseline concentrations, of whom, 1 had a positive baseline CMI response (≥20 s.f.c./million PBMCs) to peptide stimulation and none had a positive baseline response to lysate stimulation. Only 13/48 (27.1%) subjects with medium or high baseline MeV neutralizing antibodies had a positive baseline CMI result by peptide stimulation and 7/46 (15.2%) subjects had a positive baseline CMI result by lysate stimulation.

The spot-forming cells/million PBMCs were generally higher with peptide stimulation compared to lysate stimulation. At baseline, the MeV peptide mean spot-forming cells was 19.6±9.3 s.f.c./million PBMCs compared to 11.9±7.2 s.f.c./million PBMCs by lysate stimulation. At 1-month post-MMR3, the MeV peptide mean spot-forming cells was 18.5±7.6 s.f.c./million PBMCs, with 13/52 (25.0%) specimens positive by peptide stimulation, compared with 7.3±2.9 s.f.c./million PBMCs, with 5/51 (9.8%) specimens positive by lysate stimulation. At 1-year post-MMR3, the mean spot-forming cells was 29.7±15.9 s.f.c./million PBMCs, with 14/47 (29.8%) positive by peptide stimulation, compared with 10.3±6.4 s.f.c./million PBMCs, with 7/47 (14.9%) specimens positive by lysate stimulation.

Baseline MeV antibody concentrations did not correlate with baseline MeV T-cell responses to peptide stimulation (R^2=0.002, p=0.73) or lysate stimulation (R^2=0.0008, p=0.85)(Figure 4). MeV antibody concentrations at 1-month post-MMR3 correlated with MeV T-cell responses at 1 month by peptide stimulation (R^2=0.30, p<0.0001), but the correlation did not remain after removing the 2 outliers (R^2=0.05, p=0.13). There was no correlation between MeV antibody concentrations and lysate stimulation at 1 month (R^2=0.001, p=0.80), but after removing the outlier, there was a correlation (R^2=0.14, p=0.007). At 1-year post-MMR3, there was a significant correlation between MeV antibody concentrations and MeV T-cell responses by peptide stimulation (R^2=0.17, p=0.004), but no correlation by lysate stimulation (R^2=0.06, p=0.09).

Avidity

Overall, 38/59 (64.4%) subjects evaluated had MeV antibodies with high avidity at baseline (Table 2), including 7/24 (29.2%) subjects with low MeV antibody concentrations at baseline. The avidity results did not correlate with MeV antibody concentrations at baseline (R^2=0.07, p=0.07), 1-month (R^2=0.01, p=0.50) or 1-year (R^2=0.02, p=0.31) post-MMR3 (Figure 5).

Discussion

A modest but significant boost in MeV geometric mean neutralizing antibody concentrations occurred 1-month and 1-year post-MMR3 compared with baseline. However, almost all subjects were MeV seropositive prior to receiving MMR3, and subjects' antibody levels returned to near-baseline 1-year post-vaccination. Nonetheless, for the 24 (3.6%) subjects with low or negative baseline MeV antibody concentrations, 18 (75%) moved into medium or high categories at 1 month, of whom, 12 (67%) remained medium or high at 1 year. Among the subsets tested for CMI and avidity, we did not find compelling qualitative data to support a routine third dose of MMR vaccine.

The second MMR vaccine dose was recommended to provide measles immunity to individuals who failed to respond to the first dose[19]; two doses are 97% effective at preventing measles[20, 21]. Although 95% of vaccinated persons have detectable MeV antibodies 10-15 years after the second MMR dose[10, 22], waning immunity occurs after two doses[10][23], and two-dose failures have been documented[24].

Having a low or negative baseline MeV antibody concentration was the biggest risk factor for low or negative antibody concentrations 1-month and 1-year post-MMR3, suggesting that inherent biology may be partially responsible for a person's measles antibody levels[10, 25]. Although our results concurred with other reports that timing of administration of the first and second MMR doses significantly affected MeV antibody levels later in life[26, 27], our findings represented only a small proportion of the study population (only 50 [7.6%] subjects received their first dose at age 12-<15 months).

Most subjects did not have a positive CMI result at baseline, despite the majority of subjects having medium or high baseline MeV antibody concentrations. Nonetheless, low-levels of spot-forming cells generally occurred for most specimens after stimulation, suggesting T-

cell memory. However, this was not greatly boosted by MMR3. After removing outliers, we found mixed results at 1-month post-MMR3 with no correlation between MeV antibody response and MeV T-cell response by peptide stimulation, but a significant correlation by lysate stimulation. Although we did find a significant correlation between CMI response by peptide stimulation and MeV antibody concentration at 1-year post-MMR3, <1/3 of subjects had positive cell-mediated responses by peptide stimulation and even fewer had positive responses by lysate stimulation at 1 year. These findings could have been because transient increases in circulating MeV-specific T-cells were missed due to specimen collection timing (antigen-stimulated T-cell responses typically peak 2 weeks post-vaccination[28], whereas samples were taken 1-month and 1-year post-MMR3). Other studies assessing antibody and T-cell responses after a second MMR dose showed no correlation[29, 30]. Another possibility is that numbers of T-cells producing IFN-γ in response to MeV did not increase post-MMR3 due to lack of infection by vaccine virus in the presence of neutralizing antibodies.

The MeV IgG avidity results did not correlate with neutralization results. Most subjects reached an IgG avidity plateau. Typically, IgG avidity maturation for measles shifts from low to high 4 months following immunization or infection[16] which might negate additional increases in antibody avidity with subsequent doses of measles-containing vaccine. Nonetheless, only 29% of subjects with low baseline MeV neutralizing antibody concentrations had high avidity results at baseline. It could be interpreted that subjects with poor antibody response and intermediate avidity results were potentially susceptible prior to revaccination. However, the avidity results are an average of the measles IgG and should be interpreted cautiously, since whole MeV is used as the target antigen in the avidity assay, whereas the neutralization assay measures antibodies that bind MeV surface glycoproteins[31].

Our study had additional limitations. Subjects were not representative of the U.S. population. Selection bias may have occurred in Cohort 1, because MMR3 was only offered to those who had a low or negative measles, mumps, or rubella antibody concentration during the longitudinal study.

Overall, MeV neutralizing antibody concentrations initially increased after MMR3 but declined to near-baseline levels one year later. Although our findings showed that MMR3 increased antibody levels for the small percentage of subjects with low MeV neutralizing antibody concentration levels who were on the cusp of protection, the CMI and avidity results in the subset tested showed that MMR3 did not result in substantial improvements in the quality of the immune response. While a third MMR dose may successfully immunize the rare individual who failed to respond after two doses, MMR3 is unlikely to solve the problem of waning immunity in the U.S. A better strategy for maintaining U.S. measles elimination would be to improve vaccination coverage in pockets of unvaccinated individuals and maintain high two-dose coverage nationally with the current two-dose MMR recommendation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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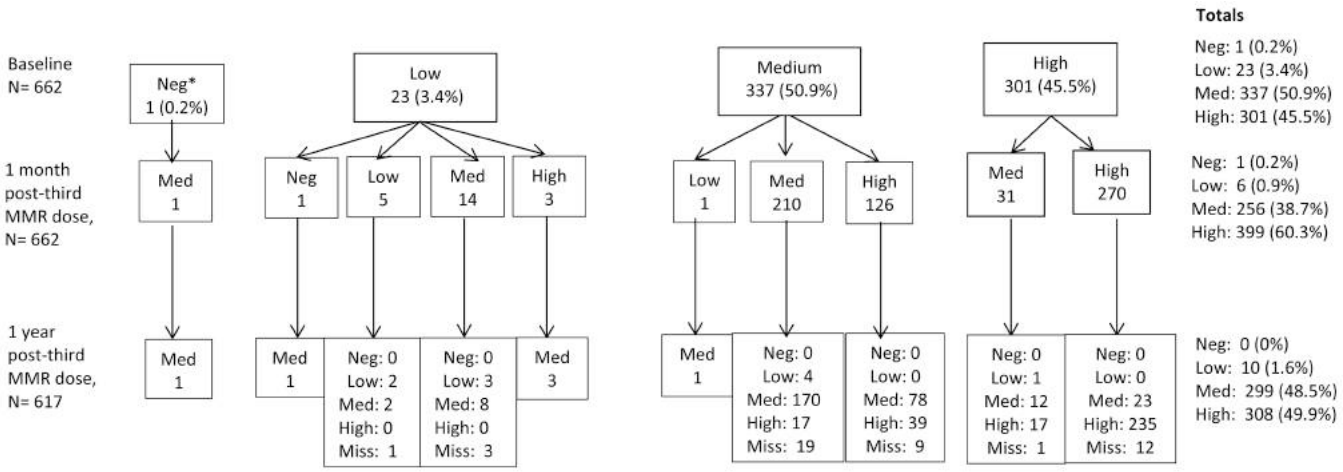

Figure 1.

Flow chart of measles virus neutralizing antibody concentration levels at baseline, 1 month, and 1 year following a third dose of MMR vaccine.

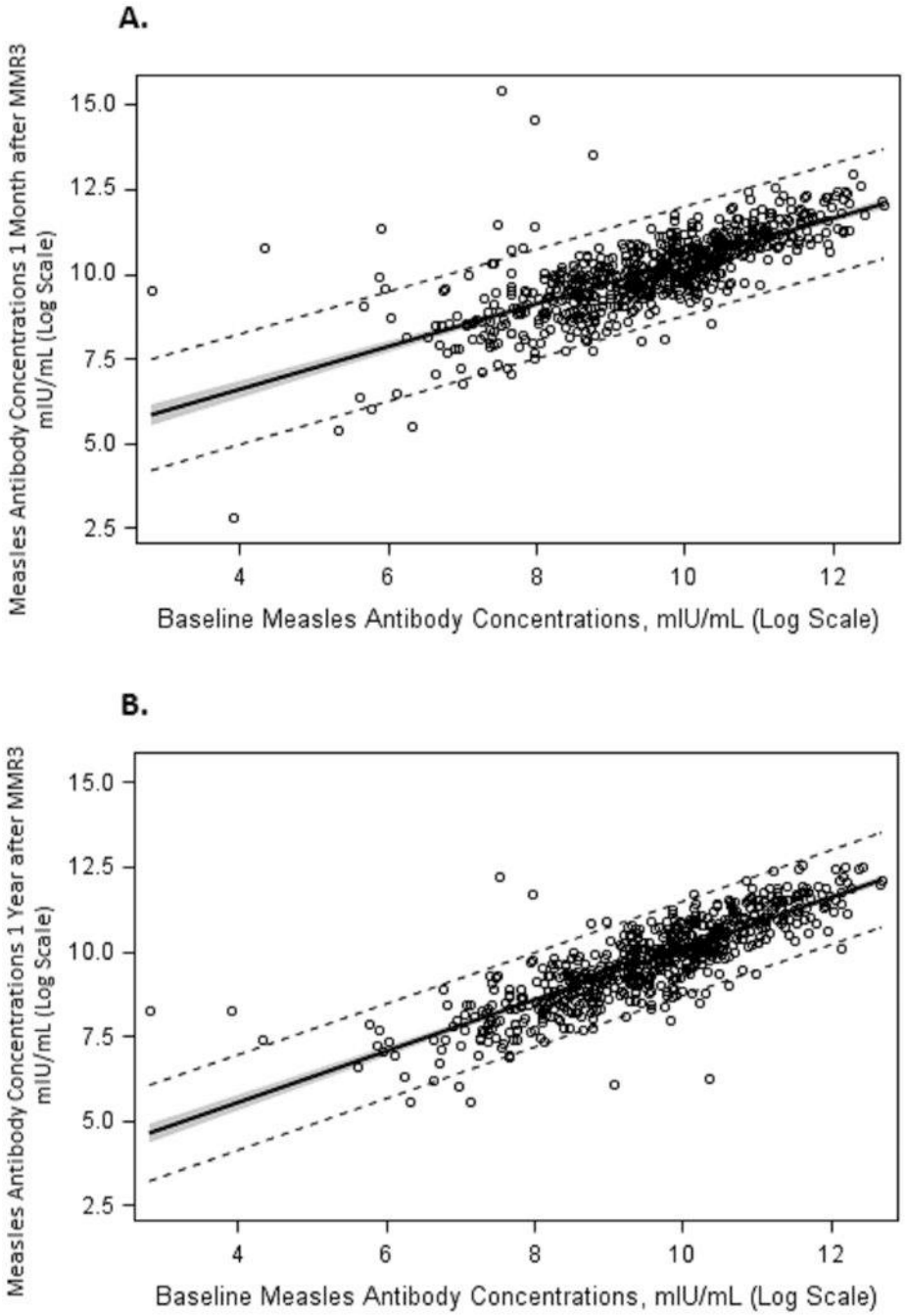

Figure 2.

A. Comparison of individual measles antibody concentration levels at baseline and 1 month following a third dose of MMR vaccine. R^2=0.54, p<0.0001. **B.** Comparison of individual measles antibody concentration levels at baseline and 1 year following a third dose of MMR vaccine. R^2=0.68, p<0.0001. For both figures, data points are represented by circles and they show the comparison result for each subject. The dark solid line represents the best-fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits.

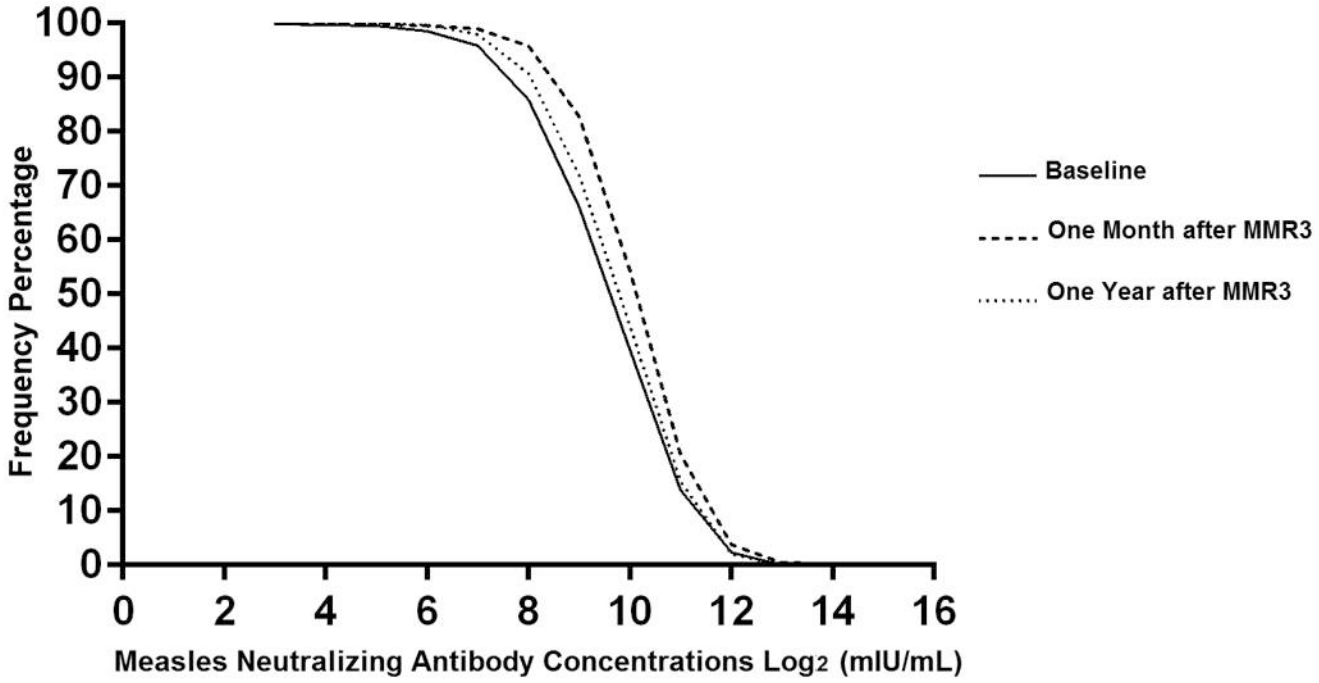

Figure 3.

Reverse cumulative distribution curve by percent of subjects who had measles virus neutralizing antibody concentrations at baseline, 1 month, and 1 year following a third dose of MMR vaccine.

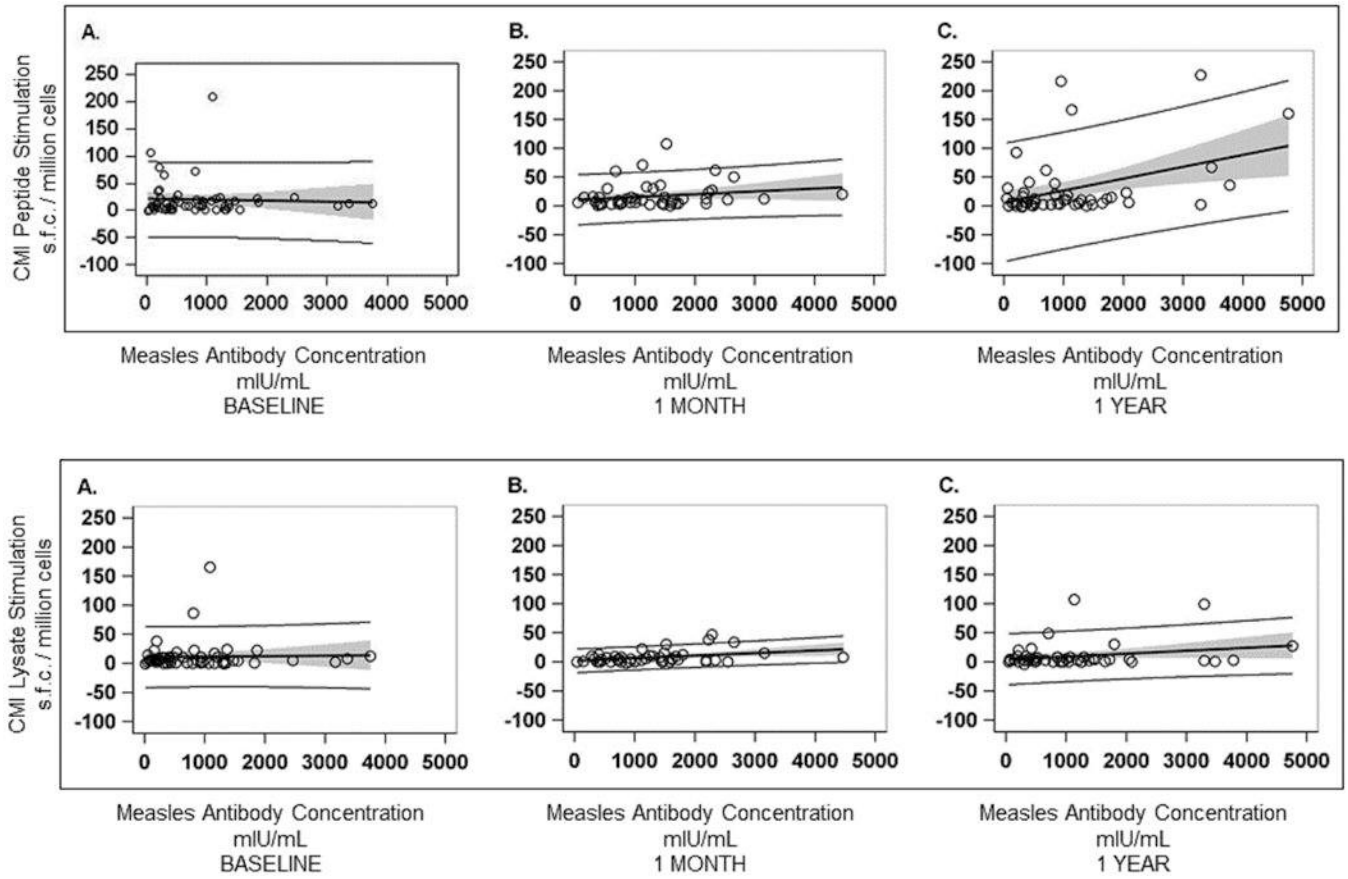

Figure 4.

Figure 4a: A. Comparison of baseline measles virus neutralizing antibody concentration levels (mIU/mL) and baseline measles virus T-cell response to measles virus peptide stimulation (spot-forming cells [s.f.c.]/ million cells), n= 53. R^2= 0.002, p=0.73. **B.** Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus peptide stimulation (s.f.c./ million cells) 1 month after receiving a third dose of MMR vaccine, n=50. R^2=0.05, p=0.13 (Note that 2 outliers were removed from the figure). When the 2 outliers were included, the results were: n=52. R^2= 0.30, p<0.0001, and the x-axis on the graph extended beyond 40,000 mIU/mL. **C.** Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus peptide stimulation (s.f.c./ million cells) 1 year after receiving a third dose of MMR vaccine, n=47. R^2= 0.17, p=0.004. For all figures, data points are represented by circles and they show the comparison result for each subject. The dark solid line represents the best-fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits.

Figure 4b: A. Comparison of baseline measles virus neutralizing antibody concentration levels (mIU/mL) and baseline measles virus T-cell response to measles virus lysate stimulation (s.f.c./ million cells), n=51. R^2= 0.0008, p=0.85. **B.** Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus lysate stimulation (s.f.c./ million cells) 1 month after receiving a third dose of MMR vaccine, n=49. R^2= 0.14, p=0.007 (Note that 1 outlier was removed from the figure;

the other outlier was already missing because of insufficient blood drawn to analyze the measles virus lysate response). When the outlier was included, the results were: n=50. R^2=0.001, p=0.80, and the x-axis on the graph extended beyond 40,000 mIU/mL. **C.** Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus T-cell response to measles virus lysate stimulation (s.f.c./ million cells) 1 year after receiving a third dose of MMR vaccine, n=47. R^2=0.06, p=0.09. For all figures, data points are represented by circles and they show the comparison result for each subject. The dark solid line represents the best-fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits.

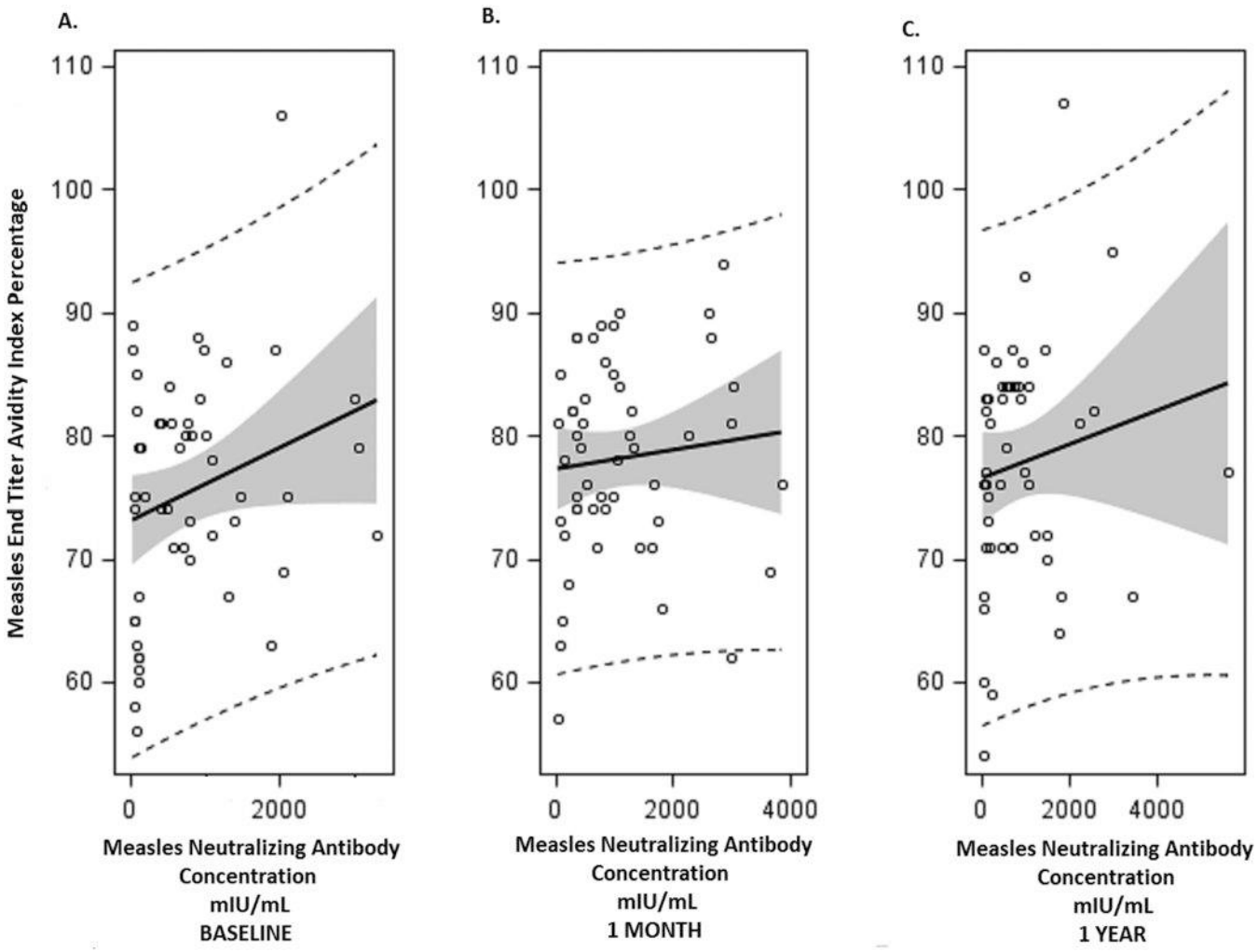

Figure 5.

A. Comparison of baseline measles virus neutralizing antibody concentration levels (mIU/mL) and baseline measles virus antibody avidity levels (end titer avidity index percentage [etAI%]), n= 51. R^2=0.07, p=0.07. **B.** Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus antibody avidity levels (etAI%) 1 month after receiving a third dose of MMR vaccine, n=51. R^2=0.01, p=0.50. **C.** Comparison of measles virus neutralizing antibody concentration levels (mIU/mL) and measles virus antibody avidity levels (etAI%) 1 year after receiving a third dose of MMR vaccine, n=47. R^2=0.02, p=0.31. For all figures, data points are represented by circles and they show the comparison result for each subject. The dark solid line represents the best-fit of the comparison. The light shading around the line represents the 95% confidence limits. The dotted lines represent 95% prediction limits.

Table 1

Risk factors for negative or low measles neutralizing antibody concentrations at baseline, 1 month, and 1 year after receiving a third dose of measles-mumps-rubella (MMR) vaccine

	Baseline N= 662				1 Month Post-MMR3 N= 662				1 Year Post-MMR3 N= 617			
	Unadjusted OR (95% CI)[1]	p-value[2]	Multivariate OR (95% CI)	Multivariate p-value	Adjusted OR[3] (95% CI)	p-value	Multivariate OR (95% CI)	Multivariate p-value	Adjusted OR (95% CI)	p-value	Multivariate OR (95% CI)	Multivariate p-value
Sex												
Female	0.56 (0.24-1.28)	0.16	0.53 (0.23- 1.23)	0.14	0.22 (0.03-1.45)	0.08	0.16 (0.02- 1.48)	0.11	0.34 (0.06-1.80)	0.04*	0.19 (0.04-0.99)	0.049*
Male	Reference		Reference		Reference		Reference		Reference		Reference	
Age at 1st MMR dose												
12- <15 months	3.47 (1.24- 9.72)	0.01*	3.94 (1.37- 11.30)	0.01*	0.83 (0.09-7.57)	0.15	—	—	1.37 (0.19-9.74)	0.90	—	—
≥15 months	Reference		Reference		Reference				Reference			
Time since 2nd MMR dose												
<15 years	0.22 (0.05- 0.93)	0.03*	0.18 (0.04-0.80)	0.02*	1.75 (0.20-15.29)	0.79	—	—	2.38 (0.49-11.61)	0.26	2.64 (0.50-14.04)	0.25
≥15 years	Reference		Reference		Reference				Reference		Reference	
Baseline antibody concentrations												
<121mIU/mL	N.A.[4]	N.A.	N.A.	N.A.	N.A.	N.A.	195.8 (21.8- >999.9)	<0.0001*	N.A.	N.A.	54.95 (10.90- 277.14)	<0.0001*
≥121 mIU/mL	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	Reference		N.A.	N.A.	Reference	

[1] OR= Odds Ratio, CI= Confidence Interval

[2] Statistical Significance at p<0.05 is represented by an asterisk.

[3] Adjusted by controlling for baseline measles antibody concentrations.

[4] N. A. means not applicable. (By default, baseline measles neutralizing antibody concentrations could not be a risk factor at baseline. We were also unable to assess baseline neutralizing antibody concentrations at 1 month or 1 year post-MMR3 during univariate analysis because this was the variable we adjusted for to account for the statistical differences between Cohort 1 and Cohort 2. This adjustment allowed us to combine the cohorts during the analysis to increase our power. However, it is of note that the *unadjusted* OR's for baseline neutralizing antibody concentrations were highly significant at 1-month and 1-year post-MMR3.)

Table 2

Measles virus neutralizing antibody geometric mean concentrations by plaque reduction neutralization in correlation with measles virus antibody avidity levels by end titer avidity index percentages at baseline, 1 month, and 1 year after receiving a third dose of measles-mumps-rubella (MMR) vaccine

	BASELINE							1 MONTH POST-MMR3						1 YEAR POST-MMR3[1]						
		Measles Neutralizing Antibody Concentrations	Avidity Index					Measles Neutralizing Antibody Concentrations	Avidity Index					Measles Neutralizing Antibody Concentrations	Avidity Index					
Quartile[2]	n	GMC (mIU/mL)[3]	Mean[4]	Neg[5] (%)	Low (%)	Int (%)	High (%)	GMC (mIU/mL)	Mean	Neg (%)	Low (%)	Int (%)	High (%)	GMC (mIU/mL)	Mean	Neg (%)	Low (%)	Int (%)	High (%)	Miss (%)
1	27	69	71	8 (29.6)	0	10 (37.0)	9 (33.3)	249	75	2 (7.4)	0	5 (18.5)	20 (74.1)	143	73	1 (3.7)	0	6 (22.2)	16 (59.3)	4 (14.8)
2	11	556	78	0	0	0	11 (100)	606	81	0	0	0	11 (100)	466	80	0	0	0	10 (90.9)	1 (9.1)
3	11	990	79	0	0	1 (9.1)	10 (90.9)	1222	78	0	0	0	11 (100)	750	79	0	0	1 (9.1)	10 (90.9)	0
4	10	2130	78	0	0	2 (20)	8 (80)	2435	78	0	0	2 (20)	8 (80)	2225	81	0	0	3 (30)	7 (70)	0
Total	59	299	75	8	0	13	38	582	77	2	0	7	50	415	77	1	0	10	43	5

[1] Five subjects were missing data at one year.

[2] Quartiles were established based on baseline plaque reduction neutralization measles antibody concentration. Subjects with the lowest baseline measles neutralizing antibody concentrations were placed in Quartile 1 and subjects with the highest baseline measles neutralizing antibody concentrations were placed in Quartile 4. The number of subjects selected from Quartile 1 is more than from the other 3 quartiles, because we tested the avidity on every subject who had a negative or low measles neutralizing antibody concentration during at least 1 time point. Of note, 24 of 27 subjects in Quartile 1 had a negative or low baseline measles antibody concentration; the remaining 3 subjects in Quartile 1 had a medium neutralizing antibody concentration at baseline (but were still in the lowest quartile).

[3] Abbreviations used: GMC means Geometric Mean Concentration, Neg means negative, Int means intermediate, Miss means missing

[4] The mean avidity index excludes the negative specimens by Captia Measles IgG enzyme immunoassay since avidity could not be run on those specimens.

[5] Negative means that at 1:21 dilution, the specimen had undetectable IgG by the Captia Measles IgG enzyme immunoassay.

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Review Article

What, in Fact, Is the Evidence That Vaccinating Healthcare Workers against Seasonal Influenza Protects Their Patients? A Critical Review

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Background and Methods. Vaccination of all healthcare workers is widely recommended by health authorities and medical institutions and support for mandatory vaccination is increasing. This paper presents the relevant literature and examines the evidence for patient benefit from healthcare worker vaccination. Articles identified by Medline searches and citation lists were inspected for internal and external validity. Emphasis was put on RCTs. The literature on self-protection from vaccination is also presented. *Results*. Published research shows that personal benefit from vaccinating healthy nonelderly adults is small and there is no evidence that it is any different for HCWs. The studies aiming to prove the widespread belief that healthcare worker vaccination decreases patient morbidity and mortality are heavily flawed and the recommendations for vaccination biased. No reliable published evidence shows that healthcare workers' vaccination has substantial benefit for their patients—not in reducing patient morbidity or mortality and not even in increasing patient vaccination rates. *Conclusion*. The arguments for uniform healthcare worker influenza vaccination are not supported by existing literature. The decision whether to get vaccinated should, except possibly in extreme situations, be that of the individual healthcare worker, without legal, institutional, or peer coercion.

1. Introduction

Vaccination of all healthcare workers (HCWs) is widely recommended by health authorities and medical institutions [1–3]. This recommendation is based on the argument that because of their proximity to patients, HCW vaccination protects themselves and their patients from influenza.

The growing pressure on HCWs to vaccinate as part of their ethical professional responsibility is illustrated by the statement by the Canadian National Advisory Committee on Immunization for the 2010-2011 season that "in the absence of contraindications, refusal of HCWs who have direct patient contact to be immunized against influenza implies failure in their duty of care to patients" [2]. The fact that this is being taken even a step further with recommendations and pressure on institutions to mandate such vaccination at the expense of individual freedom and as a condition for continued employment [4] increases the urgency of examining the evidence. Is it sufficient for such draconian measures?

The argument in favor of vaccinating all HCWs is based primarily on their obligation to protect their patients. However, in order to give a complete picture, the evidence base concerning personal HCW benefit from vaccination is also presented.

As demonstrated in a study of an educational and promotional intervention in primary care, HCWs' vaccination rates can be increased by an easy intervention [5]. However, does the evidence justify widespread implementation of such programs?

The specific questions regarding benefit from HCW vaccination examined in this paper are detailed in Section 2.

2. Methods

The possible reasons for vaccinating HCWs are presented and discussed in turn.

Proposed Reasons for Vaccinating Healthcare Workers

(i) Self-protection

(a) All adults (HCWs included) should be vaccinated against influenza.

(b) HCWs are at increased risk of infection (and thus also of secondarily infecting their family members) and, therefore, their vaccination is more beneficial.

(ii) Patient protection

(a) Patients are at increased risk of being infected by transmission from infected HCWs. HCW vaccination reduces this risk.

(b) Vaccinating HCWs increases vaccination rates among their patients.

(c) Vaccination reduces HCWs sick leave during the flu season when there is increased patient need.

The discussion of these arguments is based on critical appraisal of the few published studies, mainly randomly controlled trials, examining the effect of vaccination and on relevant systematic reviews. A Medline search was performed for studies published from 1980 to date of submission using combinations of search terms depending on the specific issue being examined, with the major terms being influenza vaccine, vaccination, immunization, health personnel, healthcare worker, absenteeism, and sick leave. The search was all inclusive with no limitation put on language or research method. Citation lists for identified studies and cited papers, including those from articles presenting evidence as to vaccination effectiveness, were also investigated, as was The Cochrane Database of Systematic Reviews. The central effort was directed at taking a fresh critical look at the trials examining patient benefit. The studies' internal validity (bias, confounding, chance effects), the correlation between their content and conclusions, and their applicability were examined.

3. Results and Discussion

3.1. Assertion: "All Adults (HCWs Included) Should Be Vaccinated against Influenza". Commonly quoted figures demonstrating the effectiveness of influenza vaccination in healthy nonelderly adults are those from a study on randomly assigned volunteers from the Minneapolis area [6]. The rates of upper respiratory infections and of sick leave were, respectively, 25% and 36% lower among vaccinated adults compared to placebo recipients. However, the results of most other studies on the effectiveness of healthy adult vaccination are not as impressive. The 2010 Cochrane review on vaccines for preventing influenza in healthy adults [7] detected a statistically significant reduction in confirmed influenza cases, the size of which depended on the degree of vaccine matching to the circulating virus. However, the reviewers point out that the small overall average absolute difference of about 1% suggests that 100 adults would need to be vaccinated to prevent one case of influenza. The review showed that vaccine reduced time off work by an average of 0.13 days. This small effect was of borderline statistical significance (95% CI 0.00–0.25). Vaccination did not have a statistically significant effect on hospitalization or complications, and no evidence was found that vaccines prevent viral transmission. As the review included industry funded trials, the authors found it necessary to include a warning as to the interpretation of its content, stressing that the association between industry funding and study conclusions and publication, as demonstrated in a systematic review of studies on the effect of influenza vaccines [8], could have biased their results and that reliable evidence on influenza vaccines is thin but there is evidence of widespread manipulation of conclusions and spurious notoriety of the studies. The reviewers suggest that although serious harm from vaccination may be rare it cannot be ignored and conclude that the results of their literature review discourage the utilization of vaccination against influenza in healthy adults as a routine measure.

3.2. Assertion: "HCWs Are at Increased Risk of Infection (and Thus Also of Secondarily Infecting Their Family Members) and, Therefore, Their Vaccination Is More Beneficial". No reliable date could be found on influenza rates in HCWs (or their families) or comparisons to the general population [9, 10].

A small number of hospital based trials examined the effect of influenza vaccination on HCWs. Weingarten et al. [11], in a season with partial matching between vaccination and outbreak strains, failed to find a significant reduction of respiratory disease or sick leave among vaccinated HCWs. Wilde et al. [12] demonstrated high effectiveness of vaccinating hospital employees in preventing serologically defined influenza infection with 13.4% of control subjects and only 1.7% of vaccine recipients developing serologic evidence of influenza. However, this did not translate into clear clinical benefit—the small mean reductions in febrile respiratory disease (0.12 days) and absence from work (0.11 days) were far from reaching statistical significance. Saxén and Virtanen [13], in pediatric hospitals, also found no reduction in respiratory disease but demonstrated a statistically significant reduction of 0.4 days of sick leave because of respiratory infection in vaccinated personnel. There was no difference in absenteeism between HCWs with or without close patient contact. It should be noted that no explanation was given as to why this datum, favoring vaccination, was presented but no data were presented as to the total, all cause, absenteeism.

In a matched cluster-randomized trial of the effect of vaccinating nursing home staff in the Paris area [14], 8.7% of HCWs reported at least one day of sick leave during the influenza season compared to 13.3% in the control arm ($P = 0.03$). It should be noted that this, and the other cluster randomized studies which are discussed later in this paper, were not blinded. Therefore, any suggested benefit in the vaccinated group was not necessarily the effect of vaccination itself but, rather, could be that of the vaccination campaign

increasing awareness of influenza and leading to the taking of other preventive measures. Data supporting this argument is presented in Section 3.3.

The small amount of data on HCW vaccination does not, therefore, support the notion that it is more effective for self-protection in this group than in the general population. No studies appear to have been performed in the setting of primary care clinics where the contact with patients is less intense. The prevailing argument that HCWs are at higher risk of infection because of their proximity to infected patients is presently theoretical. A contrary, also logical and unproven, argument could be that HCWs are more aware of the danger and take more precautions against infection, such as keeping a distance from others, hand washing, and room ventilation.

3.3. Assertion: "Patients Are at Increased Risk of Being Infected by Transmission from Infected HCWs. HCW Vaccination Reduces This Risk". The most compelling argument presented for HCW vaccination is that infection of HCW puts their patients at risk of infection and therefor HCWs are morally obliged to get vaccinated.

A number of reports have documented influenza outbreaks in hospitals [15] and nursing homes [16], showing that personnel infection preceded patient infection. This has been interpreted as proof that HCWs were the source of patient infection. However, no studies have shown that this sort of temporal relationship between staff and patient infection is more frequent than expected by chance. Furthermore, even if we were to accept that in some situations HCW infection precedes inpatient outbreaks, this is far from proving causality. The fact that infection takes longer to reach patients in a relatively closed environment is not surprising but does not show that the main vectors were HCWs (except in an isolated intensive care unit with no other contacts and no introduction of new patients), or that an infection from other sources, such as visitors or new patients, would have spread less extensively.

The heavier proof for patient benefit from HCW vaccination is considered to come from the four randomly controlled trials on elderly residents in long term care institutions [14, 17–19], comparing control homes with homes where vaccination campaigns were directed at the staff. All four studies concluded that HCW vaccination leads to a reduction in patient mortality. Because of the importance of this conclusion, these articles require special scrutiny.

The first published article [17], on geriatric long-term care hospitals in Scotland, demonstrated that staff-vaccinated hospitals had a significantly lower rate of inmate mortality (10% compared to 17% in control hospitals, OR 0.56, 95% CI 0.40–0.80) and influenza like disease. However, special inspection of the article reveals that mortality and morbidity data used for the comparison started at the end of October although the first outbreak of influenza occurred in January, over two months later. Examination of the patient mortality curves for vaccinated and unvaccinated staff shows that they diverge from the beginning of data collection, two months before the first influenza outbreak, and continue to diverge at the same rate when influenza breaks out. It is unclear why data preceding the outbreaks were included in the analysis, and the early mortality divergence clearly suggests that the difference between the two groups was unrelated to influenza. The difference could be the effect of intervention on increased awareness of the dangers of influenza, this resulting in other behavioral preventive measures which were effective also against other respiratory viruses. Further problems in the article include data inconsistencies such as a larger number of patients dying of pneumonia than the number that developed lower respiratory tract infection, the possible bias in identifying influenza-like disease by nonblinded nurses, and the fact that no difference was identified in serologic proven influenza. The article's conclusion and heading stating that "influenza vaccination of health care workers in long-term-care hospitals reduces the mortality of elderly patients" is, therefore, not supported by its content.

The second, similar, study [18] was performed two years later by the same investigating team (with some changes) in the same geographic area. Mortality during winter was 13.6% in staff-vaccinated long-term hospitals and 22.4% in control hospitals (OR 0.58, 95% CI 0.40–0.84). This is interpreted as indicating that staff vaccination substantially decreased mortality among patients. No data is presented on dates of flu outbreaks, so that the temporal relationship between influenza and mortality cannot be examined here. However, the data reveals that vaccine hospitals had both a much higher rate of vaccinated patients (48% compared to 33%) and less patient disability at baseline. Clearly, both these factors could have contributed to the lower mortality in these hospitals. A regression analysis showed that when the confounding effects of disability and patient vaccination were even partially controlled for (by using uniform average disability and vaccination scores for all residents of each hospital), the association between staff vaccination and patient mortality lost its statistical significance. Regretfully, the result of this multivariate analysis is ignored in the authors' conclusion, based on the crude data, that vaccination of HCWs is associated with a substantial decrease in patient mortality. This study, like its predecessor, did not find an association between HCW vaccination and virological proof of patient infection, despite a good match between the vaccine and outbreak influenza variants.

The third study [19] was performed in pair matched English care homes during two consecutive years. Significantly lower resident mortality (11.2% versus 15.3%, $P = 0.002$) was observed in the intervention homes in the first year but not in the second, in which influenza activity was low. Lower rates were found in the first year also for influenza like illness and admissions with influenza-like illness. However, comparison of resident characteristics demonstrates that intervention homes in that year, but not the other, had appreciably higher rates of resident vaccination (78.2% compared to 71.4%) and lower rates of highly dependent residents (36.0% and 41.4%). As in the previous study [16] this could explain much of the difference in mortality and morbidity. The article failed to examine the effect of these factors, or, as previously explained, to distinguish between the effect of vaccination and the general effect of

the intervention increasing staff awareness for preventive behavior. No serologic evidence was presented to support the claim that the differences in outcomes were related to influenza. Also, disease data could have been biased by being collected by nonblinded nurses.

The last of the four RCTs, performed on pair-matched nursing homes in Paris [14], did not show a significant difference in crude resident mortality data (5.2% in intervention and 6.0% in control homes, $P = 0.08$). However a multivariate analysis model, which included a disability score and patient vaccination status, showed that belonging to the vaccination arm was a significant predictor of resident mortality (OR 0.80, $P = 0.02$). The vaccination homes did not show lower hospitalization rates, but influenza like illness was also significantly lower. The authors point out that examining the weekly mortality rates and influenza like illness they were surprised to find that the difference between intervention and control rates was larger for a preceding RSV (respiratory syncytial virus) outbreak than for the influenza outbreak. They explain, correctly, that reduced RSV morbidity in the intervention group could not be an effect of influenza vaccination, but rather that the intervention may have made the staff in the vaccinated homes more aware of the risks of influenza, leading them to adopt general preventive measures effective also against other respiratory viruses (such as RSV). If that is the case, then why are these general effects of the intervention not accepted also as the reason for the smaller reduction in morbidity and mortality during the influenza outbreak? Examining the chart of the weekly rates in the article's supporting information shows the even more surprising information (not mentioned in the text) that most of the reduced morbidity and mortality in the study period was during the two weeks before the influenza outbreak. This difference, before the appearance of influenza, is shortly after the RSV outbreak peak and most probably related to it. Looking at the graphs, it appears that removing these two weeks, the inclusion of which remains unexplained, would cancel any substantial difference in mortality.

To summarize these four RCTs, the repeated conclusion that staff vaccination has preventive value for elderly patients in nursing homes appears to be the result of major methodological errors and wishful thinking. Even when there appears to be less morbidity and mortality in the intervention hospitals this probably resulted from other factors.

The severely biased conclusions of these articles are the crux of the "proof" presented by authorities supporting HCW vaccination. It is somewhat depressing to see the prejudiced manner in which the literature can be presented, as illustrated by the 2010 CDC advisory committee on immunization practices [20] recommendations on HCW vaccination. The above reviewed flawed studies are presented by this committee as evidence and further support is added by stating: "a review concluded that vaccination of HCP in settings in which patients also were vaccinated provided significant reductions in deaths among elderly patients from all causes and deaths from pneumonia." This statement does not correctly represent the referenced 2006 review [21] which presented the flawed data from the two studies published at that time [17, 18] but actually concluded, very differently, that "…an incremental benefit of vaccinating health-care workers for elderly people has yet to be proven in well-controlled clinical trials". This review was updated in a 2010 Cochrane systematic review [9] based on all four RCTs, which concluded that "no effect was shown for specific outcomes: laboratory proven influenza, pneumonia, and death from pneumonia. An effect was shown for nonspecific outcomes of ILI (influenza like disease), GP consultation for ILI, and all-cause mortality. These nonspecific outcomes are difficult to interpret because ILI includes many pathogens, and influenza contributes <10% of all-cause mortality in individuals >60…The identified studies are at high risk of bias…*We conclude there is no evidence that vaccinating HCWs prevents influenza in elderly patients in long term care facilities*." This important and unambiguous conclusion was disregarded by the CDC committee in their recommendations, published six months later, favoring HCW vaccination [20].

3.4. Assertion: "Vaccinating HCWs Increases Vaccination Rates among Their Patients". Studies have shown the importance of a physician's recommendation for patient vaccination [22, 23] and an association between physicians being immunized and their reported recommendations to their patient [24, 25]. Patients have more confidence in counseling from physicians who themselves demonstrate healthy behavior [26, 27] and physicians who can report that they themselves got immunized may be more successful in convincing reluctant patients. However, only a weak association was demonstrated between actual patient vaccination and their primary care physician's personal vaccination status (OR 1.08, 95% CI 1.02–1.14) in one cross-sectional study [28] and none in another [29].

The previously described RCTs on the effect of staff vaccination on patients in long term institutions did not specifically examine the effect of HCW vaccination on patient vaccination. Comparison of the crude patient vaccination data for intervention and control homes gives inconsistent results; only in one trial [18] was patient vaccination rate clearly higher in the institutions where the staff was vaccinated. Even if one were to suggest that the increased patient vaccination rate resulted from the intervention among the staff, this, as the authors themselves correctly imply, did not necessarily result from HCW vaccination but rather from the intervention raising HCWs' awareness of influenza risk in their patients. The vaccination rates in the other trials are even less supportive of a positive effect of staff vaccination: in one trial [17] the rate in the homes where the workers were vaccinated was significantly lower than in the controls, in one [14] it was similar and in another [19] it was significantly higher in only one of two years.

A controlled trial in primary care [30] failed to demonstrate a substantial association between raising staff vaccination rates and patient vaccination rates.

Staff vaccination appears, therefore, not to be an important factor in increasing patient vaccination.

3.5. Assertion: "Vaccination Reduces HCWs Sick Leave during the Flu Season When There Is Special Patient Need". This

issue was addressed in the section on HCW flu risk, showing that vaccination leads to only a small, if any, reduction in HCW sick leave. This cannot be considered to be a proven significant benefit to patients.

3.6. Is Benefit from Vaccination Uniform to All HCWs? All four RCTs supposedly showing that staff vaccination reduces patient mortality were performed in long-term nursing institutions and, therefore, even if their conclusions were valid, they would not necessarily apply to all healthcare situations. Encounters in most other situations, for example in community clinics, are of less proximity and duration and the patients are generally healthier and at lower risk. Preventive measures which may be valuable in intensive care units or geriatric nursing homes may have no significance in healthier settings such as preventive services for healthy populations or primary care clinics. People using these services are generally mobile and in repeated and close contact with others, including family, friends, at the supermarket, in the mall, at the post office, on the bus, in waiting rooms, and at the theatre. There is no evident basis to the belief that a short encounter with community clinic HCWs substantially increases the risk of contracting influenza. The importance of vaccination may also differ according to the HCW's specific activity; vaccination may be necessary for a nurse in a hospice but superfluous for a clerk in an ambulatory dermatology clinic. HCWs, like others, with chronic disease may have greater personal benefit from vaccination.

4. Conclusion

The present paper examined each of the arguments in favor of HCW influenza vaccination and showed that they are not supported by existing literature. The evidence base supporting vaccination is unsound and prejudiced.

The personal benefit from vaccinating healthy nonelderly adults is small and there is no evidence to show that it is any different for HCWs. The studies aiming to prove the widespread belief that staff vaccination has a substantial effect on patient morbidity and mortality are heavily flawed. No reliable evidence shows that HCW vaccination has noteworthy advantage to their patients—not in reducing patient morbidity or mortality, not in increasing patient vaccination, and not in decreasing HCW work absenteeism.

The finding that there is no valid evidence clearly supporting vaccination of HCWs does not mean that there cannot be some unproven benefit from vaccination. However, if substantial benefit exists it still needs to be demonstrated in valid studies.

This paper is of special importance due to the increasing pressure to mandate HCW vaccination. Such drastic action, at the expense of personal freedom, should not be accepted in the absence of very strong evidence for a very strong population benefit. The decision whether to vaccinate is, at present and in most situations, not a moral issue and should remain that of the individual HCW, preferably based on real information.

Key Points

(i) There are no studies showing that healthcare workers are at increased risk of influenza and its complications or that the vaccine is more effective in this group.

(ii) The evidence base for the claim that vaccinating healthcare workers against influenza protects their patients is heavily flawed and inconclusive at best.

(iii) The benefit from vaccinating healthcare workers, if any, may differ according to specifics of the patients, location, and worker.

(iv) At present, the decision whether to get vaccinated should, except possibly in extreme situations, be that of the individual healthcare worker, without legal, institutional, or peer coercion.

Conflict of Interests

The author declares no conflict of interests.

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ORIGINAL ARTICLE

Waning Protection after Fifth Dose of Acellular Pertussis Vaccine in Children

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ABSTRACT

BACKGROUND

In the United States, children receive five doses of diphtheria, tetanus, and acellular pertussis (DTaP) vaccine before 7 years of age. The duration of protection after five doses of DTaP is unknown.

METHODS

We assessed the risk of pertussis in children in California relative to the time since the fifth dose of DTaP from 2006 to 2011. This period included a large outbreak in 2010. We conducted a case–control study involving members of Kaiser Permanente Northern California who were vaccinated with DTaP at 47 to 84 months of age. We compared children with pertussis confirmed by a positive polymerase-chain-reaction (PCR) assay with two sets of controls: those who were PCR-negative for pertussis and closely matched controls from the general population of health-plan members. We used logistic regression to examine the risk of pertussis in relation to the duration of time since the fifth DTaP dose. Children who received whole-cell pertussis vaccine during infancy or who received any pertussis-containing vaccine after their fifth dose of DTaP were excluded.

RESULTS

We compared 277 children, 4 to 12 years of age, who were PCR-positive for pertussis with 3318 PCR-negative controls and 6086 matched controls. PCR-positive children were more likely to have received the fifth DTaP dose earlier than PCR-negative controls ($P<0.001$) or matched controls ($P=0.005$). Comparison with PCR-negative controls yielded an odds ratio of 1.42 (95% confidence interval, 1.21 to 1.66), indicating that after the fifth dose of DTaP, the odds of acquiring pertussis increased by an average of 42% per year.

CONCLUSIONS

Protection against pertussis waned during the 5 years after the fifth dose of DTaP. (Funded by Kaiser Permanente).

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Pertussis is a worldwide, cyclic infection. Before widespread vaccine coverage, up to 270,000 cases of pertussis were diagnosed in the United States annually, with as many as 10,000 deaths per year, predominantly among infants.[1] Pertussis vaccines prepared from whole *Bordetella pertussis* organisms were available from the 1940s through the 1990s, protecting infants who were 2 months of age or older.[1]

Whole-cell pertussis vaccines, when administered as part of a combined diphtheria, tetanus toxoids, and pertussis vaccine, were effective, but they were associated with adverse effects[2]; this led to the development of the diphtheria–tetanus–acellular pertussis (DTaP) vaccine.[3] Beginning in the early 1990s, the United States started to make the transition from whole-cell pertussis vaccines to DTaP, and by the late 1990s, DTaP was being used for all five recommended doses.[4] DTaP is now used in many countries.

Pertussis vaccination resulted in a marked decrease in the incidence of disease,[1,5] with diagnosed cases of pertussis reaching a nadir in 1976. However, since the 1980s, despite high levels of vaccine coverage in children, outbreaks of *B. pertussis* have occurred every 3 to 5 years, with an increase in the peak incidence with each successive outbreak.[6] The reasons for the ongoing outbreaks are not well understood and are probably multifactorial.[7-9]

Receipt of five doses of DTaP is mandatory for school entry in many states, including California, with the fifth dose usually administered in children between 4 and 6 years of age. Nonetheless, in 2010, California had a large pertussis outbreak,[10] with the highest incidence rates since 1958. After this outbreak, we sought to assess and quantify the waning of DTaP protection against pertussis over time in a highly vaccinated population of school-age children who had received only DTaP rather than whole-cell pertussis vaccines.

Methods

Databases

Kaiser Permanente Northern California is an integrated health care delivery system that provides care to approximately 3.2 million members. It operates 49 medical clinics and 19 hospitals, including pharmacies and laboratories. Databases capture vaccinations and laboratory tests, as well as inpatient, emergency department, and outpatient diagnoses.

Data on race or ethnic group were available in the medical record for approximately 75% of members. For the remainder, we imputed race or ethnic group with the use of the RAND Bayesian Imputed Surname Geocoding algorithm.[11] In members for whom we imputed values for missing data on race or ethnic group (American Indian or Alaska Native, Asian or Pacific Islander, black, Hispanic, or white), the probabilities summed to 1; a single value was not assigned. Microbiologic testing was centralized in a single laboratory that has identified *B. pertussis* and *B. parapertussis* with the use of polymerase-chain-reaction (PCR) assays since 2005. PCR kits were supplied by Roche from December 2005 through May 2009 and by Cepheid beginning in May 2009.

Kaiser Permanente Northern California first introduced DTaP for the fifth dose of pertussis vaccine in 1991 and completed the transition from whole-cell pertussis vaccines to DTaP for all five doses by 1999.

Study Oversight

The institutional review board of Kaiser Permanente Northern California approved this study and waived the requirement for informed consent.

All authors vouch for the completeness and accuracy of the data and analyses presented.

Study Design and Population

In this case–control study, we selected case patients and controls for the primary analysis from all Kaiser Permanente Northern California members who received a pertussis PCR test result between January 2006 and June 2011. PCR results were positive for *B. pertussis*, positive for *B. parapertussis*, or negative for both.

Potential case patients were all children who were positive for pertussis and negative for parapertussis on PCR testing during the study period and who received a dose of DTaP between the ages of 47 and 84 months (this dose was considered the fifth DTaP dose) before the PCR test was performed. We excluded persons born before 1999 (to limit the analyses to children who exclusively received DTaP vaccines) and persons who received a vaccine with reduced pertussis-antigen content (Tdap) or any pertussis-containing vaccine after the fifth dose but before the PCR test. We also ex-

cluded children in whom a PCR test was performed within 2 weeks after receipt of the fifth DTaP dose and children who were not members of Kaiser Permanente Northern California for more than 3 months between the fifth dose of DTaP and the PCR test.

The study included two control groups. The first group consisted of children who were PCR-negative for both pertussis and parapertussis and who received a fifth dose of DTaP before receiving a negative test result (the PCR-negative controls). The second group consisted of health-plan members who were matched to each PCR-positive child (the matched controls). Matched controls were the same sex and age (year and quarter of birth), of the same race or ethnic group (with seven groups defined: six for available data on race or ethnic group and one for imputed data on race or ethnic group, to account for missing data), and attended the same medical clinic (of 49 clinics) as the PCR-positive children and were members on the date of the PCR test in the PCR-positive children (the anchor date). We retained all matched controls (with no sampling) who received a fifth dose of DTaP before their anchor date. We applied the same exclusion criteria described above to both control groups and excluded children as controls if they had previously tested positive for pertussis.

The final study population consisted of children who were 4 to 12 years of age, 58% of whom were continuously enrolled in the health plan between 1 month of age and either the date on which PCR was performed or the seventh birthday. In this subgroup, the rate of vaccine coverage with five doses of DTaP was 99% and did not differ between PCR-positive case patients and PCR-negative controls.

STATISTICAL ANALYSIS

We assessed the waning of immunity after DTaP vaccination using two analyses. The primary analysis compared PCR-positive case patients with PCR-negative controls, and the secondary analysis compared PCR-positive case patients with matched controls. We considered the comparison with PCR-negative controls to be primary because it minimized the potential biases associated with the general propensity to use health care and the specific propensity of parents and physicians to test for pertussis.

We fit conditional logistic-regression models to estimate the effect of each additional year after receipt of the fifth DTaP dose on the odds of a positive PCR test for pertussis. For the primary analysis, we conditioned the logistic model on blocks of calendar time (yearly from 2006 through 2009 before the epidemic, quarterly for the first quarter of 2010, and then monthly thereafter during the epidemic). We included covariates to adjust for age (4 to <7, 7 to <10, and 10 to 12 years), sex, medical clinic (49 clinics aggregated into 12 service areas), and race or ethnic group (in children for whom data were available or from imputed probabilities). For the secondary analysis, we conditioned the logistic model on all the matching variables (PCR test date, quarter of birth, sex, race or ethnic group, and medical clinic), and we used imputed probabilities of race or ethnic group as covariates for additional adjustment for the strata of children with imputed data. For all analyses, we used SAS software, version 9.2 (SAS Institute).

RESULTS

INCIDENCE OF PERTUSSIS

From January 2006 through June 2011, a total of 27,912 PCR assays for *B. pertussis* were performed in members of the health plan, regardless of age; of these tests, 1512 (5.4%) had a positive result. During the period from January 2010 through June 2011, when 95% of the cases of pertussis in the study population were diagnosed, the incidence of pertussis was 115 cases per 100,000 person-years among members younger than 1 year of age, decreasing to 29 cases per 100,000 person-years at 5 years of age, sharply increasing to 226 cases per 100,000 person-years at 10 and 11 years of age, sharply decreasing until 15 years of age, and remaining low in persons 15 years of age or older (Fig. 1). Ecologic data showing the percentage of persons who had received DTaP instead of whole-cell pertussis vaccines as infants, according to their current age, are shown in Figure 1.

CHARACTERISTICS OF THE STUDY POPULATION

Our study population included 277 children between the ages of 4 and 12 years who were PCR-positive for pertussis, 3318 PCR-negative controls, and 6086 matched controls. Table 1 lists characteristics of the case patients and controls.

Older age was associated with a higher percentage of positive PCR tests: 4.5% among 6-year-old children, 12.2% among 8-year-old children, and 18.5% among 10-year-old children. Increasing time since the fifth dose of DTaP was associated with an increasing percentage of positive PCR tests (Fig. 2). The time since the fifth dose of DTaP was significantly longer for PCR-positive children (1699 days; 95% confidence interval [CI], 1627 to 1772) than for PCR-negative controls (1028 days; 95% CI, 1003 to 1053) (P<0.001); case children received their fifth dose of DTaP significantly earlier than controls.

WANING OF DTaP EFFECTIVENESS

In the primary analysis comparing PCR-positive children with PCR-negative controls, with adjustment for calendar time, age, sex, race or ethnic group, and medical service area, the odds ratio for pertussis was 1.42 per year (95% CI, 1.21 to 1.66), indicating that each year after the fifth dose of DTaP was associated with a 42% increased odds of acquiring pertussis. A secondary analysis comparing PCR-positive cases with matched controls yielded similar results (Table 2).

SEVERITY OF PERTUSSIS

Cases of pertussis were mild or moderate in severity. Within 5 days before or after the PCR test, 272 of the 277 children had an outpatient encounter (98.2%), and 261 received a prescription for azithromycin (94.2%); 219 children received a diagnosis of whooping cough, cough, or pertussis exposure (79.1%); and 45 children received related diagnoses (respiratory infection, asthma, bronchitis, croup, or unspecified viral infections) (16.2%). Within 100 days before or after the PCR test, 11 of the children (4.0%) had emergency department visits related to pertussis; there were no hospitalizations or deaths related to pertussis.

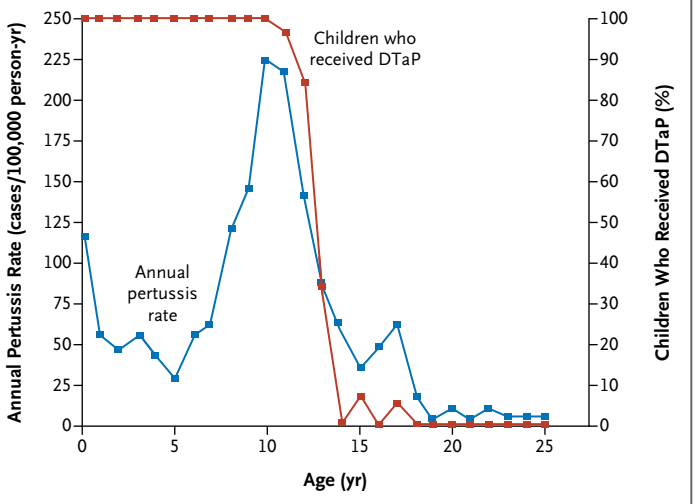

Figure 1. Annual Rate of Pertussis and Vaccination History in the Entire Health-Plan Population, According to Age, during the Pertussis Outbreak from January 2010 through June 2011.

The annual rate of pertussis (the number of cases per 100,000 person-years) for each age was calculated as follows: all cases of pertussis confirmed by a positive polymerase-chain-reaction (PCR) assay were divided by all person-years at risk and then multiplied by 100,000. Age was calculated on the date of the PCR test (for persons counted in the numerator) and on the last date of each month (for persons counted in the denominator). The percentage of members as of August 14, 2010, who were likely to have received diphtheria, tetanus, and acellular pertussis (DTaP) vaccine for all five doses (i.e., none of the doses were whole-cell pertussis vaccines) was calculated from population-based data on the timing of the transition in the health plan from diphtheria, tetanus, and whole-cell pertussis vaccines to DTaP vaccine. August 14, 2010, was the midpoint of cases (the median diagnosis date) during the 18-month period.

DISCUSSION

In the 2010 pertussis outbreak in California, a longer time since receipt of a fifth dose of DTaP was associated with an elevated risk of acquiring pertussis among children who had received all recommended acellular pertussis vaccines. In this study, the risk of pertussis increased by 42% each year after the fifth DTaP dose. If DTaP effectiveness is initially 95%, so that the risk of pertussis in vaccinated children is only 5% that of unvaccinated children, then the risk would increase after 5 years by a factor of 1.42[5] to 29% that of unvaccinated children. The corresponding decrease in DTaP effectiveness would be from 95% to 71%. The amount of protection remaining after 5 years depends heavily on the initial effectiveness. If the initial effectiveness of DTaP was 90%, it would decrease to 42% after 5 years. Regardless of the initial effectiveness, the protection from disease afforded by the fifth dose of DTaP among fully vaccinated children who had exclusively received DTaP vaccines waned substantially during the 5 years after vaccination.

The results of clinical trials evaluating the duration of protection conferred by DTaP vaccines after three or four doses suggested that protection against pertussis was sustained 5 to 6 years after

Table 1. Characteristics of PCR-Positive Children and Controls, January 2006–June 2011.*

Variable	PCR-Positive Children (N=277)	PCR-Negative Controls (N=3318)	P Value†	Matched Controls (N=6086)‡	P Value†
Male sex — no. (%)	121 (43.7)	1684 (50.8)	0.02	2659 (43.7)	1.00
Age — yr			<0.001		0.78
Mean	8.8±1.7	6.9±2.1		8.8±1.7	
Range	4–12	4–12		4–12	
Age distribution — no. (%)			<0.001		0.60
4 to <7 yr	36 (13.0)	1629 (49.1)		765 (12.6)	
7 to <10 yr	121 (43.7)	1164 (35.1)		2844 (46.7)	
10 to 12 yr	120 (43.3)	525 (15.8)		2477 (40.7)	
Year of PCR test — no. (%)			0.003		1.00
2006	2 (0.7)	97 (2.9)		44 (0.7)	
2007	1 (0.4)	102 (3.1)		22 (0.4)	
2008	6 (2.2)	107 (3.2)		132 (2.2)	
2009	6 (2.2)	155 (4.7)		132 (2.2)	
2010	201 (72.6)	2150 (64.8)		4416 (72.6)	
2011	61 (22.0)	707 (21.3)		1340 (22.0)	
Race or ethnic group — no. (%)§			<0.001		1.00
American Indian or Alaska Native	2 (0.7)	14 (0.4)		44 (0.7)	
Asian or Pacific Islander	23 (8.3)	547 (16.5)		505 (8.3)	
Black	9 (3.2)	216 (6.5)		198 (3.2)	
Hispanic	83 (30.0)	790 (23.8)		1824 (30.0)	
White	133 (48.0)	1328 (40.0)		2922 (48.0)	
Unknown and imputed	27 (9.7)	423 (12.7)		593 (9.7)	

* Plus–minus values are means ±SD. PCR denotes polymerase chain reaction.
† P values, which are based on comparisons between PCR-positive children and either PCR-negative controls or matched controls, were calculated with the use of the t-test for the continuous variable of age and with the use of the chi-square test for the rest of the variables.
‡ The controls were matched according to all the characteristics shown. The numbers and percentages in this column are weighted to indicate that the comparison of PCR-positive children with the matched controls was balanced in the analysis.
§ Race or ethnic group was determined from the medical record or was imputed in the case of missing data. The Hispanic ethnic group includes children of all races.

vaccination.[12-14] Other studies showed some waning of protection,[15-17] and several showed that increasing time since DTaP vaccination was a risk factor for vaccine failure, observations that are consistent with our findings.[17-19] Disease-free intervals after pertussis vaccination have decreased over the past two decades in Massachusetts.[20] A study in Canada showed that the transition from whole-cell pertussis vaccines to DTaP was associated with an increased incidence of pertussis among children who received only DTaP.[21] Taken together, these studies indicate that protection is less enduring with DTaP than with whole-cell pertussis vaccines.[22] The recent California epidemic provides data from a large population of children who only received acellular vaccines and for whom enough time had passed that we could quantify the extent to which DTaP protection waned.

The incidence of pertussis was highest among the population of children who were 8 to 11 years of age and who had received the full five-dose series of DTaP in childhood, suggesting that the waning efficacy of the fifth dose among school-age children played a key role in both allowing

and sustaining the recent pertussis outbreak. This observation was surprising because it is not until children reach their teenage years that they are usually considered to be a reservoir for pertussis,[23] and teenagers have been disproportionately affected in previous pertussis outbreaks.[20] Figure 1 shows that on a population basis, the incidence of pertussis decreased very sharply at 12 to 15 years of age, precisely the same ages of children who were likely to have received whole-cell pertussis vaccines as infants. These ecologic data show that the risk of pertussis was lower among older adolescents, who were likely to have previously received at least one dose of the whole-cell pertussis vaccine than among younger adolescents, who had exclusively received DTaP.

Most children in this study received their fifth dose of DTaP between 4 and 6 years of age. Thus, age and time since vaccination were highly collinear (r=0.97), and we were unable to fully separate out these two variables in the primary analysis involving PCR-negative controls. We could not entirely rule out the possibility that the incidence of pertussis among older children was higher because they were older rather than because of waning protection. The sharp increase in the incidence of pertussis among children 8 to 11 years of age, followed by a sharp decrease at 12 to 15 years (Fig. 1), is not characteristic of the epidemiology of pertussis in unvaccinated persons or in previous outbreaks. Furthermore, the secondary analyses involving controls who were closely matched for age showed that the association between the time since vaccination and the risk of pertussis was similar to that in the primary analysis. Therefore, it is more plausible to attribute the increased incidence of pertussis in children between 8 and 11 years of age to the waning effectiveness of DTaP rather than to aging.

The Centers for Disease Control and Prevention recommends routine administration of Tdap beginning at 11 years of age, with vaccination of children as young as 7 years of age in certain circumstances.[24] The limited duration of DTaP protection raises the question of whether routine administration of Tdap in younger children (e.g., 8-year-old children) is warranted. However, several issues must be clarified, including the effectiveness and duration of protection of Tdap, the possibility of increased local reactions with more frequent administration of Tdap, the increased cost and burden associated with earlier Tdap boosting (particularly since no other vaccines are routinely given at this age), and the risk of transmission to infants posed by mild-to-moderate pertussis infections that could be prevented with earlier Tdap boosting. Prevention of future outbreaks will be best achieved by developing new pertussis-containing vaccines that provide long-lasting immunity.

The large population in the health plan allowed controls to be matched to PCR-positive children on many potential confounders, and matched con-

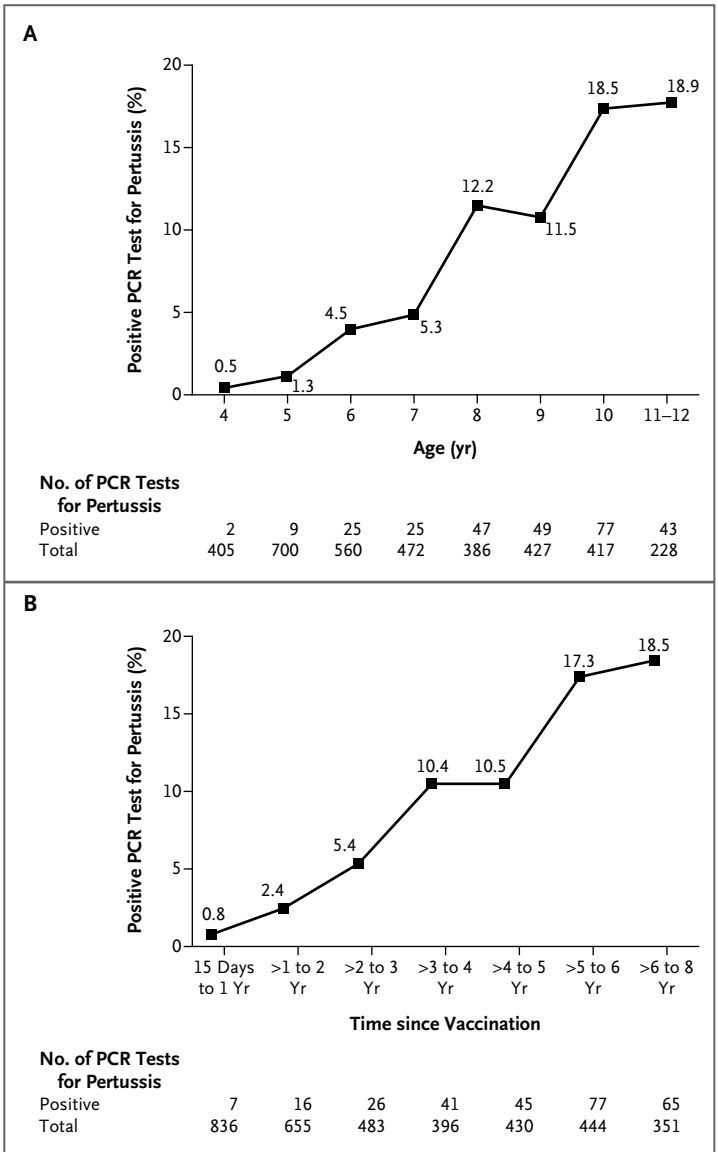

Figure 2. Percentage of PCR Tests That Were Positive for Pertussis from January 2006 through June 2011, According to Age and Time since Vaccination.

Table 2. Waning of Effectiveness per Year after Fifth Dose of DTaP Vaccine.

Group Compared with PCR-Positive Children	Odds Ratio for Pertussis (95% CI)	P Value
PCR-negative controls	1.42 (1.21–1.66)*	<0.001
Matched controls	1.50 (1.13–2.00)†	0.005

* The odds ratio was estimated on the basis of a conditional logistic-regression analysis that was stratified according to calendar time and included covariates to adjust for age, sex, race or ethnic group, and medical service area. This model deleted 10 observations for PCR-negative controls because of missing covariate data.

† The odds ratio was estimated on the basis of a conditional logistic-regression analysis that was stratified according to calendar time, age, sex, race or ethnic group, and medical clinic and included imputed probabilities of race or ethnic group as covariates to provide additional adjustment within the strata of children with imputed data.

trols were more similar to PCR-positive children than were PCR-negative controls on all measured potential confounders. However, matched controls were probably not as similar to PCR-positive children as PCR-negative controls were with respect to unmeasured potential confounders, such as the propensity to have undergone a PCR test to detect pertussis. Because we believe that such unmeasured confounders were probably a greater source of bias than the ones we were able to measure, we considered the analysis involving PCR-negative controls to be more informative.

Our study has several important strengths. One was that we compared PCR-positive children with two sets of controls and obtained similar results with each comparison. Another was that we had precise histories regarding the number of doses of vaccine received and the timing of vaccination and nearly complete demographic data for PCR-positive children and controls. Finally, we observed that older age was associated with an increasing proportion of positive PCR tests (Fig. 2); this supports our inference that the increase in the incidence of pertussis reflected a true increase in the incidence of disease rather than increased testing for pertussis.

Our study has limitations. First, although we estimated that the fifth dose of DTaP became 42% less effective each year, we could not anchor this estimate to the initial effectiveness of the vaccine because of the absence of an unvaccinated population. Second, it is possible that PCR testing misclassified a small fraction of persons (i.e., false positive and false negative tests). Since it was highly unlikely that such potential misclassification depended on the time since immunization, misclassification would imply that DTaP effectiveness may have waned even more than we estimated.

In conclusion, our evaluation of data from a large pertussis outbreak in California showed that protection from disease after a fifth dose of DTaP among children who had received only DTaP vaccines was relatively short-lived and waned substantially each year. Our findings highlight the need to develop new pertussis-containing vaccines that will provide long-lasting immunity.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Vaccine development needs a booster shot

by Liz Szabo, USA TODAY

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A new study, which finds that immunity from the whooping cough vaccine fades sharply over time, underscores the urgent need to develop new vaccines and consider additional booster shots for children, health experts say.

Authors say the study in today's New England Journal of Medicine helps explain part of the resurgence in whooping cough, or pertussis, which has sickened more than 26,000 this year -- the largest outbreak in more than 50 years.

The current vaccine, in use since the 1990s, doesn't protect people as long as previously believed, losing 42% of its effectiveness with each passing year, says author Nicola Klein, co-director of the Kaiser Permanente Vaccine Study Center in Oakland, Calif. So even some fully vaccinated children -- who have received all five doses recommended by age 4 to 6 -- would still be vulnerable to the disease by age 10.

The Centers for Disease Control and Prevention has reached similar conclusions, says Tom Clark, a CDC epidemiologist specializing in whooping cough. While the whooping cough vaccine protects about 98% of children in the first year, it protects only about 70%five years later, Clark says.

"We know the short-term protection is very good," Clark says. "But the protection is wearing off and that is the problem."

The findings shouldn't cause parents to stop vaccinating their children, however, Klein says. Even an imperfect vaccine is better than no vaccine, she says.

Whooping cough is typically more severe among unvaccinated children than among those who've had at least some of their shots, Clark says. Unvaccinated patients also tend to be sick longer and are often more contagious.

Doctors say they're most concerned about infants.

Newborns too young to be fully vaccinated -- whose airways can quickly swell shut -- are the most likely to die from whooping cough, says C. Mary Healy, a pediatric infectious-disease specialist at Texas Children's Hospital in Houston. Eleven of the 13 deaths from whooping cough this year were in infants; the other two deaths were in toddlers, according to the CDC.

Given the vaccine's limitations, Healy says, it's more important than ever to create a "cocoon" of protection around babies by vaccinating everyone around them. About 75% of babies with whooping cough contract the bacteria from a household member, such as a sibling, parent or grandparent.

"If a vaccine does not have 100% protection that's lifelong, then it's even more important that we have 'herd immunity' to stop the virus from circulating into the community," Healy says. "That's an unacceptable level of infant deaths, in the 21st century, in the richest country in the world."

Ultimately, the country needs a better vaccine, says James Cherry of the University of California-Los Angeles.

But "the business of coming up with a better vaccine is not going to be a quick fix," says Edgar Marcuse, a professor of pediatrics at Seattle Children's Hospital. "We still don't fully understand immunity from pertussis."

For example, even those naturally infected with whooping cough don't develop life-long immunity, and can come down with the bacterial infection again in 10 years or less, Marcuse says.

Infection rates today, in spite of the current outbreak, are 23 times lower than in the pre-vaccine days, Cherry says. In the pre-vaccine era, up to 270,000 Americans became sick with whooping cough each year, known as the "100-day cough," and up to 10,000 died, Klein says.

The whooping cough vaccine, available beginning in the 1940s, cut infection rates dramatically. That vaccine, known as DTP, was associated with more reactions than the current vaccine.

Most of those reactions were mild, such as increased crying or sore arms and legs. Some children developed benign -- but frightening -- fever-related seizures, which occured in about one in every 1,750 doses, says Gregory Poland, a professor of infectious disease at the Mayo Clinic in Minnesota.

An analysis by the Institute of Medicine found that DTP could cause rare but more serious problems: a dangerous brain inflammation, occurring in 1 to 10 per million doses; and an unusual, shock-like state, occurring 3 to 300 times per million doses, Poland says.

Whooping cough rates began rising after the current vaccine, known as Dtap, came into widespread use in the late 1990s, Cherry says.

The experience with DTP had far-reaching effects.

Although multiple studies show that today's vaccines are safe, many parents remain nervous about immunizations, delaying or skipping some of their children's shots -- a trend that has helped to fuel outbreaks of a number of infectious diseases, says Tom Belhorn, a pediatric infectious disease specialist at the University of North Carolina-Chapel Hill.

Until researchers produce a better vaccine -- with long-lasting immunity -- health experts could consider changing the vaccine schedule to get the most protection possible from the current shot, Poland says. Researchers would have to first carefully test the safety of any changes, he says, to avoid causing bad reactions.

For example, the CDC's Advisory Committee on Immunization Practices could consider adding an additional booster shot for teens -- who have made up a large number of whooping cough patients -- at around 16 or 17, Clark says. There's not much room in the current vaccine schedule to add extra shots for little kids, and there are currently no whooping cough vaccines licensed for children ages 7 to 10.

To better protect infants, Cherry says, researchers could test the safety of giving babies their first three vaccinations by age 3 months, instead of 6 months. Vaccinating pregnant women also helps protect babies for the first month or two of life, he says.

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The Journal of Infectious Diseases
EDITORIAL COMMENTARY

Flu Vaccine—Too Much of a Good Thing?

John Treanor

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(See the major article by Skowronski et al on pages 1059–69.)

Keywords. Influenza vaccine; sequential vaccination; antigenic distance.

Control of influenza through vaccination is a particularly difficult task because of the continued antigenic evolution of influenza viruses. Influenza viruses rapidly accumulate mutations in the antibody epitopes of the hemagglutinin (HA) and neuraminidase (NA) proteins that allow them to escape from immunity generated by prior vaccination or infection, a process known as "antigenic drift." Antigenic drift occurs in unpredictable stops and starts, and at different rates for different types and subtypes of the virus. One consequence of this phenomenon is that for optimal protection, the viruses contained in the vaccine should match the virus(es) causing the outbreaks as closely as possible, requiring comprehensive surveillance for new emerging variants, and continuous updating of the vaccine. Because at least 1 component of the vaccine is almost always updated each year, optimal protection would also require annual vaccination, which is currently recommended for all persons in the United States.

Multiple large networks have been established in the United States, Canada, Europe, and other countries to monitor the effectiveness of influenza vaccine on a yearly basis, using a now well-established methodology known as the test-negative case-control study. In this approach, individuals with acute respiratory illness are assessed, and the vaccination history of subjects with laboratory-documented influenza (test-positive cases) is compared to that of those whose tests were negative (test-negative controls). Over the last several years, many of these studies have suggested that vaccination in prior seasons can reduce the effectiveness of vaccination in the current season, a phenomenon first identified by Hoskins and colleagues in a British boarding school [1] and referred to as the "Hoskins effect."

A negative effect of prior vaccination is not a consistent finding of all studies [2, 3], and the mechanisms that might underlie this phenomenon remain unknown. However, it was suggested several years ago that the effect may depend on the antigenic relatedness of the previous vaccine to the current vaccine, and of both to the circulating virus, referred to as the antigenic distance hypothesis (ADH) [4]. According to the ADH, the biggest negative effect would be predicted to occur when the previous and current vaccines are antigenically similar, and the circulating virus is significantly drifted.

In this issue of *The Journal of Infectious Diseases*, an analysis of data over several years from the Canadian Vaccine Effectiveness (VE) network provides support for the ADH as a predictor of the possible inhibitory effect of prior vaccination. Essentially, Skowronski and colleagues [5] found that the greatest negative effect of prior vaccination occurred in the 2014–2015 season, when the prior and current vaccines were the same, and the circulating virus was a poor antigenic match. In contrast, there was no effect of prior vaccination on VE in 2010–2011, when the prior and current vaccines were distantly related and the circulating virus was also a drift variant, and an intermediate negative effect in 2012–2013, when the current and prior vaccine were similar, but not identical and the circulating virus was again drifted.

The mechanisms that might be responsible for a negative effect of prior vaccination on vaccine effectiveness are not known, but are reviewed in detail in the article. The finding that the magnitude of the negative effect depends on antigenic distance could be consistent with antigenic focusing [6]. In this case, when sequentially exposed to 2 antigenically related viruses, the immune system focuses on the shared epitopes at the expense of novel epitopes on the second virus that might be important for the protection against a third, antigenically drifted virus. In contrast, a person who has not been previously vaccinated might mount a broader response against all of the epitopes in the vaccine. Other potential mechanisms could include interference by prior immunity on antigenic presentation, or the "infection-block hypothesis." In this case, prior vaccination reduces prior infections with influenza virus, which in turn would have provided more effective protection against subsequent drifted influenza infection than the vaccine does, resulting in lower rates of influenza in subjects with infection-based immunity than in those

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with vaccine-induced immunity. As the authors point out, there is also the possibility of undetected confounding variables that impact the health-care behavior of multiply vaccinated individuals compared to unvaccinated ones. Multiple factors could all be playing a role, making a complete mechanistic understanding of the phenomenon quite difficult.

The actual measurement of antigenic distance is also challenging, particularly for influenza A subtype H3N2 viruses. Traditionally, the antigenic difference between 2 influenza viruses has been determined by the hemagglutination-inhibition (HAI) assay using ferret antiserum samples. In this test, an influenza-naive ferret is infected by the first influenza virus, and the titer of postinfection serum samples is determined in the HAI assay using 2-fold dilutions of serum samples against the infecting virus and the new virus. If the titer of the serum samples against the infecting virus is 3 or more dilutions higher than it is against the new virus (ie, an antigenic distance of 3 or more), then the 2 viruses are considered antigenically different. In the current study, antigenic distance was calculated from values of the HAI assay for the vaccine and circulating viruses reported by the World Health Organization. However, this would not necessarily be the same result that would be obtained using human sera [7], which might be more relevant to human seasonal outbreaks. In addition, recent H3N2 viruses do not grow well in the laboratory, and may need to be adapted by serial passage to develop high enough hemagglutination titers to use in this type of assay, potentially introducing additional mutations and complicating the assessment of antigenic distance. It will be important in future assessments to include new methodology that is being developed to assess antigenic differences in H3N2 viruses such as sequencing and neutralization assays.

In one year of the study, it appeared that multiply vaccinated subjects were actually more likely to develop influenza than unvaccinated subjects (that is, VE was statistically significantly less than zero). A similar effect was noted during the 2009 influenza A virus subtype H1N1 pandemic when increased rates of pandemic H1N1 were reported in patients who had previously received seasonal H1N1 vaccine in Canada [8] but not in other countries [9]. The authors speculate that this might be consistent with a disease-enhancing effect of influenza vaccine. Vaccine-enhanced disease has been recognized as a potential problem in other human infectious diseases such as dengue [10] and respiratory syncytial virus [11], and can be a significant obstacle to vaccine development. There is relatively little evidence to support any form of enhanced influenza disease in humans, although disease enhancement by low-avidity antibodies with deposition of immune complexes in the lungs was reported in the 2009 pandemic [12]. Measurements of disease severity were not reported in the current study, so it is not possible to judge whether the disease was more severe in multiply vaccinated individuals. Alternatively, it is possible that the same types of biases that might impact overall estimates could systematically lower estimates of VE in multiply vaccinated subjects, such that the estimates become negative numbers in years when true VE is close to zero.

Continued monitoring of influenza vaccine effectiveness is important in shaping vaccine policy, and it has recently resulted in major changes such as the recommendation against use of quadrivalent live attenuated influenza vaccine in the United States [13]. Because there is no current practical alternative to annual vaccination, the findings in the article by Skowronski and colleagues and others will probably not change public health recommendations. However, they are a call to further research to understand the effects of prior vaccination mechanistically and devise strategies to mitigate any inhibitory effects of prior vaccination. Such approaches might include adjuvants or high-dose vaccines. Ultimately, the answer may lie in the development of vaccines that provide broad and long-lasting protection against multiple influenza viruses, eliminating the need for annual vaccination altogether.

Notes

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http://www.burnettcountysentinel.com/news/flu-season-continues-to-be-widespread-throughout-country/article_4bd056da-0c21-11e8-b6ae-533d3d165845.html

Flu season continues to be widespread throughout country

Jonathan Richie EDITOR@BURNETTCOUNTYSENTINEL.com Feb 8, 2018

Clarification: **Last week's article "Influenza potentially made stronger by vaccines" may have been misleading. The article's main source, Anna Treague, is not a nurse with the Centers for Disease Control (CDC). Treague, who was quoted, is a nurse with Burnett County Public Health. We regret the error and apologize for any confusion, inconvenience or misunderstanding it may have caused.**

The flu season has been in full swing for a few months. The seasonal disease has mutated over the years, and professionals say it has been made stronger as medicine continues to process vaccines.

According to the Centers for Disease Control and Prevention(CDC) this season's flu is widespread in 49 states, Wisconsin and Minnesota are covered in high levels of the flu.

"I believe that the low effective rate of the vaccine this year is due to the mutations that the virus made in the processing of the vaccine itself," said Anna Treague, nurse for Public Health. "That is at least part of the reason that influenza cases are so widespread this year."

The flu or influenza is a seasonal contagious respiratory disease that is caused by influenza viruses. The CDC says the dominant strain this year is H3N2, which tends to be more severe and causes more severe symptoms than most other strains.

Treague said that symptoms include fever, chills, headache, dry cough and aching of muscles and joints. They usually appear 1 to 3 days after being infected with most people recovering within a week.

"The H3N2 strain also has proven to not be as impacted by the vaccines as other strains," Treague said.

That being said, Treague still suggests everyone should get a flu shot.

"If you are able, get the flu shot," Treague said. "Even if the flu vaccine isn't as effective as it has been in year's past it does help. Some protection is better than no protection."

A number of different influenza vaccines are produced every year. The most common uses a chicken egg to grow the virus, which is why people with an egg allergy need a special type of vaccine. Some vaccines are trivalent (containing 3 virus strains) or quadrivalent (containing 4 virus strains.)

Treague said the flu shot is a inactive/killed virus and the nasal spray from in alive, but weakened strain.

Typically production for the next year's flu shots are developed before the current season of the flu ended.

Treague explained that there are two main viruses associated with the flu, type A and type B. It spread through droplets of moisture that go from person to person when they sneeze, cough, or talk. Those droplets are then inhaled by another person and that is how it spreads.

“I think it is important that people know how serious influenza can be for certain people, especially those who are very young and the older population,” Treague said.

She said this is because at the beginning and end of one’s life their immune system is not as strong and their bodies have to work harder to fight off viruses and compensate for the symptoms of influenza.

“Fortunately, in Wisconsin to date there has been no influenza-associated pediatric deaths reported, whereas nationwide there have been 37,” Treague said.

Treague said anyone experiencing symptoms should see their doctor immediately so it can be caught in the early stages and treated with antiviral medication. She also stressed proper hand hygiene and covering one’s mouth when coughing is instrumental in not spreading the flu.

“Another thing to help avoid spreading influenza, if you are sick, stay home,” Treague said “Please take a break from daily errands and rest, don’t venture out unless needed, if you have to venture out wear a mask, to prevent the spreading of the virus through those moisture droplets.”

Herd Immunity

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An increasing, potentially measles-susceptible population over time after vaccination in Korea

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ABSTRACT

Background: In Korea, measles occurs mainly in infants <12 months of age, who are unvaccinated. In addition, vaccine populations, including adolescents and young adults, can become infected though importation. Thus, the question arises whether the current level of herd immunity in Korea is now insufficient for protecting against measles infection.
Methods: Age-specific measles seroprevalence was evaluated by performing enzyme immunoassays and plaque reduction-neutralization tests on 3050 subjects aged 0–50 years (birth cohort 1964–2014) and 480 subjects aged 2–30 years (birth cohort 1984–2012).
Results: The overall seropositivity and measles antibody concentrations were 71.5% and 1366 mIU/mL, respectively. Progressive decline in antibody levels and seropositivity were observed over time after vaccination in infants, adolescents, and young adults. The accumulation of potentially susceptible individuals in the population was confirmed by comparing data from 2010 and 2014 seroprevalence surveys. The statistical correlation between measles incidence and measles seronegativity was determined.
Conclusions: Waning levels of measles antibodies with increasing time post-vaccination suggests that measles susceptibility is potentially increasing in Korea. This trend may be related to limitations of vaccine-induced immunity in the absence of natural boosting by the wild virus, compared to naturally acquired immunity triggered by measles infection. This study provides an important view into the current measles herd immunity in Korea.

1. Introduction

Measles is a highly contagious vaccine-preventable disease caused by the measles virus. Since a vaccine against measles became available in 1963, accelerated immunization activities have reduced the global incidence and mortality of measles. Many countries have successfully eliminated measles by following a routine vaccination program [1,2].

In Korea, the measles-containing vaccine (MCV) became available in 1965, and the trivalent measles, mumps, and rubella (MMR) vaccine was introduced in early 1980s. A 2-dose MMR vaccination schedule was recommended beginning in 1997, with the first dose given at 12–15 months of age and the second dose given at 4–6 years of age. Before the introduction of a measles vaccine, large number of measles cases were reported annually in Korea. Owing to the occurrence of large, nationwide measles outbreaks with approximately 55,000 cases of measles and 7 deaths during 2000–2001, the government implemented the 5-year National Measles Elimination Plan that included the measles vaccination "catch-up campaign" and "keep-up" programs in 2001. The

Abbreviations: CIs, confidence intervals; EIA, enzyme immunoassay; GMT, geometric mean titer; KCDC, Korea Centers for Disease Control and Prevention; KNHANES, Korea National Health and Nutrition Examination Survey; MCV, measles-containing vaccine; MMR, measles, mumps, and rubella; ND_{50}, 50% neutralizing antibody end-point titers; PRNT, plaque-reduction neutralization test; WHO, World Health Organization.

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catch-up immunization program targeted 5.86 million children aged 8–16 (March 1985–February 1994 birth cohort) who did not have documented evidence of receiving the MCV vaccine, and the keep-up program maintained >95% 2-dose MCV coverage by requiring the achievement of 2-dose MMR vaccination before entering elementary school by all children aged 7 years [1,3,4]. As a result of national efforts to control measles, the reported numbers of measles cases decreased to 0.93 cases/million people during 2008–2013 and 2-dose MMR vaccination coverage had been maintained at >95% since 1996. In March 2014, the World Health Organization (WHO) verified that measles had been eliminated in Korea [1,4]. Although measles had been eliminated in Korea, the resurgence of measles outbreaks related to imported and import-associated measles cases occurred during 2013–2014. Most patients with measles were infants aged <1 year, but measles cases were also identified in patients aged 13–24 old who had received a 2-dose measles vaccination [4]. Measles outbreaks among highly vaccinated populations have been observed in many countries [5–7]. Such outbreaks in a population with high 2-dose measles vaccine coverage may be related to a vaccine-handling issue (cold chain issue), the vaccination strategy (number of doses, age of vaccination), host immunity (waning immunity, suboptimal immunity), and environmental factor (heavy exposure) [5,8,9].

By investigating the seroprevalence of measles in Korea, we provide a significant window into current measles herd immunity to better understand the prevalence of measles susceptibility underlying measles outbreaks in Korea.

2. Material and methods

2.1. Serum samples

A total of 3050 residual serum specimens were provided in 2014, including sera from 1000 patients aged <10 years by a private diagnostic laboratory, and sera from 2050 patients aged 10–50 years were obtained from the fifth Korea National Health and Nutrition Examination Survey (KNHANES VI-1st), which was conducted by the Korea Centers for Disease Control and Prevention (KCDC) [10]. The serum samples from the private diagnostic laboratory were collected for medical diagnosis and health screening, and the other samples from the KNHNES were collected to assess the health and nutritional status of Koreans. In total, 3050 sera (50 per age group, by months for infants <12 months of age and by years for healthy individuals aged 1–50 years) were stored at −20 °C until investigation. We excluded samples referred for the diagnosis of measles, mumps, rubella, or human immunodeficiency virus. Personal and confidential information were removed, except for demographic information including age and gender. Specific vaccination documents were not available for individuals in this study population.

2.2. Detection of measles virus-specific IgG antibodies in enzyme immunoassays

Measles virus-specific IgG antibodies were detected using an enzyme immunoassay (EIA) kit (Enzygnost® anti-Measles Virus/IgG, Siemens Healthcare Diagnostics, GmbH Marburg, Germany) on the BEP® III automated system (Siemens Healthcare Diagnostics), according to the manufacturer's instructions. The sample results were classified as follows: optical density (OD) >0.2 was deemed positive, 0.1–0.2 was equivocal, and <0.1 was negative. Serum samples with equivocal results were re-tested in duplicate and classified based on the results with a majority. Positive delta ODs were then converted to international units using the α-method, as specified by the manufacturer.

2.3. Analyzing neutralizing-antibody concentrations against measles virus

Measles virus neutralizing antibody titers were determined by performing a modified plaque-reduction neutralization test (PRNT) [11]. All sera were heat inactivated at 56 °C for 30 min, and serially diluted 4-fold and incubated in the presence of 25–35 plaques of Edmonston strain for 2 h at 37 °C. The virus/serum mixtures were then added in triplicate to a Vero/hSLAM cell monolayer growing in a 24-well plate, after which the plate was incubated at 35 °C for 1 h. Viral inocula were removed and overlay medium was added. The plate was incubated for an additional 4 days, the overlay medium was removed, and culture overlay medium containing neutral red was added. The plate was incubated for another 1 day, and the medium was decanted. The cells were fixed with 4% paraformaldehyde. The 50% neutralizing antibody end-point titers (ND_{50}) were calculated using the Kärber formula, and those results were standardized against the WHO 3rd International Standard (NIBSC code 97/648) with an antibody concentration of 3000 mIU/ml.

2.4. Statistical analyses

Statistical analysis and graph constructions were performed using SAS software (version 9.3; SAS Institute, Cary, NC) and Prism software (version 6.0; GraphPad software Inc., San Diego, CA). We analyzed proportions and 95% confidence internals (CIs) of measles seroprevalences in the study population. Correlations were calculated using Pearson's and Spearman's correlation coefficients. $P < 0.05$ was considered to reflect statistical significance.

3. Results

3.1. Measles seroprevalence in Korea

The seroprevalence of antibodies against measles virus was analyzed in 3050 serum samples, of which 1575 (51.6%) were from male and 1475 (48.4%) from females, by an indirect IgG EIA. The prevalence of measles IgG antibodies by age group is shown in Table 1. The overall seropositivity of measles in the study population was 71.5% (95% CI, 69.6–73.4), and 8.6% (95% CI, 5.2–12.0) were equivocal. Young children (aged 1–6 years) presented the highest seropositivity of 93.0% (95% CI, 90.0–96.0) and a GMT of 2175 mIU/mL (95% CI, 1961–2412). The lowest seropositivity and GMT values of 48.5% (95% CI, 38.6–58.4) and 478.3 mIU/mL (95% CI, 421–543.3), respectively, were detected in adolescents (aged 16–19 years) in this study (Table 1). No significant differences were observed in seropositivity rates between males and females in any age group (data not shown). The age-specific measles seropositive proportion and distribution of GMT antibodies are presented in Fig. 1. The highest seropositivity of IgG antibodies was detected in 5- and 6-year-old children. Measles seropositivity gradually decreased from 100% in children aged 5 and 6 years to 42% (95% CI, 20.9–63.1) in the 19-year-old age group. This decline recovered steadily to >80% seropositivity for measles in individuals aged 23 years and over. The GMTs of antibodies indicated a pattern similar to that found with seroprevalence, and the highest GMT level was observed in infants aged 1 year (3137.5 mIU/mL), who most likely had received 1-dose of the MMR vaccine at 12–15 months. Among young children aged 1–5 years, the GMT decreased sharply from 3137.5 mIU/mL at 1 year of age to 1464.5 mIU/mL at 5 years of age. The GMT levels displayed significant linearity ($P < 0.001$), dropping from 1786.5 mIU/mL at 7 years of age to 415.9 mIU/mL at 19 years of age, but the subsequent rates of decline were slower than those in children aged 1 to 5 years

Table 1
Seroprevalence of measles by age group, Republic of Korea, 2014.

Age group	Year of birth	No. tested	Proportion seropositive % (95% CI)	Proportion equivocal % (95% CI)	Geometric mean titer mIU/mL (95% CI)
<1	2014	550	13.3 (5.5–21.1)	6.5 (0–14.6)	643.5 (530.7–780.3)
1–6	2008–2013	300	93.0 (90.0–96.0)	0.7 (0–11.9)	2175 (1961–2412)
7–12	2002–2007	300	91.3 (88.0–94.7)	5.7 (0–16.7)	1336 (1205–1482)
13–15	1999–2001	150	66.0 (56.7–75.3)	17.3 (2.8–31.9)	840.1 (699.1–1010)
16–19	1995–1998	200	48.5 (38.6–58.4)	28.5 (16.8–40.2)	478.3 (421–543.3)
20–24	1990–1994	250	69.6 (62.8–76.4)	19.2 (8.1–30.3)	822.1 (721.6–936.6)
25–29	1985–1989	250	90.8 (87.0–94.6)	7.2 (0–19.1)	1517 (1333–1727)
30–39	1975–1984	500	88.8 (85.9–91.7)	7.2 (0–15.6)	1526 (1390–1675)
40–50	1964–1974	550	93.3 (91.1–95.4)	3.8 (0–12.0)	2065 (1895–2252)
Total	1964–2014	3050	71.5 (69.7–73.4)	8.6 (5.2–12.0)	1366 (1309–1426)

Abbreviation: CI, confidence interval.

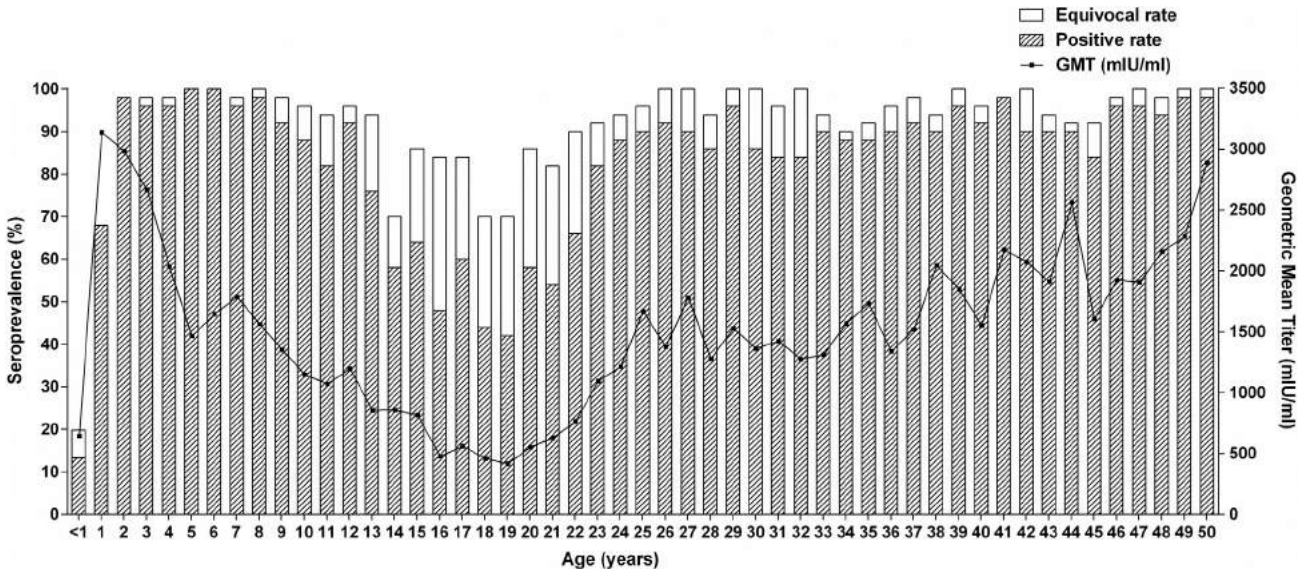

Fig. 1. Age-specific distribution of seroprevalence and GMT of measles IgG antibodies in Korea in 2014. The sample size was 50 for each age group, except for infants <12 months of age, where the sample size was 550.

after receiving the 2-dose of the MMR vaccine. These declines gradually rose back to measles antibody GMTs of >1200 mIU/mL in those aged 25 years and over (Fig. 1).

3.2. Neutralizing antibody concentrations

In total, 480 specimens were randomly selected from 3050 sera tested by EIA and analyzed by PRNT for measles neutralizing-antibody concentrations. Fig. 2 shows the distribution of neutralizing-antibody concentrations determined in the PRNT and EIA for IgG antibody titers among individuals in the study population aged 2–30 years. The potential measles-susceptibility rate was estimated using the 120 mIU/mL threshold of potential susceptibility and correlations between the values from the PRNT and EIA experiments. A high agreement was observed between IgG titers from the EIA and PRNT experiments, with Pearson's and Spearman's correlation coefficients of 0.9271 and 0.954, respectively ($P < 0.0001$ for both). The lowest neutralizing-antibody concentration (145 mIU/ml) was detected in the 19-year-old group, with 7 (35%) individuals showing potential susceptibility (≤120 mIU/mL), but only 1 being negative (<8 mIU/mL).

3.3. Comparison of seroprevalence between in 2010 and 2014

This measles seroprevalence study of samples obtained in 2014 was compared to data from a previous study conducted in 2010 by the KCDC [12], which showed an overall seropositivity for measles of 78.2% in 1400 sera from individuals aged 24–47 months (2007–2008 birth cohort) and 7–18 years (1992–2003 birth cohort). The 2010 and 2014 studies were conducted using the same methods and a comparable EIA kit in the identical laboratory. Fig. 3A and B shows changes in seropositivity and antibody titers that occurred in each age group after 4 years from 2010 to 2014. The seropositivity and measles antibody GMT for the 2014 study population was significantly lower ($P < 0.0001$ in both cases) compared to the 2010 study population, and reduction values in the 2014 and 2010 study populations were 16.4% (95% CI, 12.32–20.56) and 401.7 mIU/mL (95% CI, 225–578.5), respectively (Fig. 3A and B). Fig. 3C and D presents the distribution of seropositivity and antibody titers for each age group at the time of investigation. The lowest seropositivities and GMT were observed in different age groups, i.e., in the 15-year-old group (61%, 632.81 mIU/mL) from the 2010 data set and in the 19-year-old group (42%, 415.9 mIU/mL) from the 2014 data set (Fig. 3C and D). A potential susceptibility window was found, with seropositivity rates <80% among the group aged 11–18 years in 2010, which had shifted and expanded to the group aged 13–22, 4 years later in 2014 (Fig. 3C). The trend of decreased measles antibody titers with increasing time post-vaccination occurred in both years ($r = 0.9597$, $P < 0.0001$), but the proportion of the population with a low antibody titer was greater in 2014 than in 2010 (Fig. 3D).

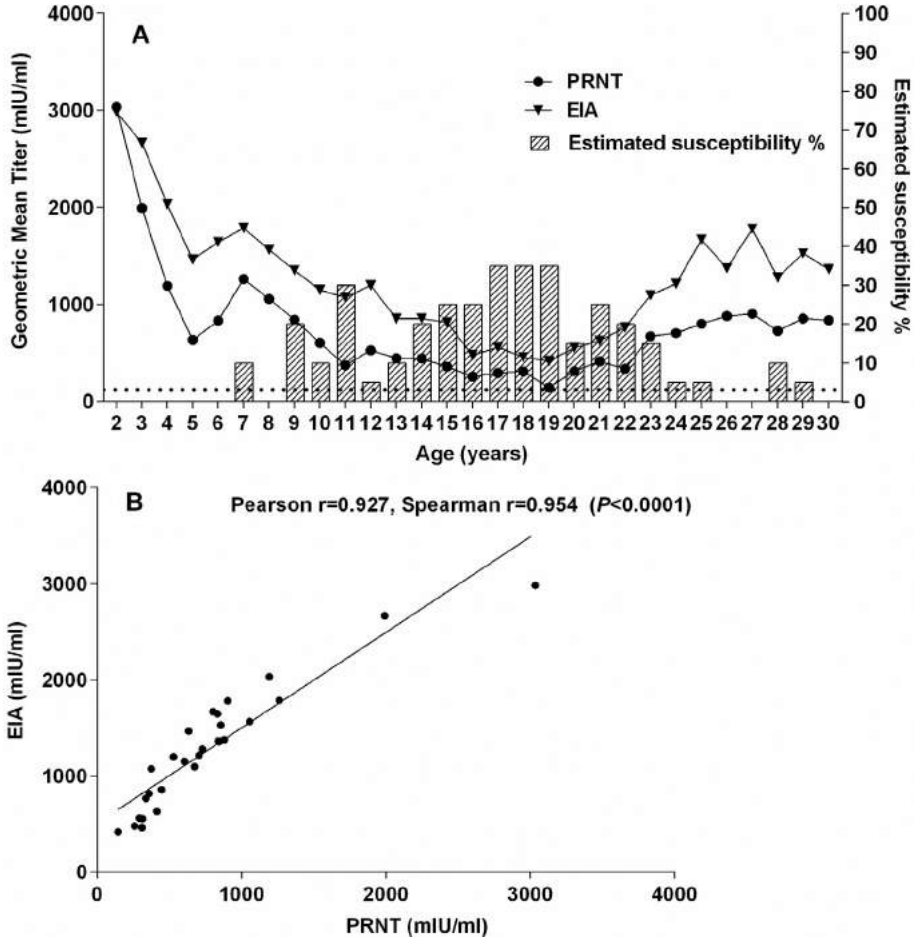

Fig. 2. Age-specific IgG levels (determined in EIAs) or neutralizing antibody titers (determined in PRNTs) versus the estimated measles susceptibility (A) and correlations between the values from the PRNT and EIA experiments calculated using Pearson's correlation coefficient (B). EIA, enzyme immunoassay; PRNT, plaque-reduction neutralization test.

3.4. Measles outbreak and seroprevalence

In 2014, a measles outbreak occurred in Korea and 442 cases of measles infection were confirmed. When classified by aged group, a significant correlation was observed between measles antibody seronegativity and the number of reported measles cases ($r = 0.8886$, $P < 0.0001$) (Fig. 4). Most cases occurred in infants, adolescents, and young adults, with 176 (39.8%) cases occurring in individuals aged $\leq$1 years and 185 (41.9%) cases among individuals aged 13–24 years. Peak seronegativity rates were observed in the age groups under 1 year (76.2%), 13–15 years (16.7%), 16–18 years (20.7%), and 19–21 years (20.7%) (Fig. 4).

3.5. Passive immunity to measles in infants during the first year of life

The infants aged under 12 months had very low seroprevalence and measles antibody GMT values of 13.3% (95% CI, 5.5–21.1) and 643.5 mIU/mL (95% CI, 788.4–1485.5), respectively. The seropositivity of IgG antibodies was 76.0% (95% CI, 62.4–89.6) in infants aged 1 month, which decreased to 8.0% (95% CI, 0–34.6) in infants aged 3 months. The measles antibody GMT was 841.0 mIU/mL (95% CI, 679.0–2155.2) in infants aged 1 month, which dropped abruptly to 369.5 mIU/mL (95% CI, 0–2010.9) in infants aged 3 months. After 4 months of age, seropositivity and measles antibody levels were very low, and detectable measles-specific antibodies were not observed after 8 months (Fig. 5).

4. Discussion

Despite high measles vaccination coverage by a successful national vaccination program, small outbreaks have occurred following the importation from other countries in recent years, even though the circulation of wild measles viruses in Korea has been stopped since 2010 [4,13]. Such outbreaks have affected mostly unvaccinated people, but they also occurred in adolescents and young adults who had been previously vaccinated against measles [4,13]. During 2010–2016 in Korea, 36.2% of individuals with confirmed measles infection were unvaccinated, 46.8% were vaccinated previously (10.5% with 1-dose, 36.2% with 2-dose), and vaccination information was not available for 17% of infected individuals (data not shown).

The existence of potential factors underlying vaccine failure, such as waning immunity, was suggested by data generated in previous studies on measles outbreaks in highly vaccinated populations [5,6,8,9]. A rise in the proportion of seronegative individuals with lowering antibody levels over time since the last vaccination was observed in our study, and this proportion had shifted and expanded towards older individuals with lowering seropositivity and antibody levels being present over time. Similar findings have been reported in other countries. For example, the antibody-avidity indexes and concentrations decreased by 8% and 58%, respectively, 22 years after a 2-dose MMR vaccination in a Finland study [14]; the measles IgG GMTs deceased to 934, 251, and 144 mIU/ml in 2001–2003, 1990–1993, and 1994–1995,

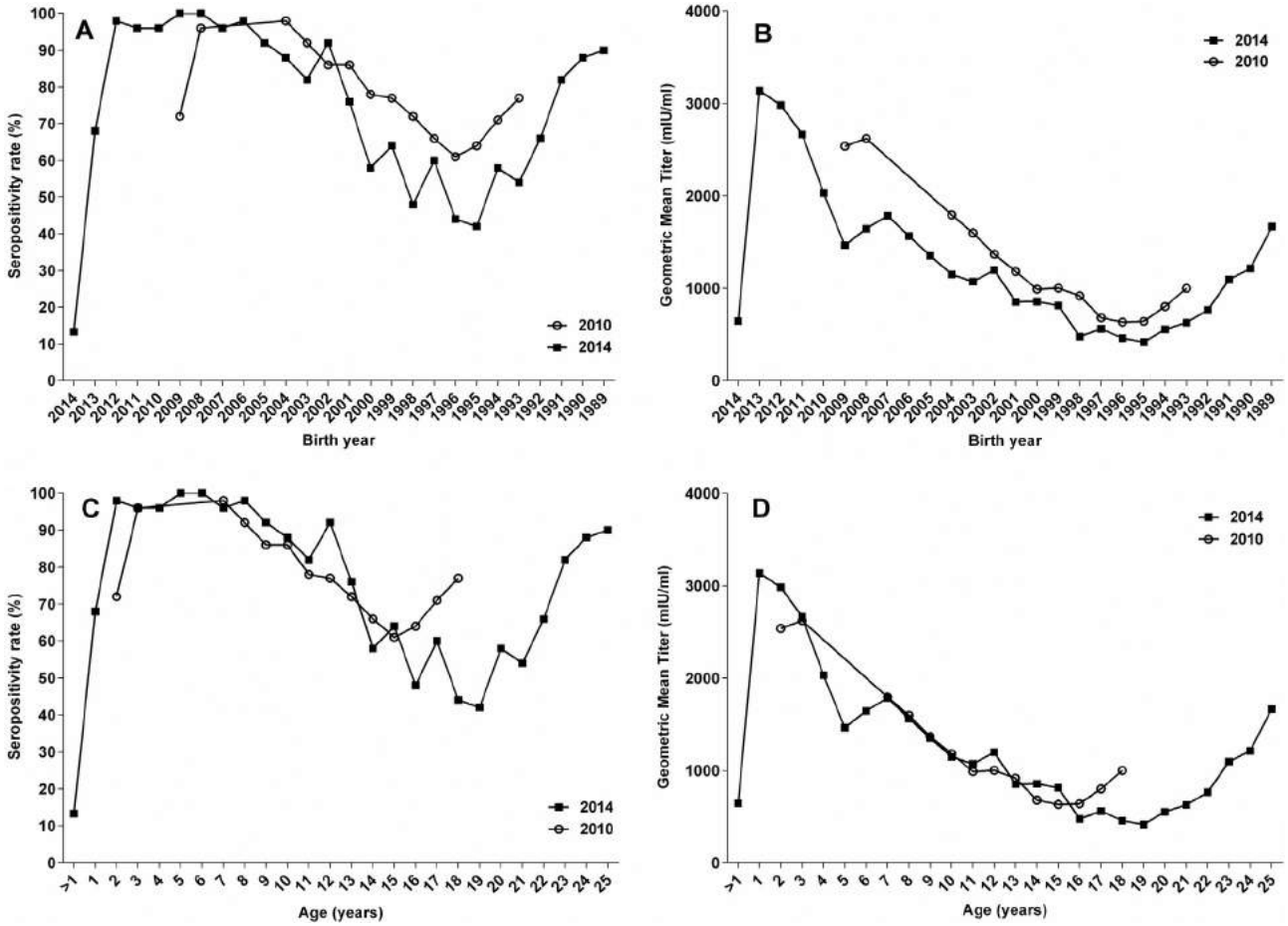

Fig. 3. Seroprevalence of measles in 2010 and 2014. The distribution of seropositive rates (A) and GMT (B) by birth cohort (1984–2014) were compared between 2010 and 2014. The seroprevalence data between 2010 and 2014 were compared for seropositivity (C) and GMT levels (D) of measles-specific antibodies, according to the age group, during this investigation.

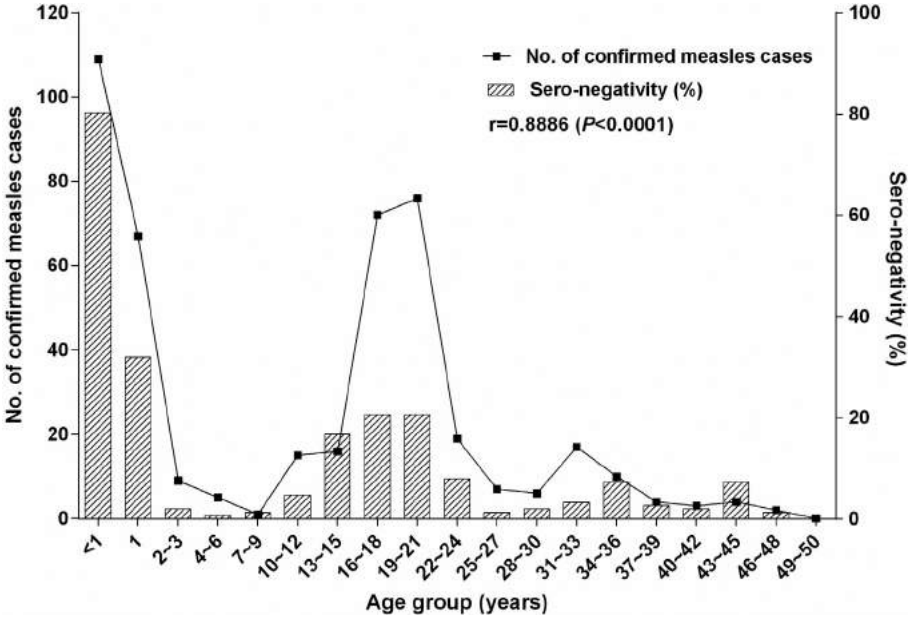

Fig. 4. Relationships between measles sero-negativity and confirmed measles cases by age groups in Korea in 2014. Correlations between sero-negativity and the number of confirmed measles cases were calculated using Pearson's and Spearman's correlation coefficients.

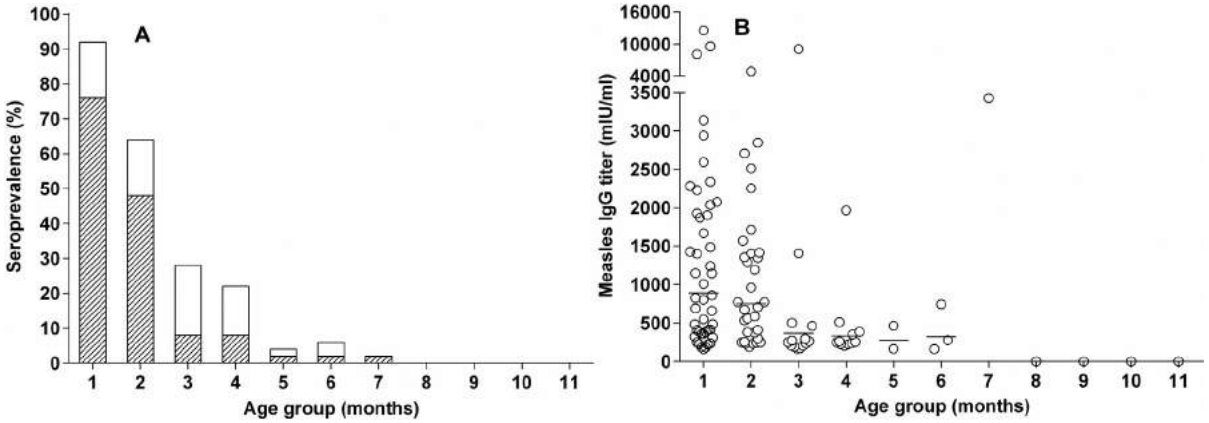

Fig. 5. Seroprevalence (A) and GMT (B) of measles IgG antibodies in infants aged <12 months.

respectively, in a study conducted in Portugal [15]; and declining neutralizing measles-antibody titers were detected in both kindergarteners and middle schoolers, 5 years and 10 years after a secondary vaccine dose (2814 decreased to 641 mIU/ml, and 1672 decreased to 737 mIU/ml, respectively) in a study conducted in the United States [16]. The neutralizing antibody titer is related to protection a measles virus infection, and PRN titers of ≥120 mIU/mL are thought to be protective against measles [17]. Age-specific, potential susceptibility was calculated using a cut-off value of ≤120 mIU/mL, and adolescents and young adults presented higher susceptibility rates compared to other age groups.

In the 20–29-year-old age group, higher seropositivity and GMT were detected than in the 13–19-year-old age group that was estimated to have been administered a measles and rubella vaccine through the catch-up vaccination program in 2001 [1,3,18]. These data can be interpreted to mean that the catch-up campaign was an appropriate strategy for progressing toward measles-elimination goals in Korea. The group aged 30–50 years, whose immunity against measles was presumably acquired naturally by previous exposure to wild measles virus, presented higher seroprevalence and antibody concentrations than did other age groups, except for the 1–12-year-old age group. Our observations that long-term and higher antibody levels were present following natural infection than after vaccination agree with data from other previously published studies [2,19].

Measles epidemics have been and still observed in other countries with low vaccination coverage, and the measles outbreaks through importation is continuously reported in countries that have eliminated measles such as Korea [4,20,21]. The measles-importation risk still exists, especially in individuals with suboptimal immunity. Several reports have warned that the susceptibility to measles infection may be rising because of waning vaccine-induced immunity over time after vaccination, in the absence of natural boosting by circulating measles viruses [22,23]. Our data showed good agreement between the incidence of measles and the susceptible age groups (adolescents and young adults) with measles seronegativity observed, suggesting the potential accumulation of measles-susceptible individuals in the population due to waning immunity, which may pose increased risk for measles outbreaks following measles importation from other endemic countries.

Cell-mediated immunity may protect against measles virus infection by promoting viral clearance, recovery from acute disease, and the persistence of long-term immunity [24–26]. Measles-avidity assays may provide valuable information for assessing the occurrence of measles in highly vaccinated populations by identifying vaccine failure [5,27,28], although these issues we not addressed in this study.

In this study, passively acquired maternal measles antibodies declined significantly and expired at 8 months after birth in Korean infants. Several studies of early waning of maternal measles antibodies in infants were published in recent years, and such waning may be related to low maternal measles titers, a limitation of vaccine-induced immunity compared with naturally developed immunity after wild measles virus infection, and the absence of natural boosters [29–33]. Because an increasing number of women have acquired immunity by vaccination instead of natural measles infection due to decreasing opportunities for wild virus exposure, the immunity gap in measles protection occurring between the loss of passive immunity derived from the mother and immunity acquired from the first vaccination can be amplified. As a consequence, the proportion of infants susceptible to measles infection increases progressively.

Earlier immunization (<12 months) has been suggested as a means for solving the problem that antibodies passively acquired from vaccine-induced maternal immunity do not persist long term [32,34,35]. Measles-endemic countries such as China, India, Philippines, and France have implemented measles vaccination programs in infants under 12 months of age [36]. Although earlier vaccination in infants before 12 months of age can reduce the susceptibility to measles, better seroconversion rates and antibody levels were observed when the MMR vaccine was administered at 12 months of age [35,37,38]. The WHO recommends administering the 1-dose of the MMR vaccine to infants aged 12 months in countries with low rates of measles transmission, but in the endemic countries, the WHO advises that the first vaccination should be given at 9 months of age and the second vaccination given at 15–18 months [39]. The waning of measles antibodies in adolescents and young adults after vaccination and in infants after birth but before the first vaccination, and the limitation of vaccine-induced immunity in measles-eliminated environments versus acquired immunity by natural infection were confirmed in our age-specific seroprevalence study. These findings suggest that an increasing proportion of measles-susceptible individuals is occurring with increasing time post-vaccination in Korea. Testing this hypothesis require further studies with data from the continuative seroprevalence survey at 3–4-year intervals to determine the accumulating measles susceptibility in Korean population. In addition, cell-medicated immune responses to measles and IgG antibody-avidity studies are needed to provide a better understanding of measles occurrences in vaccinated populations.

Ethical approval

Ethical approval (approval no. 2014-11EXP-05-P-E) for this study was obtained from the Institutional Review Board of the KCDC.

Potential conflicts of interest

None of the authors have a commercial or other association that might pose a conflict of interest.

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RESEARCH PAPER

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Long-term immunogenicity after measles vaccine vs. wild infection: an Italian retrospective cohort study

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ABSTRACT

The persistence of specific IgG after measles infection and after measles vaccination has not been sufficiently investigated. Current evidence suggests that immunity after the disease is life-long, whereas the response after two doses of measles-containing vaccine declines within 10–15 years. This study evaluated the proportion of individuals with detectable anti-measles IgG in two groups, those vaccinated with two doses of anti-MMR vaccine and those with a self-reported history of measles infection. Among the 611 students and residents who were tested, 94 (15%) had no detectable protective anti-measles IgG. This proportion was higher among vaccinated individuals (20%; GMT = 92.2) than among those with a self-reported history of measles (6%; GMT = 213.3; $p < .0001$). After one or two MMR vaccine booster doses, the overall seroconversion rate was 92%. An important proportion of people immunized for measles did not have a protective IgG titer in the years after vaccination, but among those who had a natural infection the rate was three-fold lower. This finding should be considered in the pre-elimination phase, given the resurgence of measles cases among individuals who after being vaccinated lost their circulating IgG after several years, especially if they failed to receive a natural booster.

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Introduction

Measles is an acute viral respiratory illness caused by a single-stranded, enveloped RNA virus with a single serotype (genus *Morbillivirus*, family Paramyxoviridae). Humans are the only natural host of measles virus. Patients are considered to be contagious between 4 days before and 4 days after the rash appears.[1] Common complications of measles include otitis media, bronchopneumonia, laryngotracheobronchitis, and diarrhea. One out of every 1,000 measles patients will develop acute encephalitis and 1–3/1,000 children infected with measles will die from respiratory and neurologic complications. The most dreaded complication of measles is subacute sclerosing panencephalitis, that generally develop 7–10 years after measles infection.[1]

Since the introduction of global mass vaccination, the safety,[2] cost-savings[3] and efficacy of measles-containing vaccines have been repeatedly demonstrated. Vaccination has reduced the incidence of measles by 99.9%, with >20,000,000 lives saved throughout the world.[4] Nevertheless, in the post-vaccination era, the WHO estimated almost 90,000 measles-related deaths in 2016 and reported 353,236 cases of measles in 2018.[5]

Measles virus replicates in the cytoplasm of infected human cells without the integration of the viral genome into that of the host cell. In addition, measles virus is considered sensitive to antibody-mediated clearance. Generally, measles infection and its effects on the immune system are limited to the period of viral replication, spread and clearance, during which time acute illness in the host develops. However, in natural infections of measles, the viral RNA can persist in lymphoid tissue and the immune system remains activated for many months.[6] This characteristic may explain the observed maturation of the immune response to the virus, which may be required to establish life-long protective immunity.[6] Immune activation and the proliferation of lymphocytes, particularly CD4 + T cells, is evident both during the acute phase and in the months after resolution of the rash. During this period, there is a shift in the production of cytokines to those promoting B cell maturation, thus allowing the continuous production of antibody-secreting cells.[6] The improvement over time in the quality of antibodies, as evidenced by their increasing avidity, suggests the continuous activity of follicular T-helper cells and the selection of B cells in the germinal centers of lymphoid tissue. The development of long-lived plasma cells is necessary to sustain life-long plasma antibody levels.[6]

In Italy, a single-antigen measles vaccine was introduced in the 1970s.[7] Since 2003, the national vaccination schedule has recommended universal mass vaccination consisting of two doses of measles, mumps and rubella (MMR) vaccine administered in accordance with CDC recommendations (the first dose at 12–15 months and the second at 5–6 years of age).[8] According to pre-licensure data, one dose of MMR vaccine is 93% effective and two doses are 97% effective against measles.[8] The seroconversion rate is 95–98% after a single dose and 99% after two doses.[8] The live attenuated vaccine induces both antibody and cellular immune responses that mature over a period of months.

Although the immune responses induced by the vaccine are qualitatively similar to those induced by infection, antibody

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levels are lower after vaccination. Vaccination at a young age enhances the quality and quantity of the antibody response but has a minor effect on T cell responses. However, over time, virus-specific antibodies and vaccine-induced CD4 + T cells decrease, accounting for the secondary vaccine failure rate of 5% 10–15 years after immunization.[9]

The aim of this study was to evaluate the proportion of seroprotected individuals in two populations: those vaccinated with two doses of anti-MMR vaccine and those with a history of measles infection. In addition, the GMTs were compared in the previously vaccinated and naturally infected.

The study was carried out in Apulia (southern Italy, ~4,000,000 inhabitants), where MMR vaccine coverage is ~91% (year 2017, birth cohort 2015)[10] and where in 2002/2003 a large outbreak of measles (around 20,000 cases) was documented,[11] followed by many outbreaks in subsequent years[12,13] that included documented cases of nosocomial transmission.[12-15]

Material and methods

This was a retrospective cohort study.

In accordance with the Italian Ministry of Health's recommendations,[16] in April 2014 the Hygiene Department of the Bari Policlinico University Hospital implemented a biological risk prevention program for medical students and residents of the Medical School of the University of Bari. The protocol included obtaining a medical history and the determination of measles vaccination status and measles history. To increase the accuracy of the information, the parents of the medical students and residents were to be interviewed as well.

Thus, for each student or resident participant, a 5 mL serum sample was collected to assess the measles immunity/susceptibility status, determined in a chemiluminescence (CLIA) assay using LIAISON® Measles IgG, a semi-quantitative test performed using a standardized commercial method (Diasorin). The assay's cut off value (>16.5 AU/mL) is equivalent to 175 mIU/mL (WHO Third International Standard for Anti-Measles, NIBSC code: 97/648).[16,17] Individuals with equivocal tests were retested; if their results were still equivocal, their status was classified as negative.

Vaccinated individuals who had a non-protective IgG titer received a booster dose of MMR vaccine (M-M-RVAXPRO, administered subcutaneously in the deltoid). A second blood test was performed 20–25 days thereafter to remeasure the IgG titer. If the value exceeded the cutoff, the person was classified as having seroconverted; if the titer was still negative, another vaccine dose (28 days after the first booster) was administered and the IgG titer was again measured 20–25 days later. Individuals who were still seronegative were definitively classified as "non-responders" and an evaluation for measles infection as well as immunoglobulin administration in case of measles exposure were recommended.

Individuals with a self-reported natural history of vaccination who had a non-protective IgG titer received two booster doses of MMR vaccine (M-M-RVAXPRO, administered subcutaneously in the deltoid), 4 weeks apart. IgG titers were remeasured in a new blood test 20–25 days after the second booster dose. If the value of that test exceeded the cutoff, the person was classified as seroconverted; if the results were still negative, he was treated as described for vaccinated individuals.

This management protocol was consistent with the protocols applied in some US medical schools.[18] Study participants who received booster doses underwent a 1-month follow-up to assess the development of any adverse events following vaccination.

The population considered in the present study was composed of medical students and residents who attended the Hygiene Department from April 2014 to March 2019. Informed consent was routinely collected during clinical procedures. This study was carried out according to the principles of the Helsinki Declaration.

Our survey included only those medical students and residents who at the time of study enrollment had received two doses of MMR vaccine (vaccine basal routine) or who reported a history of measles infection. The vaccination status of enrolled participants was assessed using the Regional Immunization Database (GIAVA),[19] a computerized vaccination registry that allows every Apulian inhabitant to ascertain the vaccination history.

Participants without an available vaccination history, without a history of measles and never vaccinated, with a history of measles but who were also vaccinated, who were vaccinated with a single dose or ≥2 doses of MMR vaccine at baseline were excluded.

To calculate the sample size, individuals who had been vaccinated but lacked circulating anti-measles IgG were estimated to account for 15% of the study population[20] and those who were naturally immunized but lacked circulating IgG for 4.5% (our hypothesis, since there are no studies on the topic). The groups were compared using a chi-square test, with a significance level (alpha) of 0.01 and a beta power of 95%. To improve the statistical analysis, a 1:2 allocation ratio of naturally immunized and vaccinated individuals was chosen. Thus, the preliminary sample comprised 537 participants: 358 in the vaccine group and 179 in the disease group. The two groups were matched for age, sex and chronic diseases. Since records with missing data were expected, data for 448 individuals from the vaccine group and 224 from the disease group (20% more than the minimum determined sample size) were extracted from the database. Among the extracted records, 38 from the vaccine group and 23 from the disease group were excluded due to missing data. The final sample therefore consisted of 611 individuals: 410 had been vaccinated (vaccine group) and 201 naturally immunized (disease group).

For every enrollee, a specific form was generated in which information on patient id, sex, age at enrollment, age at measles infection, dates of routine MMR vaccine, measles IgG titer, date of first booster dose, IgG titer after first booster (vaccine group), date of second booster dose and IgG titer after second booster were recorded. Data from the compiled forms were entered into a database generated using Excel and analyzed using STATA MP16 software.

Continuous variables are reported as the mean±standard deviation and range, categorical variables as proportions, with the 95% confidence interval (95%CI), when appropriate. Skewness and kurtosis test was conducted to evaluate the normality of the continuous variables; in case of a non-normal

distribution, a normalization model was established. Student's t-test for independent data (parametric) and the Wilcoxon rank sum test (non-parametric) tests were used to compare continuous variables between groups; chi-square and Fisher's exact tests were used to compare proportions.

To assess the determinants of seroprotection at the time of study enrollment, a multivariate logistic regression model was used in which seroprotection was the outcome and sex (male vs. female), age (years) at study enrollment, group assignment (vaccine vs. disease) and the presence of chronic disease (yes/no) were the determinants. The adjusted Odds Ratio (aOR) was calculated with the 95%CI. The Hosmer-Lemeshow test was used to evaluate the goodness-of-fit of the multivariate logistic regression model.

Protective antibody survival (PAS), defined as the time elapsed from the second dose of routine MMR vaccine to the evaluation of antibody titer (years) or the time elapsed between natural measles infection to the evaluation of antibody titer (years), was determined.

PAS was assessed using Kaplan-Meier curves, and the differences between groups using the log-rank test. The median PAS time as well as the incidence rate per 100 person-years of loss of seroprotection were estimated, both with their 95%CIs. The Incidence Rate Ratio (IRR), in which the number of naturally immunized individuals was the denominator and the number of vaccinated individuals the numerator, was calculated with the 95%CI.

The determinants of PAS were identified by applying a multivariate Cox semiparametric regression, in which the risk predictors were sex (male vs. female), age (years) at study enrollment, group assignment (vaccine vs. disease) and the presence of chronic disease (yes/no). The adjusted Hazard Ratio (aHR) was calculated with the 95%CI. The Schoenfeld and scaled Schoenfeld residuals test was used to evaluate the proportionality assumption of the multivariate Cox semiparametric regression model, and the Gronnesby and Borgan test to evaluate the goodness-of-fit of the model.

For all tests, a two-sided p-value<0.05 was considered to indicate statistical significance.

Results

The study sample comprised 611 medical students and residents: 201 (32.9%) in the disease group and 410 (67.1%) in the vaccine group. The characteristics of the participants at enrollment are described in Table 1.

On average, members of the disease group had contracted measles at age 5.6 ± 3.3 (range: 0–18) years. Members in the vaccine group had been given the first dose of MMR vaccine at age 17.0 ± 3.0 (range: 6–23) months and the second dose at age 10.9 ± 3.6 (range: 1–29) years.

The proportion of all participants without circulating antibodies at enrollment was 15.4% (n = 94/611; 95% CI = 12.6-18.5%). The difference between the two groups was statistically significant ($p < .0001$; Figure 1).

The average GMT of the enrollees was 92.2 (95% CI = 82.6–103.0), with a statistically significant difference between the disease group (GMT = 213.3; 95%CI = 185.4–245.5) and the vaccine group (GMT = 60.5; 95%CI = 53.0–69.1; $p < .0001$).

Following vaccination of 7 of the 12 (58.3%) non-seroprotected members of the disease group according to the vaccination protocol (two doses of MMR vaccine 4 weeks apart), the titer evaluation revealed seroconversion in all 7 (100%; 95%CI = 59.0–100.0%), with a post-administration GMT of 239.8 (95%CI = 179.5–320.5).

In the vaccine group, 54 of the 82 (65.9%) seronegative individuals received a third booster dose of MMR vaccine, which resulted in the seroconversion of 42 of 54 (77.8%; 95% CI = 64.4–88.0%); 10 of the 12 (83.3%) still seronegatives individuals received a fourth booster dose of vaccine, of whom 3 of 10 (30.0%; 95%CI = 6.7–65.2%) seroconverted (overall seroconversion rate in the vaccine group: 90.0%; 95% CI = 78.2–96.7%). The GMT of those individuals after the booster(s) was 52.9 (95%CI = 38.4–73.0).

The multivariate logistic analysis showed a statistically significant association between evidence of circulating antibodies at enrollment and the group assignment (vaccine vs. disease; aOR = 0.25; 95%CI = 0.13–0.47). There were no further associations between the outcome and the determinants in the analysis ($p > .05$; Table 2).

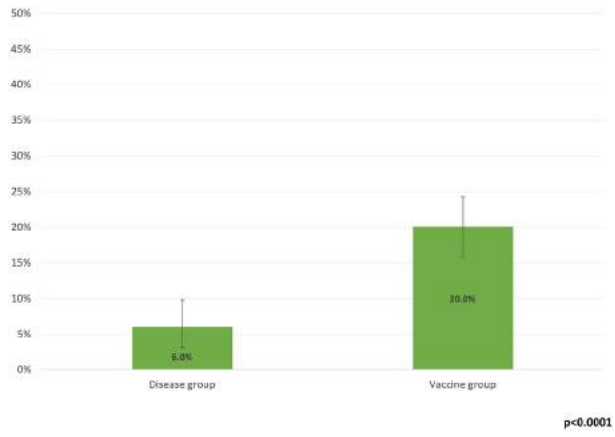

Figure 1. Proportion (%) of study participants in the vaccine and disease groups without circulating anti-measles IgG at study enrollment. p<0.0001.

Table 1. Characteristics of the two study groups at baseline.

Variable	Disease group (n = 201)	Vaccine group (n = 410)	Total (n = 611)	p-value
Age (years) at enrollment; mean±SD (range)	22.9 ± 2.6 (18–35)	22.7 ± 2.5 (18–35)	22.8 ± 2.5 (18–35)	0.458
Female; n (%)	131 (65.2)	246 (60.0)	377 (61.7)	0.216
Allergy; n (%)	59 (29.4)	127 (31.0)	186 (30.4)	0.682
Chronic disease; n (%)	26 (12.9)	60 (14.6)	86 (14.1)	0.570

Table 2. Multivariate logistic regression analysis of the determinants of seropositivity at enrollment.

Variable	aOR	95%CI	p-value
Group (vaccine vs. disease)	0.25	0.13–0.47	<0.0001
Age at enrollment (years)	1.0	0.9–1.1	0.556
Male (yes/no)	1.3	0.8–2.1	0.299
Chronic disease (yes/no)	1.6	0.8–3.2	0.202

Chi2 = 59.5; p = 0.890

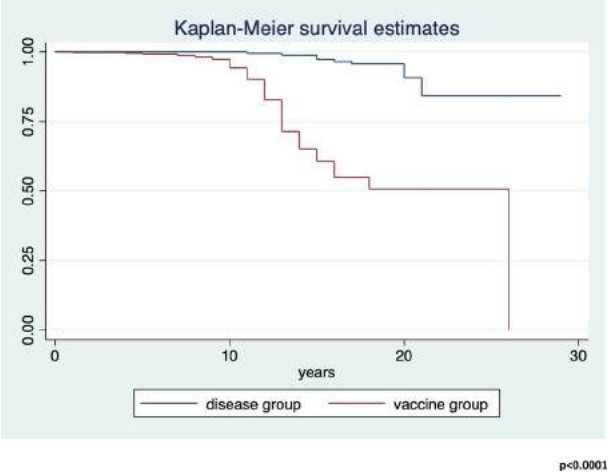

Figure 2. Kaplan-Meier PAS estimates for the vaccine and disease groups. p<0.0001.

Table 3. Multivariate cox semiparametric regression analysis of the risk predictors of PAS.

Variable	aHR	95%CI	p-value
Group (vaccine vs. disease)	11.8	6.1–22.9	<0.0001
Age at enrollment (years)	0.87	0.80–0.95	0.002
Male (yes/no)	0.88	0.57–1.35	0.549
Chronic disease (yes/no)	0.71	0.37–1.38	0.310

Chi2 = 2.0; $p = 0.160$

The average PAS time was 13.2 ± 4.4 years (range = 0–29). For seronegatives, the incidence rate ×100 person-years was 1.2 (95%CI = 1.0–1.4).

The PAS between the groups differed significantly (log-rank $p < .00001$; Figure 2). The incidence rate ×100 person-years for the loss of circulating IgG was 0.4 (95%CI = 0.2–0.7) in the disease group and 1.7 (95%CI = 1.4–2.1) in the vaccine group, with an IRR of 4.6 (95%CI = 2.5–9.3; $p < .0001$).

The multivariate analysis identified belonging to the vaccine group (aHR = 11.8; 95%CI = 6.1–22.9) and age (aHR = 0.88; 95% CI = 0.80–0.95) as determinants of the loss of circulating antibodies. There were no associations between the PAS and the other determinants in the analysis ($p > .05$; Table 3).

Discussion

Our study showed that 15% of the screened participants lacked detectable circulating anti-measles IgG and one or more booster doses was needed for seroconversion; this value is higher than the one reported in a 2020 meta-analysis[21] on Italian HCWs (equal to 9%), probably due to the young age of our sample. The difference between the two groups (20% vs. 6%) is consistent with literature reports and provides further evidence that natural immunity is more long-lasting than vaccine immunity. Additional support for this conclusion comes from the significantly higher baseline GMT in the naturally immunized group (213 vs. 61; $p < .0001$); these results are consistent with the ones highlighted by a 2020 Italian study,[22] which concluded that among subjects who received two doses of measles vaccine, the neutralizing antibody titer tended to decline over time, on contrary of natural immunized subjects.

The seroconversion rate after two doses of MMR vaccine in the disease group was 100% (95%CI = 59–100%), while in the vaccinated group it was 86% (95%CI = 73–94%). The difference in the response to the booster dose(s) may have reflected the greater persistence of immunological memory in naturally immunized individuals. Also in this case, the GMT measured after the booster(s) was significantly higher in the naturally immunized than in the vaccinated participants (240 vs. 53). The overall seroconversion after a booster(s) in subjects found seronegative after the first blood sample was 92.2% (95% CI = 80.7–97.1%).

An analysis of the determinants of seroprotection showed that the detection of circulating IgG at baseline was associated with natural immunization (aOR = 0.25; 95%CI = 0.13–0.47). The survival analysis also indicated a greater persistence of circulating antibodies in the naturally immunized. Although a stronger antibody response (titer) is induced by natural disease than by vaccination, a 1994 study[23] found that for MMR immunity, serological memory after vaccination is similar to that after natural infection. However, the second dose of the MMR vaccine is essential, as the antibody titer undergoes since a slow decline during the first 10 years after the first vaccination of the basal routine.[23] The levels of neutralizing antibodies 10 years after the second dose of vaccine remain above the level considered protective and confer long-lasting immunity, although they fall in the years thereafter.[24] A 2019 Italian study estimated that circulating anti-measles IgG antibodies decrease 10–15 years after the second dose of MMR vaccine administered according to the basal routine.[20]

A strength of our study was it large sample size. Its main limitation arose from the source of the information on the natural history of measles in the enrollees, as it relied on the historical memory of the interviewed participants (and their parents), whose recall may not have been accurate. In addition, individuals naturally immunized as children may still have been vaccinated by their pediatrician; however, if they had no memory of the event and there was no record of it, because in the past vaccination was not consistently reported, then bias may have inadvertently been introduced into the study. This problem has been discussed in the literature, although self-reported information is still considered to be a good investigative tool.[25-27] In particular, an Italian 2007 study showed that a self-reported measles history had a positive predictive value of 94.7%[28] and a 2006 survey among HCWs showed a predictive value of 92%.[29] Moreover, our results may have been partly influenced by the epidemiological change that has occurred in recent years, which has made exposure to natural boosters less frequent. Finally, the level of functional antibodies, i.e. neutralizing antibodies, measured through virus neutralization assays and cellular immunity have not been measured and so next studies must focus on these elements to achieve more robust conclusions.

A key to the interpretation of our data is to define the role of circulating antibodies and memory B cells in protecting against wild virus. Protection correlates better with the quality and quantity of the induced neutralizing antibodies, but the development of immunity against the disease is probably largely determined by T cells.[9] Studies on macaques have shown that neutralizing antibodies provide protection from the disease

(rash) but not necessarily from infection and that T cells alone do not protect against either infection or disease but instead facilitate the clearance of viral RNA.[9] Indeed, the role of cell-mediated immunity in the long-term response to the vaccine/disease (and consequent protection against measles) is discussed controversially in the scientific literature. Amanna et al., in a 2017 study,[30] conducted a prospective observational analysis of antibody titer changes in 45 individuals over a period of more than 26 years. Antigen-specific memory B cells were measured and their levels compared with those of the corresponding antibodies. The authors determined an association between the levels of memory B-cell and the concentration of antibodies against measles, based on the assumption that serum antibodies and memory B cell levels are equally stable but independently maintained. However, a direct cause-and-effect relationship could not be established.[30] A 1975 study highlighted the role of cellular immunity and postulated that the cell-associated immune system is the main host defense against measles. The findings were based on the observed responses to measles in agammaglobulinemic children and the death of these children but not those with a thymus deficiency who also contracted measles.[24] However, a 2016 study found that the contribution of T cells to protection is generally minor compared to that of neutralizing antibodies.[6] Nonetheless, field experience has shown that during measles outbreaks vaccinated individuals have been among the infected.[31,32]

While further research is needed, our study clearly showed that natural immunity is both more robust and longer-lasting than vaccine immunity. However, this finding should not lead to a questioning of the role of measles vaccination. It is well-established that the complications of measles are more frequent and more serious than any vaccine-related adverse reaction.[1,33] For example, in a recent study published in Science,[34] Mina et al. described the long-term damage to immune memory caused by measles infection. They found that measles infection can greatly diminish previously acquired immune memory, potentially leaving individuals at risk of infection by other pathogens. The same authors showed that the MMR vaccine does not impair the immune repertoire and that the loss of antibodies that occurs in measles virus infection does not appear to accompany MMR vaccination.[34] In light of this evidence, the MMR vaccine remains the most effective, safe and cost-effective tool for preventing measles.

The elimination of measles is a 20-year objective of national and international Public Health institutions.[35] The results of this study highlight the risk of a loss of antibodies over time. Thus, from now until the next 10–20 years, the vaccinated population can be expected to lose circulating antibodies such that their susceptibility to measles may increase. Moreover, since it is highly unlikely that measles will be eliminated in the immediate future, a part of individuals vaccinated several years ago will soon lose their circulating antibodies, such that outbreaks of the disease in the coming years can be expected. Recently, Kurata et al. described a cluster of measles cases, seven of which (including the index case) involved fully vaccinated individuals.[36] The confirmation of our results may lead to a revision of the mathematical algorithms used in disease elimination strategies. Current mathematical models[37] applied to reach the elimination goal consider the vaccinated population to be 100% immunized, ignoring the possibility of vaccination failure or the waning of circulating antibodies in those previously vaccinated (20% in our sample) or with a history of measles (6%).

In the absence of a revised strategy, our combined screening and vaccination approach allows safe access to healthcare environments by ensuring that HCWs are immune to circulating pathogens responsible for preventable diseases. The introduction of a third MMR dose for serosusceptible HCWs, both vaccinated and naturally immunized, showed high levels of efficacy and safety; furthermore, the above described strategy showed good compliance by health personnel, a critical determinant in the immunization of HCWs, as evidenced by many studies in literature[38,39] The benefits of our approach also include economic ones, as it will lead to a lowering of the risk of measles outbreaks and therefore their associated costs;[40] indeed, the cost of serological screening eventually followed by third MMR dose has less impact on public funds compared to the measures required in the context of epidemic outbreaks. Our screening model is applicable and implementable in a short time for HCWs in epidemiological contexts similar to that described by us; over time the results of further experiences will confirm the effectiveness of this strategy and the effects on the field could be measured with a zeroing of nosocomial clusters of measles in the structures where it is applied. Finally, a 2019 meta-analysis[21] showed a prevalence of Italian HCWs susceptible to measles equal to 12% and so firm measures of control and prevention are needed to reduce the risk of measles in nosocomial environment and its complication especially in high risk patients.

Abbreviations

CDC	Center for Disease Control and Prevention
MMR	Measles, mumps, rubella
HCWs	Healthcare workers
GIAVA	Regional Immunization Database
CLIA	Chemiluminescent immunoassay
PAS	Protective antibody survival
IRR:	Incidence rate ratio
GMT	Geometric mean titer

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed. The manuscript has not previously been presented in any meeting.

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Herd Immunity and Compulsory Childhood Vaccination: Does the Theory Justify the Law?

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ABSTRACT

Compulsory childhood vaccination is a cornerstone of U.S. public health policy. All fifty states compel children to vaccinate against many infectious diseases to achieve so-called herd immunity, a scientific theory that attempts to explain how societies protect themselves against infectious disease.

This Article explores both the theory and practice of herd immunity. The authors evaluate the scientific assumptions underlying the theory, how the theory applies in law, a game theory approach to herd immunity, and a possible framework for rational policymaking. The Article argues that *herd immunity* is unattainable for most diseases and is therefore an irrational goal. Instead, the authors conclude that *herd effect* is attainable and that a voluntary vaccination marketplace, not command-and-control compulsion, would most efficiently achieve that goal.

The Article takes on the bugaboo of the citizen "free rider" who is out to game the system, how a vaccination marketplace might work, and what factors policymakers must take into account in developing sound policies. The Article concludes that it is time for states to adopt more realistic and cost-efficient laws to achieve attainable herd effect, not illusory herd immunity.

INTRODUCTION

Many state and federal laws compel childhood vaccination based on the theory of herd immunity.[1] The theory describes a form of indirect protection in which non-immune individuals are protected from those that have acquired a disease and recovered.[2] Promoters of

Natural Immunity

[1] *See* James G. Hodge, Jr. & Lawrence O. Gostin, *School Vaccination Requirements: Historical, Social, and Legal Perspectives*, 90 KY. L.J. 831, 833 (2002) ("Each state has school vaccination laws which require children of appropriate age to be vaccinated for several communicable diseases." (citation omitted)); *see also State Information*, IMMUNIZATION ACTION COALITION, http://www.immunize.org/laws (last visited Mar. 6, 2014) (showing vaccination mandates by state, and while the Immunization Action Coalition is solely responsible for this website, its information is based on government sources, and the website is funded in part by the Centers for Disease Control and Prevention).

[2] *See, e.g.*, Paul Fine et al., *"Herd Immunity": A Rough Guide*, 52 CLINICAL INFECTIOUS DISEASES 911 (2011) [hereinafter Fine, *Rough Guide*]; Paul E.M. Fine, *Herd Immunity: History, Theory, Practice*, 15 EPIDEMIOLOGIC REVS. 265 (1993) [hereinafter Fine, *History*]; John P. Fox et al., *Herd Immunity: Basic Concept and Relevance to Public Health Immunization Practices*, 94 J. EPIDEMIOLOGY 179 (1971).

universal vaccination adopted this theory, suggesting that it applies to vaccine-induced immunity as well.[3] Today, herd immunity is the central rationale for compulsory vaccination, and the U.S. Supreme Court has long upheld the right of states to mandate vaccines under certain circumstances.[4] Vaccine proponents in the United States argue that the theory justifies vaccination of all children against vaccine-targeted diseases, except those few children with lawful exemptions.[5] Today, at or above ninety percent of all U.S. children have been vaccinated against routine childhood diseases, including measles, mumps, and pertussis.[6]

But the theory of herd immunity alone does not justify compulsion. The leap in logic from herd immunity theory to compulsory vaccination programs requires three fundamental assumptions: (1) that herd immunity is a valid and obtainable objective of vaccination policy; (2) that without compulsion, unvaccinated individuals, or their guardians, will seek to "free ride" on the immunity of the community; and (3) that individuals have an implied duty to society to be vaccinated to achieve herd immunity.[7] This Article looks at the underpinnings of the herd immunity theory and at the ties binding the theory to compulsory laws. Is herd immunity obtainable with modern vaccines? Are the assumptions of the theory relevant in the real world? Is there a free rider problem? Do members of society, and children in particular, have an obligation to accept vaccines "for the good of the herd"?

This Article concludes that herd immunity has only limited application in the world of policy. Given contemporary, imperfect vaccine technology and geographical and age-stratified vaccination mandates, herd immunity does not exist and is not attainable. Therefore, policy should seek to maximize attainable benefits, not unattainable ones, by relying on herd effect and the optimal use of scarce resources.

A game theory approach suggests that a market based on individual vaccination choices would best protect society. Game theory refutes

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[3] *Id.*

[4] *See* Jacobson v. Massachusetts, 197 U.S. 11 (1905).

[5] *See* Hodge, Jr. & Gostin, *supra* note 1.

[6] Ctrs. for Disease Control & Prevention, *National, State, and Local Area Vaccination Coverage Among Children Aged 19–35 Months—United States, 2009*, 59 MORBIDITY & MORTALITY WKLY. REP. 1171, 1171–73 (2010), *available at* http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5936a2.htm.

[7] *See* Douglas S. Diekema, *Choices Should Have Consequences: Failure to Vaccinate, Harm to Others, and Civil Liability*, 107 MICH. L. REV. FIRST IMPRESSIONS 90 (2009).

the free rider problem by showing that a unique equilibrium point exists that best balances vaccination benefits and disease harms. The Article finds that market-based, not regulatory, solutions better fit vaccination decision making.[8] This market approach suggests that in the long term, individuals will appropriately balance the relative costs of vaccination and infection, leading people to vaccinate voluntarily in light of the cost-benefit analysis. Although the equilibrium vaccination coverage is in almost all cases lower than the herd immunity threshold, "soft" regulation can achieve aggregate health benefits for society without imposing inefficient marginal costs on individuals and the healthcare system.[9] We therefore argue that personal choices in a market with adequate information would better allocate scarce healthcare resources, better protect the public health, and better respect individual autonomy. Our viewpoint may help explain why many developed countries, including those with political systems closest to our own, have only voluntary childhood vaccination programs. Vaccination uptake and disease levels in these countries, including Canada, the United Kingdom, Australia, and New Zealand, are comparable to those in the United States.[10]

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[8] Such market-based approaches have been well described in the literature of administrative and regulatory law. *See generally* OFFICE OF MGMT. & BUDGET, CIRCULAR A-4, at 7-9 (2003), *available at* http://www.whitehouse.gov/omb/circulars_a004_a-4 (outlining alternatives to federal regulation, including specification of performance as opposed to design standards, use of economic incentives, and informational measures); Bruce A. Ackerman & Richard B. Stewart, *Reforming Environmental Law*, 37 STAN. L. REV. 1333, 1336–37 (1985) (describing the "massive information-gathering burdens" on administrators attempting to impose command-and-control emissions regulations).

[9] *Cf.* Exec. Order No. 12,866 § 1(b)(3), 58 Fed. Reg. 190 (Oct. 4, 1993) ("Each agency shall identify and assess available alternatives to direct regulation, including providing economic incentives to encourage the desired behavior, such as . . . providing information upon which choices can be made by the public."); *id.* at § 1(b)(6) ("Each agency shall assess both the costs and the benefits of the intended regulation and . . . propose or adopt a regulation only upon a reasoned determination that the benefits of the intended regulation justify its costs."); *see also* Exec. Order No. 13,563 § 1(b), 76 Fed. Reg. 14 (Jan. 21, 2011) (supplementing Exec. Order No. 12,866 and reaffirming general principles of regulatory policy).

[10] There is no mandatory vaccination in the United Kingdom. *Childhood Immunisation: A Guide for Healthcare Professionals*, BRIT. MED. ASS'N (June 2003), http://www .worcslmc.co.uk/upload/Childhood_Immunisation_June_03.pdf. Scandinavia and Germany also rely on voluntary vaccination rather than compulsion. *Id.* There are some vaccination requirements in Australia, but there is a broad right of conscientious objection. *Id.* Some provinces in Canada require vaccines but allow conscientious objection, and the country as a whole does not mandate vaccination. *Vaccine Safety Frequently Asked Questions*, PUB. HEALTH AGENCY OF CAN., http://www.phac-aspc .gc.ca/im/vs-sv/vs-faq16-eng.php (last modified Aug. 27, 2012). In 2012, the United Kingdom, with a

Every state in the United States currently mandates roughly twenty-five to thirty-five doses of vaccines to preschoolers and school-aged children, with limited rights of exemption.[11] While there are other vaccination mandates in the United States for military personnel, hospital workers, and university students, to name a few, this Article focuses exclusively on state mandates for preschoolers and schoolchildren. Today, if children do not comply with state vaccination mandates and do not have valid exemptions, they lose their ability to attend school, a fundamental right and obligation of citizenship.[12] Further, state agents may charge the parents with medical neglect and potentially remove children to foster care for failure to vaccinate.[13] Even if a state offers limited medical, religious, and philosophical exemptions, we consider its vaccination mandate to be compulsory for purposes of this Article. We do so because in the majority of states, exemptions are extremely limited,[14] and even in those states where they exist, there are strong legislative efforts to curtail them.[15] We note at the outset that many vaccine-related issues are beyond the scope of this Article. While further considerations of

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population of roughly 63 million, had 0 reported cases of diphtheria, 2092 reported cases of measles, and 3178 reported cases of mumps. *WHO Vaccine-Preventable Diseases: Monitoring System. 2014 Global Summary*, WORLD HEALTH ORG., http://apps.who.int/immunization_monitoring/globalsummary/ (select "United Kingdom of Great Britain and Northern Ireland (the)" from the dropdown menu) (last updated July 15, 2014). Similarly, Australia, with a population of roughly 23 million, had 0 reported cases of diphtheria, 199 reported cases of measles, and 195 reported cases of mumps in 2012. *Id.* (select "Australia" from the dropdown menu). In 2012, the United States, where choice is more limited, with a population of roughly 317 million, had 1 reported case of diphtheria, 55 reported cases of measles, and 229 reported cases of mumps. *Id.* (select "United States of America (the)" from the dropdown menu).

11 *See States with Religious and Philosophical Exemptions from School Immunization Requirements,* NAT'L CONF. STATE LEGISLATURES (Dec. 2012), http://www.ncsl.org/research/health/school-immunization-exemption-state-laws.aspx (showing that only Mississippi and West Virginia do not have religious exemptions).

12 *See, e.g.*, N.Y. PUB. HEALTH LAW § 2164 (Consol. 2011).

13 *See* Kim Mack Rosenberg, *Forced Child Removal*, *in* VACCINE EPIDEMIC: HOW CORPORATE GREED, BIASED SCIENCE, AND COERCIVE GOVERNMENT THREATEN OUR HUMAN RIGHTS, OUR HEALTH, AND OUR CHILDREN 238 (Louise Kuo Habakus & Mary Holland eds., 2012).

14 *See* Y. Tony Yang & Vicky Debold, *A Longitudinal Analysis of the Effect of Nonmedical Exemption Law and Vaccine Uptake on Vaccine-Targeted Disease Rates*, 104 AM. J. PUB. HEALTH 371 (2014) (stating that fewer than ten percent of all children have exemptions).

15 *See, e.g.*, First Warning Letter from Jane R. Zucker, Assistant Comm'r, N.Y.C. Dep't of Health & Mental Hygiene, to Principals (Nov. 8, 2012), *available at* http://schools.nyc.gov/NR/rdonlyres/1B9F9BF4-34AE-49B9-8C45-B0176A0CA970/0/FirstWarningLetter.pdf (threatening principals if they do not achieve 98.8% vaccination compliance).

personal autonomy, vaccine safety, and vaccine injury are all critical and interrelated, we do not consider those issues in depth here.[16]

Part I defines and analyzes herd immunity and the closely related but distinct concept of herd effect. It contrasts disease eradication and elimination with control, highlighting the limits of what modern vaccination programs can achieve. It then explores the real world of disease outbreaks in vaccinated and unvaccinated populations. Part II introduces the Feudtner-Marcuse framework for "just" vaccination policy. This systematic approach highlights seven objectives of vaccination programs, including mandatory ones. Part III reviews game theory to understand the factors that drive people to choose or decline vaccination. We discuss a social equilibrium point that maximizes net public health gains. The Article ends by summarizing our conclusions and recommendations for U.S. vaccination policies.

I
HERD IMMUNITY AND ITS ASSUMPTIONS

Herd immunity depends on the time a disease persists within an infected host and the rate at which the disease spreads.[17] In a population of only susceptible individuals, the introduction of a single infected person will result in indiscriminate transmission to all others whom the infected person contacts until those infected people die or recover.[18] The average number of people in such a susceptible population who become infected is the so-called *basic reproduction number* R_0.[19] Each of those people who contracted the disease from the initial infected individual is able to transmit the disease to other susceptible contacts; this process repeats itself until the entire

16 Other sources provide more in-depth considerations of these issues. *See generally* VACCINE EPIDEMIC: HOW CORPORATE GREED, BIASED SCIENCE, AND COERCIVE GOVERNMENT THREATEN OUR HUMAN RIGHTS, OUR HEALTH, AND OUR CHILDREN, *supra* note 13; *see also* Mary Holland et al., *Unanswered Questions from the Vaccine Injury Compensation Program: A Review of Compensated Cases of Vaccine-Induced Brain Injury*, 28 PACE ENVTL. L. REV. 480 (2011).

17 *See* J.M. Heffernan et al., *Perspectives on the Basic Reproductive Ratio*, 2 J. ROYAL SOC'Y INTERFACE 281 (2005).

18 *See* Fine, *History*, *supra* note 2, at 273 fig.5 (showing one hundred percent transmission from one individual to all other individuals with whom he or she has effective contact in an entirely susceptible population).

19 Heffernan et al., *supra* note 17.

population is infected.[20] This model of disease transmission exhibits *epidemic* dynamics.[21]

A. Herd Immunity Threshold

By contrast, consider the case where a certain fraction θ of the population has immunity to the disease. If a single infected individual comes into the population, the average number of secondary infections from transmission is then $R_0(1-\theta)$.[22] If $R_0(1-\theta) < 1$, then the disease on average will not spread to other susceptible people.[23] This means that the disease is likely to die out either through the host's death or recovery before further spread.[24] The threshold θ_H of immune individuals to create these circumstances is $\theta_H = 1-1/R_0$, or the *herd immunity threshold*.[25] The underlying rationale for mass vaccination policies is to ensure that the fraction of immune individuals in society is above the herd immunity threshold, thus eliminating the disease from the population.[26] The moral of the herd immunity story, though, is that not every individual needs to be immune to provide protection to the society as a whole.[27]

B. Herd Effect

The concept of herd immunity refers to the complete removal of a disease from society; so long as *any* member of the population has immunity to the disease, however, the disease's ability to spread

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[20] *See* Fine, *History*, *supra* note 2 (showing the complete spread of infection in an entirely susceptible society).

[21] *See id.* at 269 (defining the epidemic threshold for a simple mass-action model of infectious dynamics).

[22] To derive this relationship, note that within a susceptible population of size N, a single infectious individual will infect on average R_0 persons. If N_V members of the population have immunity to the disease, however, then transmission is only possible within a subpopulation of size $N-N_V$. The resulting average number of secondary infections then decreases to $(R_0/N)(N-N_V) = R_0(1-N_V/N) = R_0(1-\theta)$.

[23] *See* Heffernan et al., *supra* note 17, at 281–87.

[24] *Id.*

[25] *See generally* Fine, *History*, *supra* note 2, at 269 (providing one example of use of the herd immunity threshold); Fine, *Rough Guide*, *supra* note 2, at 912 fig.1 (providing another example of use of the herd immunity threshold).

[26] *See* Fine, *Rough Guide*, *supra* note 2 (discussing the success of vaccination programs against measles, mumps, rubella, etc. in delaying or averting epidemics by keeping the amount of susceptible individuals below the threshold); *see also* Fine, *History*, *supra* note 2 (discussing the success of the global smallpox eradication program).

[27] *See* Fine, *History*, *supra* note 2.

lessens.[28] This decrease in the rate of epidemic transmission is the *herd effect*.[29] Even if herd immunity itself is not achievable, society still benefits from a "buffer" of immune individuals in order to mitigate disease.[30] Although the concepts of herd immunity and herd effect are sometimes interchangeable, they describe different aspects of the immunity puzzle—whereas herd immunity aims to *eliminate* a disease from society, herd effect refers to infection *control*.[31] Since the 1960s, compulsory state vaccination programs have achieved herd effects for specific diseases, but none has achieved herd immunity. We maintain the analytic distinction between these terms in the discussion below.

C. The Free Rider Problem

Why are universal mandatory vaccination policies necessary if we can achieve herd immunity by vaccinating only a fraction of the population? Proponents of compulsion argue that if vaccination is not mandatory, then herd immunity is generally unattainable due to a *free rider problem*.[32] From the perspective of an individual weighing the decision to vaccinate, it is in her best interest not to vaccinate because she is unlikely to become sick if all others are immune and are unlikely to transmit disease.[33] This decision-maker could then "free ride" on the immunity of others.[34]

If all individuals in a population attempt to free ride, then they all run the risk of illness. If the expected risks of vaccine injury outweigh those of illness, then no one will choose to vaccinate.[35] This situation

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28 *See* T. Jacob John & Reuben Samuel, *Herd Immunity and Herd Effect: New Insights and Definitions*, 16 EUR. J. EPIDEMIOLOGY 601, 601 (2000) (defining herd effect).

29 *See id.* (distinguishing herd effect and herd immunity).

30 *See* Fine, *Rough Guide*, *supra* note 2, at 912 (discussing the importance of "selective vaccination"—specifically, vaccinating groups that play an important role in transmission, either in slowing transmission or reducing incidence among the entire population).

31 *See infra* Part I.F. (discussing definitions of "control" and "elimination" in the context of vaccination policy).

32 *See* Fine, *Rough Guide*, *supra* note 2, at 914.

33 *See id.*

34 *See id.*

35 It is essential to distinguish between *perceived* and *absolute* costs of vaccination and infection. In general, individuals in society operate under limited information as to the probabilities of vaccine-related harm and infection and thus make individual estimations of expected costs consistent with such incomplete information. If all members of society had perfect information, absolute costs of vaccination and infection could be determined. In practice, such perfect information is never available. *See infra* Part III.

represents a *tragedy of the commons*, in which society loses an important benefit because of competing individual interests.[36] As the rate of infection decreases, individuals may perceive the risks of infection as declining, inducing some individuals to forego vaccination. This scenario has led some to decry that vaccines are the "victim[s] of their own success."[37] Compulsory vaccination is then one solution to the potential free rider problem because it forces all children to assume part of the collective responsibility to prevent infectious disease.[38]

D. Assumptions Underlying Herd Immunity Theory

The potential social costs of the free rider problem are severe in the face of a highly infectious, life-threatening disease and the failure to reach the herd immunity threshold.[39] Under what conditions, however, is herd immunity actually possible? Many of the underlying assumptions of herd immunity may be irrelevant in the real world, as authoritative scientists have acknowledged.[40] We address the following core assumptions of the theory[41]:

1. Population homogeneity;
2. Well-mixing of the population;
3. Random vaccination of individuals;
4. Perfect vaccine efficacy; and
5. Age uniformity in the population.

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36 *See* Chris T. Bauch et al., *Rapid Emergence of Free-Riding Behavior in New Pediatric Immunization Programs*, 5 PLOS ONE 1, 1 (2010).

37 *See* Matthew Janko, *Vaccination: A Victim of Its Own Success*, 14 VIRTUAL MENTOR 3, 4 (2012).

38 Dagobert L. Brito et al., *Externalities and Compulsory Vaccinations*, 45 J. PUB. ECON. 69, 69–70 (1991) (quoting J.E. STIGLITZ, ECONOMICS OF THE PUBLIC SECTOR 210 (2d ed. 1988)).

39 *See, e.g.*, V.A.A. Jansen et al., *Measles Outbreaks in a Population with Declining Vaccine Uptake*, 301 SCIENCE 804, 804 (2003) (relating the decline in measles vaccinations to "a number of large measles outbreaks").

40 *See* Fine, *History*, *supra* note 2, at 276.

41 *See id.* (naming an incomplete list of assumptions); *see also* Fine, *Rough Guide*, *supra* note 2, at 912–14 (discussing probable complexities that would upset the core assumptions).

1. The Assumption of Population Homogeneity

Population homogeneity involves two related but distinct concepts: (1) compositional homogeneity and (2) spatial homogeneity.[42] Compositional homogeneity means that all individuals belong to a single identifiable group.[43] Persons within this group transmit the disease among themselves as if all group members are the same.[44] Compositional homogeneity ignores racial, sociological, economic, and genetic differences, all of which in the real world may affect resistance to an infectious disease.[45]

Spatial homogeneity, by contrast, refers to the degree of uniform spread over a geographic region.[46] Spatial homogeneity assumes that people behave identically in spreading disease.[47] But if a group of people lives in a particular area, and its members spread disease differently from the rest of society, then this violates the assumption of interchangeability.[48] For the simple analysis of herd immunity to hold true, both compositional and spatial homogeneity must exist.[49]

As a practical matter, however, compositional homogeneity *never* holds. Social stratification by age, ethnicity, class, gender, race, and sexual orientation, among other factors, results in differing individual risks.[50] For example, the Centers for Disease Control and Prevention (CDC) noted that more than fifty percent of all new cases of HIV infection between 2006 and 2009 were among men who have sex

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42 *See generally* DIETRICH STOYAN ET AL., STOCHASTIC GEOMETRY AND ITS APPLICATIONS (2d ed. 1995) (discussing spatial homogeneity in the context of stochastic point processes); *see also* Fine, *Rough Guide*, *supra* note 2, at 913 (discussing models of heterogeneous populations).

43 *Id.*

44 *Id.*

45 *Id.*

46 *See generally* STOYAN ET AL., *supra* note 42.

47 Spatial homogeneity is mathematically defined by the property of translation-invariance for all probabilistic descriptors governing the spatial correlations among groups of individuals within a population, implying that the choice of origin for a Euclidean coordinate system adopted to describe the spatial region does not affect measured statistical properties.

48 Fine, *Rough Guide*, *supra* note 2, at 913.

49 *Id.* (listing heterogeneous populations as a complex problem disrupting herd immunity's core assumptions).

50 *See generally CDC Fact Sheet: Estimates of New HIV Infections in the United States, 2006–2009,* CTRS. FOR DISEASE CONTROL & PREVENTION 1, 3 (Aug. 2011), http://www.cdc.gov/nchhstp/newsroom/docs/Hiv-infections-2006-2009.pdf (providing statistics showing disparities in HIV outbreaks among differing populations).

with men.[51] Additionally, African Americans accounted for forty-four percent of new HIV infections in 2009.[52] These types of differences are compositional, relating to characteristics that distinguish population subgroups. Compositional heterogeneity increases the herd immunity threshold for the population, meaning that the minimum number of people vaccinated must be higher, because vaccination of low-risk individuals provides little marginal herd effect.

Spatial homogeneity, another bedrock assumption of herd immunity, similarly does not hold true in practice.[53] Scientists have studied the effects of clustering using network models, showing individuals as nodes on a graph with intersections indicating transmissible contacts.[54] Limiting the types and numbers of transmissible contacts can substantially change the rate at which a disease spreads through the population.[55] The existence of isolated, highly clustered groups of susceptible individuals can increase the required herd immunity threshold for the population as a whole because vaccinating people outside the clustered group provides little benefit.

Diseases spread more slowly when there is more distance between people.[56] This spatial effect can result in rapid disease spread within clustered areas, such as cities, even when disease spread is decreasing overall.[57] As travel technology continues to develop, diseases can spread quickly, both domestically and internationally. However, spatial dissemination coupled with transmission dynamics may lead to

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51 *Id.*

52 *Id.* at 4.

53 *See* Martial L. Ndeffo Mbah et al., *The Impact of Imitation on Vaccination Behavior in Social Contact Networks*, 8 PLOS COMPUTATIONAL BIOLOGY 1, 7 (2012) (noting that spatial homogeneity fails to take into account the fact that "individuals frequently imitate others").

54 *See generally* Chris T. Bauch & Alison P. Galvani, *Using Network Models to Approximate Spatial Point-Process Models*, 184 MATHEMATICAL BIOSCIENCES 101 (2003) (using network models to evaluate spatial effects on ecological and epidemiological systems); Matt J. Keeling & Ken T.D. Eames, *Networks and Epidemic Models*, 2 J. ROYAL SOC'Y INTERFACE 295 (2005) (providing an overview of the process of approximating a network); Martial L. Ndeffo Mbah et al., *supra* note 53 (using network-based models to examine the correlation between the spread of disease and social contacts).

55 Keeling & Eames, *supra* note 54, at 300–01 (contrasting networking models that account for clustering with random networks, which assume that connections are formed at random).

56 *See* Bauch & Galvani, *supra* note 54, at 102.

57 *Id.*

stationary patterns of infectious regions.[58] In sum, neither compositional nor spatial homogeneity assumptions hold true in the real world.

2. The Assumption of a Well-Mixed Population

The well-mixing assumption refers to the notion that all susceptible individuals are equally likely to become sick from an infectious individual.[59] Network models can test the well-mixing assumption and, in a well-mixed population, each node in a network model will have an intersection with every other node in that same model.[60] To understand how well-mixing affects the dynamics, consider the simple case of a population of nine individuals, three of whom are susceptible and six of whom are infected. If each infected individual contacts only one susceptible person, and if each susceptible person contacts two infected people, it follows that there are only six possible transmissible contacts in the population.

By contrast, the well-mixing assumption implies that there are eighteen transmissible contacts, overestimating the disease propagation rate by a factor of three. Isolated groups of highly connected, susceptible people may face particularly rapid disease transmission that might otherwise have spread relatively slowly through the population as a whole.[61] Clustering of susceptible individuals is again the key to understanding how to control disease dynamics. Indeed, all statewide mandates are for children and young adults, representing clusterings of susceptible individuals. No states mandate vaccination for the entire population today. The result of this

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58 This pattern-forming phenomenon arises from an identical mechanism for the formation of so-called Turing patterns in reaction-diffusion chemical systems. Such patterns, which are stationary in time but heterogeneous in space, develop when an "inhibiting" species diffuses faster in space than a competing "growth" species, resulting in local activation of dynamic transmission that is inhibited on a global scale. *See* A.M. Turing, *The Chemical Basis of Morphogenesis*, 237 PHIL. TRANSACTIONS ROYAL SOC'Y LONDON 37, 57–58 (1952).

59 James Holland Jones, *Notes on R_0*, DEP'T ANTHROPOLOGICAL SCI. STANFORD U. 1, 2 (2007), http://www.stanford.edu/~jhj1/teachingdocs/Jones-on-R0.pdf.

60 Alun L. Lloyd et al., *Infection Dynamics on Small-World Networks*, *in* MATHEMATICAL STUDIES ON HUMAN DISEASE DYNAMICS: EMERGING PARADIGMS AND CHALLENGES 209, 220–21 (Contemporary Mathematics Ser. Vol. 412, Abba B. Gumel et al. eds., 2006).

61 *See* Fine, *Rough Guide*, *supra* note 2, at 913–14.

type of clustering is that the herd immunity threshold may be higher than estimated from the well-mixing assumption.[62]

U.S. policies for hepatitis B disease prevention provide a good example of how the well-mixing assumption applies in practice.[63] Although only a small portion of the U.S. population was at risk of contracting hepatitis B, namely intravenous drug users, those who had unprotected sex with multiple partners, and infants of hepatitis B positive mothers, it proved difficult for public health authorities to gain compliance among these target groups in the 1980s.[64] As a result, even though the herd immunity threshold would be much lower for the general population than the target group, U.S. public health authorities recommended universal vaccination of infants against hepatitis B to achieve herd immunity, and forty-seven states now mandate the vaccine.[65]

3. The Assumption of Random Vaccination of Individuals

In a heterogeneous population, different subgroups may face unique risks to certain infections and vaccine injuries.[66] A vaccination program that randomly immunizes people will generally require an especially high vaccination coverage ratio to achieve herd immunity because the disease will be able to propagate efficiently among high-risk individuals.[67] One solution is therefore to target the vaccination

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62 *See id.* at 913.

63 *See* Mary Holland, *Compulsory Vaccination, the Constitution, and the Hepatitis B Mandate for Infants and Young Children*, 12 YALE J. HEALTH POL'Y L. & ETHICS 39, 41 (2012); Rui Xu & Zhien Ma, *An HBV Model with Diffusion and Time Delay*, 257 J. THEORETICAL BIOLOGY 499, 499 (noting that "it is implicitly assumed that cells and viruses are well mixed").

64 Holland, *supra* note 63, at 68–69 (citing Ctrs. for Disease Control & Prevention, *Recommendation of the Immunization Practices Advisory Committee (ACIP) Inactivated Hepatitis B Virus Vaccine*, 31 MORBIDITY & MORTALITY WKLY. REP. 317 (1982), *available at* http://www.cdc.gov/mmwr/preview/mmwrhtml/00001116.htm) (outlining recommendations of U.S. public health authorities that "higher-risk groups" receive hepatitis B vaccinations).

65 *See Hepatitis B Prevention Mandates for Daycare and K-12,* IMMUNIZATION ACTION COALITION, http://www.immunize.org/laws/hepb.asp (last updated May 26, 2011).

66 *See People at High Risk of Developing Flu-Related Complications,* CTRS. FOR DISEASE CONTROL & PREVENTION, http://www.cdc.gov/flu/about/disease/high_risk.htm (last updated Nov. 7, 2013) (listing specific subgroups that are particularly susceptible to flu-related complications).

67 *See* Fine, *Rough Guide*, *supra* note 2, at 914.

program only to those individuals who are at a highest risk of infection.[68]

Fine provides a simple example of this type of targeted vaccination program by considering a sample population composed of two equal-sized subgroups: high-risk and low-risk.[69] Following Fine's analysis, assume that each individual in the high-risk group, if infected, would infect five other high-risk members, and each low-risk individual, if infected, would infect one other low-risk member.[70] Under this idealized scheme, the high-risk and low-risk dynamics are separable because there are no transmissible contacts between groups.[71] The disease among the low-risk group is controllable without vaccination because the reproduction rate, $R_0^{(\mathrm{LR})}$, for the low-risk group is one, meaning that each person in this group would infect one other person on average.[72] This implies that the herd immunity threshold within the low-risk group is zero, and the disease will not spread, or $\theta_H^{(\mathrm{LR})} = 0$.[73]

By contrast, the disease will exhibit epidemic dynamics among the high-risk group because each high-risk individual will on average infect five others, so $R_0^{(\mathrm{HR})} = 5$ and $\theta_H^{(\mathrm{HR})} = 0.8$.[74] If vaccination is only for the high-risk group, only 80% of that group needs to receive the vaccine to induce herd immunity in the population as a whole.[75] Surprisingly, such a program targeted only at high-risk individuals would require vaccinating only 40% of the total population, representing a substantial increase in the health of society at lower financial cost and risk of vaccine injury.[76] But a vaccination program that randomly vaccinated 80% of the total population from the high-risk and low-risk groups would *not* provide herd immunity at all

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68 *See* Holland, *supra* note 63, at 68 (targeting hepatitis B vaccinations to high-risk groups).

69 Fine, *Rough Guide*, *supra* note 2, at 914.

70 *Id.*

71 *See id.*

72 *See id.*

73 Some care is required here. If $R_0 = 1$ exactly, then the disease will exist in an endemic steady state in which the number of infected individuals neither increases nor decreases on average. We therefore assume without loss of generality that the basic reproduction number is actually infinitesimally smaller than one to ensure that the disease is unable to sustain itself.

74 Fine, *Rough Guide*, *supra* note 2, at 914.

75 *Id.*

76 *See id.*

because the fractional vaccination coverage for the high-risk population would be less than its required herd immunity threshold.[77]

Although society can achieve the greatest benefits by targeting high-risk groups, such a policy imposes the full costs of vaccination on one identifiable group while the benefits diffuse to the greater population.[78] One could characterize this program as imposing a tax on specific individuals based on inherent characteristics,[79] precluding an equitable distribution of the costs and benefits to society. This policy becomes particularly troubling when its targets are children, who are low-risk subjects, selected for convenience, as in the case with mandatory vaccination of schoolchildren against hepatitis B, a sexually transmitted disease.[80] Random vaccination fails to maximize herd immunity or herd effect; only targeted or universal vaccination can achieve that result.

4. The Assumption of Perfect Vaccine Efficacy

Vaccines do not induce immunity perfectly; they usually fail in a certain fraction of people for a variety of reasons.[81] Furthermore, as a practical matter, vaccine "efficacy" is highly uncertain.[82] Scientists refer to efficacy as the relative fractional decrease in the rate of disease transmission between unvaccinated and vaccinated individuals in double-blind, randomized, clinically-controlled studies.[83] By contrast, the concept of vaccine "effectiveness" refers to the performance of the vaccine in the "real world," outside of clinical trials.[84] This distinction is not necessarily clear because the goal of

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77 *See id.*

78 *See id.* (discussing potential equal rights violations in mandating that all young children receive the hepatitis B vaccine).

79 Indeed, the Supreme Court's recent extension of the taxation power in the Court's ruling on the Affordable Care Act suggests that such a tax may be constitutional. *See* Nat'l Fed'n of Indep. Bus. v. Sebelius, 132 S. Ct. 2566, 2599 (2012) (holding that the Constitution does not protect individuals from "taxation through inactivity").

80 *See* Holland, *supra* note 63, at 41.

81 *See Flu Vaccine Effectiveness: Questions and Answers for Health Professionals*, CTRS. FOR DISEASE CONTROL & PREVENTION, http://www.cdc.gov/flu/professionals/vaccination/effectivenessqa.htm (last updated Nov. 27, 2013) (finding, for example, that influenza vaccines are less effective in people with chronic, high-risk medical conditions).

82 John Clemens et al., *Evaluating New Vaccines for Developing Countries: Efficacy or Effectiveness?*, 275 J. AM. MED. ASS'N 390, 392 (1996).

83 *See* Geoffrey A. Weinberg & Peter G. Szilagyi, *Vaccine Epidemiology: Efficacy, Effectiveness, and the Translational Research Roadmap*, 201 J. INFECTIOUS DISEASES 1607 (2010); Fine, *Rough Guide*, *supra* note 2, at 913 tbl.1; *Flu Vaccine Effectiveness: Questions and Answers for Health Professionals*, *supra* note 81.

84 Weinberg & Szilagyi, *supra* note 83, at 1608.

any vaccination policy is to control the rate of disease transmission. Nevertheless, either definition is sufficient for our discussion of herd immunity.

If a fraction, ϕ, of the vaccinated population fails to develop immunity and thus remains susceptible to infection, then the fraction of the total population that must receive the vaccine to ensure herd immunity is $\theta_H' = (1\text{-}1/R_0)/\phi = \theta_H/\phi$.[85] If the fraction of the population that fails to develop immunity is greater than the herd immunity threshold, or $\phi < \theta_H$, then herd immunity is theoretically impossible, even if the entire population is vaccinated.[86] A herd immunity threshold, θ_H, is generally high, ranging from 80%–99%.[87] For example, Fine estimates that the threshold for measles is 83%–94% and pertussis is 92%–94%.[88] As an illustration of the problem, measles vaccine has an estimated vaccine efficacy rate of 85%–95% for the first dose given to babies between 12 and 15 months.[89] This leaves unclear whether herd immunity is even theoretically achievable for measles. Thus, the assumption of perfect vaccine efficacy has limited bearing in real-world conditions.

5. The Assumption of Age Uniformity

Modern immunization programs target infants and young children for both scientific and practical reasons. Experience and science suggest that children are more vulnerable to infectious disease, but the practical reasons are also compelling.[90] Linking recommended and compulsory vaccination to "well-baby" and school check-ups provides a relatively low-cost method to oversee vaccination compliance. Adults, by contrast, lead more diverse lives and are more

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85 *See generally* Fine, *History*, *supra* note 2.

86 *Id.*

87 *See* Fine, *History*, *supra* note 2, at 268 (providing estimates of the herd immunity thresholds for the following diseases: diphtheria (85%); malaria (80%–99%); measles (83%–94%); mumps (75%–86%); pertussis (92%–94%); polio (80%–86%); rubella (83%–85%); smallpox (80%–85%)); *see also* Fine, *Rough Guide*, *supra* note 2, at 913. It should be noted that there is scientific uncertainty regarding the precise values of the herd immunity thresholds for various diseases.

88 Fine, *History*, *supra* note 2, at 268.

89 *Canadian Immunization Guide: Measles Vaccine*, PUB. HEALTH AGENCY CAN., http://www.phac-aspc.gc.ca/publicat/cig-gci/p04-meas-roug-eng.php (last modified Oct. 9, 2013); Fine, *History*, *supra* note 2, at 268 tbl.1.

90 *See* Gaston De Serres & Bernard Duval, *Pertussis Vaccination Beyond Childhood*, 365 LANCET 1015, 1015 (2005).

likely to assert autonomy rights in the courts and through political participation than young children or their parents.[91]

Children face particular problems from waning vaccine-induced immunity.[92] Immunity from vaccines generally requires several boosters to extend the period of protection. Adults, who may be less likely to receive boosters, have a greater fraction of susceptible individuals as a group than children.[93] Furthermore, unlike in prior decades, younger adults today do not have naturally acquired immunity because they never had infectious childhood diseases. Why then does the disease not produce an epidemic among adults? Are adults free riding on the vaccination programs of children?

We gain some insight into this question by comparing the differing vaccination policies for pertussis in European countries and the United States in the 1980s.[94] European countries had little or no pertussis immunization in childhood, resulting in widespread pertussis transmission among infants and children, but few adolescent or adult cases due to long-lasting natural immunity.[95] By contrast, the United States consistently administered pertussis vaccines to infants and children in the 1980s, causing an increase in pertussis cases among adults and adolescents because temporary vaccine-induced immunity had waned.[96] Therefore, while the adult population is not completely free riding on the vaccination of children, vaccinating children may have the unintended effect of increasing the average age when people become infected. For example, while chickenpox is a relatively mild disease among children, it can have extremely serious consequences in high-risk populations, including pregnant women, the elderly, and those who have compromised immunity.[97] Society may be disadvantaged by vaccinating children early, thus creating conditions

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91 *See generally* Peter A. Briss et al., *Reviews of Evidence Regarding Interventions to Improve Vaccination Coverage in Children, Adolescents, and Adults*, 18 AM. J. PREVENTATIVE MED. 97 (2000).

92 *Id.*

93 *See generally* Ctrs. for Disease Control & Prevention, *Noninfluenza Vaccination Coverage Among Adults—United States, 2011*, 62 MORBIDITY & MORTALITY WKLY. REP. 66 (2013), *available at* http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6204a2.htm?s_cid=mm6204a2_w.

94 De Serres & Duval, *supra* note 90, at 1015–16.

95 *Id.*

96 *Id.*

97 *Chickenpox (Varicella): People at High Risk for Complications*, CTRS. FOR DISEASE CONTROL & PREVENTION, http://www.cdc.gov/chickenpox/hcp/high-risk.html (last updated Nov. 16, 2011).

where older adults acquire the illness with greater risk of complications.[98]

While herd immunity assumes age uniformity, in practice this is virtually never present in real-world vaccination programs.[99] Overwhelmingly, children are the targets of mandatory vaccination programs, and this lack of age uniformity poses significant challenges given the temporary nature of vaccine protection.[100]

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In sum, the five underlying assumptions at the foundation of herd immunity—population homogeneity, well-mixing, random vaccination, perfect vaccine efficacy, and age uniformity—are of exceedingly limited practical relevance. The following cases highlight these limitations in practice.

E. Herd Immunity Theory in Practice

Recent experience shows infectious disease outbreaks in highly vaccinated populations. Such outbreaks seeming to violate the herd immunity theory have caused many researchers to reject the theory altogether. For instance, the International Medical Council on Vaccination states in its "Principles and Findings," that "[w]e find the premise of herd immunity to be a faulty theory."[101] Dr. Russell Blaylock argues that "[h]erd immunity is mostly a myth and applies only to natural immunity—that is, contracting the infection itself."[102] Dr. Suzanne Humphries argues that "[s]ince the beginning of vaccination, there is little proof that vaccines are responsible for eradicating disease even when herd immunity vaccination levels have

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98 *See* Timothy C. Reluga et al., *Optimal Timing of Disease Transmission in an Age-Structured Population*, 69 BULL. MATHEMATICAL BIOLOGY 2711, 2719 (2007) (suggesting that foregoing vaccination at a young age may provide greater aggregate social health benefits).

99 *See* Briss et al., *supra* note 91.

100 *Id.*

101 *Principles and Findings*, INT'L MED. COUNCIL ON VACCINATION, http://www.vaccinationcouncil.org/about/ (last visited Mar. 9, 2014).

102 Russell Blaylock, *The Deadly Impossibility of Herd Immunity Through Vaccination*, INT'L MED. COUNCIL ON VACCINATION (Feb. 18, 2012), http://www.vaccinationcouncil.org/2012/02/18/the-deadly-impossibility-of-herd-immunity-through-vaccination-by-dr-russell-blaylock/.

been reached."[103] Dr. Tetyana Obukhanych explains that "[t]he absence of viral epidemics in the [United States] is due to the absence of endemic viral exposure, not due to . . . herd immunity, and sporadic outbreaks . . . occur due to . . . viral exposure brought from abroad."[104]

While these researchers acknowledge that vaccinations can create short-term immunity, and that vaccines can cause herd effect, they argue that vaccination's long-term effects are often harmful to individuals and society. Despite nearly three hundred years of vaccination, scientists have not rigorously compared the long-term health outcomes of vaccinated versus unvaccinated subjects.[105] Without such critical information, some scientists are profoundly skeptical of current vaccine policies, including the goal of vaccine-induced herd immunity.[106]

Below, we consider empirical examples illustrating a range of problems with herd immunity in practice. They include: (1) primary vaccine failure—when a vaccine initially fails to induce immunity; (2) secondary vaccine failure—when the immunity the vaccine induced has waned over time and no longer offers protection; (3) mutation of the infectious virus—suggesting that the vaccine itself may have contributed to the viral shift; (4) importation of viral infections "just a plane ride away"; and (5) disease transmission, or "viral shedding," by vaccinated people who show no symptoms of disease. In addition, there have been disease outbreaks in vaccinated populations that scientists simply cannot explain. While there are many examples, we will focus on the measles and varicella vaccination programs.

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103 Suzanne Humphries, *"Herd Immunity." The Flawed Science and Failures of Mass Vaccination*, INT'L MED. COUNCIL ON VACCINATION (July 5, 2012), http://www.vaccinationcouncil.org/2012/07/05/herd-immunity-the-flawed-science-and-failures-of-mass-vaccination-suzanne-humphries-md-3/#sthash.aRBEJNVz.dpuf.

104 TETYANA OBUKHANYCH, VACCINE ILLUSION: HOW VACCINATION COMPROMISES OUR NATURAL IMMUNITY AND WHAT WE CAN DO TO REGAIN OUR HEALTH 90 (2012).

105 However, a bipartisan bill introduced in the U.S. House of Representatives on April 25, 2013, cited as the "Vaccine Safety Study Act," seeks to "conduct or support a comprehensive study comparing total health outcomes, including risk of autism, in vaccinated populations in the United States with such outcomes in unvaccinated populations in the United States." H.R. 1757, 113th Cong. (2013), *available at* https://www.govtrack.us/congress/bills/113/hr1757/text. Although this bill only has a one percent chance of being enacted according to *GovTrack.us*, its purpose is to fund science that needs to be done to compare vaccinated versus unvaccinated health outcomes. *H.R. 1757: Vaccine Safety Study Act*, GOVTRACK.US, https://www.govtrack.us/congress/bills/113/hr1757 (last visited Mar. 14, 2014).

106 *See, e.g.*, *Principles and Findings*, *supra* note 101.

1. The Case of Measles Vaccination and Immunity

Before the United States embarked on state mandates for measles vaccination, one of the leading proponents of the vaccine, Alexander Langmuir, characterized the disease as a "self-limiting infection of short duration, moderate severity, and low fatality."[107] In the same article, he noted that the disease had maintained a "remarkably stable biological balance over the centuries," and that "[t]he decline in mortality demonstrates the degree to which we have adapted to this balance and have learned to live with this parasite."[108] He explained that measles vaccination was by no means an urgent public health necessity, but rather he sought measles eradication because "it can be done."[109] In the 1960s, Langmuir seemed to believe that vaccination policies could eradicate measles in the near term.

a. Measles Outbreaks in Highly Vaccinated Populations

At that time, scientists believed the herd immunity threshold to be 70% and that one dose of the vaccine would confer long-lasting immunity.[110] Over time, however, scientists pushed the herd immunity threshold up to 95%[111] and started requiring two doses of the vaccine.[112] Evidence suggests, however, that even these policies have not been enough to create herd immunity. During a 1985 measles outbreak in a Texas high school, more than 99% of the 1806 students in the school had been vaccinated against measles.[113] Upon testing, only 4.1% of the students, or 74 of them, lacked detectable antibodies due to either primary or secondary vaccine failure.[114] The authors concluded, "outbreaks of measles can occur in secondary

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107 Alexander D. Langmuir et al., *The Importance of Measles as a Health Problem*, 52 AM. J. PUB. HEALTH 1, 1 (1962).

108 *Id.*

109 *Id.* at 3 (citation omitted) (internal quotation marks omitted).

110 Fine, *History*, *supra* note 2, at 285 (showing that as late as 1982, the World Health Organization estimated the herd immunity threshold for measles to be 70%).

111 *Id.*

112 *See Immunization Schedules: Recommended Immunization Schedule for Persons Aged 0 Through 18 Years*, CTRS. FOR DISEASE CONTROL & PREVENTION, http://www.cdc.gov/vaccines/schedules/hcp/imz/child-adolescent.html (last updated Jan. 31, 2014) (stating that children should receive two doses of the measles-mumps-rubella vaccine by six years of age).

113 Tracy L. Gustafson et al., *Measles Outbreak in a Fully Immunized Secondary-School Population*, 316 NEW ENGL. J. MED. 771, 771 (1987).

114 *Id.* at 772.

schools, even when more than 99[%] of the students have been vaccinated and more than 95[%] are immune," that is, they have measles antibodies.[115] They acknowledged that such an outbreak should have been virtually impossible but rationalized that the "[r]ates of primary vaccine failure in this range [*eds.*: 4.1%] are expected."[116]

Another measles outbreak occurred in a 100% vaccinated school population in Illinois in 1984:

> The affected high school had 276 students and was in the same building as a junior high school with 135 students. A review of health records in the high school showed that all 411 students had documentation of measles vaccination on or after their first birthday, in accordance with Illinois law.[117]

Not all students became ill, but scientists noted that those students who had received vaccines within the previous ten years were less likely to become sick than those who had been vaccinated more than ten years earlier.[118] Notably, officials could not explain how the seventeen-year-old index patient came down with the measles.[119]

The Centers for Disease Control and Prevention's editors noted several possible reasons for the outbreak, including vaccine failure due to improper storage, vaccination of infants younger than one who might be less likely to acquire protection, and other factors.[120] Still, they concluded that "these risk factors did not adequately explain the occurrence of this outbreak."[121] They further noted, "this outbreak suggests that measles transmission can occur within the 2%–10% of expected vaccine failures."[122] In other words, they acknowledged that even with 100% vaccination, they could not ensure herd immunity with existing vaccine technology and stated explicitly that "[t]his outbreak demonstrates that transmission of measles can occur within

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115 *Id.* at 771.

116 *Id.* at 773.

117 Ctrs. for Disease Control & Prevention, *Measles Outbreak Among Vaccinated High School Students–Illinois*, 33 MORBIDITY & MORTALITY WKLY. REP. 349 (1984), *available at* http://www.cdc.gov/mmwr/preview/mmwrhtml/00000359.htm [hereinafter *Measles Outbreak*]; *see generally* Benjamin M. Nkowane et al., *Measles Outbreak in a Vaccinated School Population: Epidemiology, Chains of Transmission and the Role of Vaccine Failures*, 77 AM. J. PUB. HEALTH 434 (1987) (describing a 1984 outbreak in a Massachusetts high school with a 98% immunization level, providing evidence that outbreaks may occur in highly immunized populations).

118 *Measles Outbreak*, *supra* note 117, at 350.

119 *Id.* at 349.

120 *Id.* at 350.

121 *Id.*

122 *Id.* (citations omitted).

a school population with a documented immunization level of 100%."[123]

b. Actual and Perceived Outbreaks in Unvaccinated Populations

Measles outbreaks have also occurred among the unvaccinated. A recent example happened in 2013 in a largely intentionally unvaccinated Hasidic community in Brooklyn, New York, when a teenager returned from abroad with subclinical measles.[124] Fifty-eight members of the Orthodox Jewish community became infected, the largest outbreak in the United States since 1996.[125] No one died, and no one outside the religious community became infected, but many of those who became ill had in fact been vaccinated.[126]

Sometimes, public health officials and others have blamed disease outbreaks on vaccine critics. Some have blamed Dr. Andrew Wakefield for measles outbreaks; in February 1998, he suggested that there might be a causal link between the MMR vaccine, gastrointestinal disease, and autism.[127] Having observed a new syndrome of gastrointestinal disease and autism in some children after vaccination with the MMR, he publicly recommended that parents consider using the single measles vaccine rather than the combination vaccine.[128] At the time he made the recommendation, a single measles vaccine was available. A few months later, the United Kingdom government took the single measles vaccine off the market.

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123 *Id.*

124 Ctrs. for Disease Control & Prevention, *Notes from the Field: Measles Outbreak Among Members of a Religious Community – Brooklyn, New York, March–June 2013*, 62 MORBIDITY & MORTALITY WKLY. REP. 752, 752 (2013) http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6236a5.htm; *see also* Renee Ghert-Zand, *Measles Vaccine Developer Warns Jewish Anti-Vaxxers*, TIMES OF ISRAEL (Dec. 11, 2013), http://www.timesofisrael.com/measles-vaccine-developer-warns-jewish-anti-vaxxers/.

125 *Id.*

126 *Id.*

127 A.J. Wakefield et al., *Ileal-Lymphoid-Nodular Hyperplasia, Non-Specific Colitis, and Pervasive Developmental Disorder in Children*, 351 LANCET 637 (1998), *retracted*, Feb. 2, 2010, for reasons related to patient referrals and ethics committee approvals, not scientific fraud, *available at* http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(97)11096-0/abstract (last visited Mar. 9, 2014). For a discussion of the article and subsequent retraction, see Mary Holland, *Who is Dr. Andrew Wakefield*, *in* VACCINE EPIDEMIC, *supra* note 13, at 311–19; David Lewis, *The Exoneration of Professor Walker-Smith*, *in* VACCINE EPIDEMIC, *supra* note 13, at 320–38.

128 F. Edward Yazbak, *Measles in the United Kingdom: The "Wakefield Factor,"* VACCINATION NEWS, http://www.vaccinationnews.com/measles-united-kingdom-wake field-factor (last visited Mar. 9, 2014).

Many in the media have argued vociferously that Dr. Wakefield's public statement caused measles outbreaks in the United Kingdom.[129]

There is little data to support such assertions. In a careful review of United Kingdom data on measles in the ten years preceding Dr. Wakefield's statement and the ten years after, Dr. Yazbak notes that there were 188,483 reported measles cases in the ten years before 1998, compared to 28,289 cases in the ten years after, an 85% decrease.[130] Comparing the five years before and after 1998 also showed a 67% decline, suggesting that there was little or no "Wakefield Factor" for reported measles cases.[131]

Dr. Yazbak notes that measles outbreaks were occurring at about the same time in other countries. He points out that in Saudi Arabia, where vaccination rates were between 95% and 98%, there were 4648 cases of measles in 2007 compared to 373 in 2005.[132] The rate of infection was considerably higher in Saudi Arabia than the United Kingdom, and despite media sensationalism, rates of measles infection in the United Kingdom have declined steadily overall.[133]

c. Potential Explanations for Outbreaks in Highly Vaccinated Populations

Some argue that outbreaks in highly vaccinated populations are possible because mass vaccination creates "quasi-sterile environment[s]."[134] "[C]onstant re-infection cycles have an essential role in building a stable herd immunity. In a population that is not constantly exposed to the infection . . . a serious risk of re-emerging infections may arise."[135] In other words, young children's infections play a critical role in continually boosting the entire population's immunity. On measles, Dr. Humphries observes:

> Susceptible age groups have essentially traded places since vaccinating. What used to happen with measles is that infants were protected by maternal antibodies, adults were protected by continued exposure, and infected children handled the disease normally and became immune for long periods of time. So, while

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129 *Id.*; *see also* Holland, *supra* note 127; Lewis, *supra* note 127.

130 Yazbak, *supra* note 128.

131 *Id.*

132 *Id.*

133 *Id.*

134 Humphries, *supra* note 103.

135 *Id.* (citing A.A. Navarini et al., *Long-Lasting Immunity by Early Infection of Maternal-Antibody-Protected Infants,* 40 EUR. J. IMMUNOLOGY 113 (2010)).

> measles vaccines have decreased the expression of measles infections, it has not necessarily improved the bigger picture.[136]

In sum, two doses of measles vaccine, even to one hundred percent of school populations, does not ensure societal protection from measles outbreaks. While there may be strong rationales for individuals to choose to vaccinate, there would appear to be a weak rationale to compel all children to take the vaccine if one hundred percent vaccination cannot reliably induce herd immunity.

2. The Case of Varicella Vaccination and Immunity

The U.S. varicella vaccination program provides perhaps an even more troubling example of imperfect vaccines and herd immunity. Drs. Goldman and King have surveyed this program since its inception in 1995.[137] They concluded, based on extensive data and analysis, that "rather than eliminating varicella in children as promised, routine vaccination against varicella has proven extremely costly and has created continual cycles of treatment and disease."[138]

a. The Rollout of the U.S. Varicella Program

The varicella-zoster virus (VZV) causes chickenpox or varicella as a primary infection.[139] A latency period follows the initial infection, after which the lifelong VZV can subsequently reactivate as herpes zoster (HZ), commonly known as shingles, a secondary infection. After only short-term safety and efficacy clinical trials, pharmaceutical company Merck licensed its varicella vaccine for children one year of age and older.[140] By 1996, the CDC's Advisory Committee on Immunization Practices had recommended it for universal use in children twelve to eighteen months.[141] As of

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136 *Id.*

137 G.S. Goldman & P.G. King, *Review of the United States Universal Varicella Vaccination Program: Herpes Zoster Incidence Rates, Cost-Effectiveness, and Vaccine Efficacy Based Primarily on the Antelope Valley Varicella Active Surveillance Project Data*, 31 VACCINE 1680 (2013).

138 *Id.* at 1691 (citations omitted).

139 *Id.* at 1680.

140 *Id.*

141 Ctrs. for Disease Control & Prevention, *Prevention of Varicella: Recommendations of the Advisory Committee on Immunization Practices (ACIP)*, 45 MORBIDITY & MORTALITY WKLY. REP. 1 (1996).

November 2012, all fifty states compelled varicella vaccination for preschool or schoolchildren.[142]

In cost-benefit analyses done before the start of the program, public health officials focused on chickenpox, largely disregarding possible effects on HZ epidemiology.[143] Lieu et al. modeled the cost-effectiveness of a routine varicella vaccination program, finding that vaccination was not cost effective.[144] Vaccine proponents could only justify the program by taking into account the cost of parents' absence from work due to sick children.[145]

Goldman worked as an analyst in one of the three CDC varicella surveillance sites from 1995 to 2005, so he closely observed the early rollout of the program.[146] He argues that the cost-effectiveness analysis from the beginning was based on four key but incorrect assumptions: (1) the vaccine's total cost of $40 per dose; (2) a single dose confers lifelong immunity; (3) vaccine effectiveness is between 85%–95% with negligible adverse effects; and (4) a universal varicella program has no negative impact on the incidence of HZ.[147] There were many at the prelicensure phase who questioned these optimistic assumptions, but the licensure process moved forward nonetheless.[148] After licensure, the cost of the vaccine doubled, and one dose failed to protect against disease breakthroughs.[149] An accurate preliminary cost-benefit analysis would have scratched the program.

In addition, though, the assumptions about adverse events and the influence on HZ were way off the mark. People have reported a wide range of adverse events from the varicella vaccine, which proponents had characterized as negligible. These have included problems with vision, the central nervous system, rashes, strokes, secondary transmission to others, pneumonia, breakthrough varicella, Stevens-Johnson syndrome, autoimmune disorders, and death.[150] A 2005

142 *Varicella Prevention Mandates*, IMMUNIZATION ACTION COALITION, http://www.immunize.org/laws/varicel.asp (last updated Nov. 1, 2012).

143 Goldman & King, *supra* note 137, at 1680.

144 *Id.* at 1689.

145 *Id.*

146 *Id.* at 1681.

147 *Id.* at 1685.

148 *Id.*

149 *Id.*

150 *Id.* at 1690.

study found adverse events in one-sixth of the subjects within forty-two days following vaccination.[151]

b. Herpes Zoster and Varicella Zoster Virus

Goldman observed herd effect when varicella case reports dropped precipitously after introduction of the vaccine, but saw that the surveillance sites were not capturing data on HZ prevalence. Starting in 2000, at Goldman's recommendation, his surveillance site started to track HZ incidences. After two years, HZ reports remained the same or increased in every adult category except those for adults older than seventy.[152] HZ had also increased among children who previously had chickenpox.[153] When Goldman sought to publish data about trends in HZ, his supervisor arranged for the Los Angeles County Legal Department to send him a "cease and desist" letter[154] to censor publication of the studies.[155] With a response from Goldman's lawyer, the Los Angeles Legal Department dropped its demand, and he published three articles on VZV and HZ.[156]

After widespread introduction of the vaccine in 2002, its effectiveness rate declined significantly, in large part because the boosting effects of naturally circulating varicella virus were gone.[157] Vaccine effectiveness declined rapidly and steeply, such that in several disease outbreaks, the reported vaccine effectiveness rates were between 44% and 56%.[158]

The costs and complications of varicella and HZ in adults are a different magnitude than those of chickenpox in children. Because the

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151 Gary S. Goldman, *The Case Against Universal Varicella Vaccination*, 25 INT'L J. TOXICOLOGY 313, 315–16 (2006).

152 Goldman & King, *supra* note 137, at 1681.

153 *Id.* at 1682.

154 *See Brief Summary of Chickenpox: A New Epidemic of Disease and Corruption*, DR. GOLDMAN ONLINE, http://www.drgoldmanonline.com/SummaryofChickenpoxVaccine.pdf (last visited Mar. 9, 2014) (discussing the "cease and desist" letter).

155 Goldman & King, *supra* note 137, at 1682.

156 G.S. Goldman, *Incidence of Herpes Zoster Among Children and Adolescents in a Community with Moderate Varicella Vaccination Coverage,* 21 VACCINE 4243 (2003); G.S. Goldman, *Using Capture-Recapture Methods to Assess Varicella Incidence in a Community Under Active Surveillance*, 21 VACCINE 4250 (2003); Gary S. Goldman, *Varicella Susceptibility and Incidence of Herpes Zoster Among Children and Adolescents in a Community Under Active Surveillance,* 21 VACCINE 4238 (2003).

157 Goldman, *The Case Against Universal Varicella Vaccination*, *supra* note 151, at 314.

158 Goldman & King*, supra* note 137, at 1689.

varicella vaccine's protection is short-lived, it shifted chickenpox to a more vulnerable adult population. Chickenpox in adults carries 20 times more risk of death and 10-15 times more risk of hospitalization compared to chickenpox in children.[159] A 2005 article reported that the universal varicella vaccination program caused an additional 14.6 million HZ cases, or a 42% increase among adults younger than fifty during a fifty-year period at a significant medical cost burden.[160]

The rationales for the varicella vaccination program were weak from the outset and weakened further with time. Rather than acknowledge problems and debate solutions when its weaknesses became clear, public health officials apparently made serious attempts to censor problematic information. Neither medical rationales (such as herd immunity) nor cost rationales (based on true cost-benefit analysis) seem to justify the vaccination program. Here, pursuing the objective of herd immunity created a far more costly public health problem than an elective program pursuing herd effect would have created. The varicella vaccine's apparent vaccine effectiveness rate was higher when the virus was in circulation. The marginal gains from the program have not outweighed their marginal costs. This recent example of a compulsory program to achieve herd immunity backfired; instead of herd immunity, the program created herd effect and a series of new, serious public health problems.

To be clear, vaccines have an important role in modern public health policy. Herd immunity as a theory, however, provides an irrational basis for guiding policy, leading to inefficiencies in the marketplace. Furthermore, policies based on herd immunity constrain the significant positive role that individual choice can play in furthering the public health.[161] Indeed, many of the failures noted above are a result of the modern insistence on compulsory vaccination as the only solution to the problem of infectious disease. Mandatory programs rely on unattainable herd immunity, which improperly balances the costs to individuals and the healthcare system with the marginal benefits from compulsory policies.

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159 *Id.* at 1691.

160 *Id.* at 1689.

161 In the language of administrative law, reliance on the herd immunity theory as the basis for vaccination policy must not be "arbitrary, capricious, [or] an abuse of discretion." 5 U.S.C. § 706(2)(A) (2012).

F. Eradication Versus Elimination: What Can Vaccination Policy Achieve?

Herd immunity theory rationalizes elimination of infection within a specific population, driving transmission of a disease to zero.[162] Eradication requires global coordination of disease-control programs to ensure that a pathogen is not able to reintroduce itself anywhere in the world.[163] As a result, achieving disease eradication or extinction involves huge investments of healthcare resources toward the goals of developing safe and effective vaccines, ensuring sufficient vaccination coverage to ensure herd immunity in all geographic regions, and efficiently tracking and isolating infections as they arise.[164]

Hinman and others have developed specific terminology to describe the possible objectives of vaccination policy, reproduced below[165]:

1. *Control*: Reduction of disease incidence, prevalence, morbidity, or mortality to a locally acceptable level as a result of deliberate efforts; continued intervention measures are required to maintain the reduction;
2. *Elimination of disease*: Reduction to zero of the incidence of a specified disease in a defined geographic area as a result of deliberate efforts; continued intervention measures are required;
3. *Elimination of infection*: Reduction to zero of the incidence of infection caused by a specific agent in a defined geographic area as a result of deliberate efforts; continued measures to prevent reestablishment of transmission are required;
4. *Eradication*: Permanent reduction to zero of the worldwide incidence of infection caused by a specific agent as a result of deliberate efforts; intervention measures are no longer needed;
5. *Extinction*: The specific infectious agent no longer exists in nature or the laboratory.

This hierarchy highlights the inherent geographic limitations of vaccination policy. Extinction and eradication involve *global* removal

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162 A. Hinman, *Eradication of Vaccine-Preventable Diseases*, 20 ANN. REV. PUB. HEALTH 211, 213 (1999).

163 *Id.*

164 *See generally* Fine, *History*, *supra* note 2 (detailing the efforts made throughout history toward global eradication of various diseases, including smallpox, influenza, polio, and pertussis).

165 Hinman, *supra* note 162.

of a specific pathogen from nature, whereas control and elimination, both of disease and of infection, primarily concern *local* efforts to mitigate disease.[166] Few diseases have ever been eradicated; extinction has never been achieved for any modern pathogen.[167]

Hinman identified the following factors favoring eradicability[168]:

1. A highly effective, safe, cheap, and stable vaccine;
2. Lifelong immunity after natural infection or immunization;
3. A short period of communicability;
4. A highly characteristic clinical disease syndrome;
5. An easy and reliable means of diagnosis;
6. The absence of a nonhuman or environmental reservoir of disease;
7. A genetically stable causative agent; and
8. Seasonality of occurrence.

These factors for effective disease eradication raise several issues for a "just" vaccination policy that we address in Part II below.

1. Limitations on U.S. Vaccination Policy

Can U.S. vaccination programs achieve control, elimination, or eradication of disease? Vaccine technology influences the theoretical capability to achieve any of these goals.[169] If the rate of vaccine failure exceeds the herd immunity threshold, society can *never* achieve elimination or eradication.[170] Therefore, disease control is likely the only feasible objective of vaccination programs when society possesses imperfect and potentially harmful vaccination tools.

If the harms of vaccination are high, increasing vaccination coverage imposes higher costs on society through adverse health effects.[171] When herd immunity is lacking, the marginal costs of mandates exceed their marginal benefits.[172] In the "just" vaccination framework, the results misallocate healthcare resources and fail to properly account for the individual's autonomy interest.[173]

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166 *Id.* at 213–14.

167 David H. Molyneux et al., *Disease Eradication, Elimination and Control: The Need for Accurate and Consistent Usage*, 20 TRENDS PARASITOLOGY 347, 347 (2004).

168 Hinman, *supra* note 162, at 214.

169 *See infra* Part II.D.4.

170 *See id.*

171 Steve P. Calandrillo, *Vanishing Vaccinations: Why Are So Many Americans Opting Out of Vaccinating Their Children?*, 37 U. MICH. J.L. REFORM 353, 388–93 (2004).

172 *See infra* Part II.

173 *See supra* Part I.

2. Communicability, Diagnosis, and the Problems of Contact Tracing

The capacity to control, eliminate, or eradicate a disease depends on the ability to identify cases of infection and proceed rapidly to isolate and treat them.[174] For a population lacking herd immunity, disease transmission among susceptible people is inevitable.[175] Control of infectious outbreaks then involves the process of contact tracing.[176] Contact tracing is the "backward" mapping of disease spread. Starting from any infected person or group of infected people, the problem is tracing the line of infectious contacts back to the first known "index" case, treating individuals along the chain to prevent further transmission.[177] Contact tracing is an iterative process that attempts to identify all contacts for each infected index case.[178]

If the rate of disease spread exceeds the rate at which scientists can trace cases, then the disease will spread faster than it is possible to control it, and contact tracing will fail.[179] The resulting "race to trace" involves a competition between infectious dynamics and the ability to identify and trace infectious individuals.[180] A short period of disease communicability facilitates elimination of a disease.[181] Conversely, a long period of communicability makes eradication or elimination virtually impossible.[182]

3. Disease Adaptability

To successfully eradicate infectious disease, the pathogen must be stable, and there must be no animal or other reservoir for the disease.[183] If a particular pathogen is not genetically stable, then

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174 *See* Ken T.D. Eames & Matt J. Keeling, *Contact Tracing and Disease Control*, 270 PROC. ROYAL SOC'Y LONDON 2565, 2569 (2003) (describing contact tracing as efficient means of identifying cases of infection).

175 *See* Fine, *Rough Guide*, *supra* note 2, at 913.

176 *See* Ken T.D. Eames, *Contact Tracing Strategies in Heterogeneous Populations*, 135 EPIDEMIOLOGY & INFECTION 443, 443 (2006) (discussing models of contact tracing); Eames & Keeling, *supra* note 174, at 2565 (discussing how contact tracing can efficiently be used to identify individuals with sexually transmitted diseases).

177 *See* Eames, *supra* note 176, at 444.

178 *See id.* at 446.

179 *See id.* at 448.

180 *See id.* at 450.

181 *See id.* at 448.

182 *See id.*

183 *See* David M. Morens & Anthony S. Fauci, *Emerging Infectious Diseases: Threats to Human Health and Global Stability*, 9 PLOS PATHOGENS 1, 2–3 (2013) (discussing

vaccines may not afford any protection against related strains.[184] A prime example is *Bordetella parapertussis*, which causes symptoms similar to *Bordetella pertussis*, the bacterium responsible for whooping cough.[185] Immunity to *B. pertussis* does not confer immunity against *B. parapertussis*, suggesting that the current *B. parapertussis* virus may have evolved in response to vaccination against *B. pertussis*.[186]

Diseases can also spread through animal and insect vectors.[187] For example, malaria infects humans through mosquitoes, so efforts to control malaria require insect-control programs.[188] More generally, when a pathogen can survive in nonhuman reservoirs, it can continue to infect the human population.[189] In many cases it may be impossible to identify which nonhuman repositories exist, making eradication unachievable.[190]

Disease eradication seems unattainable in the near future for all infectious childhood diseases, including measles and chickenpox.[191] Disease control seems to be the most viable goal. We consider next a framework within which to evaluate vaccination program objectives.

II
"JUST" VACCINATION POLICY AND PUBLIC HEALTH

Because herd immunity is not an appropriate objective of contemporary vaccination policy, the normative question arises as to what *should* be the correct goal. To address this issue, we adopt the Feudtner-Marcuse model of "just" vaccination policy, which identifies seven factors that must be appropriately weighted and balanced in designing vaccination programs.

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common reemergence of diseases with nonhuman reservoirs and pathogens that undergo rapid changes).

184 *See* Daniel N. Wolfe et al., *The O Antigen Enables* Bordetella Parapertussis *to Avoid* Bordetella Pertussis*-Induced Immunity*, 75 INFECTION & IMMUNITY 4972, 4978 (2007).

185 *See id.* at 4972.

186 *See* SUZANNE HUMPHRIES & ROMAN BYSTRIANYK, DISSOLVING ILLUSIONS: DISEASE, VACCINES, AND THE FORGOTTEN HISTORY 324–30 (2013) (discussing "original antigenic sin committed by vaccination"); *see also id.*

187 *See* Molyneux et al., *supra* note 167, at 351 (contemplating that insect vectors, such as mosquitoes, can infect humans with diseases).

188 *See id.* at 350 tbl.2.

189 *See id.* at 349.

190 *See id.*

191 *See infra* Part III.

Drs. Feudtner and Marcuse, who have worked extensively on U.S. vaccination programs, introduced the "just" vaccination policy framework more than a decade ago.[192] Overall, we agree with the elements of their framework; however, we draw substantially different conclusions concerning current U.S. vaccination policy.

A. Framework for "Just" Vaccination Policy

Feudtner and Marcuse's framework provides seven objectives for modern vaccination policy[193]:

1. Minimization of the deleterious effects of disease;
2. Minimization of the deleterious effects of vaccination;
3. Optimization of personal liberty to choose or to refuse vaccination;[194]
4. Maximization of an equitable distribution of benefits and burdens across members of society;
5. Promotion of the duty of families to protect children;
6. Promotion of the duty of society to protect current and future children; and
7. Prudent utilization of healthcare resources.[195]

The framework provides a reasonably comprehensive approach, although the model entirely discounts the possible benefits of contracting and overcoming disease naturally, thereby achieving long-lasting immunity. Below, we explore open questions about how to weigh the factors in "just" vaccination policy.[196]

Feudtner and Marcuse propose three types of programs: elective, recommended, and mandatory. An elective program uses public education to inform individuals about the availability, benefits, and risks of vaccination, but leaves the choice to immunize at the sole

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192 *See* Chris Feudtner & Edgar K. Marcuse, *Ethics and Immunization Policy: Promoting Dialogue to Sustain Consensus*, 107 PEDIATRICS 1158 (2001).

193 *Id.* at 1163 tbl.2.

194 Although Feudtner and Marcuse refer to the personal liberty objective in terms of "optimization," it is somewhat ambiguous whether this term is equivalent to *maximization* in the same sense as used in the other objectives or whether Feudtner and Marcuse intend this factor to carry less weight in the balancing analysis. This distinction in turn depends on the questions of how and whether to weigh these factors.

195 Feudtner & Marcuse, *supra* note 192, at 1163 tbl.2.

196 Indeed, Feudtner and Marcuse analyze their model with what amounts essentially to a tabulation of the various factors. Such an approach avoids the difficult question of weighing the policy considerations, but we also disagree with many of their conclusions concerning whether mandatory vaccination programs best achieve certain objectives.

discretion of parents in the case of childhood vaccination.[197] A recommended program, by contrast, uses public education and expert advice to induce uptake.[198] Whereas the elective program provides information to the vaccine consumer but offers no opinion, recommended programs aim to raise immunization rates.[199] Finally, mandatory programs leave almost no discretion to individuals on whether to vaccinate, with significant penalties for non-compliance.[200]

Feudtner and Marcuse argue that mandatory programs best minimize disease harms, maximize the equitable distribution of benefits and burdens within society, promote the societal duty to protect children, and use healthcare resources most prudently.[201] They acknowledge, though, that elective programs best minimize vaccine harms and optimize personal liberty.[202] Furthermore, they assert that recommended programs best promote a familial duty to protect children.[203] In the model, a simple tabulation of the seven factors suggests that mandatory programs are the most "just."[204] But to what extent does this conclusion follow? Agreeing with the model's objectives in principle, we consider each of their factors in turn.

1. Minimization of Disease Harm

A vaccination program in theory can reduce the risk of harm from infectious disease to zero if it completely eliminates the disease from circulation. The conclusion that a mandatory program best achieves this objective assumes that mandates ensure the highest level of uptake, thus reducing the rate at which disease can spread. Based on this theory, policymakers believe that minimizing individual choice necessarily reduces disease harms.[205] Imposing penalties for failure to vaccinate requires each individual to take on the burden of the collective, conceivably increasing the number of individuals willing to vaccinate.

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197 Feudtner & Marcuse, *supra* note 192, at 1161.

198 *Id.*

199 *Id.*

200 *Id.*

201 *Id.* at 1163 tbl.2.

202 *Id.* at 1163.

203 *Id.*

204 *Id.*

205 *Id.* at 1161.

This analysis fails, however, when it is possible to eliminate or sufficiently mitigate the spread of infection without requiring all individuals to vaccinate. If herd immunity is possible, then society can obtain the same benefits without imposing *unnecessary* vaccination costs. The herd immunity theory applies precisely to this situation because it predicts a unique threshold beyond which a disease can no longer sustain infection throughout the population. If enough people in society have immunity, and if either a recommended or an elective program is sufficient to achieve the herd immunity threshold, then *mandatory programs impose excessive costs with no marginal gains*. These costs include manufacturing, healthcare providers, administration, and the costs of potential injury and treatment.

2. Minimization of Vaccine Harm

Vaccine harm is zero when people do not vaccinate, making this objective the opposite of factor one's minimization of disease harms. Some balance between disease prevention and protection against vaccine harms is necessary. Mandatory programs do not necessarily reconcile these competing objectives, given the temporary protection of vaccine-induced immunity and the uncertainty about potential vaccine harms. Conversely, choosing a purely elective program may or may not reach the herd immunity threshold and sufficiently prevent disease in the broader society. Nevertheless, as Feudtner and Marcuse acknowledge, an elective program best minimizes vaccine-related harms.[206]

3. Maximization of an Equitable Distribution of Benefits and Harms

In the absence of vaccines, all people share the expected risks of disease, but they do not share them equally.[207] People of different ages and health statuses have differing levels of natural immunity.[208] Natural immunity implies that, with age, more and more people have acquired the disease, recovered from it, and subsequently become immune.[209] This is because: (1) a longer lifetime implies a greater chance of having already encountered the disease, and (2) naturally-

206 *Id.* at 1163 tbl.2.

207 *See, e.g.*, Reluga et al., *supra* note 98, at 2711–19.

208 *Id.* at 2718.

209 *See id.* at 2718–19.

acquired immunity among older individuals makes it more difficult for the disease to sustain itself among that group.[210] Thus, the result is that children are ordinarily at greater risk of infection than healthy adults.[211]

Vaccines create competing risks between infection and injury. On the one hand, requiring all children to vaccinate ensures that all children face the risks of both vaccination and disease. But such a program may not be preferable, however, if only a small portion of the population is particularly susceptible. Requiring vaccination of non-susceptible individuals forces them to accept risks without benefits, a scenario that raises the specter of constitutional equal protection violations under the Fourteenth Amendment.[212]

4. Optimization of Personal Liberty

Elective vaccination programs maximize individual choice, protecting the autonomy interest in bodily integrity.[213] How much weight should we give to this? Feudtner and Marcuse give individual liberty little or no deference, nor do other proponents of mandatory vaccination.[214]

Several commentators have recently proposed tort-based negligence liability for individuals who choose not to vaccinate and transmit disease.[215] They argue that the tort system would then force unvaccinated individuals to accept responsibility for their choice.[216] Such a proposal is another form of a mandatory program with enforcement through civil liability. Individuals then would discount the possible risks of their actions by the "detection" probability of

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210 *See id.* at 2712.

211 However, this observation is not universally true. One prominent example is rubella, which can have severe health complications on unborn children when acquired by a pregnant mother. In this case, the most severe health costs may be associated with the older subpopulation of pregnant women, which may alter the choice of a vaccination program. *See generally id.* at 2711–21.

212 *See* Holland, *supra* note 63, at 42–59, 85.

213 Feudtner & Marcuse, *supra* note 192, at 1163 tbl.2.

214 *See generally* Gregory A. Poland & Robert M. Jacobson, *The Clinician's Guide to the Anti-Vaccinationists' Galaxy*, 73 HUMAN IMMUNOLOGY 859 (2012); Susanne Sheehy & Joel Meyer, *Should Participation in Vaccine Clinical Trials be Mandated?*, 14 VIRTUAL MENTOR 35 (2012) (suggesting that the government should enforce a duty for all citizens to participate in clinical trials).

215 *See generally* Rebecca Rodal & Kumanan Wilson, *Could Parents Be Held Liable for Not Immunizing Their Children?*, 4 MCGILL J.L. & HEALTH 39 (2010); Diekema, *supra* note 7.

216 *See* Diekema, *supra* note 7, at 94.

facing a lawsuit.[217] Despite valuation problems, Feudtner and Marcuse acknowledge that elective vaccination programs best maximize liberty for parents to choose on their children's behalf.[218]

5. Promotion of a Familial Duty to Protect Children

Feudtner and Marcuse identify the familial duty to protect children as the sole objective that a recommended program best fulfills, arguing that medical professionals can best help families protect children.[219] Parents concerned about the potential harms of vaccines are often in direct conflict with their physicians, some of whom refuse to accept and retain children in their practices who fail to comply with vaccination recommendations.[220] Unfortunately, physicians who refuse to see noncompliant families may leave them without healthcare.[221] A recommended program may serve the interests of protecting children while preserving the right to informed consent for the parent, but both physician and patient are on uncertain ground.[222]

By contrast, a mandatory program gives parents no discretion to act in their own children's best interests, a situation that drives a wedge between parents and physicians.[223] This could result in a "black market" of vaccination records, providing false information, and inhibiting the capacity of state, local, and federal agencies to track and contain the spread of disease in the event of an epidemic. Just as in the cases of abortion, medical use of marijuana, and other medical prohibitions, some families simply will not comply with state public health laws as a matter of conscience.

6. Promotion of a Societal Duty to Protect Children

Feudtner and Marcuse conclude that mandatory vaccination programs, rather than recommended ones, best promote society's duty to protect children.[224] Some view mandatory programs as the best

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217 *See* Rodal & Wilson, *supra* note 215, at 63.

218 Feudtner & Marcuse, *supra* note 192, at 1162.

219 *Id.* at 1163.

220 *See* Douglas S. Diekema, *Improving Childhood Vaccination Rates*, 366 NEW ENG. J. MED. 391, 393 (2012) (noting that asking patients to seek other healthcare options is counterproductive).

221 *See id.*

222 Feudtner & Marcuse, *supra* note 192, at 1163.

223 *Id.* at 1161.

224 Feudtner & Marcuse, *supra* note 192, at 1163.

way for the state to exercise appropriate paternalism and prevent children from contracting disease. The reason for the discrepancy between society's duty and the familial one is the recognition of an *implied duty of care* between all members of society and children, not just a recognition of the state's duty to the child.[225] The legal foundation for this implied duty is suspect, because there is no clear analog in common law criminal or tort systems for a duty to rescue, even when a person can do so at small or no cost to herself.[226] If the common law is unwilling to impose liability on individuals toward strangers, Feudtner and Marcuse may be wrong as a matter of law to suggest that a mandatory program may impose a duty on all members of society to protect children.

There is a distinction between a duty to rescue and an implied duty to vaccinate. Children have a higher risk of infection than healthy adults because of their age. If vaccine-induced harm carries a relatively small risk, then there may be a basis to impose such a duty on society as a whole. However, it still does not follow that mandatory vaccination is the optimal mechanism. Under the theory of herd immunity, society need not achieve complete vaccination coverage to mitigate the spread of infection.[227] If a recommended or elective program can contain disease, then it is likely superior to a mandatory one.

7. Prudent Utilization of Healthcare Resources

Thoughtful use of resources, unlike the six factors above, refers to *implementing* a particular program rather than to theoretical tensions between liberty and collective security.[228] At first, resource allocation may appear only incidental to a "just" vaccination program; on further examination, however, it is of primary importance in balancing society's healthcare interests.[229] This factor is foremost in the discussion of vaccination choice in Part III. Society should be willing to invest healthcare resources, including funding, infrastructure, and research, in those endeavors that are likely to achieve the greatest aggregate benefit at the lowest aggregate cost.[230] Although Feudtner

225 *See id.* at 1160.

226 *See generally* Ernest J. Weinrib, *The Case for a Duty to Rescue*, 90 YALE L.J. 247 (1980) (evaluating the case for imposing a duty to rescue).

227 *See* Poland & Jacobson, *supra* note 214, at 862.

228 *See* Feudtner & Marcuse, *supra* note 192, at 1163.

229 *See id.* at 1160–61.

230 *See id.* at 1161.

and Marcuse suggest that a mandatory program best achieves the prudent use of resources,[231] this conclusion is doubtful. If the marginal benefit of a mandatory program does not exceed the marginal cost of implementation, then society can better invest its healthcare resources elsewhere.[232] This observation is particularly true for most childhood infectious diseases where herd immunity is per se unachievable because the vaccine failure rate exceeds the herd immunity threshold.[233] Undervaluing pragmatism risks exposing individuals to unnecessary harms for which there are no commensurate gains.[234] This factor is absolutely critical to ensuring efficiency in the vaccination market and therefore must play a central role in designing vaccination programs.

B. Weighing the Feudtner-Marcuse Factors

Feudtner and Marcuse's attempt to analyze the justice of vaccination policies is insightful.[235] While we do not reach the same conclusions they do, we find their measurements relevant and worthy of further examination. We may agree that a *uniform* "just" vaccination policy is impossible.[236] "Just" policies depend upon the specifics of the individual, the population, the disease, and the potential vaccine efficacy, injuries, and costs. There is no "one-size-fits-all" solution, although that seems to be the goal of most mandatory programs.

We argue that the original model undervalues considerations of individual autonomy, misapplies the notion of a social duty to vaccinate, and critically fails to provide a pragmatic use of healthcare resources for infectious disease. We claim that the proper focus of programs cannot be eradication of disease "at all costs"; indeed, Feudtner and Marcuse acknowledge this limitation by advocating prudent allocation of healthcare resources.[237] Efficiency requires taking account not only of the costs of infection, but also of the costs

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231 *Id.* at 1163.

232 *See id.* at 1161.

233 *See supra* Part I.E.1.i. (discussing measles as an example for which herd immunity is likely unattainable given the rapid rate at which the disease spreads through a population and the relatively low vaccine efficacy).

234 *See* Feudtner & Marcuse, *supra* note 192, at 1161.

235 *See id.* at 1160.

236 *See id.* at 1162.

237 *Id.* at 1160.

of the "cure."[238] In striving for unattainable herd immunity, society pays a heavy price.[239]

We conclude that the appropriate and rational objective of modern vaccination programs should be to maximize herd effect to the extent that marginal gains in vaccination coverage are not outweighed by the marginal costs to the individual, the healthcare system, and society. This objective is fully consistent with contemporary regulatory policy[240] and properly balances individual choice, direct and indirect costs to healthcare, and the real benefit that vaccines provide in protecting individuals from infectious diseases.

III
A GAME THEORY ANALYSIS OF VACCINATION DECISIONS

Proponents of mandatory policies argue that failure to vaccinate breaches an implied duty to other members of society to protect the herd.[241] Under free rider assumptions, herd immunity cannot exist without government compulsion.[242] Game theory, however, provides a useful alternative framework for examining the severity of the free rider problem. The aim of game theory is to identify optimal strategies for people in which their gains depend on others' choices.[243] Using game theory, Chris Bauch and David Earn have attempted to quantify the effect of risk perception on a person's willingness to vaccinate with perfectly efficacious vaccines.[244] Their analysis lays the foundation for market-based solutions to vaccination policy. In order to facilitate discussion, however, we will only generally review game theory and readers should refer to the original Bauch-Earn analysis for technical details.[245]

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238 *See id.* at 1163.

239 *See id.* at 1161.

240 *See* Exec. Order No. 12,866 § 1(b)(6) ("Each agency shall . . . adopt a regulation only upon a reasoned determination that the benefits of the intended regulation justify its costs."); *id.* at § 1(b)(11) ("Each agency shall tailor its regulations to impose the least burden on society, including individuals, businesses of differing sizes, and other entities . . . consistent with obtaining the regulatory objectives . . . .").

241 *See* Diekema, *supra* note 7, at 93 (suggesting that parents who do not vaccinate their children should be subject to civil negligence liability).

242 *See id.* at 91.

243 *See generally* KEN BINMORE, GAME THEORY: A VERY SHORT INTRODUCTION (2007) (discussing game theory and the way humans interact in certain cooperative scenarios).

244 *See generally* Chris T. Bauch & David J.D. Earn, *Vaccination and the Theory of Games*, 101 PROCS. NAT'L ACAD. SCI. 13391 (2004).

245 *See id.*

A. Game Theory of Vaccination Choice

The following scenario provides the framework for the Bauch-Earn "vaccination game."[246] Alice is a rational "player" in a large, homogeneous population trying to decide whether to vaccinate or to take her chances and get sick. To help her with the decision, she has in front of her a box of coins. Each coin is labeled according to the probability *P* that on any given toss it will come up heads; the coins are therefore biased, or rigged, to come up heads a specific fraction of the time. Alice can choose any coin in the box, and she will choose to vaccinate if, upon tossing the coin, it comes up heads; otherwise, she will not vaccinate. The "vaccination game" is therefore as follows: which coin should Alice choose in order to maximize her expected net health benefits, given that everyone else in the population is also playing this same game? In other words, how does Alice maximize her *individual* health benefits given the *collective* choices of others?

The "vaccination game" is a form of cost-benefit analysis, based on the information she gathers from others' "successes" in the game. Furthermore, Alice is not an automaton; her goal is not merely to decide *whether* to vaccinate but, more importantly, to pick *the best* coin, that is, the coin that will minimize her risks of both vaccination and infection. Specifically, if her coin comes up heads, then Alice will face the risks of potential vaccine injury and future booster shots to preserve immunity.[247] Conversely, if the coin lands tails, then she faces the potential but uncertain risk of infection. Alice will discount the risks of infection by the probability that she may get sick, which decreases as a function of increasing vaccination coverage.[248] At the herd immunity threshold, Alice's risks of not vaccinating are zero because she can "free ride" on herd immunity. With her biased coin

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246 *Id.* at 13394 (describing how game theory can be used to develop schemes regarding disease eradication; the coin toss game set forth here serves as an illustration of the vaccination game described by Bauch and Earn).

247 Note that this cost is an average cost over all possible "adverse" events of the vaccine, including the chance that nothing will happen. This average cost is always negative because the net benefit of the vaccine is prevention of the disease, which is not a *net* gain to the player if she does not have the disease when she starts the game.

248 Beyond the herd immunity threshold, by definition the disease cannot support itself in the population, and no individual will attain the disease regardless of vaccination status. However, the rate at which a disease is transmitted through a population will increase as the fraction of people choosing to vaccinate falls below the herd immunity threshold, meaning that the probability of any individual acquiring the disease must also increase as the vaccine coverage level decreases.

and a perceived estimate of these risks, Alice can then figure out her best strategy.

To understand how other players will affect Alice's strategy in the "vaccination game," assume that Bob is also playing the game with a biased coin that comes up heads with probability *Q*. If Alice and Bob have equal information about the risks of vaccination and infection,[249] then they will both obtain gains. However, they will discount the risks differently because they are playing with different coins.[250] Who then is doing *better* in the game by drawing a greater payoff, where the payoff is maximization of all benefits and minimization of all harms? If Bob is obtaining a greater payoff with coin *Q*, then there is no reason for Alice to play with coin *P*; the converse will be true if Alice obtains a better payoff. Furthermore, if Cindy can beat both Alice and Bob by using coin *O*, then both Alice and Bob will switch to Cindy's coin. It is through this type of information exchange based on the performance of other players that we can identify the *optimal strategy* for the vaccination game, a coin P^* with an expected payoff greater than with any other coin.[251]

B. Theoretical Optimum Vaccination Choice Strategy

There are two possible variants to the "vaccination game": (1) the vaccine is perfectly efficacious, as in the scenario considered by Bauch and Earn,[252] and (2) the vaccine is imperfect, as in the "real-world" case. The analysis of this latter scenario is original to this Article.

1. Using a Perfect Vaccine

Bauch and Earn prove that there are two possible optimal strategies for the vaccination game with the perfectly efficacious vaccine.[253] If

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249 Alice and Bob represent "average" members of the population in the sense that their estimates rely on the same information available to the public. The Bauch-Earn framework therefore faces several of the same limitations of the herd immunity theory discussed in Part II, but the results provide a useful systematic framework for evaluating the scope and direction of U.S. vaccination policy.

250 Note that all players in the vaccination game will discount the costs of vaccination by the probability that the coin comes up heads and will similarly discount the costs of infection by the probability that the coin lands tails.

251 *See* Bauch & Earn, *supra* note 244, at 13394 (Bauch and Earn prove that P^* is a stable Nash equilibrium for the vaccination game, meaning roughly that it is indeed better than any other coin that Alice could choose from her box.).

252 *Id.*

253 *Id.*

the perceived risks of vaccination are greater than the perceived risks of infection when *no one* is vaccinating,[254] then the optimal strategy is in fact never to vaccinate.[255] Indeed, this "tragedy of the commons" occurs *only* when the costs to the individual from vaccine uptake are extraordinarily high.

In the alternative case where the perceived vaccination risks are less than the worst-case infectious disease scenario, there is a *stable equilibrium point* P^* between zero and one that Bauch and Earn show is equal to the vaccination coverage θ^* necessary to exactly balance the risks of vaccination and infection.[256] To understand why this result is true, note that when the perceived vaccination risks are less than the worst-case infectious disease scenario, then there must exist a vaccination coverage level θ^* at which the expected risks of vaccination balance the risks of infection.[257] If society vaccinates below this level, then risks of infection will be greater than the risks of vaccination, and unvaccinated individuals will have an incentive to vaccinate.[258] Conversely, when society vaccinates above this level, the aggregate risks of vaccination exceed the aggregate harms of infection, and the incentive is to forego vaccination.[259] Therefore, deviations in either direction from the equilibrium coverage θ^* should return over time to this equilibrium point.[260] The question is then whether θ^* is at least equal to the herd immunity threshold θ_H, the answer to which is no in practically all cases. Indeed, herd immunity is only obtainable as an equilibrium point when there are no further risks of vaccination or infection.[261] Bauch and Earn verify this

254 If no one in the population is vaccinating, then the vaccine coverage is zero, and the expected costs of infection are maximal for the individual.

255 Bauch & Earn, *supra* note 244, at 13393.

256 *Id.* at 13394.

257 Recall that the probability of acquiring an infection decreases with increasing vaccine coverage from the "worst-case scenario" at zero coverage until it vanishes at the herd immunity threshold. Therefore, if the costs of vaccinating are below the "worst-case" level, these vaccination costs must meet with the expected infection costs at some vaccination level between zero and one.

258 Bauch & Earn, *supra* note 244, at 13393–94.

259 *Id.*

260 *Id.* at 13394.

261 The only point where the costs of infection are zero is at the herd immunity threshold, meaning that if the herd immunity threshold is an equilibrium point, the costs of vaccination must also vanish.

conclusion through simulations on model populations of susceptible, infectious, and recovered individuals.[262]

2. Using an Imperfect Vaccine

As in the real world, what if a vaccine provides imperfect immunity with an efficacy of probability η? The new setup for the vaccination game then has several important changes:

- If Alice's biased coin comes up heads, she faces the expected risks of the vaccine itself *and also* the expected risks of infection if the vaccine fails.
- The expected risks of infection exist even at the herd immunity threshold because the vaccine is imperfect, meaning that society must invest additional resources to eliminate the disease. If the vaccine efficacy η is less than the herd immunity threshold, then herd immunity is impossible to achieve.

If the perceived risks of vaccination are greater than the "worst-case scenario" when no one vaccinates, then the optimal strategy is not to vaccinate.[263] However, the vaccination risks need not be this high. Alice would still choose not to vaccinate even if the expected vaccination risks are below the "worst-case" infection risks, because she also expects to face some infection risks when she vaccinates with an imperfect vaccine. In fact, this analysis predicts this "do not vaccinate" result in all cases where the expected vaccination risks exceed the "worst-case" infection risks *discounted* by the probability of vaccine efficacy η.

C. Vaccination Choice Strategy in the "Real World"

How does the equilibrium vaccination coverage with the imperfect vaccine compare to the result for the game with the perfect vaccine? Intuitively, one might think that the equilibrium vaccination coverage with the imperfect vaccine should be less than the corresponding equilibrium coverage for the perfect vaccine. However, it turns out that this result is only true when the expected vaccination risks are high. When the expected vaccination risks are relatively low,[264] there

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262 Bauch & Earn, *supra* note 244, at 13392.

263 *Id.* at 13393.

264 The notion of "relatively low" can be made quantitative by comparing the infectious cost curves for the perfect and imperfect vaccines and by noting that there exists a "cross-over" point at a certain level of vaccine coverage due to the longer tail on the cost distribution for the imperfect vaccine.

is a greater risk of infection than risk of vaccine harm.[265] When a vaccine provides even incomplete protection to infection, the marginal benefit of using it may be perceived to be relatively large.[266]

So what are the results of elective vaccination programs? A follow-up article by Perisic and Bauch in 2009 suggests that they work.[267] As with the herd immunity analysis in Part I, the game theory model assumes population homogeneity.[268] Utilizing a network population model, in which individuals in the population only interact with neighbors with whom they share a connection, Perisic and Bauch show that altruism develops within tightly connected "neighborhoods" of individuals, decreasing the total spread of disease.[269] Within small neighborhoods, people will voluntarily vaccinate with a relatively safe vaccine.[270] As the neighborhood size increases, however, the infection is more likely to escape to infect the larger population, thereby approaching the disease dynamics in a homogeneous population.[271]

Reluga, Medlock, Poolman, and Galvani have also shown that age stratification can affect optimal strategy.[272] They show that because vaccination at a young age increases the average age of initial infection, it may be better for people to acquire natural immunity through infection at a young age rather than to risk greater harm from waning vaccine-induced immunity at a later age.[273] Game theory suggests that a market will best balance vaccine and infection risks and benefits.

Although not the conventional wisdom, evidence suggests that individual choice is not at odds with public health benefits from vaccines. To the extent that individuals contribute to herd effect both through vaccine-induced and natural immunity, "soft" regulation of the market can create the same or higher levels of public health more efficiently than compulsion. Indeed, Drs. Yang and Debold have recently demonstrated that for several diseases, there is no statistically

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265 Bauch & Earn, *supra* note 244, at 13393–94.

266 *Id.*

267 Ana Perisic & Chris T. Bauch, *Social Contact Networks and Disease Eradicability Under Voluntary Vaccination*, 5 PLOS COMPUTATIONAL BIOLOGY 1, 2 (2009).

268 *Id.*

269 *Id.*

270 *Id.*

271 *Id.*

272 Reluga et al., *supra* note 98.

273 *Id.* at 2718–19.

significant relationship, at the ninety-five percent confidence level, between measures of non-medical childhood disease exemptions and disease incidence rates in the fifty states.[274] Although several open issues of their study remain for the scientific literature to consider,[275] their empirically-based study results strongly reinforce the view that herd immunity should not be the *de facto* objective of vaccination policy.

A voluntary approach to maximizing herd effect ensures efficiency of the vaccination marketplace and preserves individual choice. Policymakers should reconsider the appropriate level of regulation of the vaccination market, explicitly balancing the costs of vaccination coverage with the expected benefits from a particular vaccination program.[276]

CONCLUSION AND RECOMMENDATIONS

Herd immunity is generally unattainable in the real world because key assumptions, like population homogeneity, do not exist and because current vaccine technology is imperfect. Vaccination programs should therefore aim to achieve herd effect, not herd immunity and concomitantly, disease control rather than eradication.

The free rider problem is a red herring. The Bauch-Earn game theory analysis and experience suggest that it does not drive individual decision making in the real world.[277] If safe and effective vaccines are available, most people will voluntarily accept the risks of vaccination rather than the potential risks of serious infectious disease.

Market forces will naturally lead to an equilibrium point for vaccination; mandates to increase coverage above the equilibrium point yield little or no marginal gains in the absence of obtainable herd immunity. Vaccination programs should therefore focus on "soft" regulation by investing in safer and more efficacious vaccine

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274 Yang & Debold, *supra* note 14, at 374–76.

275 *Id.* at 375.

276 *See* OFFICE OF MGMT. & BUDGET, *supra* note 8, at 9–10 (noting that an agency "should also perform a [benefit-cost analysis] for major health and safety rulemakings to the extent that valid monetary values can be assigned to the primary expected health and safety outcomes[,]" and that even "[i]f the non-quantified benefits and costs are likely to be important, [the agency] should recommend which of the non-quantified factors are of sufficient importance to justify consideration in the regulatory decision").

277 Bauch & Earn, *supra* note 244, at 13393–94.

technology, ensuring informed consent and opening lines of communication between parents, physicians, and policymakers.

These conclusions lead to the following specific recommendations for U.S. federal and state vaccine policy makers. First, federal and state vaccination programs should acknowledge that the goal of vaccine policy is to control disease, not eradicate it. Effective programs should focus on creating herd effect, not herd immunity, and take into account all the economic costs and health risks of vaccination.

Second, states should experiment with market-based approaches to vaccination, freeing resources otherwise devoted to compliance to other healthcare needs. States can change mandates to recommended or elective programs with relative ease and observe what consequences follow. States can start by removing those vaccination mandates that have inadequate public health rationales, such as the mandate for tetanus, which is non-contagious, and for hepatitis B, which is primarily sexually transmitted and a disease for which children are at low risk.

Third, states should ensure that vaccine consumers receive complete information to make rational choices. States can impose higher informational requirements than current federal law. Under federal law, parents are required to receive only minimal information on vaccination benefits and risks.[278] States should require that parents or guardians receive all the information they would otherwise obtain with any prescription drug.

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Parents can and should be able to determine their own children's best interests and voluntarily choose vaccines based on complete and accurate information. Prior, free, and informed consent is the hallmark of modern ethical medicine.[279] The "choice" between fulfilling a child's vaccination mandates or foregoing her education is

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[278] 42 U.S.C. § 300aa—26 (2012) (describing the Vaccine Information Statements that the CDC now produces); *see Vaccine Information Statements*, CTRS. FOR DISEASE CONTROL & PREVENTION, http://www.cdc.gov/vaccines/hcp/vis/index.html?s_cid=cs_000 (last updated June 11, 2014).

[279] *Universal Declaration on Bioethics and Human Rights*, UNITED NATIONS EDUC., SCIENTIFIC, AND CULTURAL ORG. (UNESCO), at art. 6 (2005), unesdoc.unesco.org /images/0014/001461/146180e.pdf ("Any preventive, diagnostic and therapeutic medical intervention is only to be carried out with the prior, free and informed consent of the person concerned, based on adequate information.").

scarcely a voluntary choice; it is a coerced choice at best. Because public health policies have not attained herd immunity for any childhood disease despite sixty years of compulsory policies and intensive effort, it seems both logical and wise to recalculate our policies. It is time to abandon the illusion of herd immunity through compulsion and to adopt realistic and respectful policies to achieve herd effect based on parents' informed choices.

Outbreaks in Vaccinated Populations

N Engl J Med. 1987 Mar 26;316(13):771-4. doi: 10.1056/NEJM198703263161303.

Measles outbreak in a fully immunized secondary-school population

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Abstract

An outbreak of measles occurred among adolescents in Corpus Christi, Texas, in the spring of 1985, even though vaccination requirements for school attendance had been thoroughly enforced. Serum samples from 1806 students at two secondary schools were obtained eight days after the onset of the first case. Only 4.1 percent of these students (74 of 1806) lacked detectable antibody to measles according to enzyme-linked immunosorbent assay, and more than 99 percent had records of vaccination with live measles vaccine. Stratified analysis showed that the number of doses of vaccine received was the most important predictor of antibody response. Ninety-five percent confidence intervals of seronegative rates were 0 to 3.3 percent for students who had received two prior doses of vaccine, as compared with 3.6 to 6.8 percent for students who had received only a single dose. After the survey, none of the 1732 seropositive students contracted measles. Fourteen of 74 seronegative students, all of whom had been vaccinated, contracted measles. In addition, three seronegative students seroconverted without experiencing any symptoms. We conclude that outbreaks of measles can occur in secondary schools, even when more than 99 percent of the students have been vaccinated and more than 95 percent are immune.

Can J Public Health. 1991 May-Jun;82(3):189-90.

[Major measles epidemic in the region of Quebec despite a 99% vaccine coverage]

[Article in French]

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Affiliations

PMID: 1884314

Abstract

The 1989 measles outbreak in the province of Quebec has been largely attributed to an incomplete vaccination coverage. In the Quebec City area (pop. 600,000) 1,363 confirmed cases of measles did occur. A case-control study conducted to evaluate risk factors for measles allowed us to estimate vaccination coverage. It was measured in classes where cases did occur during the outbreak. This population included 8,931 students aged 5 to 19 years old. The 563 cases and a random sample of two controls per case selected in the case's class were kept for analysis. The vaccination coverage among cases was at least 84.5%. Vaccination coverage for the total population was 99.0%. Incomplete vaccination coverage is not a valid explanation for the Quebec City measles outbreak.

Pediatrics. 2004 Mar;113(3 Pt 1):455-9. doi: 10.1542/peds.113.3.455.

Chickenpox outbreak in a highly vaccinated school population

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Abstract

Objective: We investigated a chickenpox outbreak that started in an Oregon elementary school in October 2001, after public schools began phasing in a varicella vaccination requirement for enrollment. We sought to determine the rate of varicella vaccination and effectiveness and risk factors for breakthrough disease.

Methods: A chickenpox case was defined as an acute maculopapulovesicular rash without other explanation occurring from October 30, 2001 through January 27, 2002 in a student without a prior history of chickenpox. We reviewed varicella vaccination records and history of prior chickenpox, and we calculated vaccine effectiveness. We evaluated the effects of age, gender, age at vaccination, and time since vaccination on risk of breakthrough disease (ie, chickenpox occurring >42 days after vaccination).

Results: Of 422 students, 218 (52%) had no prior chickenpox. Of these, 211 (97%) had been vaccinated before the outbreak. Twenty-one cases occurred in 9 of 16 classrooms. In these 9 classrooms, 18 of 152 (12%) vaccinated students developed chickenpox, compared with 3 of 7 (43%) unvaccinated students. Vaccine effectiveness was 72% (95% confidence interval: 3%-87%). Students vaccinated >5 years before the outbreak were 6.7 times (95% confidence interval: 2.2-22.9) as likely to develop breakthrough disease as those vaccinated </=5 years before the outbreak (15 of 65 [23%] vs 3 of 87 [3%]).

Conclusions: A chickenpox outbreak occurred in a school in which 97% of students without a prior history of chickenpox were vaccinated. Students vaccinated >5 years before the outbreak were at risk for breakthrough disease. Booster vaccination may deserve additional consideration.

Mumps Outbreaks in Vaccinated Populations: Are Available Mumps Vaccines Effective Enough to Prevent Outbreaks?

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Increased reports of mumps in vaccinated populations prompted a review of the performance of mumps vaccines. The effectiveness of prior vaccination with 1 dose of vaccine ranged from 72.8% to 91% for the Jeryl Lynn strain, from 54.4% to 93% for the Urabe strain, and from 0% to 33% for the Rubini strain. Vaccine effectiveness after 2 doses of mumps vaccine was reported in 3 outbreaks and ranged from 91% to 94.6%. There was evidence of waning immunity, which is a likely factor in mumps outbreaks, aggravated by possible antigenic differences between the vaccine strain and outbreak strains. Inadequate vaccine coverage or use of the Rubini vaccine strain accounted for the majority of outbreaks reviewed; however, some outbreaks could not be prevented, despite high vaccination coverage with 2 doses of the Jeryl Lynn vaccine strain. Our findings indicate the need for more-effective mumps vaccines and/or for review of current vaccination policies to prevent future outbreaks.

Mumps is an acute communicable disease characterized by fever, headache, and lethargy, followed by painful swelling of the salivary glands, typically the parotid. In the prevaccine era, mumps was a leading cause of viral meningitis and the most common cause of unilateral acquired sensorineural deafness in children [1]. Use of mumps vaccine in routine pediatric immunization schedules has significantly reduced the incidence of mumps, although outbreaks can occur even among highly vaccinated populations. In the United States, the incidence of mumps decreased from >100 cases per 100,000 population in most years in the prevaccine era (before 1967) to 10 cases per 100,000 population in 1977 [2, 3]. After the 1989 institution of a 2-dose measles, mumps, and rubella vaccine schedule, the number of reported mumps cases further decreased to 1 case per 100,000 population in 1992 and to 0.1 case per 100,000 population in 2001 [4] (figure 1). On the basis of the success of the mumps vaccination program, a national health objective to eliminate indigenous transmission of the virus by 2010 was instituted [5]. Although similar success in the control of mumps has been achieved in other countries through high vaccine coverage [6, 7], the recent resurgence of mumps in the United States, where outbreaks have occurred in the context of high 2-dose vaccination coverage [8–10], raised the question of whether available mumps vaccines are sufficiently effective to prevent outbreaks and achieve disease elimination. In this review, we summarize the data to date on outbreaks of mumps in vaccinated populations to evaluate the effectiveness of 1 and 2 doses of different mumps vaccine strains and aim to provide a balanced assessment of factors potentially impacting vaccine effectiveness.

METHODS

Published studies of mumps outbreaks among vaccinated populations were identified through a comprehensive search of the PubMed and EMBASE databases with use of the search term "mumps" in conjunction with "mumps vaccine" or "measles-mumps-rubella vaccine" and "epidemic" or "outbreak." Only articles about outbreak investigations with information on the proportion of cases that occurred among vaccinated persons or on vaccine effectiveness were selected for the analysis.

The following information was abstracted from the selected articles: year, place, setting (e.g., school), number of cases, percentages of persons who received 1 and 2 doses of vaccine,

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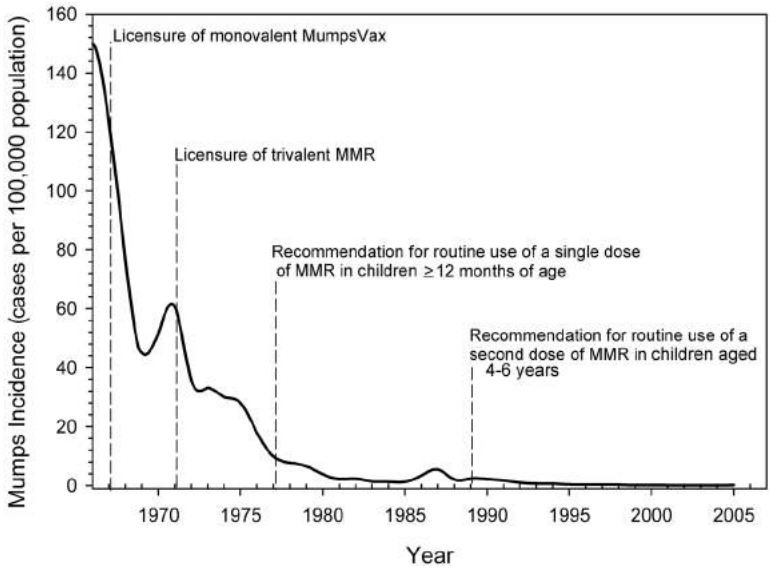

Figure 1. Mumps incidence in the United States over the past 40 years. Incidence rates adapted from the Centers for Disease Control and Prevention [3] and McNabb et al. [4]. MMR, measles-mumps-rubella vaccine.

vaccine effectiveness, type of vaccine used, genotype of circulating viruses, and vaccination coverage for the setting. When available, the percentage of vaccinated persons with documented number of doses was considered. In addition, information on time since vaccination was also collected to assess possible occurrence of waning immunity. Rates of primary vaccine failure were determined on the basis of published studies of mumps vaccine immunogenicity trials, which were identified in PubMed with use of the search terms "measles-mumps-rubella vaccine" or "mumps vaccine" in conjunction with "antibodies" or "immunogenicity." The search was limited to studies involving at least 25 initially seronegative children who were tested for neutralizing antibody 4–8 weeks after vaccination.

Studies that examined antigenic differences between mumps virus strains were identified and reviewed through a comprehensive search in PubMed with use of the search term "mumps" in conjunction with "neutralization," "variation," "genotype," or "antigenic." Only studies reporting neutralizing antibody titers against different mumps virus strains in serum samples from vaccinated persons were included.

For all literature searches, no language, article type, or date restriction was imposed. A manual search was also performed for references cited in relevant articles.

RESULTS

The search produced 47 articles on mumps outbreaks among vaccinated populations. Three articles described 2 outbreaks; therefore, information on 50 outbreaks is presented. Of these, 13 outbreaks occurred among populations vaccinated only with the Jeryl Lynn strain (table 1); 14 outbreaks occurred among populations vaccinated with multiple strains, including Jeryl Lynn, Urabe, Rubini, Toitsukabu, and Torii (table 2); and 21 outbreaks occurred among populations vaccinated with a vaccine strain that could not be identified (table 3). There were no evaluable reports of mumps outbreaks among recipients of other vaccines. The studies included outbreaks in the United States (11 outbreaks), Canada (5), Europe (29, occurring in the United Kingdom, Switzerland, Italy, Spain, Austria, Belgium, Sweden, Ireland, Czech Republic, and Moldova), and Asia (5, occurring in Singapore, Korea, and Japan). The outbreaks included in this review occurred during the past 31 years (1977–2008). Articles reporting 27 (54.0%) of the 50 outbreaks contained information on populations vaccinated with 2 doses; of these 27 outbreaks, 10 (37.0%) involved the Jeryl Lynn vaccine strain, 13 (48.1%) involved a vaccine strain that was not identified, and 4 (14.8%) involved multiple vaccine strains.

The percentage of total cases among individuals previously vaccinated with 1 dose of vaccine was highest (98.7%) in an outbreak in Kansas [15], where vaccination coverage in schools in the county where the outbreak occurred was 99.8%. In general, the proportion of cases among vaccinated patients tended to increase with higher vaccination coverage rates. In outbreaks involving patients vaccinated with different vaccine strains, the percentage of cases among vaccinated patients was highest among those vaccinated with the Rubini strain and lowest among those vaccinated with Urabe strain (table 2). The percentage of cases among individuals vaccinated with 2 doses was generally lower; however, in 1 investigation of this outbreak at a large university in Kansas, the percentage of cases among patients vaccinated with 2 doses was 99.3% (when counting

Table 1. Characteristics of the outbreaks involving patients vaccinated with Jeryl Lynn strain vaccine.

Study (outbreak year)	Setting	No. of cases	No. of cases assessed	Proportion of patients who had received 2 doses of vaccine	Proportion of patients who had received 1 dose of vaccine	Vaccine effectiveness, %		Vaccination coverage,[a] %	Virus genotype isolated
						2 doses	1 dose		
Lewis et al. [11] (1977)	Ontario, Canada (school)	84	84	NA	9.5	NA	NR	20.5	NR
Sullivan et al. [12] (1981)	Ohio (middle school)	62	57	NA	54.4	NA	72.8[b]	81.7	NR
Chaiken et al. [13] (1983)	New Jersey (school)	63	24	NA	20.8	NA	91	68	NR
Wharton et al. [14] (1986)	Tennessee (high school)	332	194	NA	17	NA	78	48[c]	NR
Hersh et al. [15] (1988–1989)	Kansas (school, community)	269	79	1.3	98.7	NR[d]	83	99.8	NR
Cheek et al. [16] (1990)	Texas (high school)	54	54	1.9	96.3	NR	82	99.7	NR
Briss et al. [17] (1991)	Tennessee (high school)	68	68	5.9	92.6	NR	NR	97.6	NR
Whitman et al. [18] (1997–1998)	New York City (community)	119	111	62.2	29.7	NR[e]	81	⩾1 dose: 98; 2 doses: 62	NR
Schaffzin et al. [19] (2005)	New York (summer camp)	31	29	55.2	13.8	91.6	79.7	2 doses: 86.8; ⩾1 dose: 95.9	None
Boxall et al. [51] (2005–2006)	Czech Republic (community)	5998	5933	70.6	1.1	NR	NR	99.6 in a highly affected cohort[f]	NR
Schmid et al. [20] (2006)	Austria (community)	214	169	10.7	40.2	NR	NR	NR	NR
CDC [21] (2006)	Iowa (community)	1798	1252	70.6	19.6	NR	NR	NR	G
Cortese et al. [10] and Bitsko et al. [22] (2006)	Kansas (university)	174	140	99.3[g]	0.7	NR	NR	2 doses: ⩾95	G
Dayan et al. [9] (2006)	United States (community)	6584	3115[h]	62.5[g]	24.8	NR	NR	2 doses: 87[i]	G

NOTE. CDC, Centers for Disease Control and Prevention; NA, not applicable; NR, not reported.

[a] Vaccination coverage with 1 dose, unless otherwise specified.
[b] Vaccine effectiveness was 81.2% when persons with a history of mumps were excluded from the analysis.
[c] Among control subjects with provider or written parental record.
[d] Recipients of 2 doses of vaccine were at lower risk than were recipients of 1 dose.
[e] One-third greater effectiveness with 2 doses, compared with 1 dose.
[f] Vaccine coverage by no. of doses not available.
[g] Data are for persons who received ⩾2 doses.
[h] Only cases in patients from Iowa, Illinois, Kansas, Minnesota, Missouri, Nebraska, South Dakota, and Wisconsin.
[i] Does not include vaccination coverage among all cohorts of the outbreak.

Table 2. Characteristics of the outbreaks involving patients vaccinated with multiple-strain vaccines or with strains other than the Jeryl Lynn strain.

Study (outbreak year)	Setting	No. of cases	No. of cases assessed	Proportion of patients who had received 2 doses of vaccine (strain)	Proportion of patients who had received 1 dose of vaccine (strain)	Vaccine effectiveness, % (strain)		Vaccination coverage,[a] %	Virus genotype isolated
						2 doses	1 dose		
Paccaud et al. [23] (1991)	Switzerland (kindergarten)	9	9	NA	77.7 (R), 11.1 (U)	NA	22 (R), 93 (U)	96.2[b]	NR
Paccaud et al. [23] (1992–1993)	Switzerland (community)	112	112	NA	44.6 (R), 0.9 (other)	NA	33 (R), NR (other)	61.2	NR
Germann et al. [24] (1992–1993)	Switzerland (community)	102	102	NA	77.5 (R), 6.9 (JL)	NA	NR	61	NR
Oda et al. [25] (1994–1995)	Japan (school, community)	236	215	NA	6.5 (T/T)	NA	NR	21.6	NR
Mussini et al. [26] (1995)	Italy (community)	152	152	NA	78.9 (R), 3.3 (U), 3.9 (other)	NA	NR	79[b]	NR
Limon et al. [27] (1997)	Spain (community)	256	135	NA	31.1 (R), 7.4 (JL), 34.8 (other)	NA	59[c]	87[c]	NR
Goh et al. [28] (1998)	Singapore (community)	592	592	NA	24.3 (R), 7.1 (JL), 1.5 (U), 10.6 (other)	NA	0 (R)[d]	91.4[d]	NR
Pons et al. [29] (1998–1999)									
Study 1	Spain (community)	106	105	NA	83.8 (R), 11.4 (U, JL)	NA	<0 (R), 85 (U, JL)	95.9	NR
Study 2	Spain (community)	43	38	NA	94.7 (R)	NA	40 (R)	96.8	NR
Ong et al. [30] (1999)	Singapore (school)	235	235	NA	63.8 (R), 3.4 (JL), 2.1 (U), 15.7 (other)	NA	−55.3 (R), 80.7 (JL), 54.4 (U)	87.1	NR
Montes et al. [31] (2000)	Spain (school)	35	35	5.7[e]	77.1 (R), 8.6 (JL), 8.6 (other)	NR	NR	⩾1 dose: 100; 2 doses: 16.4	H
Barroso et al. [32] (2005)	Spain (community)	145	109	10.1 (R), 8.3 (JL), 30.3 (R, JL)	10.1 (R), 11.0 (JL)	NR	NR	2 doses: 48.9[f]	G1
Gerstel et al. [33] (2006)	Spain (community)	19	12	50 (R),[g] 33.3 (other)	0	NR	NR	NR	NR
Ortuondo et al. [34] (2006)	Spain (school, community)	63	52	1.9 (R), 5.8 (JL)	38.5 (R),[h] 53.8 (JL)[h]	NR	NR	NR	G1
Schlegel et al. [35] (NR)	Switzerland (community)	66	66	NA	80.3 (R), 7.6 (JL), 4.5 (U)	NA	−4 (R), 78 (JL), 87 (U)	95	NR

NOTE. JL, Jeryl Lynn vaccine; NA, not applicable; NR, not reported; R, Rubini vaccine; T/T, Toitsukabu or Torii strain; U, Urabe vaccine.

[a] Vaccination coverage with 1 dose, unless otherwise specified.

[b] For R, vaccination coverage was 36% in the 1991 study by Paccaud et al. [23] and 77% in the study by Mussini et al. [26].

[c] For an estimated 87% vaccination coverage.

[d] Calculated among 2304 children attending 5 childcare centers.

[e] The percentage of cases in vaccinated patients, by strain, was not available.

[f] Does not include vaccination coverage among all cohorts of the outbreak.

[g] R was administered as the second dose

[h] First dose of the 2-dose schedule.

Table 3. Characteristics of the outbreaks involving patients vaccinated with an unidentified vaccine strain.

Outbreak year	Setting	No. of cases	No. of cases assessed	Proportion of patients who had received 2 doses of vaccine	Proportion of patients who had received 1 dose of vaccine	Vaccine effectiveness, %		Vaccination coverage,[a] %	Virus genotype isolated
						2 doses	1 dose		
Arnedo et al. [36] (1987)	Spain (school, community)	104	95	NA	4.2	NA	89.6	0.0–77.9 among different age groups	NR
Guimbao et al. [37] (1989–1990)	Spain (community)	52	28	NA	32.1	NA	74.7	NR	NR
Brianti et al. [38] (1994–1995)	Italy (community)	466	466	NA	51.7	NA	41.1	64.5	NR
Vandermeulen et al. [39] (1995–1996)	Belgium (school)	105	105	NA	81	NA	61	91.8	NR
Visser et al. [40] (1996)	Spain (school, community)	897	897	NA	13	NA	97.7 and 69.4[b]	98.7 and 80.0[b,c]	NR
Lopez et al. [41] (1997)	Spain (school, community)	283	81	NR	90.1	NR	49	94.7	NR
Van Den Bosch et al. [42] (1998–1999)	England (community)	144	137	2.2	46.7	NR	NR	1–2 doses: 67–86	C
Savard et al. [43] (1998–1999)	Canada (school, community)	37	37	NR	59[d]	NR	NR	NR	NR
Lee et al. [44] (1999)	Korea (school)	736	344	39.5	53.2	NR	NR	NR	H
Reaney et al. [45] (1999–2000)	Northern Ireland (community)	729	316	0.9	58.2	NR	NR	>94[c]	NR
Pugh et al. [46] (2000)	England (community)	200	200	18.5	49.5	NR	NR	87.1[c]	NR
Sartorius et al. [6] (2004)	Sweden (community)	42	23	NR	NR	91	65	>90[c]	NR
Mackenzie et al. [47] (2004)	Scotland (school)	50	20	10	45	NR	NR	NR	G2
Cohen et al. [48] (2004–2005)	England (community)	312	312	31.1	16.7	94.6	87.8	1 dose: ⩾59.4; 2 doses: ⩾55.8	NR
CDC [49] (2004)	England and Wales (community)	16367	NR	3.3	30.1	NR	NR	1 dose: 82;[c] 2 doses: 75[c]	NR
Watson-Creed et al. [50] (2005)									
Study 1	Canada (school)	13	13	69.2	30.8	NR	NR	NR	G
Study 2	Canada (university)	19	19	5.3	94.7	NR	NR	NR	G
Park et al. [52] (2006)	Korea (school)	15	15	20	73.3	NR	NR	⩾1 dose: 95.1; 2 doses: 12.2	I
Castilla et al. [53] (2006–2007)	Spain (community)	>1300	143	78.3	NR	NR	NR	2 doses: >90[c]	NR
NACI [54] (2007)	Canada (community)	555	284	9	75	NR	NR	NR	G
Bernard et al. [55] (2007–2008)	Moldova (community)	13853	9223	3.8	92.2	NR	NR	⩾94 in highly affected cohorts[c]	G5

NOTE. CDC, Centers for Disease Control and Prevention; NA, not applicable; NACI, National Advisory Committee on Immunization; ND, not reported.

[a] Vaccination coverage with 1 dose, unless otherwise specified.

[b] Among persons aged 1–5 years and 5–10 years, respectively.

[c] Does not include vaccination coverage among all cohorts of the outbreak.

[d] Patients who had been vaccinated or were indicated as having been vaccinated.

only patients with complete records) or 96.4% (when counting patients with incomplete or missing immunization records as unvaccinated) [10].

Vaccine effectiveness was reported in 23 (46.0%) of the reviewed outbreaks. Vaccine effectiveness after 1 dose ranged from 72.8% to 91% for the Jeryl Lynn strain vaccine, from 54.4% to 93% for the Urabe strain vaccine, and from negative values to 33% for the Rubini strain vaccine (tables 1 and 2). Among the outbreaks in which the strain of the vaccine could not be identified, vaccine effectiveness of a single dose ranged from 41.1% to 97.7% (table 3). The effectiveness of 2 doses of vaccine was reported in 3 articles, and overall, the effectiveness of 2 doses was higher than that of 1 dose (91.6% vs. 79.7% [19], 94.6% vs. 87.8% [48], and 91% vs. 65% [6]), although no statistically significant differences were determined.

Although some studies did not find an association between time since vaccination and increased risk of disease [11, 12, 14, 16], other studies conducted in the United States [15, 17, 19] found persons vaccinated >5 years before the outbreak to be at higher risk of developing disease than persons vaccinated ⩽5 years before the outbreak, suggestive of waning immunity. In a recent study conducted at a university in Kansas during an outbreak in 2006, case patients were more likely than their roommates without mumps to have been last vaccinated with the second dose ⩾10 years earlier [10]. In addition, studies conducted in the United Kingdom and Europe revealed lower vaccine effectiveness in older cohorts and an increased risk of developing mumps with increased time after vaccination [39, 40, 48].

The genotype of the mumps viruses associated with the reviewed outbreaks was reported in 14 (28.0%) of the outbreaks. Genotype G was isolated in outbreaks in the United States [9, 22, 56], the United Kingdom [47], Canada [50, 54], Spain [34], and Moldova [55]. Genotype C was isolated in 1 outbreak in the United Kingdom [42]. Genotype H was isolated in Korea [44] and Spain [31], and genotype I was isolated in Korea [52].

The extent to which primary vaccine failure (i.e., no seroconversion after vaccination) may contribute to mumps outbreaks was assessed through a review of 30 different studies of neutralizing antibody responses in initially seronegative children after vaccination with the Jeryl Lynn, RIT-4385, Urabe, or L-Zagreb mumps virus strains. Data were not available to adequately assess virus neutralizing antibody activity after vaccination with the Rubini strain or other vaccine strains. Mean rates of primary vaccine failure did not significantly differ for the Jeryl Lynn, RIT-4385, Urabe, and L-Zagreb vaccine strains, ranging from 5.4% to 8.8% (table 4). Although neutralizing antibody responses after vaccination with the Rubini strain have not been adequately reported, ELISA-based data suggest much higher rates of primary vaccine failure. In studies reported by Schwarzer et al. [72, 73], 103 (62.0%) of 166 individuals did not experience seroconversion after Rubini vaccination; this result was similar to that obtained from a prospective sampling of vaccinated persons in 2 small towns in Cadiz, Spain, where 29 (59.2%) of 49 Rubini vaccine strain recipients were mumps virus antibody seronegative when assessed by ELISA 18–34 months after vaccination [74].

Table 4. Rates of primary mumps vaccine failure after a single dose of vaccine.

Vaccine	No. of studies	Reference(s)	Mean PVF rate, % (95% CI)	Overall PVF rate, % (95% CI)
L-Zagreb	1	[57]	8.8	5.2 (3.6–6.8)
Urabe-AM9	6	[58–62]	7.9 (3.0–12.7)	…
Jeryl Lynn	22	[59–61, 63–70]	5.4 (2.4–8.4)	…
RIT-4385	1	[71]	6.0	…

NOTE. The above data were derived from studies involving at least 25 initially seronegative children who were tested for neutralizing antibody 4–8 weeks after vaccination. All such studies were identified using the following search terms in PubMed: "measles-mumps-rubella vaccine" or "mumps vaccine" and "antibodies." PVF, primary vaccine failure.

A total of 8 publications were identified that reported neutralization of heterologous wild-type mumps viruses in recipients of different vaccines (Jeryl Lynn, Urabe, Hoshino, and Leningrad-3) [75–82]. In all of these studies, neutralization titers against the wild-type viruses were lower than those to the homologous vaccine virus. In a few instances, serum samples were capable of neutralizing the homologous vaccine virus but not the heterologous wild-type viruses, although this mostly occurred in serum samples from persons with low response to the vaccine.

DISCUSSION

In the outbreaks examined, the effectiveness of 1 dose of the Jeryl Lynn vaccine strain was similar to that of the Urabe vaccine strain and was lowest for the Rubini vaccine strain. These values were similar to those reported in other studies not included in our review (because they were not outbreak investigations), with a vaccine effectiveness ranging from 61.6% to 70% for the Jeryl Lynn strain, from 73.1% to 75.8% for the Urabe strain, and from 0% to 12.4% for the Rubini strain [83–85]. Reviewed articles indicated that the effectiveness of 2 doses of mumps vaccine is higher than that of 1 dose; these results are similar to those from a case-control study conducted in England that revealed vaccine effectiveness of 69% for 1 dose and 88% for 2 doses [86].

Although vaccine effectiveness during outbreaks was lower than that reported during controlled clinical trials, there is no doubt that mumps vaccines confer protection. Compared with attack rates of 31.8%–42.9% among unvaccinated individuals, attack rates among recipients of 1 dose and 2 doses of the Jeryl

Lynn vaccine strain were 4%–13.6% and 2.2%–3.6%, respectively [10, 12, 13, 15, 19].

Other than outbreaks linked to use of the poorly protective Rubini vaccine [23, 26, 27, 30, 85], the major factor in most of the outbreaks reviewed here appeared to be incomplete vaccine coverage. For example, nearly 70% of the 16,367 notified mumps cases in the United Kingdom in 2004 occurred in unvaccinated individuals [49] who had not been targeted by the vaccination program and remained susceptible because of low circulation of mumps caused by high levels of vaccination in younger cohorts. Similarly, a 2004 outbreak in Sweden—a country maintaining a 2-dose vaccine coverage rate of >90% for the past 20 years—occurred almost exclusively among unvaccinated individuals not targeted by the vaccination program [6]. Outbreaks in Canada in 2007 were mostly linked to use of only 1 of the 2 recommended doses of vaccine [87]; however, mumps outbreaks have also occurred among populations with high 2-dose coverage. For example, in 2006, a series of mumps outbreaks occurred in the United States, despite 2-dose vaccination coverage >95%, and in some investigations, >99% of patients had been vaccinated with 2 doses of vaccine [10]. Interestingly, the vaccine strain involved in those outbreaks, Jeryl Lynn, had been responsible for the near elimination of mumps in the United States until that time.

Although the causes of Rubini vaccine failures have not been firmly established, serological studies strongly suggest inadequate seroresponses to vaccination [72–74]. In contrast, robust antibody responses after vaccination with Jeryl Lynn and other vaccine strains have been measured, and primary vaccine failure is relatively uncommon. Furthermore, nearly all of the individuals who failed to produce measurable neutralizing antibody after the first dose of vaccine will experience seroconversion after a second dose [88, 89]; thus, primary vaccine failure in recipients of 2 doses of vaccine appears to be an unlikely cause of mumps outbreaks among vaccinees.

Although the high potential for transmission in densely packed environments (e.g., university campuses) was certainly a factor in recent large-scale outbreaks, our review suggests additional factors, including waning immunity in older vaccinated persons and antigenic variation among mumps viruses. Although a few studies included in our review did not find an association between time after vaccination and increased risk of disease, others revealed age-specific decreases in vaccine effectiveness (for both 1 and 2 doses) [48], increased risk of development of mumps with time after vaccination [10, 39], and higher attack rates with time since vaccination [15, 17, 19]. Furthermore, there are numerous studies documenting decreases in antimumps virus antibody levels with time since vaccination [78, 89–92] and, in some cases, complete loss of seropositivity, even in recipients of 2 doses of vaccine [89, 90, 93]. In 1 study, 28.9% of persons who received 1 dose of vaccine and 8.4% of persons who received 2 doses of vaccine were seronegative 18–20 and 6–9 years after vaccination, respectively [94]. During the mumps resurgence in the United States in 2006, most cases occurred in cohorts in which the most recent vaccination (second dose) was likely to have been administered ⩾10 years earlier [9]. Of note, attack rates are not expected to continue to increase in older cohorts, because older individuals are likely to have been repeatedly exposed to wild-type mumps viruses earlier in life, before the dramatic decreases in virus transmission that resulted from implementation of national childhood immunization programs. It is important to mention that a decrease in antibody titer or even an inability to detect antibody does not necessarily imply a loss of immunity. Functional antibody may exist at levels below assay detection limits, and cell-mediated immune responses, which may be protective, have been measured up to 21 years after vaccination, even in seronegative vaccinated persons [63, 95, 96].

Perhaps aggravating the effect of decreasing levels of antibody over time on mumps susceptibility is antigenic variation among mumps viruses. This was most clearly demonstrated in antibody cross-neutralization studies, in which antibody titers to heterologous mumps viruses were often considerably lower than corresponding titers to the homologous virus [75, 76, 78, 79, 81, 82, 97]. Of note, viruses isolated from recent mumps outbreaks differed phylogenically and, possibly, antigenically from the vaccine viruses used. For example, the Jeryl Lynn, RIT-4384, and Rubini vaccine strains are genotype A viruses, whereas wild-type viruses associated with outbreaks occurring in countries using these vaccines belong to genotype groups B, C, D, G, H, and I [31, 32, 52, 56, 98–101]. Likewise, the Urabe, Hoshino, and Torii vaccine strains are genotype B viruses, and viruses isolated during outbreaks in countries using these vaccines have been identified mostly as genotypes C, D, G, J, K, and L (although genotype B viruses have also been isolated) [80, 99, 101, 102]. In individuals responding to vaccination with only nominal levels of neutralizing antibody or in individuals for whom immune responses have waned with time after vaccination, this mismatch between the vaccine genotype and that of circulating mumps virus strains may facilitate immune escape. Of note, these genotype designations are based on sequence variation within the small-hydrophobic gene [103]. Although the small-hydrophobic gene does not play a role in protective immunity, sequence variation in the small-hydrophobic gene is reflective of the virus's overall genetic and antigenic variability, including the hemagglutinin-neuraminidase gene [75, 79, 102], which encodes the major cell-surface target of neutralizing antibody [104–106]. Despite clear evidence of decreasing vaccine antibody levels over time and of reduced vaccine antibody potency to heterologous virus strains, in the absence of a known protective level of neutralizing an-

tibody, whether such quantitative differences are clinically meaningful cannot be asserted.

Our review has several limitations. The data from the articles reviewed was collected and analyzed using different methodologies; therefore, the comparisons made between studies presented here should be interpreted with caution. In many articles, the type of vaccine used before the outbreak was not reported; therefore, conclusions regarding the vaccine strain could not be made. Vaccination coverage was not always available for all of the cohorts involved in the outbreak; therefore, the relationship between vaccination coverage and protection could not be assessed in all cases. When available, we considered the percentage of vaccinated persons with documented numbers of doses. Had we assumed that individuals with undocumented vaccination were unvaccinated, the percentage of vaccinated persons might have been lower.

The cause of mumps outbreaks among vaccinated populations remains unclear, but several potential contributing factors may be involved, as documented in this review. That outbreaks have recently occurred in populations with >95% 2-dose vaccine coverage strongly suggests that long-term prevention of mumps outbreaks with use of current vaccines and vaccination schedules may not be feasible. Mathematical modeling including populations highly vaccinated with 2 doses would be important in assessing different vaccination schedules. Additional research is needed to develop more immunogenic and effective mumps vaccines and/or to review current vaccination policies.

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Whooping cough outbreak on Long Island

By abc7NY

Wednesday, June 22, 2011

SMITHTOWN, N.Y.
Thirteen students in three schools in Smithtown have been confirmed with the contagious bacterial infection.

Health officials in Suffolk County said Tuesday they had alerted area pediatricians and had given advice to school officials on how to control the outbreak.

Pertussis causes an uncontrollable, violent cough lasting several weeks or even months. It may begin with cold-like symptoms or a dry cough that progress to episodes of severe coughing. It is spread from person to person.

Officials say all of the Smithtown students had been immunized, helping to reduce the severity of their illness.

The three schools with ill children are St. James Elementary School, Tackan Elementary School and Nesaquake Middle School.

Fordham University mumps outbreak jumps campuses

By abc7NY

Friday, February 21, 2014

NEW YORK

There are now 13 reported cases, 12 at the Rose Hill campus in the Bronx and one at the Lincoln Center campus in Manhattan.

The symptoms are similar to the flu, but the virus can also cause painful swelling.

All of the infected patients had the mumps vaccine, but doctors say it's not 100 percent effective.

Infected students have been either isolated or sent home.

- UHS saw 1 case in January, 4 cases on Feb. 18; 3 cases on Feb. 19 and 5 cases on Feb. 20.

- All the students with suspected mumps infections have either returned home or have been isolated from other residents during the infectious phase of the illness.

- All Fordham students are required to have full vaccinations before attending the University, including the vaccination for mumps, measles, and rubella (MMR).

- All of the students who were tentatively diagnosed with mumps had been vaccinated. Vaccinations do not offer 100 percent protection, however, vaccination is still strongly recommended.

- Typically mumps patients are contagious for two days prior to the outbreak of symptoms and five days after.

Mumps is a viral infection. The symptoms are:

- Fever

- Headache

- Muscle aches

- Tiredness

- Loss of appetite

- Swollen and tender salivary glands under the ears or jaw on one or both sides of the face (parotitis)

Mumps is spread from person to person through contact with respiratory secretions, e.g., saliva and sneeze droplets, from an infected person. Items used by an infected person, such as cups, utensils, etc., can also be contaminated with the virus and should not be shared.

NEWS HEALTH

Measles Outbreak Traced to Fully Vaccinated Patient for First Time

"Measles Mary" raises questions about how long vaccine-given immunity lasts

11 APR 2014 • BY NSIKAN AKPAN

Get the measles vaccine, and you won't get the measles—or give it to anyone else. Right? Well, not always. A person fully vaccinated against measles has contracted the disease and passed it on to others. The startling case study contradicts received wisdom about the vaccine and suggests that a recent swell of measles outbreaks in developed nations could mean more illnesses even among the vaccinated.

When it comes to the measles vaccine, two shots are better than one. Most people in the United States are initially vaccinated against the virus shortly after their first birthday and return for a booster shot as a toddler. Less than 1% of people who get both shots will contract the potentially lethal skin and respiratory infection. And even if a fully vaccinated person does become infected—a rare situation known as "vaccine failure"—they weren't thought to be contagious.

That's why a fully vaccinated 22-year-old theater employee in New York City who developed the measles in 2011 was released without hospitalization or quarantine. But like Typhoid Mary, this patient turned out to be unwittingly contagious. Ultimately, she transmitted the measles to four other people, according to a recent report in *Clinical Infectious Diseases* that tracked symptoms in the 88 people with whom "Measles Mary" interacted while she was sick. Surprisingly, two of the secondary patients had been fully vaccinated. And although the other two had no record of receiving the vaccine, they both showed signs of previous measles exposure that should have conferred immunity.

A closer look at the blood samples taken during her treatment revealed how the immune defenses of Measles Mary broke down. As a first line of defense against the measles and other microbes, humans rely on a natural buttress of IgM antibodies. Like a wooden shield, they offer some protection from microbial assaults but aren't impenetrable. The vaccine (or a case of the measles) prompts the body to supplement this primary buffer with a stronger armor of IgG antibodies, some of which are able to neutralize the measles virus so it can't invade cells or spread to other patients. This secondary immune response was presumed to last for decades.

By analyzing her blood, the researchers found that Measles Mary mounted an IgM defense, as if she had never been vaccinated. Her blood also contained a potent arsenal of IgG antibodies, but a closer look revealed that none of these IgG antibodies were actually capable of neutralizing the measles virus. It seemed that her vaccine-given immunity had waned.

Although public health officials have assumed that measles immunity lasts forever, the case of Measles Mary highlights the reality that "the actual duration [of immunity] following infection or vaccination is unclear," says Jennifer Rosen, who led the investigation as director of epidemiology and surveillance at the New York City Bureau of Immunization. The possibility of waning immunity is particularly worrisome as the virus surfaces in major U.S. hubs like Boston, Seattle, New York, and the Los Angeles area. Rosen doesn't believe this single case merits a change in vaccination strategy—for example, giving adults booster shots—but she says that more regular surveillance to assess the strength of people's measles immunity is warranted.

If it turns out that vaccinated people lose their immunity as they get older, that could leave them vulnerable to measles outbreaks seeded by unvaccinated people—which are increasingly common in the United States and other developed countries. Even a vaccine failure rate of 3% to 5% could devastate a high school with a few thousand students, says Robert Jacobson, director of clinical studies for the Mayo Clinic's Vaccine Research Group in Rochester, Minnesota, who wasn't involved with the study. Still, he says, "The most important 'vaccine failure' with measles happens when people refuse the vaccine in the first place."

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Influenza Outbreak in a Vaccinated Population — USS Ardent, February 2014

Theodore L. Aquino, DO[1], Gary T. Brice, PhD[2], Sherry Hayes, MPH[3], Christopher A. Myers, PhD[2], Jaqueline McDowell, MD[3], Brenda White, MSPH, MPH[2], Rebecca Garten, PhD[4], Daniel Johnston[5] (Author affiliations at end of text)

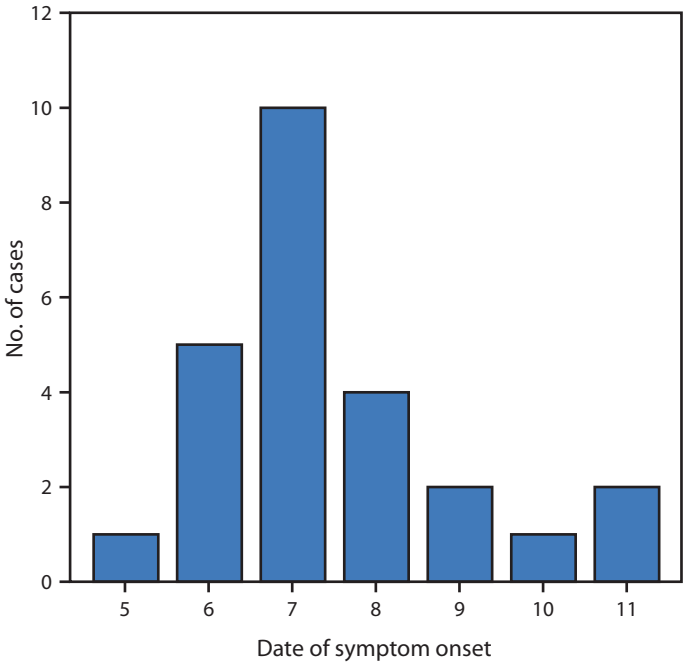
On February 10, 2014, the USS Ardent, a U.S. Navy minesweeper, was moored in San Diego, California, while conducting training. Over the course of 3 days, 25 of 102 crew members sought medical care because of influenza-like illness (ILI). Nasal swab specimens were collected from each patient, and initial rapid influenza testing indicated 16 cases of influenza A. Ultimately, polymerase chain reaction (PCR) testing conducted by the Naval Health Research Center determined that 20 specimens were influenza A, of which 18 were subtype H3N2. Two specimens could not be subtyped. The HA gene sequence of an outbreak isolate was 99% identical to strains circulating during the 2013–14 influenza season and antigenically similar to the H3N2 component of the 2013–14 influenza vaccine. At the time of the outbreak, 99% of the crew had received influenza vaccine. Through the duration of the outbreak, the minesweeper squadron medical officer collaborated with Navy Environmental and Preventive Medicine Unit Five, higher-level Navy authorities, and County of San Diego Public Health Services to implement the outbreak response, which included disseminating outbreak information to surrounding Navy units, disinfecting the ship, sending home infected crew members, identifying family members at high risk, and providing antiviral medications and guidance. No crew member had onset of symptoms >6 days after the first crew member became ill. This outbreak highlights the risk for an H3N2 influenza outbreak among vaccinated and otherwise healthy young persons.

ILI was defined as illness with two or more of the following symptoms: fever >100.4°F (>38.0°C), chills, sore throat, cough, shortness of breath, congestion, headache, body aches, and nausea. Twenty crew members reported sick on February 10, one on February 11 and four more on February 12. Symptom onset dates were February 5–11 (Figure). All ILI patients were interviewed and examined aboard ship by both an independent duty corpsman (i.e., shipboard medical provider) and a physician. Two nasal swab specimens were taken from each ILI patient by staff members from the Naval Health Research Center. Nasal swab specimens and influenza A and B rapid influenza tests were used for immediate influenza testing. The remaining nasal swab specimens were screened by the Naval Health Research Center for influenza A and B using the CDC PCR assay (*1*), and DNA sequencing of the HA1 portion of the hemagglutinin gene was performed as previously described (*2*). Data on demographics and symptomatology were collected using questionnaires and personal interviews.

All 25 crew members with ILI symptoms were otherwise healthy men aged 21–44 years. ILI cases occurred in all ranks, departments, job types, and work shifts. The ship had been in port since being transported from Bahrain to San Diego 2 months before the outbreak. No sailors reported any recent travel. Rapid influenza testing indicated 16 cases of influenza A and nine negative results. Nasal swab specimens from 20 of the 25 ILI patients were positive by PCR for influenza A, with 18 specimens confirmed as A (H3) and two as A (untyped). Influenza A virus was isolated from seven of 11 nasal swab specimens selected for viral culture. These seven specimens had HA1 protein sequences that were identical to each other and differed from the 2013–14 influenza A (H3N2) A/Texas/50/2012 vaccine strain by 5 amino acid substitutions (N128A, R142G, N145S, P198S, and V347K). Sequence analysis (*3*) of the HA1 portion of the hemagglutinin gene showed 99% homology to typical H3N2 strains circulating in the United States and worldwide during the 2013–14 northern hemisphere influenza season and were found to be antigenically similar to A/Texas/50/2012 (*4*). Ninety-nine of 102 USS Ardent crew members, 24 of the 25 with ILI symptoms, and 17 of 18 crew members with confirmed influenza A (H3N2) infection had received the 2013–14 influenza vaccine ≥3 months before the outbreak. Vaccinations had been administered at local naval health clinics and at a vaccination fair conducted by Naval Medical Center San Diego. Of the 25 crew members with ILI symptoms, 16 were vaccinated via intradermal injection, eight via intranasal mist, and one had not received vaccination.

Interviews revealed a possible source of the outbreak to be an Ardent crew member (patient A), aged 26 years, who had been evaluated at a local emergency room for fever and cough on January 30, 11 days before the first ILI case was diagnosed. A chest radiograph and computed tomographic scan were performed because of suspicion of pulmonary embolism; both were negative. The patient had been receiving treatment for pyelonephritis, and the clinical impression was that the cough was related to the pyelonephritis. No testing for influenza was performed, and the patient was discharged. Patient A's roommate in a shore apartment, also a USS Ardent sailor, experienced ILI symptoms on February 5. Because patient A's roommate was the first of the 25 crew members to experience

FIGURE. Number of cases (N = 25) of influenza-like illness, by date of symptom onset — USS Ardent, February 5–11, 2014



What is already known on this topic?

The single best way to prevent influenza infection is to receive vaccination every year. Some organizations have a mandatory vaccination policy. Despite this, influenza outbreaks can occur in highly vaccinated populations, especially in confined settings.

What is added by this report?

In February 2014, a total of 25 of the 102 crew members of a U.S. Navy minesweeper sought medical care because of influenza-like illness attributed to an influenza A (H3N2) virus antigenically similar to the H3N2 component of the 2013–14 vaccine. Among the crew members, 99% had received influenza vaccination, including 24 of 25 ill persons. Outbreak management included use of an antiviral medication, exclusion of the ill from the ship for 48 hours, disinfection, hand washing, and cough etiquette. No crew member had onset of symptoms >6 days after the first crew member had symptoms.

What are the implications for public health practice?

This influenza outbreak highlights the risk for an outbreak of influenza A (H3N2) in a cohort of vaccinated and otherwise healthy young persons.

ILI, and no other probable cause for the outbreak was found, it is possible that patient A actually had influenza. Since patient A did not board USS Ardent because he was ill, it is likely he infected his roommate, who then spread influenza to other USS Ardent crew members.

In an effort to reduce spread and impact of disease, oseltamivir (75 mg twice a day for 5 days) was prescribed to each ILI patient who reported that symptoms had developed within 48 hours of their medical visit, regardless of their vaccination status and rapid influenza testing results. In addition to antiviral medication, rapid identification of the influenza outbreak, and immediate isolation of affected persons (crew members with ILI symptoms were sent off ship to their homes for 48 hours), additional steps to control the outbreak were taken: thorough cleaning of spaces throughout the ship by the crew and use of the ship's public address system to instruct personnel to wash hands frequently, use hand sanitizer, cover their mouths when coughing, and report for medical evaluation if they were experiencing ILI symptoms. Similar announcements were made aboard three other minesweepers sharing the same pier as USS Ardent. Following a policy implemented by the independent duty corpsman, all patients experiencing ILI symptoms were required to wear an N95 filtering facepiece respirator while shipboard until 5 days after onset of symptoms. Cleaning of spaces was done by regularly disinfecting all commonly touched surfaces with disinfecting wipes and mopping all decks with an iodophor disinfectant diluted to 150 ppm of iodine. E-mails and reports regarding the outbreak, with an emphasis on rapidly identifying patients with ILI, were distributed to all ships on Naval Base San Diego and to high-level Navy officials and County of San Diego Public Health Services. No additional cases were identified after February 14. A total of 43 working days were lost by the 25 ILI patients.

Discussion

USS Ardent, an Avenger class minesweeper, is one of the smallest ships in the U.S. Navy. It has one shared space in which the entire crew eats meals. Work areas are spread throughout the ship, and there are nine sleeping spaces. Military populations, especially those living and working in confined settings, are susceptible to respiratory disease outbreaks (*5*). Shipboard personnel are at especially high risk because of constant close quarter exposure to a large number of crew members (*6*). Virtually all areas onboard ships are shared, and movement frequently requires touching handrails, door knobs, and other objects that can be contaminated with nasal secretions. In addition, ventilation systems can circulate infectious pathogens throughout a ship (*7*).

As the ship was moored in San Diego, the entire crew worked onboard during the day, and 25% remained onboard through each night. The roster of crew members who remained onboard at night rotated daily. There were 16 cases of confirmed influenza A (H3N2)infection in San Diego County (Brit H. Colanter, MPH, Health and Human Services Agency County of San Diego, personal communication, 2014) during the 6 weeks leading to the ship outbreak, making it likely that the virus was acquired from the local community.

Since the 1950s, a policy of mandatory annual vaccination against influenza for active duty personnel has been largely successful in limiting influenza epidemics in the military (*8*). The current U.S. Department of Defense influenza vaccination policy mandates that all uniformed personnel receive seasonal influenza vaccination, unless medically exempt, or face punishment under the Uniform Code of Military Justice. The policy specifically directs all Navy operational units to be at least 90% vaccinated. However, despite vaccination measures, influenza outbreaks can still occur in highly vaccinated military populations (*9,10*).

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LA Countywide Outbreak Of Whooping Cough Hits Exclusive Harvard-Westlake Hard

KCAL NEWS

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STUDIO CITY (CBSLA) – An exclusive private school has been hit with dozens cases of whooping cough, which has sickened a large number of teenagers across Los Angeles County.

Health officials say they are monitoring three large clusters of highly contagious whooping cough among 11- to 18-year-olds. The county Department of Health issued a health alert to pediatricians and other health care providers about the uptick in whooping cough last week.

Harvard-Westlake, which has campuses in Studio City and Beverly Crest, was hit particularly hard, with 30 students coming down with whooping cough since November, according to the Hollywood Reporter.

Of about 1,600 students attend Harvard-Westlake, where tuition is close to $40,000 a year, only 18 opted out of vaccinations for medical reasons. None of the 30 students who contracted whooping cough were not vaccinated.

School officials say they have done all they can to control the outbreak, including sending students home, sanitizing classrooms, and implementing a new protocol that requires students who stay home sick must be tested at a hospital for whooping cough before they can return to class.

Whooping cough, also known as pertussis, gets its name from the distinctive cough that sounds like a whoop. It is highly contagious and can be fatal for infants.

Parents are being urged to take students with flu-like symptoms to get them tested at a hospital before allowing them to return back to school.

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NECESSITY OF VACCINATION

Before the Vaccines

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DIPLOMA IN PATHOLOGY.—M. Y. Ali, B. C. Bhattacharyya, H. N. Harrison, T. Manners, T. K. Narayanan, Don S. P. S. V. J. Wijesekera.

DIPLOMA IN PUBLIC HEALTH.—S. L. Adesuyi, Lily Arratoon, L. H. Brearley, P. Changtrakul, E. Darabian, J. M. Deka, Lilian Kerr, Christine Kirby, W. G. Lewis, K. M. A. Malazie, Esther E. Simpson, M. F. X. Slattery, D. J. Stephen, G. C. Young.

DIPLOMA IN TROPICAL MEDICINE AND HYGIENE.—K. K. Appaji, G. Gaire, D. H. Melville-Swarries, Hnin Yee.

DIPLOMA IN CHILD HEALTH.—T. P. Linham, K. Tharmaraiah.

ROYAL COLLEGE OF PHYSICIANS OF IRELAND

At a meeting of the College held on September 29, 1958, Major-General G. T. L. Archer was admitted to the Fellowship of the College.

On November 7, 1958, T. E. Lear, S. Lourdenadin, J. S. McCormick, and G. B. Plunkett were admitted to the Membership.

At a meeting of the College held on December 5, 1958, with the President, Dr. P. T. O'Farrell, in the chair, Dr. J. J. Cockburn was admitted to the Licence and Membership of the College.

The following were admitted to the Licence in Medicine and Midwifery:

Y. M. Ali, R. G. R. Bobart, R. J. Christmas, Margaret M. Day, K. A. Docrat, Noreen M. Duffy, Mearl A. Fenwick, M. N. Fitzgibbon, H. Holmes, S. N. Jeawon, O. C. Parry-Jones, H. A. Marcelin, Louisa E. Moran, M. A. Q. Muhairez, J. McAleer, J. P. McCusker, R. H. Narozny, J. C. Okoye, P. R. Panniker, Janina Pisko-Dubienski, D. L. Scawn, M. E. Seedat, Y. Z. Shah, A. L. Tawiah.

Vital Statistics

MEASLES

REPORTS FROM GENERAL PRACTITIONERS

We are much indebted to the general practitioners whose names appear below for the following notes on the present outbreak of measles.

Dr. G. F. WATSON (Peaslake, Surrey) writes: Measles was introduced just before Christmas by a child from Petworth. He went to school, coughing, on December 15–17, 1958, and to the school party that afternoon, after which he developed his rash. In school and at the party he was in contact with 52 children, 25 of whom were said to be susceptible. Of these, 21 (84%) developed measles, 2 on December 27, 2 on the 28th, 6 on the 29th, 9 on the 30th, 3 on the 31st, 3 on January 1, 1959, and 2 on the 2nd. The shortest incubation was thus 12 days and the longest 16 until the rash appeared. Out of 27 other children who were said to have had measles or were doubtful, 6 (22%) developed it. One child's mother said he was 3 months old when previously affected, which suggests confusion wtih roseola infantum.

Treatment of Attack.—No drugs are given for either the fever or the cough; if pressed, I dispense mist. salin. *B.N.F.* as a placebo. Glutethimide 125 mg. may be given in the afternoon if the child is restless when the rash develops; 250 mg. in single or divided doses at bedtime ensures a good night's sleep in spite of coughing. I encourage a warm humid atmosphere in the room by various methods: some electric fires and most electric toasters allow an open pan of water to rest on top; an electric kettle blows off too much steam to be kept on for more than short periods. Parents, conscious of the need to darken the room and to forbid reading, may carry this to an unnecessary extreme, starting even before the rash appears. To save a mother some demands, the wireless is a boon to children in darkened rooms. They are allowed up when the rash fades from the abdomen—usually the fourth or fifth day—and may go outside on the next fine day. Apart from fruit to eat, solid food is avoided on the day the rash is appearing; fruit drinks or soups are all they appear to want.

Complications.—So far few complications have arisen. Four cases of otitis media occurred in the first 25 children, but only one had pain. No case of pneumonia has occurred, but one child had grossly abnormal signs in the chest for a few days after the fever subsided, uninfluenced by oral penicillin. One girl had a tear-duct infection and another an undue blepharitis. Of three adult males with the disease, two have been more severely affected than any of the children.

Treatment of Complications.—For otitis media with or without pain oral penicillin in therapeutic doses is given four times a day. Dacryocystitis was treated with an oral mixture of penicillin and sulphonamide.

Interesting Features.—The invasion phase of measles this year seems to be more drawn out than previously. Several children have been febrile for a week, one for nine days before the rash appeared. In two boys measles was tentatively excluded: the first developed no catarrhal signs in spite of his fever, and then mumps appeared; the second, who was coughing, had an evening temperature of 102° F. (38.9° C.) for three nights running, before signs of primary atypical pneumonia appeared in the right lung. Two children have had transient rashes on the trunk before the typical rash appeared on the face. One girl, who was given gamma globulin as an infant when her elder brother had measles, was on this occasion a house contact of a younger brother with a typical attack; in due course she developed a low fever and transient catarrh but no rash, at the same time that her younger sister developed a typical attack of measles. In a neighbouring practice a baby of 9 months developed fever and catarrh, but no rash, at the same time as two older children in the house developed typical attacks of measles. A girl of 2 years who has not had measles in the past failed to develop it from house contact with her father, although her younger sister had a typical attack. A girl of 8 was not infected at the school party, though she nursed the ailing victim on her knee, but later took the disease from her sisters, who were infected at the party.

LATE START

Dr. F. H. STAINES (Callington, Cornwall) writes: This practice had a large epidemic of measles from July to October, 1957 (overlapping with the Asian influenza), and a small epidemic in April, 1958, occurring in a village that was bypassed by the 1957 infection. The current epidemic has not yet reached here, and in this practice only one of the last five epidemics has started early in the New Year, the others all starting in spring or summer.

BED REST

Dr. R. E. HOPE SIMPSON (Cirencester, Glos) writes: We make no attempt to prevent the spread of measles, and would only use gamma globulin to mitigate the severity of the disease in the case of the exposure of a susceptible adult or child who is already severely debilitated. Bed rest, for seven days for moderate and severe cases and of five to six days in mild cases, seems to cut down the incidence of such complications as secondary bacterial otitis media and bronchopneumonia. We have not been impressed by the prophylactic or therapeutic use of antibiotics and

sulphonamides in the first week of the disease. As soon as the patient is out of bed we allow him out of doors almost regardless of the weather.

Otitis Media and Bronchopneumonia.—These conditions often appear so early, sometimes even before the rash, that in such cases one can only conclude that the responsible agent is the virus itself. Despite their initial alarming severity, they tend to resolve spontaneously, and treatment apart from first principles seems useless. When, on the other hand, otitis media or bronchopneumonia comes on after the subsidence of the initial symptoms of measles, it is probably due to a secondary bacterial invader, and we find antibiotics or sulphonamides useful if the severity of the complication demands them.

Staphylococcal Infections.—Styes and blepharitis commonly develop within six weeks of measles and can be dramatically severe. They often persist as a recurrent nuisance for months or even years. In the long view local applications are conspicuously unsuccessful, as are courses of antibiotics. Prolonged use of sulphonamides, on the other hand, often seems to stop the cycle of recurrences, and heartening results are achieved by the old-fashioned iron tonics or their vitamin-and-iron successors.

Experience bears out the expectation that children under 2 years old usually have mild attacks, and under 6 months often escape the disease altogether. These mild attacks in infancy do not appear to give a solid immunity, and such children are often subject to a second attack when they reach school age. One wonders if the same principle applies to attacks modified by gamma globulin.

Less Severe.—The present outbreak in this area is not distinguished by any peculiar characteristics except that it seems less severe than usual.

MILD AILMENT

DR. JOHN FRY (Beckenham, Kent) writes: The expected biennial epidemic of measles appeared in this region in early December, 1958, just in time to put many youngsters to bed over Christmas. To date there have been close on 150 cases in the practice, and the numbers are now steadily decreasing. Like previous epidemics, the primary cases have been chiefly in the 5- and 6-year-olds, with secondary cases in their younger siblings. No special features have been noted in this relatively mild epidemic. It has been mild because complications have occurred in only four children. One little girl aged 2 suffered from a lobular pneumonia, and three others developed acute otitis media following their measles. In the majority of children the whole episode has been well and truly over in a week, from the prodromal phase to the disappearance of the rash, and many mothers have remarked "how much good the attack has done their children," as they seem so much better after the measles.

A family doctor's approach to the management of measles is essentially a personal and individual matter, based on the personal experiences of the doctor and the individual character and background of the child and the family. In this practice measles is considered as a relatively mild and inevitable childhood ailment that is best encountered any time from 3 to 7 years of age. Over the past 10 years there have been few serious complications at any age, and all children have made complete recoveries. As a result of this reasoning no special attempts have been made at prevention even in young infants in whom the disease has not been found to be especially serious.

Treatment.—In the acute phase non-specific symptomatic measures such as aspirin and linctus have been the basis of treatment, and without the routine use of antibiotics or sulphonamides the rate of complications has not exceeded 3%. Even in the possibly susceptible "catarrhal children" with previous histories of recurrent ear and chest infections antibiotics have not been used in attempting to prevent complications; if and when these did occur they were treated on their merits. The few complications that did arise—namely, otitis media and chest infections—were either allowed to settle naturally on non-specific treatment, or, when severe enough, were treated with intramuscular injections of penicillin. In the present epidemic the one child with pneumonia and two of the children with acute otitis media were the only ones who required specific antibiotics. In all the others the disease followed a relatively uneventful course with complete and spontaneous resolution.

I would like to express my thanks to Dr. G. E. H. Callebaut, who has worked with me during this time.

NO PERMANENT DISABILITIES

DR. R. M. MCGREGOR (Hawick, Roxburghshire) writes: In Scotland measles is not a notifiable disease except in the case of certain ports. Information concerning incidence, therefore, is known only to the family doctor and to a lesser extent the school authorities. In this area since 1948 serious outbreaks have occurred in the autumn of 1950, in March and April of 1953, and in June and July of 1955. In the intervening periods, and since the last serious outbreak, sporadic cases have occurred without causing an epidemic. At present we enjoy a complete freedom from this disease, and it is hoped that the act of writing on the subject will not incur the penalty of a visitation.

Scanning the notes of the previous epidemics, it is evident that the 1955 episode was one of low virulence. Indeed, many of the cases were sufficiently mild as to make diagnosis difficult. The follow-up of all the epidemics reveals that the patients have not suffered any permanent disabilities. This could be due to the treatment given being satisfactory or to the excellent recuperative powers of a sturdy population.

It is conspicuous that the 5–15-years age group contained the vast majority of the cases. No effort was made to prevent the spread of the disease, except the ordinary precaution of not permitting juvenile visitors. Gamma globulin to thwart the onset of the disease was never used, since the few cases seen affecting the adults have always been severe. It is felt advisable to get the infection over in childhood and thus avoid this hazard in later life.

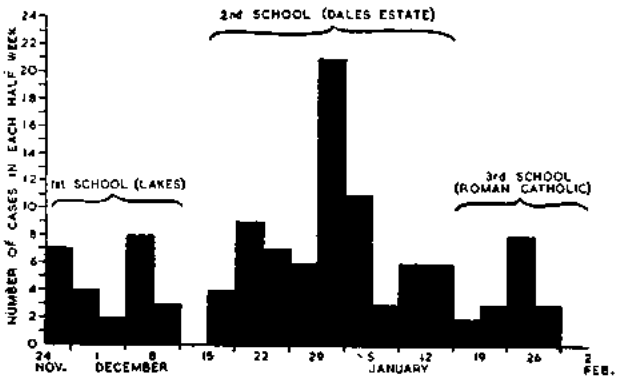
In these epidemics no serious complications were encountered. A troublesome cough for a few weeks after the infection was fairly frequent. In the 1955 episode only two cases of concomitant otitis media were seen, and in both cases it was a recrudescence of a previous attack. Contrariwise three of the cases had otitis media a few months before, and did not have a flare-up during the measles infection. In one case, as the rash of measles was fading, typical spots of chicken-pox were seen to develop. This superimposed infection did not prolong the convalescence.

The treatment given in all cases was sulphadimidine. In the older children it was dispensed in the form of tablets. In the younger children and in those that complained of difficulty in swallowing, the suspension was used. When the sulphadimidine was stopped, a sedative mixture was given to those who complained of a troublesome cough.

IMPORTANCE OF VISITS

DR. KEITH HODGKIN (Redcar, Yorks) writes: If the present measles epidemic of nearly 100 cases is compared with the two previous epidemics (250 cases), no obvious differences are observed. Several clinical observations were made which influence early diagnosis and treatment: (1) In *all* cases the classic triad of cough, Koplik's spots, and rash was found. (2) The cough began 1–5 (usually 3) days before the rash in over 80% of cases. (3) Koplik's spots were never observed more than 2 days before the onset of rash. Extensive Koplik infiltration appearing as a diffuse red granularity over the inside of the cheeks indicated the likelihood of a severe illness. (4) A stage of pulmonary catarrh as judged by moist adventitious sounds was observed in 54% of cases. This stage always appeared 1–3 days after the appearance of rash—i.e., just as the clinical condition was improving. These catarrhal sounds had disappeared in most cases four days later.

Complications.—Only three complications were observed: (1) In 6% of cases the illness followed its normal course but was unusually severe. These cases developed severe prostration and rapid respiration while the rash was at its



height. Extensive Koplik infiltration usually proceeded these developments. (2) In 3% of cases the stage of pulmonary catarrh progressed to a pneumonitis, or bronchopneumonia, with persistent fever and localizing pulmonary adventitious sounds. (3) In 6% of cases there was acute otitis media which appeared to be related to the cough.

Prophylaxis.—Isolation is a practical impossibility. Gamma globulin was used successfully to protect weakly susceptibles on three occasions.

Treatment and Prevention of Complications.—Adequate bed rest, fluids, soluble aspirin, and a cough linctus were the routine treatment in all cases. Penicillin V was used in 12% of cases when there was clinical evidence of one of the three complications mentioned above. In a further 12% penicillin was used as an "umbrella" to protect chesty children.

In the three epidemics there were no deaths and no admissions to hospital, and in no case did pulmonary complications persist long enough to show on an *x*-ray when the child was well. Pneumonia is most likely to supervene during the stage of pulmonary catarrh when the child is improving clinically. At this stage parents, especially those in overcrowded homes, are apt to allow children to get up or even to go out. The most important measure is to insist on absolute bed rest until fever and chest signs have disappeared. *Visiting on alternate days to ensure that parents carry this out is essential.*

It is suggested that the many good results claimed for different forms of therapy in measles may be artificial, and that it is the frequent visiting by the interested clinician and not the therapy which produces the good results.

Week Ending January 24

Infectious diseases were more prevalent in England and Wales during the week ending January 24. The rises in the numbers of notifications included 1,038 for measles, from 12,671 to 13,709, 355 for dysentery, from 839 to 1,194, 325 for scarlet fever, from 1,103 to 1,428, 150 for whooping-cough, from 508 to 658, 93 for food-poisoning, from 107 to 200, and 84 for acute pneumonia, from 527 to 611.

The largest rises in the incidence of measles were 201 in Middlesex, from 640 to 841 (Harrow M.B. 102, Ealing M.B. 88, Wembley M.B. 80), 153 in Bedfordshire, from 272 to 425 (Luton M.B. 180), 152 in Hampshire, from 178 to 330, 122 in Yorkshire West Riding, from 1,223 to 1,345 (Sheffield C.B. 214, Leeds C.B. 197, York C.B. 106), 116 in Essex, from 1,047 to 1,163 (Ilford M.B. 195, West Ham C.B. 148), and 104 in Warwickshire, from 402 to 506 (Birmingham C.B. 188, Coventry C.B. 140); the largest exceptions to an increased incidence were falls of 133 in Lincolnshire, from 452 to 319, and 77 in Staffordshire, from 441 to 364. No large fluctuations were recorded in the local returns of whooping-cough. The largest increases in the number of notifications of scarlet fever were 45 in Yorkshire West Riding, from 137 to 182, and 36 in Hertfordshire, from 47 to 83. 4 cases of diphtheria were notified, being 1 more than in the preceding week.

The notifications of acute poliomyelitis numbered 18 and were 7 fewer for paralytic and 1 fewer for non-paralytic cases than in the preceding week. The largest returns were 3 cases in Essex and in Cheshire.

Another 40 cases were notified from the outbreak of *Salmonella limete* paratyphoid fever in Nottingham C.B., where 28 cases were notified in the preceding week.

The largest rise in dysentery was 72 cases in Glamorganshire. The chief centres of infection were Glamorganshire 201 (Cardiff C.B. 84, Barry M.B. 62, Rhondda M.B. 37), Yorkshire West Riding 175 (Leeds C.B. 107, Bradford C.B. 24), Lancashire 109 (Liverpool C.B. 36, Eccles M.B. 16, Manchester C.B. 12), Lincolnshire 87 (Grimsby C.B. 38, Boston M.B. 15, Scunthorpe M.B. 10), London 79 (Wandsworth 18, Bermondsey 15), Essex 76 (Walthamstow M.B. 48), Warwickshire 66 (Coventry C.B. 55, Birmingham C.B. 10), Nottinghamshire 60 (Carlton U.D. 46), Yorkshire East Riding 43 (Kingston upon Hull C.B. 30), Hampshire 35 (Southampton C.B. 21), Staffordshire 35 (Litchfield R.D. 11, Stoke on Trent C.B. 10), Durham 25 (South Shields C.B. 12), Middlesex 24, and Northumberland 21 (Newcastle upon Tyne C.B. 21).

Venereal Diseases

In England and Wales during the quarter ending September 30, 1958, 1,032 new cases of syphilis were reported as attending the clinics, as compared with 1,240 the previous year. Of these, 170 were classified as primary, secondary, or latent in the first year of infection. 7 cases of congenital syphilis in children aged under 1 year were reported, and 96 cases in persons over that age. New cases of gonorrhoea (with corresponding 1957 figures in parentheses) numbered 7,986 (7,155), of chancroid 65 (66), and of non-gonococcal urethritis (males only) 5,197 (4,408).—*Monthly Bulletin of the Ministry of Health*, January, 1959.

Influenza

In the week ending January 24, 55 deaths from influenza were reported in England and Wales. This total was 22 more than in the previous week, but it is only a quarter of the total in the corresponding week last year, when Asian influenza was epidemic. Pneumonia notifications remain low for the time of year (see graph). In the week ending January 24 there were 875 deaths from pneumonia, compared with 992 in the corresponding week last year. Influenza-like illness has been reported in a few scattered districts, and serological evidence of the Asian strain has been obtained in some cases. In Birmingham for about three weeks there has been a sustained demand for hospital beds for infants with acute respiratory disease.

Industrial Accidents and Diseases

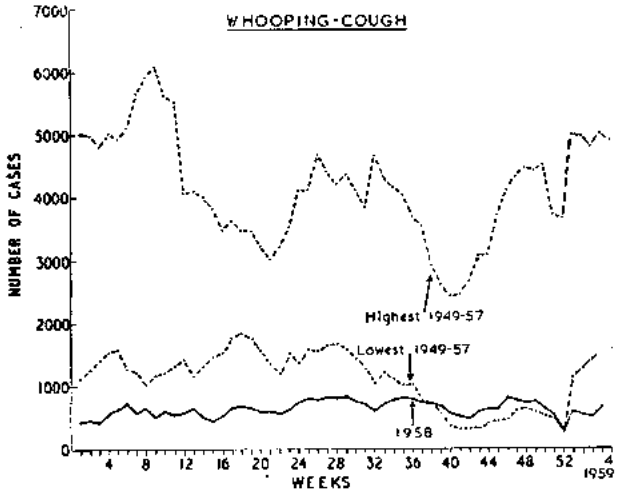
A total of 1,183 workpeople died from notifiable accidents in the course of their employment in Great Britain during 1958, compared with 1,272 in 1957. The number of cases of industrial diseases reported was 469, of which 17 were fatal; the numbers for 1957 were 518 and 15. The 1958 deaths were as follows: epitheliomatous ulceration due to mineral oil 11 and due to pitch and tar 5; toxic jaundice 1.

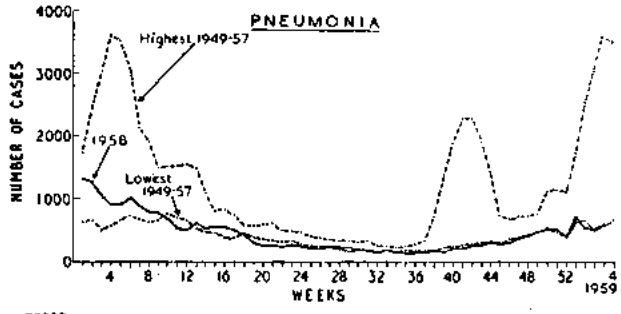
The number of workpeople (other than seamen) in the United Kingdom whose deaths from accidents in the course of their employment were reported in December, 1958, was 101, compared with 118 in the previous month and 119 in December, 1957.

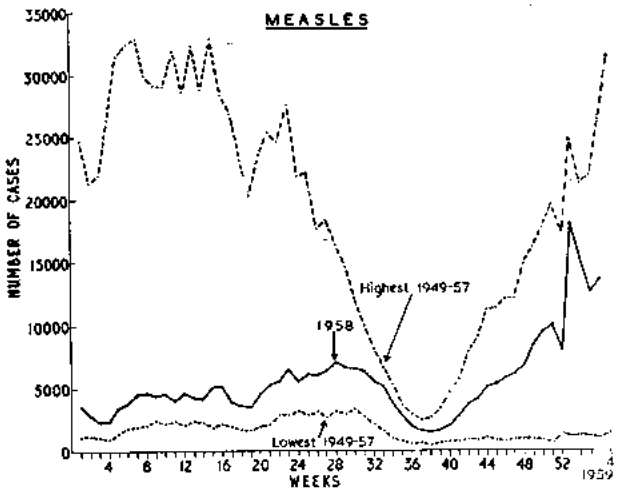
The numbers of cases of industrial diseases in the United Kingdom reported during December, 1958, were as follows: Lead poisoning 12, mercurial poisoning 3, compressed air illness 1, anthrax 1, epitheliomatous ulceration 30, chrome ulceration 20; total 67. There were eight deaths from epitheliomatous ulceration, 3 due to pitch and tar and 5 due to mineral oil.—*Ministry of Labour Gazette*, January, 1959.

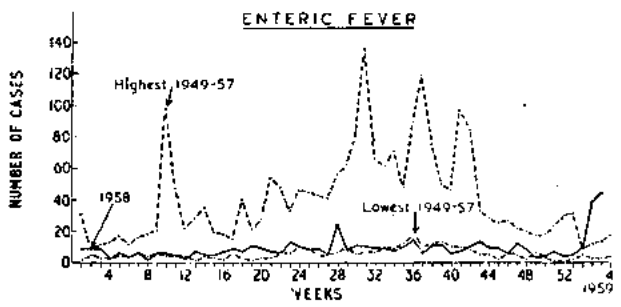
Graphs of Infectious Diseases

The graphs below show the uncorrected numbers of cases of certain diseases notified weekly in England and Wales. Highest and lowest figures reported in each week during the years 1949–57 are shown thus - - - - - -, the figures for 1958–9 thus ————. Except for the curves showing notifications in 1958–9, the graphs were prepared at the Department of Medical Statistics and Epidemiology, London School of Hygiene and Tropical Medicine.









INFECTIOUS DISEASES AND VITAL STATISTICS

Summary for British Isles for week ending **January 17** (No. 2) and corresponding week 1958.

Figures of *cases* are for the countries shown and London administrative county. Figures of *deaths* and *births* are for the whole of England and Wales (London included), London administrative county, the 17 principal towns in Scotland, the 10 principal towns in Northern Ireland, and the 14 principal towns in Eire.

A blank space denotes disease not notifiable or no return available.

The table is based on information supplied by the Registrars-General of England and Wales, Scotland, N. Ireland, and Eire, the Ministry of Health and Local Government of N. Ireland, and the Department of Health of Eire.

CASES	1959 Eng. & Wales	1959 Lond.	1959 Scot.	1959 N. Ire.	1959 Eire	1958 Eng. & Wales	1958 Lond.	1958 Scot.	1958 N. Ire.	1958 Eire
Diphtheria	5	2	2	0	4	7	1	6	0	4
Dysentery	839	69	177	12	1	574	119	114	7	4
Encephalitis, acute	3	0		0		3	0		0	
Enteric fever:										
Typhoid	1	1	1	0		2	0	0	0	1
Paratyphoid	37	2	0	0		7	0	1 (B)	0	
Food-poisoning	107	14	12	0		139	14	26	0	
Infective enteritis or diarrhoea under 2 years				7	15				11	11
Measles*	12,671	1001	536	89	615	2,888	39	90	83	13
Meningococcal infection	24	0	13	1	2	34	3	15	0	
Ophthalmia neonatorum	17	5	4	0		22	1	12	0	
Pneumonia†	527	39	350	13	2	1,292	123	568	6	21
Poliomyelitis, acute:										
Paralytic	21	1	1	1		28	1	2	0	6
Non-paralytic	5	0				8	0			
Puerperal fever§	172	24	11	0	1	235	64	22	1	
Scarlet fever	1,103	63	88	22	22	501	34	69	12	6
Tuberculosis:										
Respiratory	481	61	75	20		566	68	82	30	
Non-respiratory	48	6	5	2		50	6	8	8	
Whooping-cough	508	18	48	52	101	460	17	38	4	6

DEATHS	1959 Eng. & Wales	1959 Lond.	1959 Scot.	1959 N. Ire.	1959 Eire	1958 Eng. & Wales	1958 Lond.	1958 Scot.	1958 N. Ire.	1958 Eire
Diphtheria	0	0	0	0	1	No comparable figures available.	0	0	0	0
Dysentery	2	0		0			0		0	
Encephalitis, acute		0			0		0			
Enteric fever	0	0	0	0			0	0	0	
Infective enteritis or diarrhoea under 2 years	6	0	0	0	2		0	0	0	1
Influenza	33	1	4	1	1		21	2	0	4
Measles		0	1	0	0		0	0	0	0
Meningococcal infection		0	0				1	0		
Pneumonia	800	77	41	19	12		117	61	12	11
Poliomyelitis, acute	4	0		0	0		1		0	0
Scarlet fever		0	0	0	0		0	0	0	0
Tuberculosis:										
Respiratory	113	11	14	3	5		10	9	1	3
Non-respiratory		1	0	0	1		2	1	0	0
Whooping-cough	1	0	0	1	0		0	0	0	0
Deaths 0–1 year	352	34	42	4	23		43	58	8	11
Deaths (excluding stillbirths)	13,259	1098	872	164	215		1254	839	134	202
LIVE BIRTHS	14,554	1241	1048	254	362		1313	1142	238	353
STILLBIRTHS	324	25	29				26	28		

* Measles not notifiable in Scotland, whence returns are approximate.
† Includes primary and influenzal pneumonia.
§ Includes puerperal pyrexia.

Poliomyelitis

Epidemiology and Prevention of Vaccine-Preventable Diseases
The Pink Book: Course Textbook - 12th Edition (April 2011)

On this Page

The words polio (grey) and myelon (marrow, indicating the spinal cord) are derived from the Greek. It is the effect of poliomyelitis virus on the spinal cord that leads to the classic manifestation of paralysis.

Records from antiquity mention crippling diseases compatible with poliomyelitis. Michael Underwood first described a debility of the lower extremities in children that was recognizable as poliomyelitis in England in 1789. The first outbreaks in Europe were reported in the early 19th century, and outbreaks were first reported in the United States in 1843. For the next hundred years, epidemics of polio were reported from developed countries in the Northern Hemisphere each summer and fall. These epidemics became increasingly severe, and the average age of persons affected rose. The increasingly older age of persons with primary infection increased both the disease severity and number of deaths from polio. Polio reached a peak in the United States in 1952, with more than 21,000 paralytic cases. However, following introduction of effective vaccines, polio incidence declined rapidly. The last case of wild-virus polio acquired in the United States was in 1979, and global polio eradication may be achieved within the next decade.

Poliomyelitis

- First described by Michael Underwood in 1789
- First outbreak described in U.S. in 1843
- More than 21,000 paralytic cases reported in the U.S. in 1952
- Global eradication within next decade

Poliovirus

Poliovirus is a member of the enterovirus subgroup, family Picornaviridae. Enteroviruses are transient inhabitants of the gastrointestinal tract, and are stable at acid pH. Picornaviruses are small, ether-insensitive viruses with an RNA genome.

There are three poliovirus serotypes (P1, P2, and P3). There is minimal heterotypic immunity between the three serotypes. That is, immunity to one serotype does not produce significant immunity to the other serotypes.

The poliovirus is rapidly inactivated by heat, formaldehyde, chlorine, and ultraviolet light.

Poliovirus

- Enterovirus (RNA)
- Three serotypes: 1, 2, 3
- Minimal heterotypic immunity between serotypes
- Rapidly inactivated by heat, formaldehyde, chlorine, ultraviolet light

Pathogenesis

The virus enters through the mouth, and primary multiplication of the virus occurs at the site of implantation in the pharynx and gastrointestinal tract. The virus is usually present in the throat and in the stool before the onset of illness. One week after onset there is less virus in the throat, but virus continues to be excreted in the stool for several weeks. The virus invades local lymphoid tissue, enters the bloodstream, and then may infect cells of the central nervous system. Replication of poliovirus in motor neurons of the anterior horn and brain stem results in cell destruction and causes the typical manifestations of poliomyelitis.

Poliomyelitis Pathogenesis

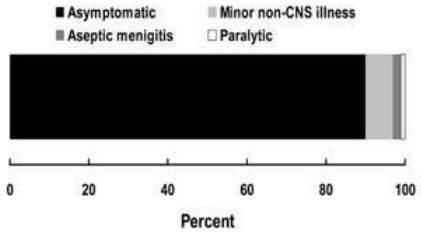
- Entry into mouth
- Replication in pharynx, GI tract, local lymphatics
- Hematologic spread to lymphatics and central nervous system
- Viral spread along nerve fibers
- Destruction of motor neurons

Clinical Features

The incubation period for poliomyelitis is commonly 6 to 20 days with a range of 3 to 35 days.

The response to poliovirus infection is highly variable and has been categorized on the basis of the severity of clinical presentation.

Outcomes of poliovirus infection



Up to 95% of all polio infections are inapparent or asymptomatic. Estimates of the ratio of inapparent to paralytic illness vary from 50:1 to 1,000:1 (usually 200:1). Infected persons without symptoms shed virus in the stool and are able to transmit the virus to others.

Approximately 4%–8% of polio infections consist of a minor, nonspecific illness without clinical or laboratory evidence of central nervous system invasion. This clinical presentation is known as abortive poliomyelitis, and is characterized by complete recovery in less than a week. Three syndromes observed with this form of poliovirus infection are upper respiratory tract infection (sore throat and fever), gastrointestinal disturbances (nausea, vomiting, abdominal pain, constipation or, rarely, diarrhea), and influenza-like illness. These syndromes are indistinguishable from other viral illnesses.

Nonparalytic aseptic meningitis (symptoms of stiffness of the neck, back, and/or legs), usually following several days after a prodrome similar to that of minor illness, occurs in 1%–2% of polio infections. Increased or abnormal sensations can also occur. Typically these symptoms will last from 2 to 10 days, followed by complete recovery.

Fewer than 1% of all polio infections result in flaccid paralysis. Paralytic symptoms generally begin 1 to 10 days after prodromal symptoms and progress for 2 to 3 days. Generally, no further paralysis occurs after the temperature returns to normal. The prodrome may be biphasic, especially in children, with initial minor symptoms separated by a 1- to 7-day period from more major symptoms. Additional prodromal signs and symptoms can include a loss of superficial reflexes, initially increased deep tendon reflexes and severe muscle aches and spasms in the limbs or back. The illness progresses to flaccid paralysis with diminished deep tendon reflexes, reaches a plateau without change for days to weeks, and is usually asymmetrical. Strength then begins to return. Patients do not experience sensory losses or changes in cognition.

Many persons with paralytic poliomyelitis recover completely and, in most, muscle function returns to some degree. Weakness or paralysis still present 12 months after onset is usually permanent.

Paralytic polio is classified into three types, depending on the level of involvement. Spinal polio is most common, and during 1969–1979, accounted for 79% of paralytic cases. It is characterized by asymmetric paralysis that most often involves the legs. Bulbar polio leads to weakness of muscles innervated by cranial nerves and accounted for 2% of cases during this period. Bulbospinal polio, a combination of bulbar and spinal paralysis, accounted for 19% of cases.

The death-to-case ratio for paralytic polio is generally 2%–5% among children and up to 15%–30% for adults (depending on age). It increases to 25%–75% with bulbar involvement.

*Less than 1% of all polio infections result in "paralvtic polio". Of those cases, 2-5% result in death among children.

Laboratory Diagnosis

Viral Isolation

Poliovirus may be recovered from the stool or pharynx of a person with poliomyelitis. Isolation of virus from the cerebrospinal fluid (CSF) is diagnostic, but is rarely accomplished.

If poliovirus is isolated from a person with acute flaccid paralysis, it must be tested further, using oligonucleotide mapping (fingerprinting) or genomic sequencing, to deter-mine if the virus is “wild type” (that is, the virus that causes polio disease) or vaccine type (virus that could derive from a vaccine strain).

Serology

Neutralizing antibodies appear early and may be at high levels by the time the patient is hospitalized; therefore, a fourfold rise in antibody titer may not be demonstrated.

Cerebrospinal Fluid

In poliovirus infection, the CSF usually contains an increased number of white blood cells (10–200 cells/mm3, primarily lymphocytes) and a mildly elevated protein (40–50 mg/100 mL).

Epidemiology

Occurrence

At one time poliovirus infection occurred throughout the world. Transmission of wild poliovirus was interrupted in the United States in 1979, or possibly earlier. A polio eradication program conducted by the Pan American Health Organization led to elimination of polio in the Western Hemisphere in 1991. The Global Polio Eradication Program has dramatically reduced poliovirus transmission throughout the world. In 2009, only 1,579 confirmed cases of polio were reported globally and polio was endemic in four countries.

Reservoir

Humans are the only known reservoir of poliovirus, which is transmitted most frequently by persons with inapparent infections. There is no asymptomatic carrier state except in immune deficient persons.

Transmission

Person-to-person spread of poliovirus via the fecal-oral route is the most important route of transmission, although the oral-oral route may account for some cases.

Temporal Pattern

Poliovirus infection typically peaks in the summer months in temperate climates. There is no seasonal pattern in tropical climates.

Communicability

Poliovirus is highly infectious, with seroconversion rates among susceptible household contacts of children nearly 100%, and greater than 90% among susceptible household contacts of adults. Persons infected with poliovirus are most infectious from 7 to 10 days before and after the onset of symptoms, but poliovirus may be present in the stool from 3 to 6 weeks.

Poliovirus Epidemiology

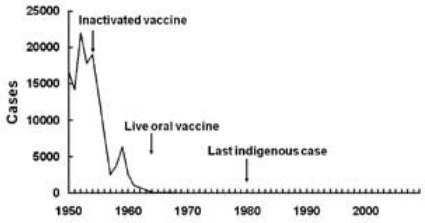
- Reservoir - Human
- Transmission - Fecal-oral
  Oral-oral possible
- Communicability - 7-10 days before onset
  Virus present in stool 3 to 6 weeks

Secular Trends in the United States

Before the 18th century, polioviruses probably circulated widely. Initial infections with at least one type probably occurred in early infancy, when transplacentally acquired maternal antibodies were high. Exposure throughout life probably provided continual boosting of immunity, and paralytic infections were probably rare. (This view has been recently challenged based on data from lameness studies in developing countries).

In the immediate prevaccine era, improved sanitation allowed less frequent exposure and increased the age of primary infection. Boosting of immunity from natural exposure became more infrequent and the number of susceptible persons accumulated, ultimately resulting in the occurrence of epidemics, with 13,000 to 20,000 para-lytic cases reported annually.

Poliomyelitis - United States, 1950-2009

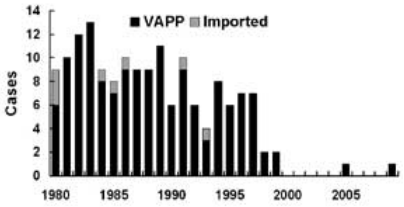


In the early vaccine era, the incidence dramatically decreased after the introduction of inactivated polio vaccine (IPV) in 1955. The decline continued following oral polio vaccine (OPV) introduction in 1961. In 1960, a total of 2,525 paralytic cases were reported, compared with 61 in 1965.

The last cases of paralytic poliomyelitis caused by endemic transmission of wild virus in the United States were in 1979, when an outbreak occurred among the Amish in several Midwest states. The virus was imported from the Netherlands.

From 1980 through 1999, a total of 152 confirmed cases of paralytic poliomyelitis were reported, an average of 8 cases per year. Six cases were acquired outside the United States and imported. The last imported case was reported in 1993. Two cases were classified as indeterminant (no poliovirus isolated from samples obtained from the patients, and patients had no history of recent vaccination or direct contact with a vaccine recipient). The remaining 144 (95%) cases were vaccine-associated paralytic polio (VAPP) caused by live oral polio vaccine.

Poliomyelitis - United States, 1980-2009



In order to eliminate VAPP from the United States, ACIP recommended in 2000 that IPV be used exclusively in the United States. The last case of VAPP acquired in the United States was reported in 1999. In 2005, an unvaccinated U.S. resident was infected with polio vaccine virus in Costa Rica and subsequently developed VAPP. A second case of VAPP from vaccine-derived poliovirus was reported in 2009. Also in 2005, several asymptomatic infections with a vaccine-derived poliovirus were detected in unvaccinated children in Minnesota. The source of the vaccine virus has not been determined, but it appeared to have been circulating among humans for at least 2 years based on genetic changes in the virus. No VAPP has been reported from this virus.

Poliovirus Vaccines

Inactivated poliovirus vaccine (IPV) was licensed in 1955 and was used extensively from that time until the early 1960s. In 1961, type 1 and 2 monovalent oral poliovirus vaccine (MOPV) was licensed, and in 1962, type 3 MOPV was licensed. In 1963, trivalent OPV was licensed and largely replaced IPV use. Trivalent OPV was the vaccine of choice in the United States and most other countries of

Poliovirus Vaccine

- 1955 - Inactivated vaccine
- 1961 - Types 1 and 2 monovalent OPV
- 1962 - Type 3 monovalent OPV
- 1963 - Trivalent OPV

the world after its introduction in 1963. An enhanced-potency IPV was licensed in November 1987 and first became available in 1988. Use of OPV was discontinued in the United States in 2000.

- 1987 - Enhanced-potency IPV (IPV)

Characteristics

Inactivated poliovirus vaccine

Two enhanced forms of inactivated poliovirus vaccine are currently licensed in the U.S., but only one vaccine (IPOL, sanofi pasteur) is actually distributed. This vaccine contains all three serotypes of polio vaccine virus. The viruses are grown in a type of monkey kidney tissue culture (Vero cell line) and inactivated with formaldehyde. The vaccine contains 2-phenoxyethanol as a preservative, and trace amounts of neomycin, streptomycin, and polymyxin B. It is supplied in a single-dose prefilled syringe and should be administered by either subcutaneous or intramuscular injection.

Inactivated Polio Vaccine

- Contains 3 serotypes of vaccine virus
- Grown on monkey kidney (Vero) cells
- Inactivated with formaldehyde
- Contains 2-phenoxyethanol, neomycin, streptomycin, polymyxin B

Oral poliovirus vaccine

Trivalent OPV contains live attenuated strains of all three serotypes of poliovirus in a 10:1:3 ratio. The vaccine viruses are grown in monkey kidney tissue culture (Vero cell line). The vaccine is supplied as a single 0.5-mL dose in a plastic dispenser. The vaccine contains trace amounts of neomycin and streptomycin. OPV does not contain a preservative.

Oral Polio Vaccine

- Contains 3 serotypes of vaccine virus
- Grown on monkey kidney (Vero) cells
- Contains neomycin and streptomycin
- Shed in stool for up to 6 weeks following vaccination

Live attenuated polioviruses replicate in the intestinal mucosa and lymphoid cells and in lymph nodes that drain the intestine. Vaccine viruses are excreted in the stool of the vaccinated person for up to 6 weeks after a dose. Maximum viral shedding occurs in the first 1–2 weeks after vaccination, particularly after the first dose.

Vaccine viruses may spread from the recipient to contacts. Persons coming in contact with fecal material of a vaccinated person may be exposed and infected with vaccine virus.

Immunogenicity and Vaccine Efficacy

Inactivated poliovirus vaccine

IPV is highly effective in producing immunity to poliovirus and protection from paralytic poliomyelitis. Ninety percent or more of vaccine recipients develop protective antibody to all three poliovirus types after two doses, and at least 99% are immune following three doses. Protection against paralytic disease correlates with the presence of antibody.

Inactivated Polio Vaccine

- Highly effective in producing immunity to poliovirus
- 90% or more immune after 2 doses
- At least 99% immune after 3 doses
- Duration of immunity not known with certainty

IPV appears to produce less local gastrointestinal immunity than does OPV, so persons who receive IPV are more readily infected with wild poliovirus than OPV recipients.

The duration of immunity with IPV is not known with certainty, although it probably provides protection for many years after a complete series.

Oral poliovirus vaccine

OPV is highly effective in producing immunity to poliovirus. A single dose of OPV produces immunity to all three vaccine viruses in approximately 50% of recipients. Three doses produce immunity to all three poliovirus types in more than 95% of recipients. As with other live-virus vaccines, immunity from oral poliovirus vaccine is probably lifelong. OPV produces excellent intestinal immunity, which helps prevent infection with wild virus.

Oral Polio Vaccine

- Highly effective in producing immunity to poliovirus
- Approximately 50% immune after 1 dose
- More than 95% immune after 3 doses
- Immunity probably lifelong

Serologic studies have shown that seroconversion following three doses of either IPV or OPV is nearly 100% to all three vaccine viruses. However, seroconversion rates after three doses of a combination of IPV and OPV are lower, particularly to type 3 vaccine virus (as low as 85% in one study). A fourth dose (most studies used OPV as the fourth dose) usually produces seroconversion rates similar to three doses of either IPV or OPV.

Vaccination Schedule and Use

Trivalent OPV was the vaccine of choice in the United States (and most other countries of the world) since it was licensed in 1963. The nearly exclusive use of OPV led to elimination of wild-type poliovirus from the United States in less than 20 years. However, one case of VAPP occurred for every 2 to 3 million doses of OPV administered, which resulted in 8 to 10 cases of VAPP each year in the United States (see Adverse Reactions section for more details on VAPP). From 1980 through 1999, VAPP accounted for 95% of all cases of paralytic poliomyelitis reported in the United States.

Polio Vaccination Recommendations, 1996-1999

- Increased use of IPV (sequential IPV-OPV schedule) recommended in 1996
- Intended to *reduce* the risk of vaccine-associated paralytic polio (VAPP)
- Continued risk of VAPP for contacts of OPV recipients

In 1996, ACIP recommended an increase in use of IPV through a sequential schedule of IPV followed by OPV. This recommendation was intended to *reduce* the occurrence of vaccine-associated paralytic polio. The sequential schedule was expected to eliminate VAPP among vaccine recipients by producing humoral immunity to polio vaccine viruses

with inactivated polio vaccine prior to exposure to live vaccine virus. Since OPV was still used for the third and fourth doses of the polio vaccination schedule, a risk of VAPP would continue to exist among contacts of vaccinees, who were exposed to live vaccine virus in the stool of vaccine recipients.

The sequential IPV–OPV polio vaccination schedule was widely accepted by both providers and parents. Fewer cases of VAPP were reported in 1998 and 1999, suggesting an impact of the increased use of IPV. However, only the complete discontinuation of use of OPV would lead to complete elimination of VAPP. To further the goal of complete elimination of paralytic polio in the United States, ACIP recommended in July 1999 that inactivated polio vaccine be used exclusively in the United States beginning in 2000. OPV is no longer routinely available in the United States. Exclusive use of IPV eliminated the shedding of live vaccine virus, and eliminated any indigenous VAPP.

A primary series of IPV consists of three doses. In infancy, these primary doses are integrated with the administration of other routinely administered vaccines. The first dose may be given as early as 6 weeks of age but is usually given at 2 months of age, with a second dose at 4 months of age. The third dose should be given at 6–18 months of age. The recommended interval between the primary series doses is 2 months. However, if accelerated protection is needed, the minimum interval between each of the first 3 doses of IPV is 4 weeks.

The final dose in the IPV series should be administered at 4 years of age or older. A dose of IPV on or after age 4 years is recommended regardless of the number of previous doses. The minimum interval from the next-to-last to final dose is 6 months.

When DTaP-IPV/Hib (Pentacel) is used to provide 4 doses at ages 2, 4, 6, and 15-18 months, an additional booster dose of age-appropriate IPV-containing vaccine (IPV or DTaP-IPV [Kinrix]) should be administered at age 4-6 years. This will result in a 5-dose IPV vaccine series, which is considered acceptable by ACIP. DTaP-IPV/Hib is not indicated for the booster dose at 4-6 years of age. ACIP recommends that the minimum interval from dose 4 to dose 5 should be at least 6 months to provide an optimum booster response.

Shorter intervals between doses and beginning the series at a younger age may lead to lower seroconversion rates. Consequently, ACIP recommends the use of the minimum age (6 weeks) and minimum intervals between doses in the first 6 months of life only if the vaccine recipient is at risk for imminent exposure to circulating poliovirus (e.g., during an outbreak or because of travel to a polio-endemic region).

Only IPV is available for routine polio vaccination of children in the United States. A polio vaccination schedule begun with OPV should be completed with IPV. If a child receives both types of vaccine, four doses of any combination of IPV or OPV by 4–6 years of age is considered a complete poliovirus vaccination series. A minimum interval of 4 weeks should separate all doses of the series.

There are three combination vaccines that contain inactivated polio vaccine. Pediarix is produced by GlaxoSmithKline and contains DTaP, hepatitis B and IPV vaccines. Pediarix is licensed for the first 3 doses of the DTaP series among children 6 weeks through 6 years of age. Kinrix is also produced by GSK and contains DTaP and IPV. Kinrix is licensed only for the fifth dose of DTaP and fourth dose of IPV among children 4 through 6 years of age. Pentacel is produced by sanofi pasteur and contains DTaP, Hib and IPV. It is licensed for the first four doses of the component vaccines among children 6 weeks through 4 years of age. Pentacel is not licensed for children 5 years or older. Additional information about these combination vaccines is in the Pertussis chapter of this book.

Polio Vaccination Schedule

Age	Vaccine	Minimum Interval
2 months	IPV	--
4 months	IPV	4 weeks
6-18 months	IPV	4 weeks
4-6 years	IPV	6 months

Polio Vaccination Recommendations

- Exclusive use of IPV recommended in 2000
- OPV no longer routinely available in the United States
- Indigenous VAPP eliminated

Schedules That Include Both IPV and OPV

- Only IPV is available in the United States
- Schedule begun with OPV should be completed with IPV
- Any combination of 4 doses of IPV and OPV by 4-6 years of age constitutes a complete series

Combination Vaccines That Contain IPV

- Pediarix
  - DTaP, Hepatitis B and IPV
- Kinrix
  - DTaP and IPV
- Pentacel
  - .DTaP, Hib and IPV

Polio Vaccination of Adults

- Routine vaccination of U.S. residents 18 years of age and older not necessary or recommended
- May consider vaccination of travelers to polio-endemic countries and selected laboratory workers

Polio Vaccination of Adults

Routine vaccination of adults (18 years of age and older) who reside in the United States is not necessary or recommended because most adults are already immune and have a very small risk of exposure to wild poliovirus in the United States.

Some adults, however, are at increased risk of infection with poliovirus. These include travelers to areas where poliomyelitis is endemic or epidemic (currently limited to South Asia, the eastern Mediterranean, and Africa), laboratory workers handling specimens that may contain polioviruses, and healthcare personnel in close contact with patients who may be excreting wild polioviruses. In addition, members of specific population groups with a current disease caused by wild polioviruses (e.g., during an outbreak) are also at increased risk.

Recommendations for poliovirus vaccination of adults in the above categories depend upon the previous vaccination history and the time available before protection is required.

Polio Vaccination of Unvaccinated Adults

- For unvaccinated adults (including adults without a written record of prior polio vaccination) at increased risk of exposure to poliomyelitis, primary immunization with IPV is recommended. The recommended schedule is two doses separated by 1 to 2 months, and a third dose given 6 to 12 months after the second dose. The minimum interval between the second and the third doses is 6 months.

- Use standard IPV schedule if possible (0, 1-2 months, 6-12 months)
- May separate first and second doses by 4 weeks if accelerated schedule needed
- The minimum interval between the second and third doses is 6 months

In some circumstances time will not allow completion of this schedule. If 8 weeks or more are available before protection is needed, three doses of IPV should be given at least 4 weeks apart. If 4 to 8 weeks are available before protection is needed, two doses of IPV should be given at least 4 weeks apart. If less than 4 weeks are available before protection is needed, a single dose of IPV is recommended. In all instances, the remaining doses of vaccine should be given later, at the recommended intervals, if the person remains at increased risk.

- Adults who have previously completed a primary series of 3 or more doses and who are at increased risk of exposure to poliomyelitis should be given one dose of IPV. The need for further supplementary doses has not been established. Only one supplemental dose of polio vaccine is recommended for adults who have received a complete series (i.e., it is not necessary to administer additional doses for subsequent travel to a polio endemic country).
- Adults who have previously received less than a full primary course of OPV or IPV and who are at increased risk of exposure to poliomyelitis should be given the remaining doses of IPV, regardless of the interval since the last dose and type of vaccine previously received. It is not necessary to restart the series of either vaccine if the schedule has been interrupted.

Polio Vaccination of Previously Vaccinated Adults

- Previously complete series
  - administer one dose of IPV
- Incomplete series
  - administer remaining doses in series
  - no need to restart series

Contraindications and Precautions to Vaccination

Severe allergic reaction (anaphylaxis) to a vaccine component, or following a prior dose of vaccine, is a contraindication to further doses of that vaccine. Since IPV contains trace amounts of streptomycin, neomycin, and polymyxin B, there is a possibility of allergic reactions in persons sensitive to these antibiotics. Persons with allergies that are not anaphylactic, such as skin contact sensitivity, may be vaccinated.

Moderate or severe acute illness is a precaution for IPV.

Polio Vaccine Contraindications and Precautions

- Severe allergic reaction to a vaccine component or following a prior dose of vaccine
- Moderate or severe acute illness

Breastfeeding does not interfere with successful immunization against poliomyelitis with IPV. IPV may be administered to a child with diarrhea. Minor upper respiratory illnesses with or without fever, mild to moderate local reactions to a prior dose of vaccine, current antimicrobial therapy, and the convalescent phase of an acute illness are not contraindication for vaccination with IPV.

Contraindications to combination vaccines that contain IPV are the same as the contraindications to the individual components (e.g., DTaP, hepatitis B).

Adverse Reactions Following Vaccination

Minor local reactions (pain, redness) may occur following IPV. No serious adverse reactions to IPV have been documented. Because IPV contains trace amounts of streptomycin, polymyxin B, and neomycin, allergic reactions may occur in persons sensitive to these antibiotics.

Polio Vaccines Adverse Reactions

- Rare local reactions (IPV)
- No serious reactions to IPV have been documented
- Paralytic poliomyelitis (OPV)

Vaccine-Associated Paralytic Poliomyelitis

Vaccine-associated paralytic polio is a rare adverse reaction following live oral poliovirus vaccine. Inactivated poliovirus vaccine does not contain live virus, so it cannot cause VAPP. The mechanism of VAPP is believed to be a mutation, or reversion, of the vaccine virus to a more neurotropic form. These mutated viruses are called revertants. Reversion is believed to occur in almost all vaccine recipients, but it only rarely results in paralytic disease. The paralysis that results is identical to that caused by wild virus, and may be permanent.

VAPP is more likely to occur in persons 18 years of age and older than in children, and is much more likely to occur in immunodeficient children than in those who are immunocompetent. Compared with immunocompetent children, the risk of VAPP is almost 7,000 times higher for persons with certain types of immunodeficiencies, particularly B-lymphocyte disorders (e.g., agammaglobulinemia and hypogammaglobulinemia), which reduce the synthesis of immune globulins. There is no procedure available for identifying persons at risk of paralytic disease, except excluding older persons and screening for immunodeficiency.

Vaccine-Associated Paralytic Polio

- Increased risk in persons 18 years and older
- Increased risk in persons with immunodeficiency
- No procedure available for identifying persons at risk of paralytic disease
- 5-10 cases per year with exclusive use of OPV
- Most cases in healthy children and their household contacts

From 1980 through 1998, 152 cases of paralytic polio were reported in the United States; 144 (95%) of these cases were VAPP, and the remaining eight were in persons who acquired documented or presumed wild-virus polio outside the United States. Of the 144 VAPP cases, 59 (41%) occurred in healthy vaccine recipients (average age 3 months). Forty-four (31%) occurred in healthy contacts of vaccine recipients (average age 26 years), and 7 (5%) were community acquired (i.e., vaccine virus was recovered but there was no known contact with a vaccine

recipient). Thirty-four (24%) of VAPP cases occurred in persons with immunologic abnormalities (27 in vaccine recipients and 7 in contacts of vaccine recipients). None of the vaccine recipients were known to be immunologically abnormal prior to vaccination.

The risk of VAPP is not equal for all OPV doses in the vaccination series. The risk of VAPP is 7 to 21 times higher for the first dose than for any other dose in the OPV series. From 1980 through 1994, 303 million doses of OPV were distributed and 125 cases of VAPP were reported, for an overall risk of VAPP of one case per 2.4 million doses. Forty-nine paralytic cases were reported among immunocompetent recipients of OPV during this period. The overall risk to these recipients was one VAPP case per 6.2 million OPV doses. However, 40 (82%) of these 49 cases occurred following receipt of the first dose, making the risk of VAPP one case per 1.4 million first doses. The risk for all other doses was one per 27.2 million doses. The reason for this difference by dose is not known with certainty, but it is probably because the vaccine virus is able to replicate longer in a completely nonimmune infant. This prolonged replication increases the chance of the emergence of a revertant virus that may cause paralysis. The situation is similar for contacts. A nonimmune child may shed virus longer, increasing the chance of exposure of a contact.

Vaccine-Associated Paralytic Polio (VAPP) 1980-1998

- Healthy recipients of OPV - 41%
- Healthy contacts of OPV recipients - 31%
- Community acquired - 5%
- Immunodeficient - 24%

The last case of VAPP acquired in the United States was reported in 1999. As noted previously, a U.S. resident with VAPP was reported in 2005, but the vaccine virus infection was acquired in Costa Rica.

Vaccine Storage and Handling

IPV may be shipped without refrigeration provided it is delivered within 4 days. It should be maintained at 35°–46°F (2°–8°C). The vaccine should be clear and colorless. Any vaccine showing particulate matter, turbidity, or change in color should be discarded.

Outbreak Investigation and Control

Collect preliminary clinical and epidemiologic information (including vaccine history and contact with OPV vaccines) on any suspected case of paralytic polio. Notify CDC, (404-639-8255) after appropriate local and state health authorities have been notified. Intensify field investigation to verify information and collect appropriate specimens for viral isolation and serology.

A single case of paralytic poliomyelitis demands immediate attention. If the evidence indicates vaccine-associated disease, no outbreak control program is needed. If, however, evidence indicates wild virus (for example, two cases in a community), all unvaccinated persons in the epidemic area who are 6 weeks of age and older and whose vaccine histories are uncertain should be vaccinated.

Polio Eradication

- Last case in United States in 1970
- Western Hemisphere certified polio free in 1994
- Last isolate of type 2 poliovirus in India in October 1999
- Global eradication goal

Polio Eradication

Following the widespread use of poliovirus vaccine in the mid-1950s, the incidence of poliomyelitis declined rapidly in many industrialized countries. In the United States, the number of cases of paralytic poliomyelitis reported annually declined from more than 20,000 cases in 1952 to fewer than 100 cases in the mid-1960s. The last documented indigenous transmission of wild poliovirus in the United States was in 1979.

Wild Poliovirus 1988

In 1985, the member countries of the Pan American Health Organization adopted the goal of eliminating poliomyelitis from the Western Hemisphere by 1990. The strategy to achieve this goal included increasing vaccination coverage; enhancing surveillance for suspected cases (i.e., surveillance for acute flaccid paralysis); and using supplemental immunization strategies such as national immunization days, house-to-house vaccination, and containment activities. Since 1991, when the last wild-virus–associated indigenous case was reported from Peru, no additional cases of poliomyelitis have been confirmed despite intensive surveillance. In September 1994, an international commission certified the Western Hemisphere to be free of indigenous wild poliovirus. The commission based its judgment on detailed reports from national certification commissions that had been convened in every country in the region.

In 1988, the World Health Assembly (the governing body of the World Health Organization) adopted the goal of global eradication of poliovirus by the year 2000. Although this goal was not achieved, substantial progress has been made. One type of poliovirus appears to have already been eradicated. In 1988, an estimated 350,000 cases of paralytic polio occurred, and the disease was endemic in more than 125 countries. By 2006, fewer than 2,000 cases were reported globally—a reduction of more than 99% from 1988—and polio remained endemic in only four countries. In addition, one type of poliovirus appears to have already

been eradicated. The last isolation of type 2 virus was in India in October 1999.

The polio eradication initiative is led by a coalition of international organizations that includes WHO, the United Nations Children's Fund (UNICEF), CDC, and Rotary International. Other bilateral and multilateral organizations also support the initiative. Rotary International has contributed more than $600 million to support the eradication initiative. Current information on the status of the global polio eradication initiative is available on the World Health Organization website .

Wild Poliovirus 2008

Postpolio Syndrome

After an interval of 30–40 years, 25%–40% of persons who contracted paralytic poliomyelitis in childhood experience new muscle pain and exacerbation of existing weakness, or develop new weakness or paralysis. This disease entity is referred to as postpolio syndrome. Factors that increase the risk of postpolio syndrome include increasing length of time since acute poliovirus infection, presence of permanent residual impairment after recovery from the acute illness, and female sex. The pathogenesis of postpolio syndrome is thought to involve the failure of oversized motor units created during the recovery process of paralytic poliomyelitis. Postpolio syndrome is not an infectious process, and persons experiencing the syndrome do not shed poliovirus.

For more information, or for support for persons with post-polio syndrome and their families, contact:

Post-Polio Health International
4207 Lindell Boulevard #110
St. Louis, MO 63108-2915
314-534-0475

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The Present Status of Polio Vaccines.

Author(s) : Ratner, H., Moderator ; Cox, H. R. ; Greenberg, B. G. ; Kleinman, H. ; Meier, P., Panelists
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Abstract : A panel discussion on the present status of poliovaccines was held in Chicago on May 26th, 1960. 2 of the panellists, Dr. Ratner and Professor Meier, have been critics of Salk poliovaceine since its inception. Professor Greenberg and Dr. Kleinman have been concerned with the evaluation of poliovaceine effectiveness and Dr. Cox is responsible for the development of attenuated polio-virus vaccine. Kleinman has also been concerned with field trials of Lederle attenuated poliovaceine. The burden of the argunient is that killed poliovaceine has been a failure and that a change to a living vaccine should be made.

Ratner notes that poliomyelitis incidence has increased from the 1957 level in 1958 and 1959, and that substantial numbers of cases occur in the triply vaccinated. Greenberg points out some fallacies in the assessment of poliovaceine effectiveness. First, the requirements have altered to increase safety but it is thought that polio-vaccine potency decreases following the introduction of a second filtration step. [In the reviewer's experience of making killed vaccine it is clear that the quality of the filtration vitally affects safety but that with suitable precautions no potency need be lost.] The report of the Poliomyelitis Surveillance Unit of December 7th, 1955, is severely criticized because the numbers of children were taken from the 1950 census and no allowance was made for increases in the population. Also, children were considered as vaccinated regardless of whether they were vaccinated early or late in the year. This diminishes the rate in the vaccinated owing to swelling the vaccinated population with those who were vaccinated late in the year after having escaped clinical infection earlier. Differences in diagnostic criteria for non-paralytic and paralytic cases introduced as a result of the 1954 killed polio-vaccine trial are thought to be the major cause of the fall in incidence of reported poliomyelitis m 1957. [Most analyses of killed poliovaceine effectiveness allowing for these factors have showed it to be at least 80% effective.

Kleinman, who had previously estimated killed polio-vaccine to be very effective, is now dubious because of the increase in numbers of paralytic poliomyelitis. He confesses himself unable to decide whether the vaccine is or is not effective. Meier reiterates his early fears about killed poliovaceine safety and the adequacy of the tests. He is uneasy about the propaganda effort to promote killed polio-vaccine. Experts, he contends, have doubts but in the newspapers the killed vaccine is represented as safe and very effective.

Cox and Kleinman describe the development and use of attenuated poliovaceine mainly in Latin America and Minnesota. They believe the vaccine to be safe and effective in producing antibodies and therefore most likely to be effective in preventing poliomyelitis. Ratner, summing up, concludes that, if killed polio vaccine is safe and highly effective, licensing of living vaccine is not urgent. The panel's view is that this proposition is not proven and that a living vaccine is an urgent necessity.

[The failure of killed poliovaceine has mainly been due to failure to achieve satisfactory acceptance of the vaccine. It is hoped that this problem will be overcome by an oral vaccine. One argument used in favour of living vaccine is the uniform satisfactory results with living vaccines in the veterinary field. A review by PRIER, *J. Amer. Vet. Med. Ass.*, 1960, v. 137, 577, is not quite so enthusiastic.] *A. J. Beale*.

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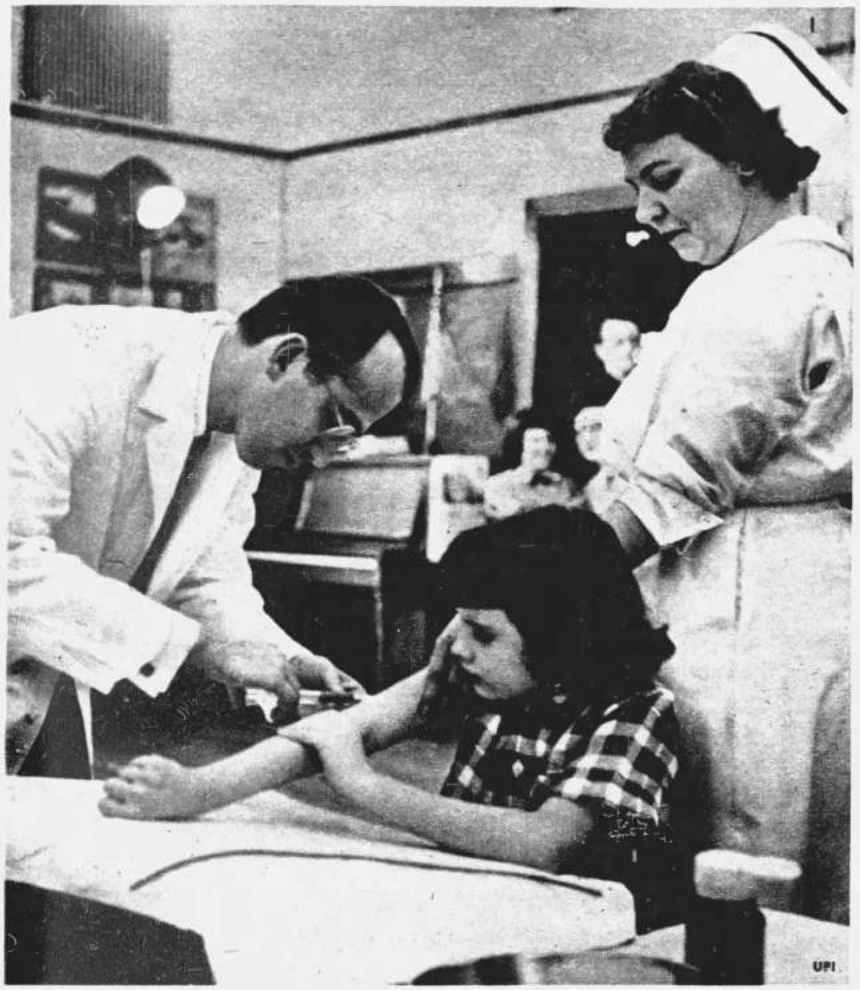
The Truth About

Do Salk Shots Really Prevent Polio? Should We Keep Using Salk Inoculations? How Good Are the New Oral Vaccines? Here Are the Facts:



Dr. Jonas E. Salk injects a volunteer with his vaccine in 1954 field trials. Controversial from the first, the Salk vaccine is still a topic hotly debated.

By Joan Beck

BEHIND GLOWING reports of the Salk polio vaccine's success and even rosier predictions about the new, live, oral Sabin vaccine rages a storm of medical controversy that seldom reaches the ears of parents.

Many serious criticisms have been leveled at the Salk vaccine. These are now being acknowledged—at least indirectly—in announcements praising and promoting the new oral vaccines.

Yet all is not yet sweetness and accord among developers of the live, oral vaccines, either. At least three different types have been developed and—according to their producers—proved safe and effective in tests, chiefly in foreign countries, but also in the United States.

One of these new oral vaccines, developed by Dr. Albert Sabin with National Foundation research funds, has been OK'd by the United States public health service for manufacture. But there are problems remaining to be solved in its production and, according to a committee of experts headed by Dr. Roderick Murray of the National Institute of Health, dangers to be considered in its use by the general public (altho it has been given to a reported 77 million Russians and to at least 300,000 Americans. Russian Prof. Mikhail Chumakov, who directed a two year program of inoculation with the Sabin vaccine, says he is convinced polio epidemics have been eliminated in the Soviet Union). Licensing is not expected until this spring. Quantities of the vaccine are not expected to be available for community-wide use until November.

"Both 'live' (Sabin) and 'killed' (Salk) poliovirus vaccines will be needed to combat poliomyelitis in the near future, United States public health officials declared at the A. M. A. clinical meeting," the Journal of the American Medical association reported in December, 1960. "The new oral poliomyelitis vaccine developed by Dr. Albert Sabin and approved for future use in this country will not be the complete solution as far as can be predicted now, the public health service experts said."

Evaluating the true effectiveness of the Salk vaccine and the new oral vaccines has been difficult for several reasons. Polio is a relatively rare disease in the United States. Because so few persons get it in its paralyzing form, success of an immunizing agent is hard to determine.

The definition of polio also has changed in the last six or seven years. Several diseases which were often diagnosed as polio are now classified as aseptic meningitis or illnesses caused by one of the Coxsackie or Echo viruses. The number of polio cases in 1961 cannot accurately be compared with those in, say 1952, because the criteria for diagnosis have changed.

Even the Salk vaccine itself is not a constant, standard product. Since the first field trials of 1954, the vaccine has been changed several times. The first alterations were aimed at increasing the vaccine's safety by changing the method of killing the polio virus and by adding an extra filtration step. Newer changes are intended to increase the vaccine's effectiveness. The success of the Salk vaccine necessarily varies, depending upon which Salk vaccine is being considered.

Ever since the public was first informed about the Salk vaccine in the Francis report of April 12, 1955, the National Foundation has praised its effectiveness and urged parents to have themselves and their children vaccinated. Altho some physicians remained skeptical about the original theories behind the vaccine, about the techniques used in its evaluation, and about its success in combating polio, these objections seldom reached the general public. With the resurgence of paralytic polio in 1958 and 1959, the criticisms increased.

These views were summed up by five experts in a panel discussion on the "Present Status of Polio Vaccines" presented before the Illinois State Medical society in Chicago, in May, 1960, and published in the August and September issues of the Illinois Medical Journal. To make parents aware of the controversy about the Salk vaccine and the problems involved in developing an effective oral vaccine against polio, here is a report of that discussion:

Moderator of the panel was Herbert Ratner, M. D., director of public health in Oak Park, and associate clinical professor of preventive medicine and public health, Stritch School of Medicine, Chicago.

Dr. Ratner noted the upward trend in polio, particularly in the paralytic form, in the United States during 1958 and 1959. He quoted Dr. Alexander Langmuir, in charge of polio surveillance for the United States public health service, as saying this resurgence is "cause for immediate concern."

"In the fall of 1955, Dr. Langmuir had predicted that by 1957 there would be less than 100 cases of paralytic polio in the United States," commented Dr. Ratner. "Four years and 300 million doses of Salk vaccine later, we had in 1959 approximately 6,000 cases of paralytic polio, 1,000 of which were in persons who had received three and more shots of Salk vaccine. Salk vaccine hasn't lived up to expectations."

Dr. Sabin says the number of cases in 1960 was less than in 1959, but that 23 per cent are now occurring in persons who have had three or more doses of Salk vaccine.

Dr. Ratner next reviewed some basic facts about polio. Paralytic polio occurs in cycles and was in a natural decline when the Salk vaccine was introduced in 1955, he pointed out.

Prior to the introduction of the Salk vaccine, the National Foundation defined an epidemic as 20 or more cases of polio per year, per 100,000 population. Now, an epidemic is defined as 35 cases per year per 100,000. This change has resulted in a statistical —but not necessarily a real—drop in polio epidemics.

For every case of known paralytic polio, there are about a thousand "subclinical polio infections," so mild they pass unnoticed, Dr. Ratner explained. These mild cases account for the high degree of natural immunity in adults. You can have a polio infection in the intestines without having paralytic polio or nonparalytic polio with enough sympt ns to be diagnosed.

The theory of the Salk vaccine, made with killed polio virus, is that it will produce enough antibodies

the Polio Vaccines

Dr. Herald R. Cox has another oral vaccine.

Dr. Hilary. Koprowski . . . he wants fair play.

circulating in the blood to neutralize poliovirus before it can reach the central nervous system. But "one of the major disappointments of the killed vaccine" is that these circulating antibodies do not protect an individual against getting a polio infection in the intestines, nor its breakthru into the circulatory system, said Dr. Ratner. Protection against paralytic polio depends upon the presence of enough circulating antibodies to offset the virus, he explained.

Discussing the "very misleading way" in which the Salk vaccine data has been handled, was Bernard G. Greenberg, Ph. D., head of the department of biostatistics of the University of North Carolina, school of public health, and former chairman of the committee on evaluation and standards of the American Public Health association.

"There has been a rise during the last two years in the incidence rates of paralytic poliomyelitis in the United States," stressed Dr. Greenberg. "The rate in 1958 was about 50 per cent higher than that for 1957, and in 1959 about 80 per cent higher than that in 1958. If 1959 is compared with the low year of 1957, the increase is about 170 per cent.

"As a result of this trend in paralytic poliomyelitis, various officials in the public health service, official health agencies, and one large voluntary health organization have been utilizing the press, radio, and television and other media to sound an alarm bell in an heroic effort to persuade more Americans to take advantage of the vaccination procedures available to them," said Dr. Greenberg.

"Altho such a program might be desirable until live virus vaccines are available to us on more than an experimental basis, the misinformation and unjustified conclusions about the cause of this rise in incidence give concern to those interested in a sound program based on logic and fact rather than personal opinion and prejudice.

"One of the most obvious pieces of misinformation being delivered to the American public is that the 50 per cent rise in paralytic poliomyelitis in 1958 and the real accelerated increase in 1959 have been caused by persons failing to be vaccinated. This represents a certain amount of double talk and an unwillingness to face facts and to evaluate the true effectiveness of the Salk vaccine," said Dr. Greenberg.

The number of persons over 2 years of age in 1960 who have not been vaccinated cannot be more and must be considerably less than the number who had no vaccination in 1957, Dr. Greenberg pointed out. Then how can it be claimed that it is the large number of unvaccinated persons who are causing the increase in polio, when there were a larger number of unvaccinated individuals in 1957 when the vaccine was given credit for reducing rates of the disease.

"A scientific examination of the data and the manner in which the data was manipulated will reveal that the true effectiveness of the present Salk vaccine is unknown and greatly overrated," Dr. Greenberg stressed.

Why was there such a tremendous reduction in reported rates of paralytic polio in 1955, 1956, and 1957? Much of this highly publicized decrease was a statistical illusion, said Dr. Greenberg.

Prior to 1954, any physician who reported a case of paralytic poliomyelitis was doing his patient a favor because funds were available to help pay his medical expenses. At that time, most health departments used a definition of paralytic poliomyelitis which specified "partial or complete paralysis of one or more muscle groups, detected on two examinations at least 24 hours apart." Laboratory confirmation and the presence of residual paralysis were not required.

In 1955, these criteria were changed. Now, unless there is paralysis lasting at least 60 days after the onset of the disease, it is not diagnosed as paralytic polio.

During this period, too, "Coxsackie virus infections and aseptic meningitis have been distinguished from paralytic poliomyelitis," explained Dr. Greenberg. "Prior to 1954, large numbers of these cases undoubtedly were mislabeled as paralytic polio."

Thus, because the definition of the disease was changed and two similar diseases virtually ruled out, the number of cases of polio reported was sure to decrease in the 1955-57 period, vaccine or not. Then, too, physicians are reluctant today to diagnose paralytic poliomyelitis in a vaccinated child without thoro laboratory tests, thus eliminating most of the false positive cases commonly reported in the pre-1954 period.

"As a result of these changes in both diagnosis and diagnostic methods, the rates of paralytic poliomyelitis plummeted from the early 1950s to a low in 1957," said Dr. Greenberg. The recent increase in the disease, despite improved diagnostic methods, he believes, is due to a long term, increasing trend in the occurrence of polio.

"Without doubt, the increasing trend has been reduced to some extent by the Salk vaccine," explained Dr. Greenberg. "Nevertheless, the Salk vaccine has limited effectiveness in its ability further to reduce this trend. . . . Any future substantial reduction in this trend will require a more potent vaccine, not simply vaccinating more people.

"Today it may be a serious mistake to be ultra-conservative in accepting the various new live vaccines under the impression that there is no hurry because an almost equivalent immunizer exists in the Salk vaccine. A delay in accepting and promoting better vaccines will be a costly one. There must be immediate pressure applied to determine whether or not the new vaccines are more effective, so that we do not cling, for sentimental or personal reasons, to an older vaccine whose true effectiveness is today unknown."

The most accurate way we have of determining the effectiveness of vaccine (except by direct exposure to the disease) is to measure the levels of neutralizing antibodies in the blood, explained Herald R. Cox, Sc. D., director of virus research at Lederle Laboratories and president elect of the Society of American Bacteriologists. We do not know, he said, the exact level of antibodies necessary to protect against paralytic polio.

Herman Kleinman, M. D., an epidemiologist from the Minnesota department of health, pointed out that in antibody studies on children who have received three or more doses of Salk vaccine, he has found more than half do not have antibodies to two of the three types of polio strains used in the Salk vaccine. Twenty per cent lack antibodies to a third type.

"This is a very disturbing fact," said Dr. Kleinman. "If polio antibodies mean anything in respect to protection, then I am forced to conclude that much of the Salk vaccine we have been using is useless."

Dr. Kleinman also commented on the "changing concept of polio" and said physicians were reluctant to diagnose the disease without overwhelming evidence. He called the insistence on a 60 day duration

(Continued on Page 11)

Dr. Albert Sabin works on a culture for his live, oral vaccine. It has been used widely in Russia, but the United States public health service has ruled that it is not yet ready for licensing in this country.

Is the Killer Still with Us?

(Continued from Page 9)

of paralysis in defining paralytic polio "silly."

Dr. Cox, who has worked in the virus field since 1929 and was the first person to prove that a killed vaccine could be made, commented on some of the problems of producing a potent, killed-virus vaccine.

"We are now learning, not only in the United States, but in Israel, England, and Denmark, that the killed product does a fairly good job of producing antibodies against Type II poliovirus," said Dr. Cox. "But Type II represents only about 3 per cent of paralytic cases thruout the world. The killed vaccine does a poor job against Type I, however, which causes 85 per cent of paralytic cases, and against Type III, which causes about 12 per cent.

"In other words, the killed vaccine is doing its best job against the least important type. It took time to find this out. It was proven in Israel in 1958, when it had its big Type I epidemic. They did not see any difference in protection between the vaccinated and the unvaccinated. Last year in Massachusetts during a Type III outbreak, there were more paralytic cases in the triple vaccinates than in the unvaccinated."

There have been problems, too, in the production of the killed Salk vaccine. An extra filtration step was added in November, 1955, Dr. Cox said, "because the amount of formalin used did not inactivate the poliovirus. We found residual live virus for as long as 42 consecutive days of inactivation."

Dr. Cox went on to assert that the second filtration step was "picked out of thin air with no experimentation to back it up," and that the extra filtration cut down on the effectiveness of the vaccine.

Mass vaccination with the Salk product started in April, 1955, and by April 26, there were reports of paralytic polio among vaccinated children, with deaths occurring in Idaho and California. Then came cases of polio among family members of vaccinated children. Live virus was discovered in the supposedly killed vaccine, altho it had been produced by the Salk procedure.

Dr. Ratner cited numerous instances in which live viruses were found in vaccine which was presumably safe, even in Dr. Salk's own standard vaccines. "It should be stressed that safety testing was inadequate when Dr. Salk developed the vaccine and when the vaccine was commercially prepared for the field trials of 1954 and for licensing and use in 1955," said Dr. Ratner. He added that in current vaccine, potency has been sacrificed for safety and that "at present, epidemiologic methods employed by the United States public health service to assure safety of the vaccine are inadequate."

Should the Salk vaccine continue to be used?

"There is no known way of preventing polio with a licensed product at the present time except thru the use of the Salk vaccine," answered Dr. Kleinman. "While I am an agnostic about the effectiveness of the Salk vaccine, I still believe it does something in preventing paralysis. So we owe it to the public to recommend its use. On the other hand, if we are going to act not only as public health physicians but as scientists we must continue our investigations into the truth about the Salk vaccine. On the basis of the facts as I know them, we must look for something better."

Other panel members agreed, pointing out that because all of the facts about the Salk vaccine have not been made public, physicians and public health officials find it difficult to resist the great pressures of public opinion built up thru an unprecedented publicity campaign urging the public to be vaccinated.

"Since nothing else is available, there seems to be no alternative but to push the use of it," commented Dr. Greenberg. "I don't think we should do so in ignorance, nor too complacently, believing that as long as we have something partially effective, there is no need to have something better. By being more cautious, we may make a mistake by accepting a better polio vaccine too slowly."

Dr. Salk (left) and Dr. Sabin clashed in 1955 hearings about the use of the Salk vaccine.

"When measured against its killed counterpart, a live virus vaccine (using modified virus which stimulate the production of anti-bodies but do not cause the disease) is always a superior vaccine," asserted Dr. Cox. He said it invariably costs much less. And it gives a higher degree of longer-lasting immunity. Dr. Cox has developed a live vaccine which was tested on thousands of school children and adults last year in Dade county, Fla., and also on thousands of persons in foreign countries.

Another live, oral polio vaccine has been developed by Dr. Hilary Koprowski, of Philadelphia's Wistar institute and has been tested on approximately 9 million individuals.

Dr. Koprowski has challenged the United States public health service decision last August to grant approval only to the Sabin vaccine. In a letter in the Jan. 14 Journal of the American Medical association, he said, "Altho it is a step forward that the principle of live virus immunization in poliomyelitis has at last been officially accepted, I am taking strong exception to this exclusive indorsement of one set of strains. In my opinion, such an indorsement should evoke a protest from individuals who believe that fair scientific judgment should be the basis for decisions affecting the physical welfare of man."

Amplifying his letter, Dr. Koprowski said, "It is my belief that government decisions, which are not based on proper evaluation of scientific data, are prompted by either poor choice of scientific advisers or by cryptic reasoning and that such ill-advised decisions could lead to development of an unhealthy climate in which scientists will see their contributions trampled upon by administrative agencies."

Discussing the development of live, oral vaccines, Dr. Cox explained, "Polio is unique because many more people get the infection than the disease." The problem in producing a live vaccine is to modify, or tame, the virus so that they will produce a mild infection strong enough to stimulate the formation of antibodies, but not the disease itself. A complicating factor in taming polio virus, is that three separate, tamed strains have to be developed to produce antibodies against the three chief types of polio.

A killed vaccine, such as the Salk, does not immunize an individual against an infection of polio virus in the intestines and, altho it can induce antibodies in the blood, this does not prevent the individual from becoming a carrier and spreading poliovirus, explained Dr. Cox.

Individuals receiving the live, modified, oral vaccines also eliminate poliovirus from their bodies for several days or several weeks after vaccination, but these are the tame, modified strains. Family contacts and even other individuals in the neighborhood can also acquire an immunity from these tame virus, altho they have never received the vaccine themselves.

However, some experts still fear that one of these strains may revert to its virulent type as it is passed from one individual to another, according to a report by Dr. Roderick Murray's committee, quoted in the Oct. 15, 1960, issue of "Modern Medicine." One solution, the committee suggested, might be to give the oral vaccine to entire communities in a brief time. This is a problem which must be solved before the Sabin vaccine is licensed.

Dr. Cox stated that using a live vaccine is the only way to eliminate wild, virulent polio strains in nature. Immunization with live vaccine probably would not protect a person for life, he added, but it would be cheap enough so you could afford it once a year.

Dr. Ratner compared Dr. Cox's vaccination figures with the 1954 field trials of the Salk vaccine. "The Cox live poliovirus has now been used by many investigators in over 2.5 million people, the other two live virus vaccines under study have been used in additional millions," he said. "Safety has been paramount in the minds of these investigators."

"On the other hand, Salk vaccine was used in only 400,000 persons in a single field trial which assumed safety and was primarily designed to determine effectiveness.

"An objective and fearless evaluation of the Salk vaccine is needed, for this is the necessary ingredient of an intelligent decision as to when the live virus vaccine should be licensed," Dr. Ratner continued. "Obviously, if the Salk vaccine is safe and highly effective, the United States public health service can take its time about licensing the live virus vaccine.

"If, on the other hand, polio and polio epidemics remain with us and children become paralyzed despite three, four, five, and six inoculations of Salk vaccine and vaccinees die, we cannot take our time."

What should parents do?

Take the advice of their pediatrician or family doctor and not be stampeded by TV commercials or overly-enthusiastic claims for vaccines. It is the individual physician who must decide which vaccine is safe and effective in what circumstances. But physicians must have honest, impartial, fully scientific information available to make this decision.

Currently, most physicians are still giving Salk vaccine shots. A few doctors do not. Some give them only if patients insist.

Once a live, oral vaccine is fully approved, it will be more effective than the killed Salk vaccine. Because of the doubt about the potency and effectiveness of the Salk vaccine in the past, a full course of the new vaccine will undoubtedly be recommended for everyone, regardless of how many Salk shots each individual has had.

Decline in Disease Mortality

The Questionable Contribution of Medical Measures to the Decline of Mortality in the United States in the Twentieth Century

JOHN B. McKINLAY AND SONJA M. McKINLAY

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". . . by the time laboratory medicine came effectively into the picture the job had been carried far toward completion by the humanitarians and social reformers of the nineteenth century. Their doctrine that nature is holy and healthful was scientifically naive but proved highly effective in dealing with the most important health problems of their age. When the tide is receding from the beach it is easy to have the illusion that one can empty the ocean by removing water with a pail."

R. Dubos, Mirage of Health,
New York: Perennial Library, 1959, p. 23

Introducing a Medical Heresy

The modern "heresy" that medical care (as it is traditionally conceived) is generally unrelated to improvements in the health of populations (as distinct from individuals) is still dismissed as unthinkable in much the same way as the so-called heresies of former times. And this is despite a long history of support in popular and scientific writings as well as from able minds in a variety of disciplines. History is replete with examples of how, understandably enough, self-interested individuals and groups denounced popular customs and beliefs which appeared to threaten their own domains of practice, thereby rendering them heresies (for example, physicians' denunciation of midwives as witches, during the Middle Ages). We also know that vast institutional resources have often been deployed to neutralize challenges to the assumptions upon which everyday organizational activities were founded and legitimated (for example, the Spanish Inquisition). And since it is usually difficult for organizations themselves to directly combat threatening

"heresies," we often find otherwise credible practitioners, perhaps unwittingly, serving the interests or organizations in this capacity. These historical responses may find a modern parallel in the way the everyday practitioners of medicine, on their own altruistic or "scientific" grounds and still perhaps unwittingly, serve present-day institutions (hospital complexes, university medical centers, pharmaceutical houses, and insurance companies) by spearheading an assault on a most fundamental challenging heresy of our time: *that the introduction of specific medical measures and/or the expansion of medical services are generally not responsible for most of the modern decline in mortality.*

In different historical epochs and cultures, there appear to be characteristic ways of explaining the arrival and departure of natural viscissitudes. For salvation from some plague, it may be that the gods were appeased, good works rewarded, or some imbalance in nature corrected. And there always seems to be some person or group (witch doctors, priests, medicine men) able to persuade others, sometimes on the basis of acceptable evidence for most people at that time, that they have *the* explanation for the phenomenon in question and may even claim responsibility for it. They also seem to benefit most from common acceptance of the explanations they offer. It is not uncommon today for biotechnological knowledge and specific medical interventions to be invoked as *the major reason* for most of the modern (twentieth century) decline in mortality.[1] Responsibility for this decline is often claimed by, or ascribed to, the present-day major beneficiaries of this prevailing explanation. But both in terms of the history of knowledge and on the basis of data presented in this paper, one can reasonably wonder whether the supposedly more sophisticated explanations proffered in our own time (while seemingly distinguishable from those accepted in the past) are really all that different from those of other cultures and earlier times, or any more reliable. Is medicine, the

[1]It is obviously important to distinguish between (a) advances in knowledge of the cause and natural course of some condition and (b) improvements in our ability to effectively treat some condition (that is, to alter its natural course). In many instances these two areas are disjoint and appear at different stages of development. There are, on the one hand, disease processes about which considerable knowledge has been accrued, yet this has not resulted (nor necessarily will) in the development of effective treatments. On the other hand, there are conditions for which demonstrably effective treatments have been devised in the absence of knowledge of the disease process and/or its causes.

physician, or the medical professsion any more entitled to claim responsibility for the decline in mortality that obviously has occurred in this century than, say, some folk hero or aristocracy of priests sometime in the past?

Aims

Our general intention in this paper is to sustain the ongoing debate on the questionable contribution of specific medical measures and/or the expansion of medical services to the observable decline in mortality in the twentieth century. More specifically, the following three tasks are addressed: (a) selected studies are reviewed which illustrate that, far from being idiosyncratic and/or heretical, the issue addressed in this paper has a long history, is the subject of considerable attention elsewhere, attracts able minds from a variety of disciplines, and remains a timely issue for concern and research; (b)age- and sex-adjusted mortality rates (standardized to the population of 1900) for the United States, 1900–1973, are presented and then considered in relation to a number of specific and supposedly effective medical interventions (both chemotherapeutic and prophylactic). So far as we know, this is the first time such data have been employed for this particular purpose in the United States, although reference will be made to a similar study for England and Wales; and (c) some policy implications are outlined.

Background to the Issue

The beginning of the serious debate on the questionable contribution of medical measures is commonly associated with the appearance, in Britain, of Talbot Griffith's (1967) *Population Problems in the Age of Malthus.* After examining certain medical activities associated with the eighteenth century—particularly the growth of hospital, dispensary, and midwifery services, additions to knowledge of physiology and anatomy, and the introduction of smallpox inoculation—Griffith concluded that they made important contributions to the observable decline in mortality at that time. Since then, in Britain and more recently in the United States, this debate has continued, regularly engaging scholars from economic history, demography, epidemiology, statistics, and other disciplines. Habakkuk

(1953), an economic historian, was probably the first to seriously challenge the prevailing view that the modern increase in population was due to a fall in the death rate attributable to medical interventions. His view was that this rise in population resulted from an increase in the birth rate, which, in turn, was associated with social, economic, and industrial changes in the eighteenth century.

McKeown, without doubt, has pursued the argument more consistently and with greater effect than any other researcher, and the reader is referred to his recent work for more detailed background information. Employing the data and techniques of historical demography, McKeown (a physician by training) has provided a detailed and convincing analysis of the major reasons for the decline of mortality in England and Wales during the eighteenth, nineteenth, and twentieth centuries (McKeown et al., 1955, 1962, 1975). For the eighteenth century, he concludes that the decline was largely attributable to improvements in the environment. His findings for the nineteenth century are summarized as follows:

> . . . the decline of mortality in the second half of the nineteenth century was due wholly to a reduction of deaths from infectious diseases; there was no evidence of a decline in other causes of death. Examination of the diseases which contributed to the decline suggested that the main influences were: (a) rising standards of living, of which the most significant feature was a better diet; (b) improvements in hygiene; and (c) a favorable trend in the relationship between some micro-organisms and the human host. *Therapy made no contributions, and the effect of immunization was restricted to smallpox which accounted for only about one-twentieth of the reduction of the death rate.* [Emphasis added. McKeown et al., 1975, p. 391]

While McKeown's interpretation is based on the experience of England and Wales, he has examined its credibility in the light of the very different circumstances which existed in four other European countries: Sweden, France, Ireland, and Hungary (McKeown et al., 1972). His interpretation appears to withstand this cross-examination. As for the twentieth century (1901–1971 is the period actually considered), McKeown argues that about three-quarters of the decline was associated with control of infectious diseases and the remainder with conditions not attributable to micro-organisms. He distinguishes the infections according to their modes of transmission (air- water- or food-borne) and isolates three types of influences which figure during the period considered: medical measures (specific therapies and immunization), reduced exposure to infection, and improved nutrition. His conclusion is that:

> the main influences on the decline in mortality were improved nutrition on air-borne infections, reduced exposure (from better hygiene) on water- and food-borne diseases and, less certainly, immunization and therapy on the large number of conditions included in the miscellaneous group. Since these three classes were responsible respectively for nearly half, one-sixth, and one-tenth of the fall in the death rate, it is probably that the advancement in nutrition was the major influence. [McKeown et al., 1975, p. 422]

More than twenty years of research by McKeown and his colleagues recently culminated in two books—*The Modern Rise of Population* (1976a) and *The Role of Medicine: Dream, Mirage or Nemesis* (1976b)—in which he draws together his many excellent contributions. That the thesis he advances remains highly newsworthy is evidenced by recent editorial reaction in *The Times* of London (1977).

No one in the United States has pursued this thesis with the rigor and consistency which characterize the work by McKeown and his colleagues in Britain. Around 1930, there were several limited discussions of the questionable effect of medical measures on selected infectious diseases like diptheria (Lee, 1931; Wilson and Miles, 1946; Bolduan, 1930) and pneumonia (Pfizer and Co., 1953). In a presidential address to the American Association of Immunologists in 1954 (frequently referred to by McKeown), Magill (1955) marshalled an assortment of data then available—some from England and Wales—to cast doubt on the plausibility of existing accounts of the decline in mortality for several conditions. Probably the most influential work in the United States is that of Dubos who, principally in *Mirage of Health* (1959), *Man Adapting* (1965), and *Man, Medicine and Environment* (1968), focused on the nonmedical reasons for changes in the health of overall populations. In another presidential address, this time to the Infectious Diseases Society of America, Kass (1971), again employing data from England and Wales, argued that most of the decline in mortality for most infectious conditions occurred prior to the discovery of either "the cause" of the disease or some purported "treatment" for it. Before the same society and largely on the basis of clinical experience with infectious diseases and data from a single state (Massachusetts), Weinstein (1974), while conceding there are some effective

treatments which seem to yield a favorable outcome (e.g., for poliomyelitis, tuberculosis, and possibly smallpox), argued that despite the presence of supposedly effective treatments some conditions may have increased (e.g., subacute bacterial endocarditis, streptococcal pharyngitis, pneumococcal pneumonia, gonorrhea, and syphilis) and also that mortality for yet other conditions shows improvement in the absence of any treatment (e.g., chickenpox). With the appearance of his book, *Who Shall Live?* (1974), Fuchs, a health economist, contributed to the resurgence of interest in the relative contribution of medical care to the modern decline in mortality in the United States. He believes there has been an unprecedented improvement in health in the United States since about the middle of the eighteenth century, associated primarily with a rise in real income. While agreeing with much of Fuchs' thesis, we will present evidence which seriously questions his belief that "beginning in the mid '30s, major therapeutic discoveries made significant contributions independently of the rise in real income."

Although neither representative nor exhaustive, this brief and selective background should serve to introduce the analysis which follows. Our intention is to highlight the following: (a) the debate over the questionable contribution of medical measures to the modern decline of mortality has a long history and remains topical; (b) although sometimes popularly associated with dilettantes such as Ivan Illich (1976), the debate continues to preoccupy able scholars from a variety of disciplines and remains a matter of concern to the most learned societies; (c) although of emerging interest in the United States, the issue is already a matter of concern and considerable research elsewhere; (d) to the extent that the subject has been pursued in the United States, there has been a restrictive tendency to focus on a few selected diseases, or to employ only statewide data, or to apply evidence from England and Wales directly to the United States situation.

How Reliable are Mortality Statistics?

We have argued elsewhere that mortality statistics are inadequate and can be misleading as indicators of a nation's overall health status (McKinlay and McKinlay, forthcoming). Unfortunately, these are the only types of data which are readily accessible for the examination of time trends, simply because comparable morbidity

and disability data have not been available. Apart from this overriding problem, several additional caveats in the use of mortality statistics are: (a) difficulties introduced by changes in the registration area in the United States in the early twentieth century; (b) that often no single disease, but a complex of conditions, may be responsible for death (Krueger, 1966); (c) that studies reveal considerable inaccuracies in recording the cause of death (Moriyama et al., 1958); (d) that there are changes over time in what it is fashionable to diagnose (for example, ischaemic heart disease and cerebrovascular disease); (e) that changes in disease classifications (Dunn and Shackley, 1945) make it difficult to compare some conditions over time and between countries (Reid and Rose, 1964); (f) that some conditions result in immediate death while others have an extended period of latency; and (g) that many conditions are severely debilitating and consume vast medical resources but are now generally non-fatal (e.g., arthritis and diabetes). Other obvious limitations could be added to this list.

However, it would be foolhardy indeed to dismiss all studies based on mortality measures simply because they are possibly beset *with known limitations.* Such data are preferable to those the limitations of which are either unknown or, if known, cannot be estimated. Because of an overawareness of potential inaccuracies, there is a timorous tendency to disregard or devalue studies based on mortality evidence, even though there are innumerable examples of their fruitful use as a basis for planning and informed social action (Alderson, 1976). Sir Austin Bradford Hill (1955) considers one of the most important features of Snow's work on cholera to be his adept use of mortality statistics. A more recent notable example is the study by Inman and Adelstein (1969) of the circumstantial link between the excessive absorption of bronchodilators from pressurized aerosols and the epidemic rise in asthma mortality in children aged ten to fourteen years. Moreover, there is evidence that some of the known inaccuracies of mortality data tend to cancel each other out.[2] Consequently, while mortality statistics may be unreliable for

[2]Barker and Rose cite one study which compared the ante-mortem and autopsy diagnoses in 9,501 deaths which occurred in 75 different hospitals. Despite lack of a concurrence on *individual* cases, the *overall* frequency was very similar in diagnoses obtained on either an ante-mortem or a post-mortem basis. As an example they note that clinical diagnoses of carcinoma of the rectum were confirmed at autopsy in only 67 percent of cases, but the incorrect clinical diagnoses were balanced by an almost identical number of lesions diagnosed for the first time at autopsy (Barker and Rose, 1976).

use in individual cases, when pooled for a country and employed in population studies, they can reveal important trends and generate fruitful hypotheses. They have already resulted in informed social action (for example, the use of geographical distributions of mortality in the field of environmental pollution).

Whatever limitations and risks may be associated with the use of mortality statistics, they obviously apply equally to all studies which employ them—both those which attribute the decline in mortality to medical measures and those which argue the converse, or something else entirely. And, if such data constitute acceptable evidence in support of the presence of medicine, then it is not unreasonable, or illogical, to employ them in support of some opposing position. One difficulty is that, depending on the nature of the results, double standards of rigor seem to operate in the evaluation of different studies. Not surprisingly, those which challenge prevailing myths or beliefs are subject to the most stringent methodological and statistical scrutiny, while supportive studies, which frequently employ the flimsiest impressionistic data and inappropriate techniques of analysis, receive general and uncritical acceptance. Even if all possible "ideal" data were available (which they never will be) and if, after appropriate analysis, they happened to support the viewpoint of this paper, we are doubtful that medicine's protagonists would find our thesis any more acceptable.

The Modern Decline in Mortality

Despite the fact that mortality rates for certain conditions, for selected age and sex categories, continue to fluctuate, or even increase (U.S. Dept. HEW, 1964; Moriyama and Gustavus, 1972; Lilienfeld, 1976), there can be little doubt that a marked decline in overall mortality for the United States has occurred since about 1900 (the earliest point for which reliable national data are available).

Just how dramatic this decline has been in the United States is illustrated in Fig. 1 which shows age-adjusted mortality rates for males and females separately.[3] Both sexes experienced a marked

[3]All age and sex adjustments were made by the "direct" method using the population of 1900 as the standard. For further information on this method of adjustment, see Hill (1971) and Shryock et al. (1971).

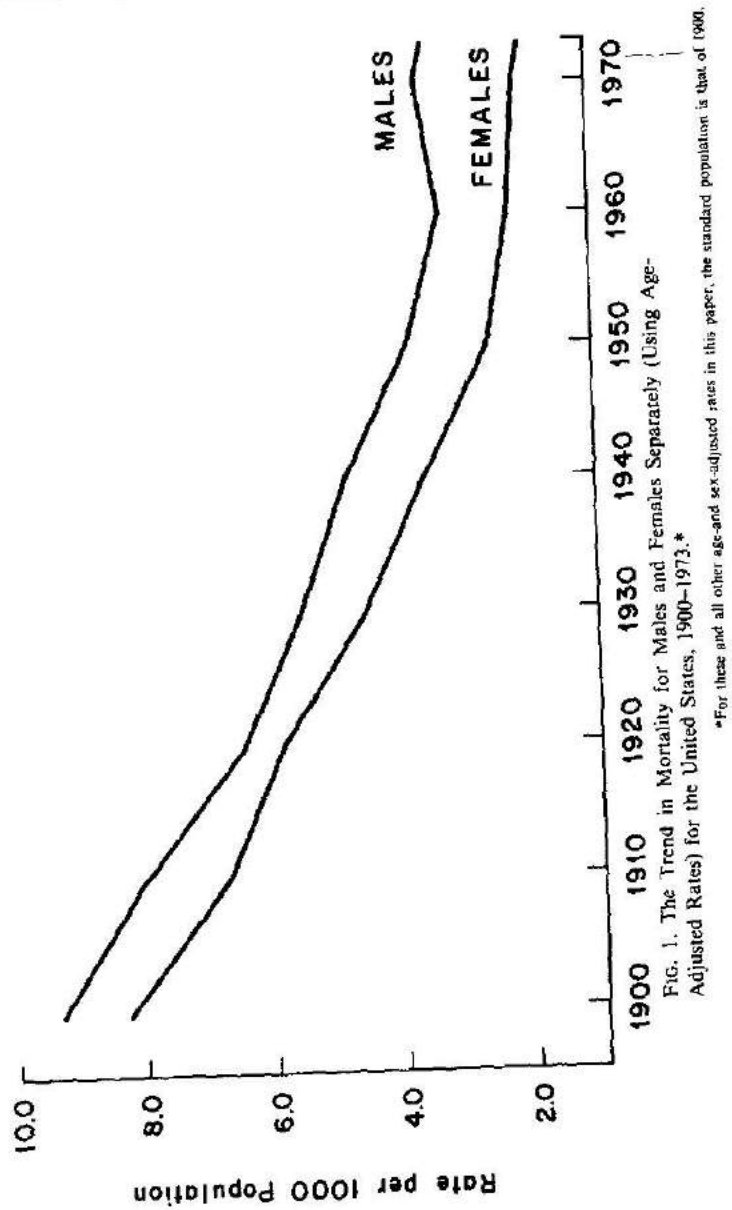

FIG. 1. The Trend in Mortality for Males and Females Separately (Using Age-Adjusted Rates) for the United States, 1900–1973.*

*For these and all other age-and sex-adjusted rates in this paper, the standard population is that of 1900.

decline in mortality since 1900. The female decline began to level off by about 1950, while 1960 witnessed the beginning of a slight increase for males. Figure 1 also reveals a slight but increasing divergence between male and female mortality since about 1920.

Figure 2 depicts the decline in the overall age- and sex-adjusted rate since the beginning of this century. Between 1900 and 1973, there was a 69.2 percent decrease in overall mortality. The average annual rate of decline from 1900 until 1950 was .22 per 1,000, after which it became an almost negligible decline of .04 per 1,000 annually. Of the total fall in the standardized death rate between 1900 and 1973, 92.3 percent occurred prior to 1950. Figure 2 also plots the decline in the standardized death rate *after* the total number of deaths in each age and sex category has been reduced by the number of deaths attributed to the eleven major infectious conditions (typhoid, smallpox, scarlet fever, measles, whooping cough, diphtheria, influenza, tuberculosis, pneumonia, diseases of the digestive system, and poliomyelitis). It should be noted that, although this latter rate also shows a decline (at least until 1960), its slope is much more shallow than that for the overall standardized death rate. A major part of the decline in deaths from these causes since about 1900 may be attributed to the virtual disappearance of these infectious diseases.

An absurdity is reflected in the third broken line in Fig. 2 which also plots the increase in the proportion of the Gross National Product expended annually for medical care. *It is evident that the beginning of the precipitate and still unrestrained rise in medical care expenditures began when nearly all (92 percent) of the modern decline in mortality this century had already occurred.*[4]

Figure 3 illustrates how the proportion of deaths contributed by infectious and chronic conditions has changed in the United States since the beginning of the twentieth century. In 1900, about 40 percent of all deaths were accounted for by eleven major infectious diseases, 16 percent by three chronic conditions, 4 percent by accidents, and the remainder (37 percent) by all other causes. By 1973, only 6 percent of all deaths were due to these eleven infectious

[4]Rutstein (1967), although fervently espousing the traditional view that medical advances have been largely responsible for the decline in mortality, discussed this disjunction and termed it "The Paradox of Modern Medicine." More recently, and from a perspective that is generally consistent with that advanced here, Powles (1973) noted the same phenomenon in England and Wales.

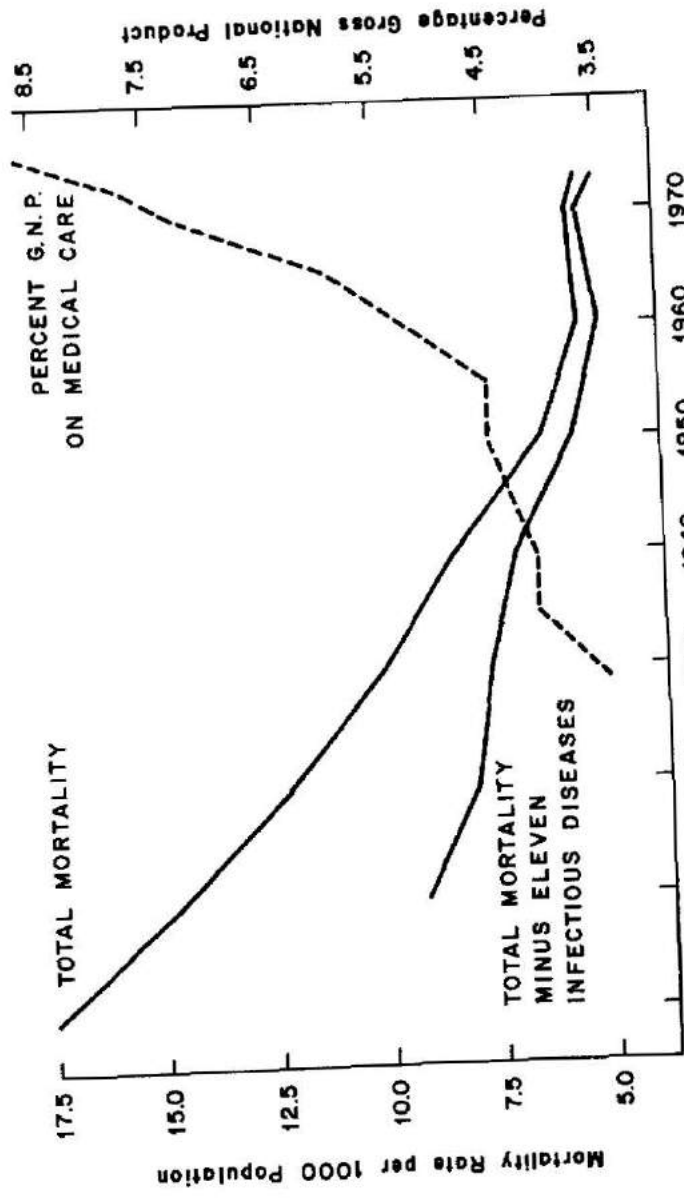

FIG. 2. Age-and Sex-Adjusted Mortality Rates for the United States 1900–1973, Including and Excluding Eleven Major Infectious Diseases, Contrasted with the Proportion of the Gross National Product Expended on Medical Care.

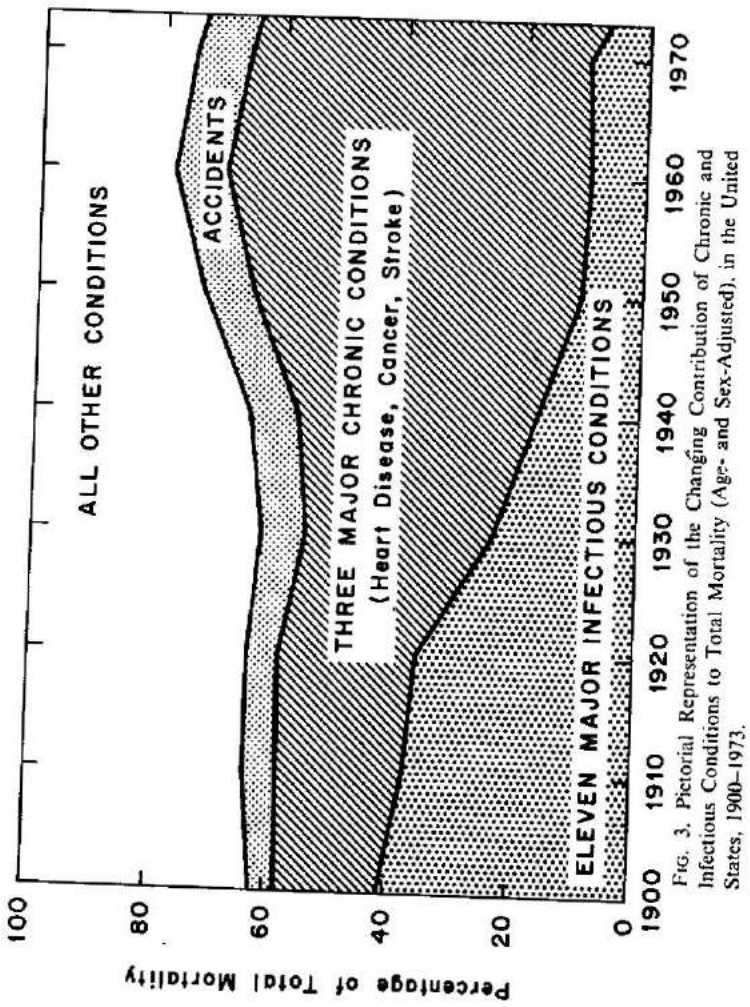

FIG. 3. Pictorial Representation of the Changing Contribution of Chronic and Infectious Conditions to Total Mortality (Age- and Sex-Adjusted), in the United States, 1900–1973.

diseases, 58 percent to the same three chronic conditions, 9 percent to accidents, and 27 percent were contributed by other causes.[5]

Now to what phenomenon, or combination of events, can we attribute this modern decline in overall mortality? Who (if anyone), or what group, can claim to have been instrumental in effecting this reduction? Can anything be gleaned from an analysis of mortality experience to date that will inform health care policy for the future?

It should be reiterated that a major concern of this paper is to determine the effect, if any, of specific medical measures (both chemotherapeutic and prophylactic) on the decline of mortality. It is clear from Figs. 2 and 3 that most of the observable decline is due to the rapid disappearance of some of the major infectious diseases. Since this is where most of the decline has occurred, it is logical to focus a study of the effect of medical measures on this category of conditions. Moreover, for these eleven conditions, there exist clearly identifiable medical interventions to which the decline in mortality has been popularly ascribed. No analogous interventions exist for the major chronic diseases such as heart disease, cancer, and stroke. Therefore, even where a decline in mortality from these chronic conditions may have occurred, this cannot be ascribed to any specific measure.

The Effect of Medical Measures on Ten Infectious Diseases Which Have Declined

Table 1 summarizes data on the effect of major medical interventions (both chemotherapeutic and prophylactic) on the decline in the age- and sex-adjusted death rates in the United States, 1900–1973, for ten of the eleven major infectious diseases listed above. Together, these diseases accounted for approximately 30 percent of all deaths at the turn of the century and nearly 40 percent of the total decline in the mortality rate since then. The ten diseases were selected on the following criteria: (a) some decline in the death rate had occurred in the period 1900–1973; (b) significant decline in the death rate is commonly attributed to some specific medical

[5]Deaths in the category of chronic respiratory diseases (chronic bronchitis, asthma, emphysema, and other chronic obstructive lung diseases) could not be included in the group of chronic conditions because of insurmountable difficulties inherent in the many changes in disease classification and in the tabulation of statistics.

TABLE 1

The Contribution of Medical Measures (Both Chemotherapeutic and Prophylactic) to the Fall in the Age and Sex-Adjusted Death Rates (S.D.R.) of Ten Common Infectious Diseases, and to the Overall Decline in the S.D.R., for the United States, 1900–1973

Disease	*Fall in S.D.R. per 1,000 Population, 1900–1973 (a)*	*Fall in S.D.R as % of the Total Fall in S.D.R. (b)=[(a)/12.14]×100%*	*Year of Medical Intervention (Either Chemotherapy or Prophylaxis)*	*Fall in S.D.R per 1,000 Population After Year of Intervention (c)*	*Fall in S.D.R After Intervention as % of Total Fall for the Disease (d)=[(c)/(a)]×100*	*Fall in S.D.R. After Intervention as % of Total Fall in S.D.R for All Causes (e)=[(b)(c)%/(a)]*
Tuberculosis	2.00	16.48	Izoniazid/ Streptomycin, 1950	0.17	8.36	1.38
Scarlet Fever	0.10	0.84	Penicillin, 1946	0.00	1.75	0.01
Influenza	0.22	1.78	Vaccine, 1943	0.05	25.33	0.45
Pneumonia	1.42	11.74	Sulphonamide, 1935	0.24	17.19	2.02
Diptheria	0.43	3.57	Toxoid, 1930	0.06	13.49	0.48
Whooping Cough	0.12	1.00	Vaccine, 1930	0.06	51.00	0.51
Measles	0.12	1.04	Vaccine, 1963	0.00	1.38	0.01
Smallpox	0.02	0.16	Vaccine, 1800	0.02	100.00	0.16
Typhoid	0.36	2.95	Chloramphenicol, 1948	0.00	0.29	0.01
Poliomyelitis	0.03	0.23	Vaccine, Salk/ Sabin, 1955	0.01	25.87	0.06

TABLE 2

Pair-Wise Correlation Matrix for 44 Countries, Between Four Measures of Health Status and Three Measures of Medical Care Input

	Variable	*Matrix of Coefficients*							
1.	Infant Mortality Rate (1972)								
2.	Crude Mortality Rate (1970–1972)	-0.14							
3.(a)	Life Expectancy (Males) at 25 Years	-0.14	-0.12						
3.(b)	Life Expectancy (Females) at 25 Years	-0.12	0.04	0.75					
4.(a)	Life Expectancy (Males) at 55 Years	-0.01	0.10	0.74	0.93				
4.(b)	Life Expectancy (Females) at 55 Years	-0.13	0.01	0.75	0.98	0.95			
5.	Population per Hospital Bed (1971–1973)	0.64	-0.30	0.05	-0.02	0.17	0.0		
6.	Population per Physician (1971–1973)	0.36	-0.30	0.11	0.04	0.16	0.07	0.70	
7.	Per Capita Gross National Product: In $U.S. Equivalent (1972)	-0.66	0.26	0.16	0.18	0.07	0.22	-0.56	-0.46
	Variable (by number)	1	2	3a	3b	4a	4b	5	6

Sources:

1. *United Nations Demographic Yearbook: 1974*, New York, United Nations Publications, 1975. (For the Crude and Infant Mortality Rates).
2. *World Health Statistics Annual:* 1972, Vol. I, Geneva, World Health Organization, 1975, pp. 780–783. (For the Life Expectancy Figures).
3. *United Nations Statistical Yearbook, 1973 and 1975*. New York, United Nations Publications, 25th and 27th issues, 1974 and 1976. (For the Population bed/physician ratios).
4. *The World Bank Atlas*. Washington, D.C., World Bank, 1975. (For the per capita Gross National Product).

measure for the disease; and (c) adequate data for the disease over the period 1900–1973 are available. The diseases of the digestive system were omitted primarily because of lack of clarity in diagnosis of specific diseases such as gastritis and enteritis.

Some additional points of explanation should be noted in relation to Table 1. First, the year of medical intervention coincides (as nearly as can be determined) with the first year of widespread or commercial use of the appropriate drug or vaccine.[6] This date does *not* necessarily coincide with the date the measure was either first discovered, or subject to clinical trial. Second, the decline in the death rate for smallpox was calculated using the death rate for 1902 as being the earliest year for which this statistic is readily available (U.S. Bureau of the Census, 1906). For the same reasons, the decline in the death rate from poliomyelitis was calculated from 1910. Third, the table shows the contribution of the decline in each disease to the total decline in mortality over the period 1900–1973 (column b). The overall decline during this period was 12.14 per 1,000 population (17.54 in 1900 to 5.39 in 1973). Fourth, in order to place the experience for each disease in some perspective, Table 1 also shows the contribution of the relative fall in mortality after the intervention to the overall fall in mortality since 1900 (column e). In other words, the figures in this last column represent the percentage of the total fall in mortality contributed by each disease after the date of medical intervention.

It is clear from column b that only reductions in mortality from tuberculosis and pneumonia contributed substantially to the decline in total mortality between 1900 and 1973 (16.5 percent and 11.7 percent, respectively). The remaining eight conditions *together* accounted for less than 12 percent of the total decline over this period. Disregarding smallpox (for which the only effective measure had been introduced about 1800), only influenza, whooping cough, and poliomyelitis show what could be considered substantial declines of 25 percent or more after the date of medical intervention. However, even under the somewhat unrealistic assumption of a constant (linear) rate of decline in the mortality rates, only whooping cough and poliomyelitis even approach the percentage which would have been expected. The remaining six conditions (tuberculosis, scarlet

[6]In determining the dates of intervention we relied upon: (a) standard epidemiology and public health texts; (b) the recollections of authorities in the field of infectious diseases; and (c) recent publications on the same subject.

fever, pneumonia, diphtheria, measles, and typhoid) showed negligible declines in their mortality rates subsequent to the date of medical intervention. The seemingly quite large percentages for pneumonia and diphtheria (17.2 and 13.5, respectively) must of course be viewed in the context of relatively early interventions—1935 and 1930.

In order to examine more closely the relation of mortality trends for these diseases to the medical interventions, graphs are presented for each disease in Fig. 4. Clearly, for tuberculosis, typhoid, measles, and scarlet fever, the medical measures considered were introduced at the point when the death rate for each of these diseases was already negligible. Any change in the rates of decline which may have occurred subsequent to the interventions could only be minute. Of the remaining five diseases (excluding smallpox with its negligible contribution), it is only for poliomyelitis that the medical measure appears to have produced any noticeable change in the trends. Given peaks in the death rate for 1930, 1950 (and possibly for 1910), a comparable peak could have been expected in 1970. Instead, the death rate dropped to the point of disappearance after 1950 and has remained negligible. The four other diseases (pneumonia, influenza, whooping cough, and diphtheria) exhibit relatively smooth mortality trends which are unaffected by the medical measures, even though these were introduced relatively early, when the death rates were still notable.

It may be useful at this point to briefly consider the common but dubious practice of projecting estimated mortality trends (Witte and Axnick, 1975). In order to show the beneficial (or even detrimental) effect of some medical measure, a line, estimated on a set of points observed prior to the introduction of the measure, is projected over the period subsequent to the point of intervention. Any resulting discrepancy between the projected line and the observed trend is then used as some kind of "evidence" of an effective or beneficial intervention. According to statistical theory on least squares estimation, an estimated line can serve as a useful predictor, but the prediction is only valid, and its error calculable, within the range of the points used to estimate the line. Moreover, those predicted values which lie at the extremes of the range are subject to much larger errors than those nearer the center. It is, therefore, probable that, even if the projected line was a reasonable estimate of the trend after the intervention (which, of course, it is not), the

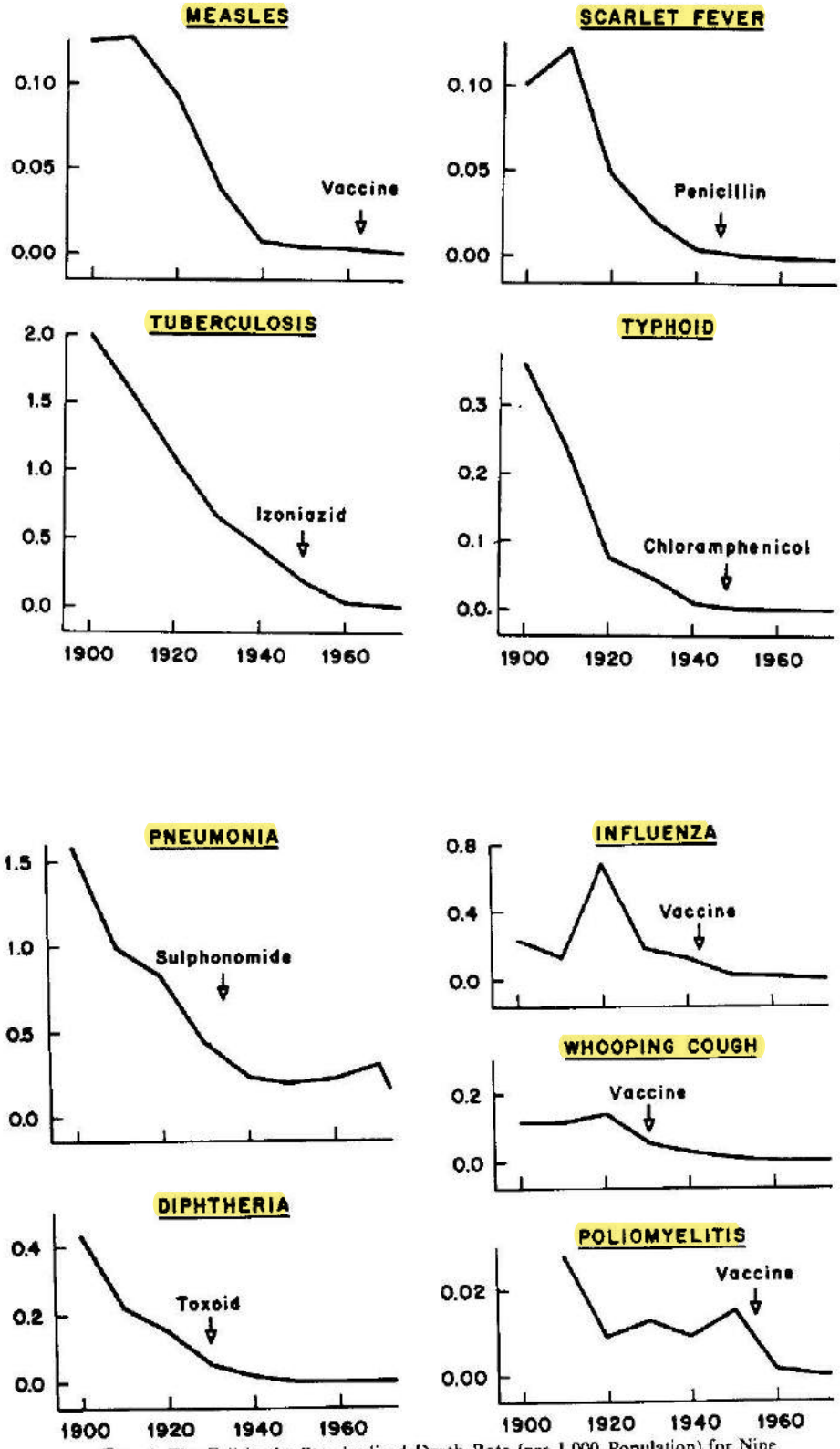

FIG. 4. The Fall in the Standardized Death Rate (per 1,000 Population) for Nine Common Infectious Diseases in Relation to Specific Medical Measures, for the United States, 1900–1973.

divergent observed trend is probably well within reasonable error limits of the estimated line (assuming the error could be calculated), as the error will be relatively large. In other words, this technique is of dubious value as no valid conclusions are possible from its application, and a relatively large prediction error cannot be estimated, which is required in order to objectively judge the extent of divergence of an observed trend.

With regard to the ten infectious diseases considered in this paper, when lines were fitted to the nine or ten points available over the entire period (1900–1973), four exhibited a reasonably good fit to a straight line (scarlet fever, measles, whooping cough, and poliomyelitis), while another four (typhoid, diphtheria, tuberculosis, and pneumonia) showed a very good quadratic fit (to a curved line). Of the remaining two diseases, smallpox showed a negligible decline, as it was already a minor cause of death in 1900 (only 0.1 percent), and influenza showed a poor fit because of the extremely high death rate in 1920. From Fig. 4 it is clear, however, that the rate of decline slowed in more recent years for most of the diseases considered—a trend which could be anticipated as rates approach zero.[7]

Now it is possible to argue that, given the few data points available, the fit is somewhat crude and may be insensitive to any changes subsequent to a point of intervention. However, this can be countered with the observation that, given the relatively low death rates for these diseases, any change would have to be extremely marked in order to be detected in the overall mortality experience. Certainly, from the evidence considered here, only poliomyelitis appears to have had a noticeably changed death rate subsequent to intervention. Even if it were assumed that this change was entirely due to the vaccines, then only about one percent of the decline following interventions for the diseases considered here (column d of Table 1) could be attributed to medical measures. Rather more conservatively, if we attribute some of the subsequent fall in the death rates for pneumonia, influenza, whooping cough, and diphtheria to medical measures, then perhaps 3.5 percent of the fall in the overall death rate can be explained through medical intervention in the major infectious diseases considered here. Indeed, given that it is precisely for these diseases that medicine claims most success in lowering mortality, 3.5 percent probably represents a reasonable upper-limit estimate of the total contribution of medical measures to the decline in mortality in the United States since 1900.

[7]For this reason, a negative exponential model is sometimes used to fit a curved line to such data. This was not presented here as the number of points available was small and the difference between a simple quadratic and negative exponential fit was not, upon investigation, able to be detected.

Conclusions

Without claiming they are definitive findings, and eschewing pretentions to an analysis as sophisticated as McKeown's for England and Wales, one can reasonably draw the following conclusions from the analysis presented in this paper:

In general, medical measures (both chemotherapeutic and prophylactic) appear to have contributed little to the overall decline in mortality in the United States since about 1900—having in many instances been introduced several decades after a marked decline had already set in and having no detectable influence in most instances. More specifically, with reference to those five conditions (influenza, pneumonia, diphtheria, whooping cough, and poliomyelitis) for which the decline in mortality appears substantial after the point of intervention—and on the unlikely assumption that all of this decline is attributable to the intervention—it is estimated that at most 3.5 percent of the total decline in mortality since 1900 could be ascribed to medical measures introduced for the diseases considered here.

These conclusions, in support of the thesis introduced earlier, suggest issues of the most strategic significance for researchers and health care legislators. Profound policy implications follow from either a confirmation or a rejection of the thesis. If one subscribes to the view that we are slowly but surely eliminating one disease after another because of medical interventions, then there may be little commitment to social change and even resistance to some reordering of priorities in medical expenditures. If a disease X is disappearing primarily because of the presence of a particular intervention or service Y, then clearly Y should be left intact, or, more preferably, be expanded. Its demonstrable contribution justifies its presence. But, if it can be shown convincingly, and on commonly accepted grounds, that the major part of the decline in mortality is unrelated to medical care activities, then some commitment to social change

and a reordering of priorities may ensue. For, if the disappearance of X is largely unrelated to the presence of Y, or even occurs in the absence of Y, then clearly the expansion and even the continuance of Y can be reasonably questioned. Its demonstrable ineffectiveness justifies some reappraisal of its significance and the wisdom of expanding it in its existing form.

In this paper we have attempted to dispel the myth that medical measures and the presence of medical services were primarily responsible for the modern decline in mortality. The question now remains: if they were not primarily responsible for it, then how is it to be explained? An adequate answer to this further question would require a more substantial research effort than that reported here, but is likely to be along the lines suggested by McKeown which were referred to early in this paper. Hopefully, this paper will serve as a catalyst for such research, incorporating adequate data and appropriate methods of analysis, in an effort to arrive at a more viable alternative explanation.

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Smith, J. 1961. Title of the article. *Journal of Communication* 125: 965–72.
Jones, J. S. 1953. *Title of the book.* Boston: Timely Press.
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History of Drinking Water Treatment

A Century of U.S. Water Chlorination and Treatment: One of the Ten Greatest Public Health Achievements of the 20th Century

American drinking water supplies are among the safest in the world. The disinfection of water has played a critical role in improving drinking water quality in the United States. In 1908, Jersey City, New Jersey was the first city in the United States to begin routine disinfection of community drinking water. Over the next decade, thousands of cities and towns across the United States followed suit in routinely disinfecting their drinking water, contributing to a dramatic decrease in disease across the country (Fig 1).

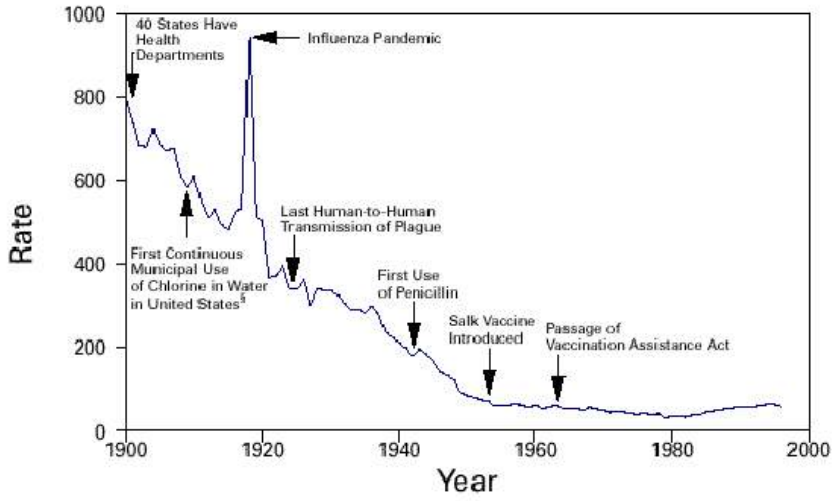


Figure 1. Crude death rate* for infectious diseases - United States, 1900-1996

*Per 100,000 population per year.

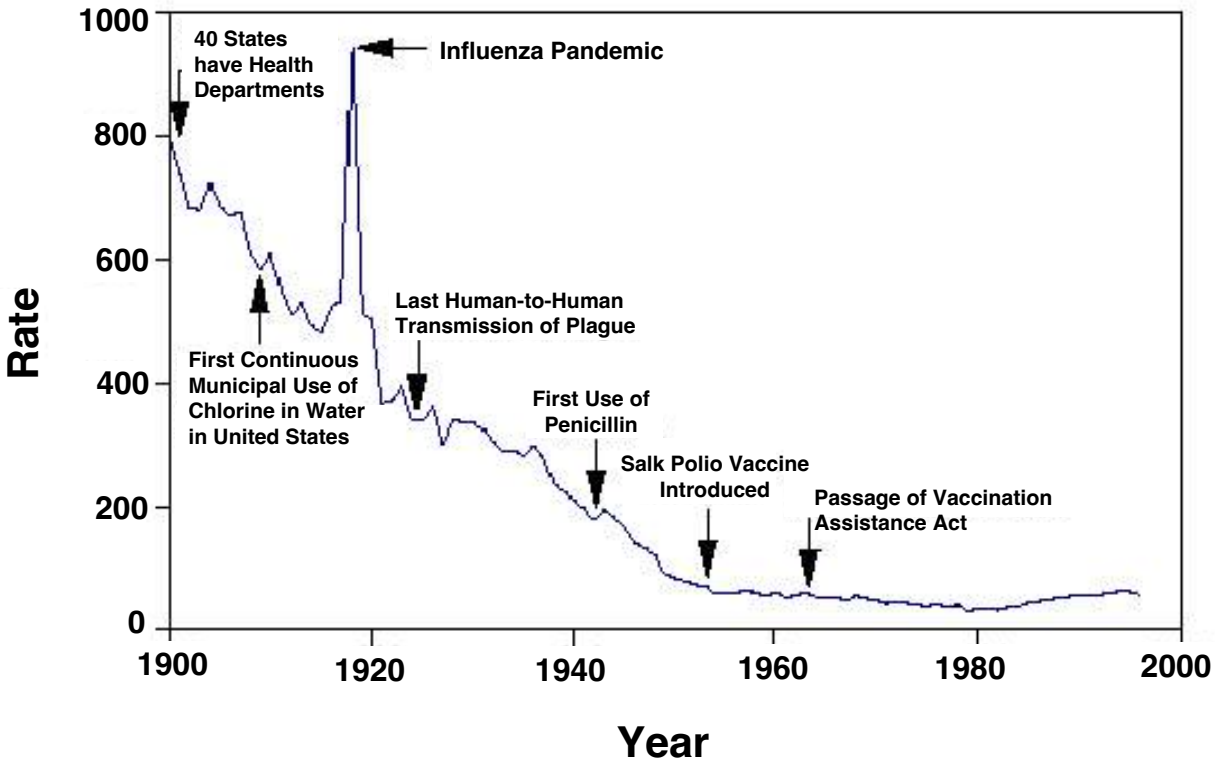
The occurrence of diseases such as cholera and typhoid dropped dramatically. In 1900, the occurrence of typhoid fever in the United States was approximately 100 cases per 100,000 people. By 1920, it had decreased to 33.8 cases per 100,000 people. In 2006, it had decreased to 0.1 cases per 100,000 people (only 353 cases) with approximately 75% occurring among international travelers. Typhoid fever decreased rapidly in cities from Baltimore to Chicago as water disinfection and treatment was instituted. This decrease in illness is credited to the implementation of drinking water disinfection and treatment, improving the quality of source water, and improvements in sanitation and hygiene.

It is because of these successes that we can celebrate over a century of public drinking water disinfection and treatment – one of the greatest public health achievements of the 20th century.

CDC. A Century of U.S. Water Chlorination and Treatment: One of the Ten Greatest Public Health Achievements of the 20th Century. Morb Mortal Wkly Rep. 1999;48(29):621-9.

Page last reviewed: November 26, 2012

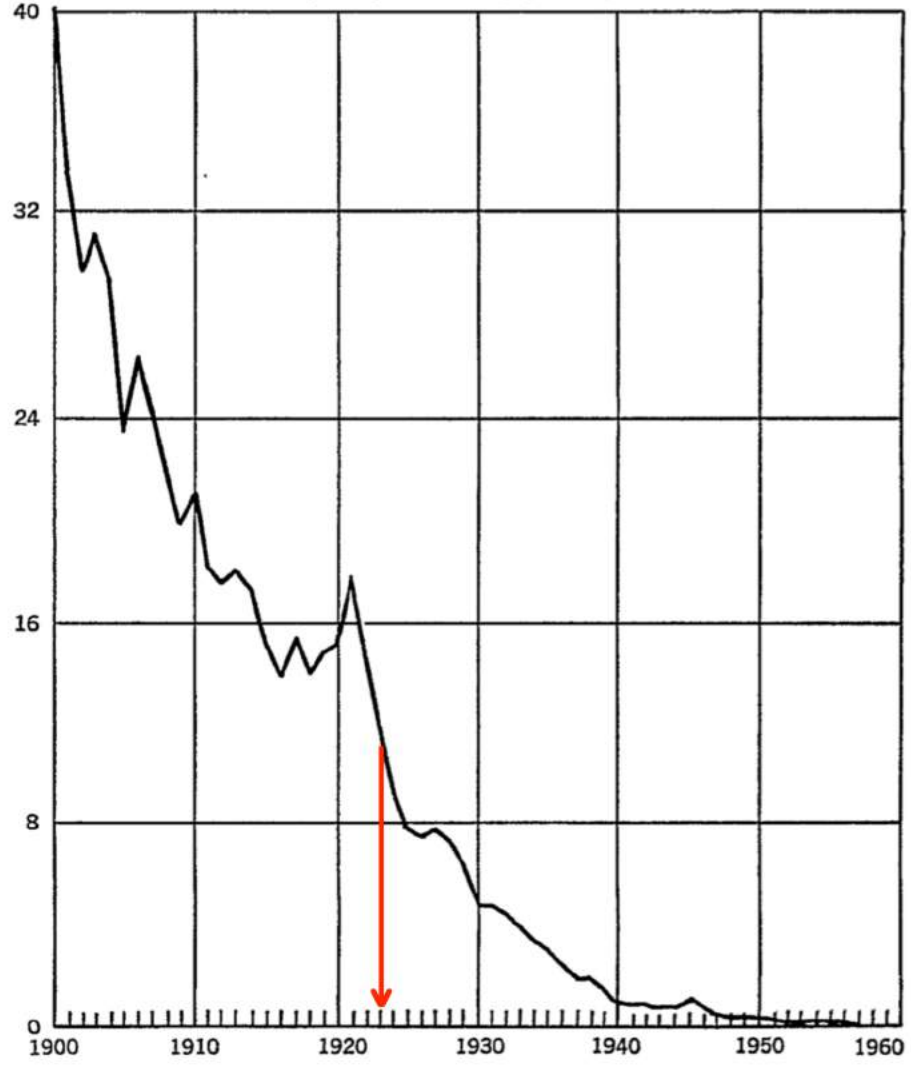
Figure 1. Crude Death Rate* for Infectious Diseases - United States, 1900-1996



*Per 100,000 population per year.

Figure 18.—Death Rates for Diphtheria: Death-registration States, 1900–32, and United States, 1933–60

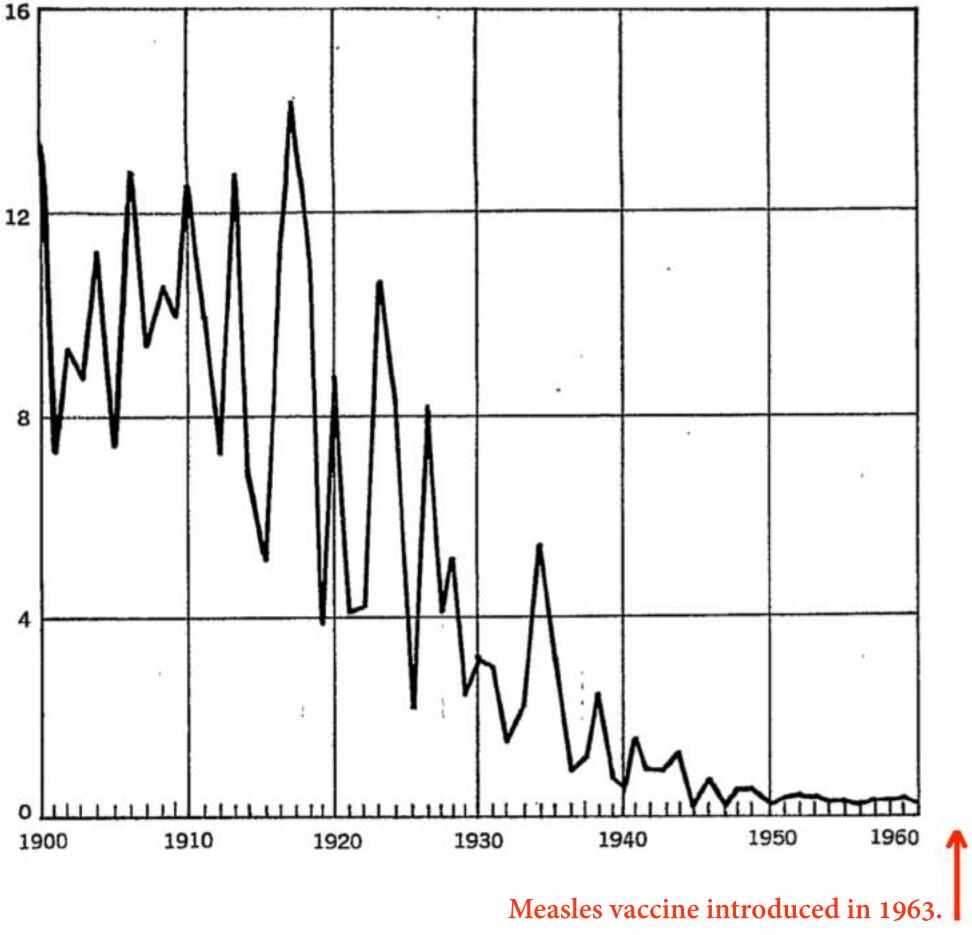
(Rates per 100,000 population)



Diphtheria vaccine introduced in 1923,
but not widely used until the 1930s.

Figure 19.—Death Rates for Measles: Death-registration States, 1900–32, and United States, 1933–60

(Rates per 100,000 population)



Measles vaccine introduced in 1963.

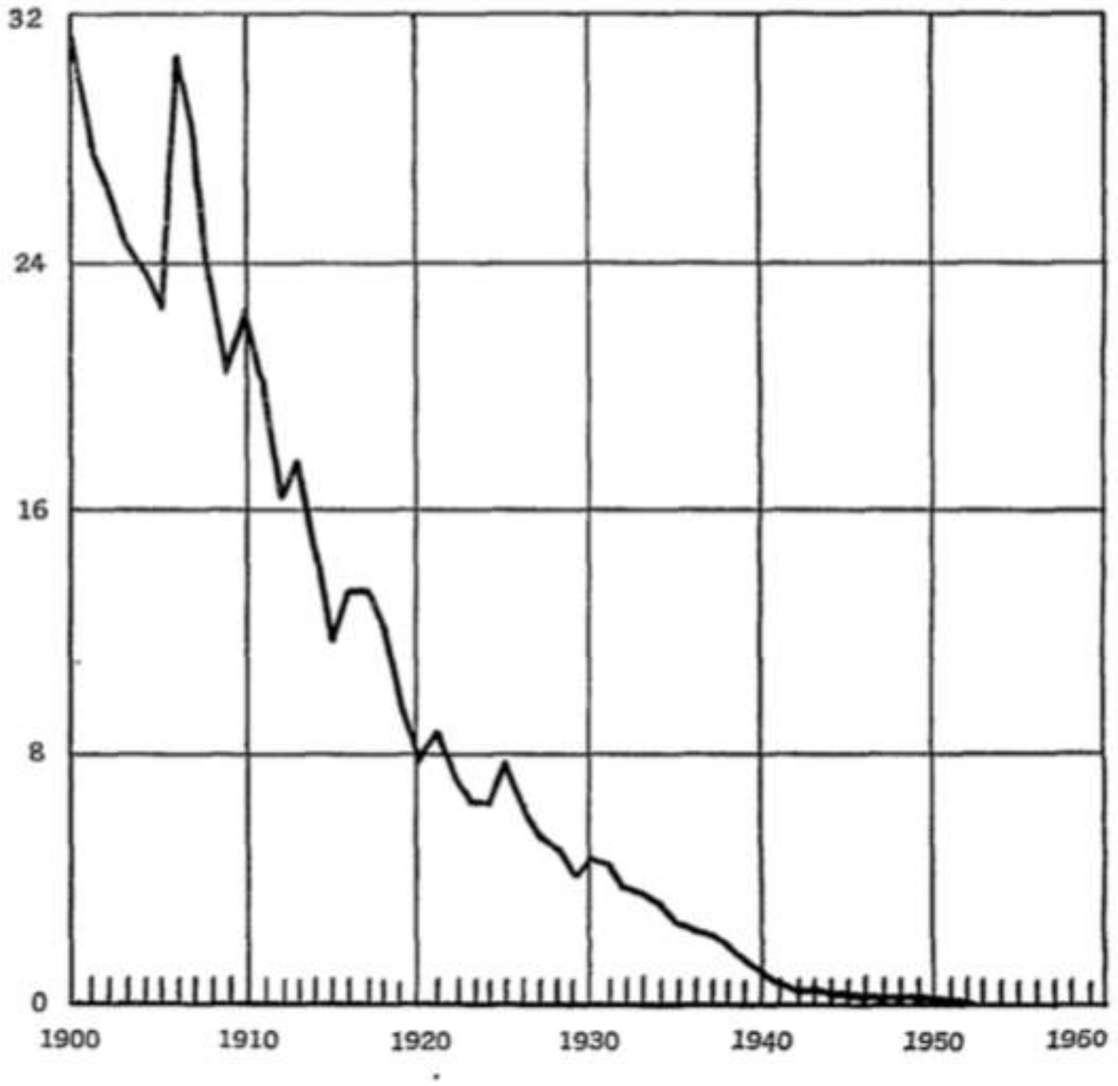
Source: https://www.cdc.gov/nchs/data/vsus/vsrates1940_60.pdf

Figure 14.—Death Rates for Tuberculosis, All Forms: Death-registration States, 1900–32, and United States, 1933–60

(Rates per 100,000 population)

Figure 16.—Death Rates for Typhoid Fever: Death-registration States, 1900–32, and United States, 1933–60

(Rates per 100,000 population)



No widespread vaccination, similar decline.

JAMA. 1993 Jan 13;269(2):221-6.

Decline of childhood Haemophilus influenzae type b (Hib) disease in the Hib vaccine era

W G Adams [1], K A Deaver, S L Cochi, B D Plikaytis, E R Zell, C V Broome, J D Wenger

Affiliations
PMID: 8417239

Abstract

Objective: Effective Haemophilus influenzae type b (Hib) conjugate vaccines were first licensed for use in US children at least 18 months old in December 1987 and for infants at least 2 months old in October 1990. We evaluated trends in Hib disease associated with licensure of Hib conjugate vaccines.

Design: Data from two sources, an intensive laboratory-based active surveillance system and the National Bacterial Meningitis Reporting System (NBMRS), were used separately to evaluate disease incidence. Data from vaccine manufacturers on Hib vaccine doses distributed in the United States were compared with trends in Hib disease incidence.

Results: The age-specific incidence of Hib disease among children less than 5 years old decreased by 71% from 37 per 100,000 persons in 1989 to 11 per 100,000 persons in 1991 (active surveillance data). Haemophilus influenzae meningitis incidence decreased by 82% between 1985 and 1991 (NBMRS data). Increases in doses of Hib vaccine distributed in the United States coincided with steep declines in Hib disease. Both surveillance systems showed decreased rates of Hib disease in infants less than 1 year old before vaccine was licensed for use in this age group. Haemophilus influenzae type b disease incidence in persons at least 12 years old and pneumococcal meningitis incidence in children less than 5 years old did not change substantially during the same period; therefore, decreased Hib disease in children less than 5 years old is not likely to be explained solely by changes in surveillance sensitivity or decreases in bacterial disease due to changes in medical practice.

Conclusion: Our data suggest that conjugate vaccines have already had a marked impact on the incidence of Hib disease in the United States, preventing an estimated 10,000 to 16,000 cases of Hib disease in 1991. The decline of disease in infants less than 1 year old before licensure for this age group warrants further investigation.

July 28, 1995 / 44(29);545-550

Progress Toward Elimination of Haemophilus influenzae Type b Disease Among Infants and Children -- United States, 1993-1994

Before effective vaccines were available, Haemophilus influenzae type b (Hib) was the most common cause of bacterial meningitis among children in the United States. Since the introduction of Hib conjugate vaccines in 1988, the incidence of invasive Hib infection has declined by at least 95% among infants and children (1,2). As part of the Childhood Immunization Initiative (CII), the Public Health Service has included Hib disease among children aged less than 5 years as one of the vaccine-preventable diseases targeted for elimination in the United States by 1996 (3). This report summarizes provisional data about invasive Hi disease during 1993-1994 based on information from three surveillance systems: the National Notifiable Diseases Surveillance System (NNDSS), the National Bacterial Meningitis and Bacteremia Reporting System (NBMBRS), and a multistate laboratory-based surveillance system. National Surveillance

State health agencies reported weekly provisional notifiable disease data to NNDSS through the National Electronic Telecommunications System for Surveillance (NETSS) (4,5). Because the primary purpose of NNDSS is timely nationwide surveillance, the information transmitted included only basic demographic data about persons with invasive Hi disease. The capacity for the electronic transmission of critical supplemental information (e.g., the type of clinical illness, serotype causing disease, Hib vaccination status, and clinical outcome) for cases of Hi disease is available through NETSS and is used consistently by approximately half of the states. NBMBRS is a collaborative effort initiated in 1977 by CDC, state health departments, and the Council of State and Territorial Epidemiologists to collect information about invasive bacterial diseases in the United States. NBMBRS includes detailed information about each case identical to the supplemental information transmitted through NETSS. Approximately 20 states participate consistently in reporting through the NBMBRS.

From 1993 to 1994, the incidence of invasive Hi disease among children aged less than 5 years reported to the NNDSS decreased 29% (from 2.4 cases per 100,000 to 1.7 cases per 100,000, respectively), a trend similar to that reported for 1992-1993 Figure 1 (2). However, the total number of cases among children aged less than 5 years reported during the first 4 months of 1995 (105) is similar to that during the same period in 1994 (104).

Supplemental case information was reported to CDC by 35 states and was obtained on request from the remaining states. Of the 340 cases of invasive Hi disease among children aged less than 5 years reported in 1994, supplemental information was available for 259 (76%). Of these, serotype data were available for 139 (54%) -- 41% of all reported cases. Hib accounted for 82 (59%) of the isolates for which serotype was known. Of the 60 (73%) cases of Hib disease for which information on age and vaccination status was available, none of the 12 children aged greater than 15 months had received four doses of Hib vaccine Table 1. Two of the 19 children aged 7-15 months had received three vaccine doses, while most (17) had not completed the recommended primary series. Nearly half (29) were aged less than or equal to 6 months, below the age recommended for completion of the full three-dose primary series of the most commonly used Hib vaccines; of these, five had received two doses of vaccine. Laboratory-Based Surveillance

The laboratory-based system coordinated by CDC includes surveillance projects with a total population of 10.4 million persons in four areas (three counties in the San Francisco Bay area, eight counties in metropolitan Atlanta, four counties in Tennessee, and the state of Oklahoma). Information routinely obtained for all cases of invasive Hi disease included serotype, clinical syndrome, outcome, vaccination status, and demographic information. Because blacks were overrepresented in the surveillance population, rates were race-adjusted to the 1990 age-specific U.S. population.

The incidence of Hib disease among children aged less than 5 years declined from 1989 to 1993 but was stable from 1993 to 1994 (1.5 and 1.4 cases per 100,000, respectively) Figure 2. Information about vaccination status was available for eight of the 10 children aged less than 5 years with invasive Hib disease reported in 1994. None of the infants had received two or more doses of vaccine, although three were aged 8 months and should have received three doses. The two children for whom vaccination information was not available were aged greater than 16 months.

Based on a projection of these age-specific and race-adjusted incidence rates, an estimated 280 cases of Hib disease occurred among children aged less than 5 years in 1994 compared with an estimated 290 cases in 1993. During 1993 and 1994, Hib accounted for 37% of all the Hi isolates obtained from children aged less than 5 years. Reported by: G Rothbrock, Bur of Disease Control, Oakland, California. L Smithee, MS, Oklahoma State Dept of Health. M Rados, MS, Dept of Preventive Medicine, Vanderbilt Medical Center, Nashville, Tennessee. W Baughman, MSPH, Veterans' Administration Medical Svcs, Atlanta. National Immunization Program; National Center for Infectious Diseases; Epidemiology Program Office, CDC.

Editorial Note

Editorial Note: The goal to eliminate Hib disease among children aged less than 5 years is feasible because of the availability of Hib conjugate vaccines that are efficacious in children and reduce carriage of the organism, thereby interrupting transmission of infection. During 1988-1992, the incidence of invasive Hib disease declined rapidly among children; however, the findings in this report indicate that, since 1992, the rate of decline among children has slowed. This report also underscores two barriers to the elimination of invasive Hib disease among children: 1) the absence of accurate national surveillance for Hib incidence because of the lack of serotype information for most invasive Hi disease cases among children, and 2) the continued occurrence of disease among undervaccinated children and among infants too young to have completed the primary series of Hib vaccination.

Serotype information for cases of invasive Hi disease is essential to evaluate the changing epidemiology of Hib disease during a period of low disease incidence. Surveillance data indicate that a decreasing proportion of Hi cases are caused by Hib -- which in the past was responsible for greater than 90% of all Hi disease. Thus, the decline in the incidence of Hi disease among children observed in NNDSS data for 1994 may not have resulted from a reduction in Hib disease; data from laboratory-based surveillance suggests that, during 1993-1994, incidence of Hib disease remained stable. Because serotype information could be obtained for only 41% of cases reported to the NNDSS in 1994, the true incidence of Hib disease among children in the United States cannot be estimated from these data. In the national surveillance data, the higher proportion of Hib among Hi isolates of known serotype probably reflects incomplete serotyping information and preferential reporting of Hib cases in the national data.

Both national and laboratory-based surveillance findings indicate that Hi disease now occurs primarily among undervaccinated children and among infants too young to have completed the primary series of vaccination. However, based on the findings from CDC's National Health Interview Survey, the quarterly levels of coverage with three or more doses of Hib vaccine among children aged 19-35 months increased significantly from the third quarter of 1993 (60%) to the second quarter of 1994 (76%) (6). Although overall Hib vaccination coverage may be increasing, population groups with low levels of vaccination coverage probably contribute to the ongoing occurrence of disease (7).

The findings in this report indicate that no cases of vaccine failure were identified through laboratory-based surveillance in a population of 10.5 million. The small proportion of Hib cases reported through national surveillance among children who had received at least three doses of Hib vaccine suggests vaccine failure occurs infrequently, but is still consistent with previous reports showing extremely high efficacy of current vaccines (8-10). As a larger proportion of Hib cases is detected and investigated, more complete evaluations of cases among fully vaccinated persons will be possible.

To meet the 1996 CII objectives to eliminate invasive Hib disease among children aged less than 5 years, CDC recommends two measures. First, national surveillance for Hi should be strengthened. To optimize surveillance efforts, case reports should satisfy four criteria: 1) because Hib vaccines protect against Hi serotype b organisms only, serotyping should be obtained for all cases of invasive Hi disease -- state health departments are encouraged to identify laboratories to ensure that serotyping is available for all Hi isolates; 2) to improve characterization of groups at risk for undervaccination and Hib disease, vaccination status of all children with invasive Hib disease should be assessed; 3) to ensure continued high levels of vaccine effectiveness and to enable systematic evaluation of factors associated with vaccine failure in persons with Hib disease, the date, vaccine manufacturer, and lot number for each Hib vaccination should be reported; and 4) important indicators of the severity of Hi infections should be reported, including the type of clinical syndrome, specimen source (e.g., cerebrospinal fluid, blood, or joint fluid), and clinical outcome. Second, timely vaccination and vaccine coverage should be increased. Because conjugate vaccines reduce Hib carriage and interrupt transmission of the organism, timely vaccination of all children also should eliminate disease among infants who are too young to be completely vaccinated.

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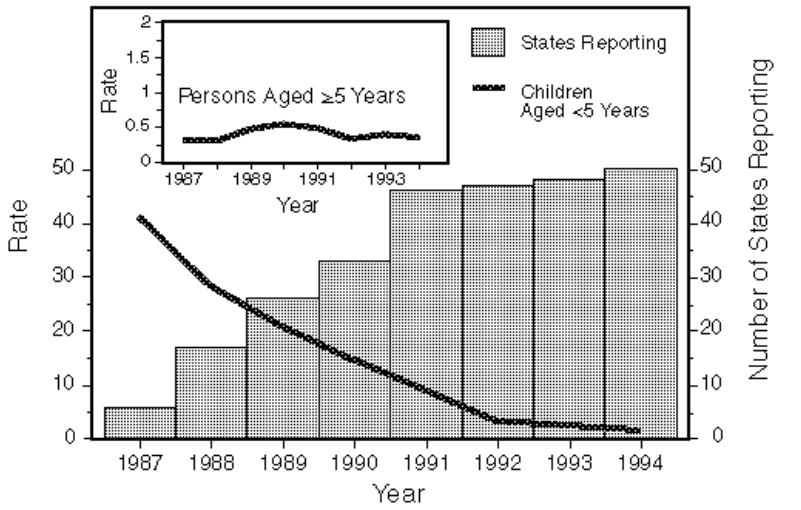
Figure_1

FIGURE 1. Incidence rate* of invasive *Haemophilus influenzae* (Hi) disease among children aged <5 years, incidence rate† of invasive Hi among persons aged ≥5 years, and number of states reporting Hi surveillance data — United States, National Notifiable Diseases Surveillance System, 1987–1994§



*Per 100,000 children aged <5 years.
†Per 100,000 persons aged ≥5 years.
§Because of the low number of states reporting surveillance data during 1987–1990, rates for those years were race-adjusted using the 1990 U.S. population.

Table_1

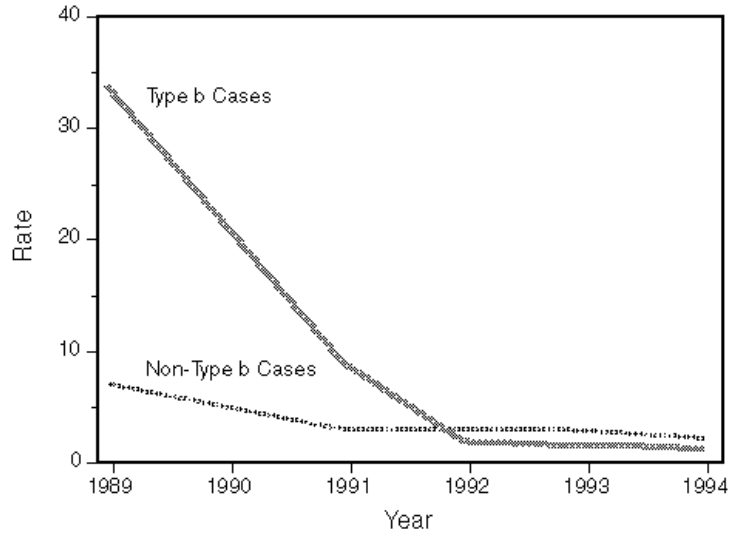
TABLE 1. Number of children aged <5 years with invasive Haemophilus influenzae type B (Hib) disease, by age group and number of Hib vaccine doses received -- United States, 1994 *

Age group (mos)	No. vaccine doses + 0	1	2	3	Total
0– 3	9	8	0	0	17
4– 6	1	6	5	0	12
7–15	6	5	6	2	19
16–59	7	1	0	4 &	12
Total	23	20	11	6	60

* Reported through the National Notifiable Diseases Surveillance System and the National Bacterial Meningitis and Bacteremia Reporting System.
\+ Doses administered within 10 days of onset of illness were not included.
& These children were aged 2 years (two), 3 years (one), and 4 years (one).

Figure_2

FIGURE 2. Race-adjusted incidence rate* of invasive *Haemophilus influenzae* type b and non-type b disease detected through laboratory-based surveillance† among children aged <5 years — United States, 1989–1994



*Per 100,000 population.
†The surveillance area population is 10.4 million in four areas (three counties in the San Francisco Bay area, eight counties in metropolitan Atlanta, four counties in Tennessee, and the state of Oklahoma).

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This page last reviewed 5/2/01

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Vaccination Coverage of 2-Year-Old Children -- United States, 1992- 1993

The principal goal of the Childhood Immunization Initiative (CII) is to increase, by 1996, vaccination levels for 2-year-old children to at least 90% for the most critical doses in the vaccination series (i.e., one dose of measles-mumps-rubella vaccine {MMR} and at least three doses each of diphtheria and tetanus toxoids and pertussis vaccine {DTP}, oral poliovirus vaccine {OPV}, and Haemophilus influenzae type b vaccine {Hib}) and to at least 70% for at least three doses of hepatitis B vaccine (Hep B) (1). Since 1991, annual national estimates of vaccination coverage levels of preschool-aged children have been available through the National Health Interview Survey (NHIS) conducted by CDC (2,3). This report presents vaccination coverage levels of children aged 19-35 months for 1992 and provisional estimates of vaccination coverage for the combined first and second quarters of 1993 (Table 1). Vaccination coverage increased for three vaccines from 1992 to 1993: for three or more doses of Hib, from 28.0% to 49.9% (p less than 0.05); for three or more doses of poliomyelitis vaccine, from 72.4% to 78.4% (p less than 0.05); and for three or more doses of DTP/ diphtheria and tetanus toxoids (DT), from 83.0% to 87.2% (p greater than 0.05). Coverage with measles-containing vaccine decreased from 82.5% to 80.8% (p greater than 0.05). Among 19- 35-month-olds, 12.7% had received three or more doses of Hep B. From 1992 to 1993, the proportion of children who had received a combined series of four or more doses of DTP/DT, three or more doses of polio vaccine, and one dose of MMR increased from 55.3% to 64.8% (p less than 0.05), primarily because of increased coverage with the fourth DTP/DT dose (from 59.0% to 71.1% {p less than 0.05}).

Reported by: National Immunization Program; Div of Health Interview Statistics, National Center for Health Statistics, CDC.

Editorial Note

Editorial Note: In 1993, processing of the NHIS was modified to produce national vaccination coverage estimates for each quarter. The findings in this report represent the first provisional quarterly estimates and indicate substantial progress in efforts to attain the 1996 antigen-specific vaccination goals for DTP and polio vaccine. However, coverage with measles-containing vaccines has not improved since 1991, when 82.0% of 2-year-old children were reported to be vaccinated. Although the coverage levels for Hib and hepatitis B remain suboptimal, the levels described in this report may underestimate coverage because many children were born before the recommendations for universal infant vaccination that were promulgated in October 1990 (4) and November 1991 (5). Less than 1% of 19-35-month-old children surveyed during January-June 1993 were born after recommendations for universal infant vaccination against hepatitis B went into effect. Similarly, only approximately two thirds of the children aged 19-35 months included in this survey were born after October 1990 -- when Hib was approved for infants. Provisional results from NHIS for the first two quarters of 1993 indicate that the combined efforts of public and private health-care providers at local, state, and national levels have facilitated progress toward both the 1996 CII goal and the year 2000 national health objective to increase vaccination levels for 2-year-olds to 90% (objective 20.11) for the complete series of recommended vaccine doses against all nine diseases (i.e., four or more doses of DTP, three or more doses of OPV, three or more doses of Hib, one dose of MMR, and three or more doses of Hep B) (6). However, based on the reported 1993 coverage levels, approximately 1.25 million children require at least one dose of OPV, and 1.12 million require a dose of measles-containing vaccine; approximately 740,000 children have not received at least three doses of DTP/DT. These findings emphasize the need for public and private health-care providers and local, state, and national public health officials to collaborate on implementation of the CII to achieve higher levels of vaccination coverage among 2-year-olds.

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Table_1

TABLE 1. Vaccination levels of children aged 19-35 months, by selected vaccines -- United States, 1992 and 1993 *

Vaccine	1992 %	1992 (95% CI +)	1993 %	1993 (95% CI)
DTP/DT &				
>=3 doses	83.0	(80.8-85.2)	87.2	(84.3-90.4)
>=4 doses	59.0	(56.1-61.9)	71.1	(67.1-75.1)
Poliomyelitis				
>=3 doses	72.4	(70.1-74.7)	78.4	(74.8-82.0)
Haemophilus influenzae type b				
>=3 doses	28.2	(25.6-30.9)	49.6	(45.4-53.8)
Measles-containing	82.5	(80.2-84.8)	80.8	(77.2-84.4)
Hepatitis B				
>=3 doses	--	--	12.7	(9.4-16.0)
3 DTP/3 polio/1 MMR @	68.7	(66.2-71.2)	72.0	(68.1-75.9)
4 DTP/3 polio/1 MMR	55.3	(52.5-58.1)	64.8	(60.6-68.9)

* Provisional data based on first and second quarters.
+ Confidence interval.
& Diphtheria and tetanus toxoids and pertussis vaccine/Diphtheria and tetanus toxoids.
@ Measles-mumps-rubella vaccine.

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Health Benefits of Temporary Infections

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Common infections in the history of cancer patients and controls

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PMID: 2066354 DOI: 10.1007/BF01630717

Abstract

The association between the frequency of manifest infectious diseases and cancer risk was investigated in a case-control study at Heidelberg, FRG. A total of 255 cases with carcinomas of the stomach, colon, rectum, breast, and ovary, as well as 255 population controls and 230 hospital controls were interviewed using a standard questionnaire. Controls were matched to the cases for age, sex, and region of residence at the time of the interview. A history of common colds or gastroenteric influenza prior to the interview was found to be associated with a decreased cancer risk. Thus the odds ratios for "three or more common colds per year (on average)" versus "no common cold within the last 5 years prior to the interview" were 0.18 (95% CI = 0.05-0.69) and 0.23 (95% CI = 0.06-0.89) relative to population controls and hospital controls, respectively. There was no apparent relationship between childhood infections or other diseases reported in the earlier history, and cancer risk. While the findings are supported by previous studies and fit well into the results of other fields of cancer research, a conclusive interpretation and biological explanation cannot yet be given.

Med Hypotheses. 1998 Oct;51(4):315-20. doi: 10.1016/s0306-9877(98)90055-x.

Febrile infectious childhood diseases in the history of cancer patients and matched controls

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Abstract

The present study was designed to investigate the hypothesis that febrile infectious childhood diseases (FICDs) are associated with a lower cancer risk in adulthood, since biographical considerations are of great importance in anthroposophic medicine. Cancer patients and control patients of 35 anthroposophic general practitioners in Switzerland were matched with respect to gender, age and physician. All patients completed a questionnaire on their FICD. We collected 424 cases; of these we could analyze 379 matched pairs. The study consistently revealed a lower cancer risk for patients with a history of FICD. The strongest associations were found between patients with non-breast cancers and rubella respectively chickenpox. A strong association was also found with the overall number of FICD both 'classical' (measles, mumps, rubella, pertussis, scarlet-fever and chickenpox) and 'other'. None of these associations was apparent for patients with breast cancer. Unexpectedly, we found that cancer was diagnosed significantly earlier in life in cancer patients with a history of FICD compared to those without FICD. Our retrospective study showed a significant association between FICD and the risk of developing cancer. The number of FICD decreased the cancer risk, in particular for non-breast cancers. The relationship with tumor site seems to be important also, but can only be addressed in a larger study.

Review Neuroimmunomodulation. 2001;9(2):55-64. doi: 10.1159/000049008.

Fever, cancer incidence and spontaneous remissions

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Abstract

Objective: Accumulating evidence exists for (1) an inverse correlation between the incidence of infectious diseases and cancer risk and (2) an inverse correlation between febrile infections and remissions of malignancies. This review is part of an effort of the Office of Alternative Medicine at the National Institutes of Health to examine this evidence.

Methods: A review of the literature to a key word search was undertaken, using the following key words: fever, infectious diseases, neoplasm, cancer incidence and spontaneous remission.

Results: The data reviewed in this article support earlier observations on the topic, i.e. that the occurrence of fever in childhood or adulthood may protect against the later onset of malignant disease and that spontaneous remissions are often preceded by feverish infections.

Conclusion: Pyrogenic substances and the more recent use of whole-body hyperthermia to mimic the physiologic response to fever have successfully been administered in palliative and curative treatment protocols for metastatic cancer. Further research in this area is warranted.

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Infectious diseases in the first year of life, perinatal characteristics and childhood acute leukaemia

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The objective of the present study was to investigate the role of early common infections and perinatal characteristics in the aetiology of childhood common leukaemia. A case–control study was conducted from 1995 to 1998 in France, and included 473 incident cases of acute leukaemia (AL) (408 acute lymphoblastic leukaemia (ALL), 65 acute myeloid leukaemia (AML) age-, sex- and region-matched with 567 population-based controls. Data on the medical history of the child and his/her environment were collected using self-administered questionnaires. Analyses were conducted using nonconditional logistic regression. A slight negative association with early infections was observed (OR = 0.8; 95% CI (0.6–1.0)). The association was stronger for early gastrointestinal infections. Early day-care was found to be associated with a decreased risk of AL (OR = 0.6; 95% CI (0.4–0.8) and OR = 0.8; 95% CI (0.5–1.2) for day-care starting before age 3 months and between 3 and 6 months, respectively). No association with breast-feeding was observed, irrespective of its duration. A birth order of 4 or more was associated with a significantly increased risk of AL (OR = 2.0; 95% CI (1.1–3.7) with ALL). A history of asthma was associated with a decreased risk of ALL (OR 0.5; 95% CI (0.3–0.90). Although the results regarding birth order and breast-feeding do not fit with Greaves' hypothesis, the study supports the hypothesis that early common infections may play a protective role in the aetiology of childhood leukaemia, although this effect was not more marked for common ALL.
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Keywords: childhood; leukaemia; infections; perinatal; epidemiology

Epidemiology

Childhood leukaemia is the most common cancer of childhood and only a few cases can be explained by known risk factors, such as ionising radiation, cancer chemotherapy or Down's syndrome.

Greaves has formulated the hypothesis that delayed exposure to common infections leads to an increased risk of childhood leukaemia, especially common pre-B acute lymphoblastic leukaemia (ALL), which has an incidence peak between ages 2 and 6 years. Childhood ALL is considered to be a rare response to common infections (Greaves, 1988; Greaves and Alexander, 1993; Greaves, 1997). The pathogenesis of leukaemia is believed to occur in two phases. The first genetic event is considered to take place during pregnancy, during the expansion of B-cell precursors. The second genetic event is thought to occur in the same mutant clone, following an immune stress, such as a common infection. The delayed exposure to infection is considered to increase the number of target cells with the 'first hit' present at older ages. On the basis of this hypothesis, a child isolated from infectious agents at the beginning of his/her life would be at a higher risk of ALL, while a high birth order value, early common infections and early day-care would be protective factors.

The present study investigated Greaves' hypothesis in a population-based case–control study by analysing the relations between childhood acute leukaemia (AL) and early common infections, day-care attendance and breast-feeding, paying particular attention to ALL. Perinatal characteristics and childhood medical history were also investigated.

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SUBJECTS AND METHODS

Subjects

A population-based case–control study was conducted from 1995 to 1998. Cases were derived from the National Registry of Childhood Leukaemia and Lymphoma (NRCL), which registers all the cases of leukaemia among children less than 15 in mainland France since 1990. Thus, to be eligible in the study, cases were required to be under 15 years old and be a resident in mainland France at the time of diagnosis. In addition, the mother had to be

able to fill out a questionnaire and the doctor had to authorise contact with the mother. Cases in four regions that were already involved in a hospital-based case–control study (Perrillat *et al*, 2002a, b), and the cases in four other regions in which the oncology department could not contribute to the study for practical reasons were excluded.

During the period 1995–1998, the NRCL registered 786 cases of AL in the 14 regions. Of those cases, 646 were eligible and 140 were not eligible: 25 were not known at the time of the study, two were known not to have parents, three had parents who were unable to fill out the questionnaire for linguistic (1) or social (2) reasons, 110 were too sick for their parents to be interviewed (28 of them died before the physician could pass on the questionnaire). The serious condition of the cases was a particular reason for noneligibility before the age of 1 year, when 17 out of the 33 registered cases were eligible. The overall participation rate, relative to all registered cases, was thus 60.2% (473 out of 786), and the response rate was 73.2% (473 out of 646).

The controls were randomly selected from the general population with stratification respecting the age, gender and regional distribution of the cases. Both the case and control mothers completed a self-administered questionnaire, distributed by the child's physician for cases, and by mail for controls. Controls were randomly selected using age, sex and region quotas from a sample of 30 000 phone numbers representative of the French population with respect to area of residence and municipality size categories. The control distribution was determined *a priori*, on the basis of the expected age, sex and region distribution of the cases derived from the previous years of registration. The study was designed with the same number of cases as controls with a frequency matching on age, sex and region.

A total of 805 controls were eligible. The mothers of 574 controls completed the self-administered questionnaire. Five controls were excluded because they were adopted and two because the questionnaires had too many missing values (only the first page, i.e. circumstances of birth, was completed). Thus, a total of 567 controls were included in the study. The response rate was 71% for the controls.

Data collection

On average, the questionnaire was completed 10 months after the diagnosis (before 6 months for 212 cases, between 6 and 12 months for 113 cases, between 12 and 18 months for 76 cases, after 18 months for 72 cases). It was completed within 6 months for the controls.

Details on the diagnosis of leukaemia were collected from the medical records by the Registry investigators. Data on the perinatal period, child's medical history and environment were collected using a standardised self-administered questionnaire. The questions on medical history were closed questions. The data on early infections included the history of ear, nose or throat (ENT), gastrointestinal (GI) and other infections, and the frequency of each type of infection ($\geqslant 1$ per month; <1 per month and $\geqslant 1$ per quarter; <1 per quarter and $\geqslant 1$ per year, less often) for the age groups: <1; 1–2; 3–4; 5 years and more.

The data on factors promoting infections included birth order of the index child, duration of breast-feeding, and history of day-care attendance.

Statistical analysis

Odds ratios (OR) were estimated using an unconditional logistic regression model including stratification variables, that is, gender, age and region, using the SAS® software package.

The analyses of day-care attendance, early infections and breast-feeding were restricted to children aged over 1 year in order to be certain that infections before age 1 had already taken place in both the cases and the controls.

The children with Down's syndrome (10 cases and two controls) were excluded from most of the analyses.

RESULTS

The cases and controls were very similar with respect to age, gender and region of residence at the time of diagnosis (Table 1). In total, 48% of the cases and 45% of the controls contributed to the age groups 2–3 and 4–5 years, corresponding to the peak of incidence of leukaemia, and 12 cases (3%) and 35 controls (6%) were younger than 1 year.

There was no difference between the cases and controls with respect to the distribution of parental socioprofessional category, or maternal or paternal educational level.

Table 1 Sample description for the cases and controls

	Cases (%) ($N=473$)	Controls (%) ($N=567$)	*P*
Gender			NS
Male	260 (55)	326 (57)	
Age at diagnosis (years)			NS
0–1	45 (10)		
2–3	115 (24)	78 (14)	
4–5	110 (23)	148 (26)	
6–9	113 (24)	109 (19)	
10–15	90 (19)	130 (23)	
		102 (18)	
Region of residence at diagnosis			NS
Alsace	28 (6)	29 (5)	
Aquitaine	59 (12)	42 (7)	
Bretagne	45 (10)	70 (12)	
Centre	51 (11)	62 (11)	
Champagne Ardennes	22 (5)	33 (6)	
Franche-Comté	28 (6)	31 (5)	
Languedoc-Roussillon	31 (7)	48 (8)	
Limousin	8 (2)	14 (2)	
Midi-Pyrénées	33 (7)	42 (7)	
Basse-Normandie	29 (6)	28 (5)	
Haute-Normandie	29 (6)	27 (5)	
Pays de Loire	48 (10)	75 (13)	
Picardie	31 (7)	36 (6)	
Poitou-Charentes	31 (7)	30 (5)	
Socioprofessional categories	NS		
Without employment	15 (3)	8 (1)	
Craftsmen and factory workers	132 (28)	118 (21)	
Farmers and agricultural workers	14 (3)	29 (5)	
Sales and service workers	49 (10)	80 (14)	
Administrative employees	66 (14)	105 (19)	
Intermediate profession	95 (20)	104 (19)	
Intellectual and scientific jobs	55 (12)	64 (11)	
Managers	43 (9)	54 (10)	
X[a]	4	5	
Maternal education			NS
<high school	337 (74)	415 (74)	
>high school	120 (26)	144 (26)	
X[a]	16	8	
Paternal education			NS
<high school	337 (77)	424 (79)	
>high school	102 (23)	113 (21)	
X[a]	34	30	

[a]Missing values.

Table 2 Perinatal characteristics and childhood leukaemia

	ALL				AML			
	Cases ($N=408$)	Controls ($N=567$)	OR[a]	95% CI	Cases ($N=65$)	Controls ($N=567$)	OR[a]	95% CI
Mean birth weight (g)								
Mean	3322	3314			3294	3314		
s.d.[b]	(514)	(522)			(396)	(522)		
Birth weight (g)								
<2500	22	31	1.0	(0.6–1.9)	1	31	0.3	(0.03–2.1)
2500–2999	73	96	1.1	(0.7–1.6)	12	96	0.9	(0.4–1.9)
3000–3499	160	217	1.0	Ref	32	217	1.0	Ref
3500–3999	97	172	0.8	(0.6–1.1)	14	172	0.6	(0.3–1.2)
⩾4000	40	37	1.4	(0.8–2.3)	3	37	0.8	(0.2–2.8)
X[c]	16	14			3	14		
Term of pregnancy								
<37	41	45	1.4	(0.9–2.2)	5	45	1.4	(0.5–4.1)
37–38	80	144	0.7	(0.5–1.0)	13	144	1.0	(0.5–2.1)
39–40	220	304	1.0	Ref	32	304	1.0	Ref
⩾41	32	52	0.9	(0.5–1.5)	7	52	1.3	(0.5–3.3)
X[c]	35	22			8	22		
Birth order								
1	180	267	1.0	Ref	35	267	1.0	Ref
2	131	199	0.9	(0.7–1.2)	12	199	0.5	(0.2–1.0)
3	64	73	1.1	(0.8–1.7)	12	73	1.3	(0.6–2.8)
4+	32	22	2.0	(1.1–3.7)	5	22	2.5	(0.8–7.5)
X[c]	1	6			1	6		
		P-trend = 0.07				*P-trend* > 0.10		
Maternal age at birth								
<25	84	93	1.4	(1.0–2.1)	11	93	0.9	(0.4–2.0)
25–29	154	241	1.0	Ref	30	241	1.0	Ref
30–34	107	169	0.9	(0.7–1.3)	14	169	0.7	(0.3–1.3)
⩾35	57	56	1.6	(1.0–2.5)	8	56	1.3	(0.5–3.1)
X[c]	6	8			2	8		
Previous foetal losses								
0	313	436	1.0	Ref	49	436	1.0	Ref
1	74	88	1.2	(0.8–1.7)	11	88	1.1	(0.5–2.3)
⩾2	16	28	0.8	(0.4–1.6)	2	28	0.6	(0.1–2.8)
X[c]	5	15			3	15		
Down's syndrome								
No	400	565	1.0	Ref	63	565	1.0	Ref
Yes	8	2	4.4	(0.9–2.2)	2	2	11.7	(1.3–108.5)

[a]Adjusted for stratification variables (gender, age at diagnosis, region of residence at diagnosis). [b]s.d. = standard deviation. [c]X = missing values.

No association between childhood leukaemia and birth weight, term of pregnancy, maternal age at birth and history of previous foetal losses was observed (Table 2).

A statistically significant association between birth order and childhood ALL was observed (*P*-trend = 0.07 – OR = 2.0; CI (3.1–3.7) for children born fourth). A similar association was observed with AML.

No association between breast-feeding, irrespective of its duration, and childhood AL was observed (Table 3).

The results for early infections are shown in Table 4. The mothers of 122 cases (104 ALL and 18 AML) and of 172 controls declared at least four common infections in the first year of their child's life. ENT infections were highly predominant, while infections other than ENT were reported at lower frequency: 44 cases and 52 controls reported only one GI infection during the first year and 15 cases and 26 controls reported only one infection other than ENT or GI during the first year. A statistically significant negative association between common infections before age 1 year and childhood ALL (OR = 0.8; CI (0.6 – 1.0)) was observed. This association was not observed with AML. The association was stronger for early GI infections (OR = 0.1; CI (0.03 – 0.6)), but this finding was based on only two cases and 18 controls.

In order to evaluate the potential influence of the missing values on the results presented in Table 4, we also estimated the OR associated with total ENT or GI infections either by including the missing values for cases and controls in the category of the least infected children or by including the missing values of cases and controls in the category of the most frequently infected children. The OR remained significantly less than 1 if missing values were assigned to the unexposed group, and increased at most to 1.0 if, conversely, the missing values were assigned to the group of children who had more than four infections in their first year of life.

Taken as a whole, day-care attendance was associated with childhood AL (OR = 0.7; CI (0.6 – 1.0) for ALL) as shown in Table 5. The association was only observed when day-care started before age 6 months (OR = 0.6; CI (0.4 – 0.8) for age less than 3 months;

Table 3 Association between childhood acute leukaemia and breast-feeding (analysis restricted to children older than 1 year)

	ALL				AML			
	Cases ($N=393$)	Controls ($N=530$)	OR[a]	95% CI	Cases ($N=59$)	Controls ($N=530$)	OR[a]	95% CI
Breast-feeding								
No	216	307	1.0	Ref	29	307	1.0	Ref
Yes	176	222	1.1	(0.9–1.5)	30	222	1.4	(0.8–2.5)
X[b]	1	1			0	1		
Breast-feeding duration								
0	216	306	1.0	Ref	29	306	1.0	Ref
<3 months	86	105	1.2	(0.8–1.7)	14	105	1.4	(0.7–2.9)
3–6 months	57	75	1.1	(0.7–1.6)	12	75	1.7	(0.8–3.7)
>6 months	29	29	1.4	(0.8–2.5)	2	29	0.5	(0.1–2.1)
X[b]	5	15			2	15		

[a]ORs adjusted for stratification variables: gender, age at diagnosis, region of residence at diagnosis. [b]X = missing values.

Table 4 Association between childhood acute leukaemia and common early infections (analysis restricted to children older than 1 year)

	ALL				AML			
	Cases ($N=393$)	Controls ($N=530$)	OR[a]	95% CI	Cases ($N=59$)	Controls ($N=530$)	OR[a]	95% CI
⩾four infections in the 1st year of life								
No	230	309	1.0	Ref	31	309	1.0	Ref
Yes	104	172	0.8	(0.6–1.0)	18	172	1.4	(0.7–2.6)
X[b]	59	49			10	49		
⩾4 ENT[c] infections in the 1st year of life								
No	257	340	1.0	Ref	35	340	1.0	Ref
Yes	101	166	0.8	(0.6–1.1)	17	166	1.3	(0.7–2.4)
X[b]	35	24			7	24		
⩾4 GI[d] infections in the 1st year of life								
No	342	456	1.0	Ref	47	456	1.0	Ref
Yes	2	18	0.1	(0.03–0.6)	0	18	—	
X[b]	49	56			12	56		

[a]ORs adjusted for stratification variables: gender, age at diagnosis, region of residence at diagnosis. [b]X = missing values. [c]ENT = ear, nose throat. [d]GI = gastrointestinal.

OR = 0.8; CI (0.5–1.2) for age 3–6 months). The trend with respect to the age of starting day-care was statistically significant.

After exclusion of children with Down's syndrome, 221 cases (210 ALL and 11 AML) and 255 controls belonged to the 2–6 years age bracket corresponding to the incidence peak, while 199 cases (161 ALL and 38 AML) and 232 controls were 6 years old or more. Of the 393 ALL after age one, 304 were of the common B-cell type, 54 were of the T-cell type, and four were B mature.

Data were also analysed separately depending on the age at diagnosis (2–6 years *vs* older) and on the subtype of ALL (common B-cell *vs* other ALL). The association between early frequent common infections and AL was restricted to the 2–6 years age group, but not to the common B-cell ALL subtype (Table 6). The associations with day-care were specific neither to the common B cell ALL subtype nor to the age bracket 2–6 years.

As shown in Table 7, a statistically significant negative association between asthma and childhood ALL was observed (OR = 0.5; CI (0.3–0.9)). The association was even stronger for asthmatic children regularly treated with bronchodilators (OR = 0.3; CI (0.1–0.7)).

No significant association with a history of chickenpox, mumps or glandular fever was observed (Table 7). In contrast, ALL was associated with a history of measles (OR = 1.7; CI (1.0–2.9)) and rubella (OR = 2.4; CI (1.4–4.1)). A history of viral hepatitis was only reported for two cases and one control (OR = 3.3; CI (0.3–37.0)).

DISCUSSION

One of the main objectives of the present population-based study was to test whether early common infections were associated with a reduced risk of AL. Slight negative associations between ALL and common early infections and day-care were observed. No association with breast-feeding, irrespective of duration, was observed. A birth order of four or more was associated with an increased risk of AL. A history of two or more infantile viral diseases was positively associated with ALL while a history of asthma was negatively associated with ALL.

The data were collected from a standardised self-administered questionnaire. The response rates for the cases and controls were very similar and the nonrespondent controls did not differ from the respondent controls in terms of age, gender or region of residence. The percentage mortality rates, obtained from the NRCL, for the respondent and nonrespondent cases were very similar (12 and 8%, respectively) making a strong survival bias unlikely. The exhaustiveness of the NRCL is close to 99%, making unlikely a selection through the process of cases' identification.

Recalling common infections may be difficult, and this may explain the rather high number of missing values in the questionnaires. However, when the missing values were assigned either exposed or unexposed status (with the hypothesis of a nondifferential bias), the OR remained less than unity. An OR of 1.0 was only obtained when all the missing values were classed as

Table 5 Association between childhood acute leukaemia and day-care attendance (analysis restricted to children older than 1 year)

	ALL				AML			
	Cases ($N=393$)	Controls ($N=530$)	OR[a]	95% CI	Cases ($N=59$)	Controls ($N=530$)	OR[a]	95% CI
Day-care								
No	220	259	1.0	Ref	34	259	1.0	Ref
Yes	167	266	0.7	(0.6–1.0)	24	266	0.8	(0.5–1.5)
X[b]	6	5			1	5		
Type of day-care								
None	220	259	1.0	Ref	34	259	1.0	Ref
Nurse only	120	198	0.7	(0.5–1.0)	17	198	0.8	(0.4–1.6)
Full time day-care centre only	29	34	1.1	(0.6–1.8)	5	34	1.3	(0.4–3.7)
Occasional day-care centre only	6	15	0.4	(0.2–1.2)	1	15	0.7	(0.1–6.4)
More than one type of day-care	12	19	0.7	(0.3–1.5)	1	19	0.6	(0.1–5.1)
X[b]	6	5			1	5		
Age at start of day-care (nurse or day-care centre)								
<3 months	61	120	0.6	(0.4–0.8)	12	120	1.0	(0.5–2.2)
3–6 months	53	82	0.8	(0.5–1.2)	6	82	0.6	(0.2–1.5)
6–12 months	25	25	1.2	(0.6–2.1)	4	25	1.3	(0.4–4.1)
⩾12 months	21	25	1.0	(0.5–1.8)	1	25	0.3	(0.04–2.8)
Never	220	259	1.0	Ref	34	259	1.0	Ref
X[b]	13	19			2	19		
			P-trend < 0.05					
Age at start of full-time day-care centre								
Never	348	475	1.0	Ref	52	475	1.0	Ref
1–3 months	10	23	0.6	(0.3–1.3)	3	23	1.5	(0.4–5.6)
4–6 months	9	10	1.4	(0.5–3.5)	3	10	2.5	(0.6–10.2)
7–12 months	8	6	2.1	(0.7–6.2)	0	6		
⩾12 months	11	9	1.6	(0.6–4.0)	0	9		
X[b]	7	7			1	7		

[a]ORs adjusted for stratification variables: gender, age at diagnosis, region of residence at diagnosis. [b]X = missing values.

Table 6 Association between childhood acute leukaemia and factors implicated in Greaves' hypothesis, according to age at diagnosis and ALL subtype

	Age				ALL subtype[a]			
	2–6 years		6–15 years		Common B-cell ALL		Other ALL	
	OR[b]	95% CI	OR[b]	95% CI	OR[b]	95% CI	OR[b]	95% CI
⩾4 infections in the 1st year of life								
Any infection	0.7	(0.5–1.0)	1.0	(0.6–1.6)	0.8	(0.6–1.1)	0.7	(0.4–1.1)
ENT	0.7	(0.5–1.0)	1.0	(0.6–1.6)	0.8	(0.6–1.1)	0.7	(0.4–1.2)
Digestive	0		0.5	(0.1–2.8)	0.1	(0.01–0.7)	0.3	(0.04–2.5)
Day-care								
Yes *vs* no	0.8	(0.5–1.2)	0.7	(0.5–1.1)	0.8	(0.6–1.0)	0.5	(0.3–0.9)
Age at start of day-care (any type)								
<3 months	0.6	(0.4–1.0)	0.7	(0.4–1.2)	0.6	(0.4–0.9)	0.4	(0.2–0.8)
3–6 months	0.9	(0.5–1.6)	0.8	(0.4–1.3)	0.8	(0.6–1.3)	0.5	(0.2–1.1)
>6 months	1.3	(0.7–2.4)	0.7	(0.4–1.6)	1.1	(0.7–1.8)	1.0	(0.5–2.1)
Never	1.0	Ref	1.0	Ref	1.0	Ref	1.0	Ref
Birth order								
1	1.0	Ref	1.0	Ref	1.0	Ref	1.0	Ref
2	0.8	(0.6–1.3)	0.7	(0.4–1.1)	0.9	(0.6–1.2)	1.1	(0.6–1.8)
3	1.4	(0.8–2.4)	0.7	(0.4–1.3)	1.2	(0.8–1.9)	0.8	(0.4–1.7)
⩾4	1.5	(0.7–3.3)	2.4	(0.9–6.3)	2.0	(1.1–3.8)	2.3	(0.9–6.1)
Breast-feeding								
Yes *vs* no	1.0	(0.7–1.5)	1.4	(0.9–2.0)	1.2	(0.9–1.5)	1.1	(0.7–1.8)
Breast-feeding duration								
Never	1.0	Ref	1.0	Ref	1.0	Ref	1.0	Ref
<3 months	0.8	(0.5–1.3)	1.7	(1.0–2.9)	1.2	(0.8–1.7)	1.1	(0.6–1.9)
⩾3 months	1.4	(0.8–2.2)	1.0	(0.6–1.6)	1.2	(0.8–1.7)	1.3	(0.7–2.2)

[a]Analysis restricted to children older than 1 year. [b]ORs adjusted for stratification variables: gender, age at diagnosis, region of residence at diagnosis.

Table 7 Medical history of asthma or infantile diseases and risk of childhood acute leukaemia

	ALL				AML			
	Cases ($N=400$)	Controls ($N=565$)	OR[a]	95% CI	Cases ($N=63$)	Controls ($N=565$)	OR[a]	95% CI
Asthma								
Asthma	17	44	0.5	(0.3–0.9)	2	44	0.4	(0.1–1.7)
Asthma and/or bronchodilators (BD)								
No asthma and no BD	358	501	1.0	Ref	59	501	1.0	Ref
Asthma or BD	25	31	1.0	(0.6–1.8)	2	31	0.7	(0.1–3.0)
Asthma and BD	6	29	0.3	(0.1–0.7)	1	29	0.3	(0.03–2.3)
X[b]	11	4			1	4		
Infantile diseases before the diagnosis								
Measles	41	38	1.7	(1.0–2.9)	8	38	1.3	(0.5–3.1)
Rubella	37	24	2.4	(1.4–4.1)	3	24	1.1	(0.3–3.9)
Mumps	24	20	1.9	(1.0–3.6)	6	20	1.6	(0.6–4.5
Chickenpox	220	323	0.9	(0.6–1.2)	33	323	0.7	(0.3–1.4)
Any of the above infantile diseases								
None	147	222	1.0	Ref	26	222	1.0	Ref
1	178	272	0.9	(0.7–1.3)	24	272	0.7	(0.3–1.6)
2 or more	53	50	1.6	(1.0–2.8)	7	50	0.7	(0.2–2.1)

[a]ORs adjusted for stratification variables: gender, age at diagnosis, region of residence at diagnosis. [b]X = missing values.

'most often infected', which is very unlikely. Case mothers may have declared minor health problems less often, and thus introduced a differential recall bias. However, the self-administered questionnaire contained closed and very precise questions. Over-reporting by control mothers was possible, but unlikely. Conversely, a nondifferential recall bias was more likely, given that the questionnaire was self-administered, and may have reduced the association with common early infections.

Few papers have addressed the role of early common infections yet. All but one (Dockerty *et al*, 1999) found a negative association with early common infections. Van Steensel-Moll *et al* (1986) observed a significant negative association between common colds before age 1 year and the risk of ALL. Neglia *et al* (2000) observed a significant trend towards a reduction in the risk of AL with an increase in the number of episodes of otitis before age 1 year. The trend was stronger for ALL. Perrillat *et al* (2002b) found a negative association between the risk of ALL and a history of four or more episodes of otitis before age 2 years, on the one hand, and a history of ENT surgical procedures before age 2 years, on the other hand.

Day-care attendance can be considered a surrogate of early contact with infections. In our study, day-care was slightly negatively associated with ALL when initiated early. However, curiously, the association did not concern full-time day-care centres. All the authors who have studied the association between day-care and leukaemia have reported OR of less than one (Petridou *et al*, 1993, 1997; Neglia *et al*, 2000). However, only four studies found significant negative associations (Infante-Rivard *et al*, 2000; Rosenbaum *et al*, 2000; Ma *et al*, 2002; Perrillat *et al*, 2002b). When age at the start of day-care was studied, the negative association was stronger for the youngest.

With regard to breast-feeding, recall bias is difficult to imagine since the mother was asked to indicate the duration of breast-feeding. The questionnaire did not distinguish between mixed feeding and complete breast-feeding. Over-reporting of long-duration breast-feeding by case mothers cannot be ruled out since some mothers may experience feelings of guilt with regard to breast-feeding. However, such a recall bias is likely to concern mothers who only breastfed for a short duration rather than those who did not breast-feed at all, and is probably insufficient to explain the absence of a negative association. Confounding by birth order is possible, since the older children were less often breastfed and received breast-feeding for shorter durations than the controls. However, adjustments for birth order did not modify the association and there was no interaction between the two variables. The majority of the studies investigating breast-feeding have found a negative association with childhood AL (Davis *et al*, 1988; Petridou *et al*, 1997; Infante-Rivard *et al*, 2000; Rosenbaum *et al*, 2000), which was more marked for prolonged breast-feeding (Magnani *et al*, 1988; Dockerty *et al*, 1999; Shu *et al*, 1999; Smulevich *et al*, 1999; Bener *et al*, 2001; Hardell and Dreifaldt, 2001; Perrillat *et al*, 2002a).

The positive association with birth order observed in the present study was unexpected and did not seem to be explained by sociodemographic characteristics or by the other variables under study. Control mothers with the largest families may have been counter-selected, for instance, because they would have been less available to answer the questionnaire. Although first-born status was included in Greaves' hypothesis as a risk factor, many studies have not observed any association between birth order and ALL or AL (Kaye *et al*, 1991; Petridou *et al*, 1993, 1997; Roman *et al*, 1997; Westergaard *et al*, 1997; McKinney *et al*, 1999; Shu *et al*, 1999; Neglia *et al*, 2000). Only three authors have observed a significant negative association between the risk of leukaemia (or ALL) and birth order (Van Steensel-Moll *et al*, 1986; Schuz *et al*, 1999a; Dockerty *et al*, 2001), in line with Greaves' hypothesis. In contrast, three studies have found a significant positive association between the risk of AL and birth order (Savitz and Ananth, 1994; Infante-Rivard *et al*, 2000; Shu *et al*, 2002).

The negative relation with asthma and bronchodilators may be fortuitous. However, several authors have already pointed out the possibility of a negative association with asthma and other allergic diseases (Magnani *et al*, 1990; Petridou *et al*, 1997; Schuz *et al*, 1999b; Wen *et al*, 2000), and the association deserves further investigation.

In conclusion, although the results regarding birth order and breast-feeding do not fit with Greaves' hypothesis, the study supports the hypothesis that early common infections may play a protective role in the aetiology of childhood leukaemia, although this effect was not more marked for common ALL.

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Day care, childhood infections, and risk of neuroblastoma

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Abstract

Neuroblastoma is the most common cancer in infants worldwide but little is known about its etiology. Infectious etiologies involving the immune system have been hypothesized for some childhood cancers, especially leukemia, but the role of infectious agents in neuroblastoma has not been fully investigated. We used data from a large case-control study conducted by the Children's Oncology Group over the period 1992–1994 in United States or Canada to investigate if there was any relation between day care attendance, childhood infections, allergies and neuroblastoma. We interviewed mothers of 538 case and 504 age-matched control children by telephone about several factors including pregnancy, medical history, lifestyle, and childhood medical conditions and exposures. Our results suggested decreased risks associated with day care attendance (odds ratio (OR) = 0.81; 95% confidence interval ([CI]: 0.56–1.17), childhood infectious diseases (chickenpox, mumps, red and German measles) (OR = 0.60;CI: 0.39–0.93) and allergies (OR = 0.68; CI: 0.44–1.07). We found reduced neuroblastoma risk associated with markers of potential childhood infections, which suggests a possible role of infectious agents in neuroblastoma etiology. Future epidemiologic studies should incorporate more direct infection data.

Keywords: Adult, Birth Order, Breast Feeding, statistics & numerical data, Canada, epidemiology, Case-Control Studies, Child, Child Day Care Centers, utilization, Child, Preschool, Communicable Diseases, complications, epidemiology, immunology, Female, Humans, Hypersensitivity, complications, Immunocompetence, Infant, Life Style, Logistic Models, Male, Neuroblastoma, epidemiology, microbiology, Population Surveillance, Questionnaires, Risk Assessment, Risk Factors, Socioeconomic Factors, United States, epidemiology

Keywords: Neuroblastoma, Day Care, Infection, Allergy, Childhood

Neuroblastoma is an embryonal malignancy of the sympathetic nervous system that derives from primordial neural crest cells. It is the third most common cancer in children and the most common tumor in infants (1). In an analysis of United States Surveillance Epidemiologic End Results (SEER) incidence data, 41 percent

of infant neuroblastomas were diagnosed during the first 3 months of life (2). Little is known about the etiology of neuroblastoma and the relatively young age at onset has led researchers to investigate parental factors before conception or during gestation. These factors have included occupation, smoking and alcohol consumption, medication use during pregnancy, pregnancy history, and birth characteristics (3). Associations between these factors and neuroblastoma risk have been inconsistent (2, 3).

Infections are suspected to play a role in the etiology of some childhood cancers, especially childhood acute leukemia and Hodgkin disease (4–6). Kinlen postulated that childhood leukemia is a rare response to a specific infection, and the risk of infection increases through the mixing of populations (7–9). Greaves hypothesized that childhood leukemia may result from a two-step process, with a first step possibly an in utero mutation in a small population of cells. The second step, a postnatal event, may be an additional mutation or proliferation of the initially mutated cell population. It has been suggested that the second event may result from exposure to an infectious agent. By contributing to the normal maturation of the immune system and the establishment of immunocompetence, early common infections or factors that favor infections in early childhood would protect the child against leukemia, while relative isolation would make the child more vulnerable (10, 11). In fact, several studies observed that the risk of childhood leukemia might be reduced by day care attendance (12–14), breast-feeding (6, 13, 15–19), early common infections (14, 20, 21), or population mixing (22–26). Moreover, a recent analysis of data from the present neuroblastoma study found a reduced odds ratio for breast-feeding (27). In this context, factors that influence children's immune systems are of special interest. To our knowledge, the relationship between neuroblastoma and factors related to the immune system have never been investigated fully. This paper focuses on markers of childhood infections and immune response, including day care attendance, birth order, childhood infections and allergies in relation to neuroblastoma.

MATERIALS AND METHODS

Study population

Details of this study have been published elsewhere (28). Cases were children and young adults under 19 years old who were newly diagnosed with neuroblastoma between May 1, 1992 and April 30, 1994 at any of 139 participating hospitals in the United States and English-speaking Canada. The hospitals were members of one of two collaborative pediatric clinical trials groups, the Children's Cancer Group and the Pediatric Oncology Group (29). The two groups merged to form the Children's Oncology Group. Treating physicians gave us permission to approach parents of patients about participation in the study. Criteria for inclusion of eligible cases were availability of the biological mother for interview, a telephone in the home, and the ability of the mother to speak English or Spanish. Among 741 potentially eligible cases, 538 (73 percent) case mothers were interviewed successfully. Reasons for nonparticipation of mothers included physician refusal (n=90; 12 percent) mother's refusal (n=57; eight percent), not traceable (n=44; six percent), and other reasons (n=12; two percent).

One control was selected for each case using a random-digit dialing method based on the first eight digits of the case's telephone number (30). Controls were individually matched to cases by telephone number and on the date of birth (within 6 months older or younger for cases diagnosed at younger than 3 years old, within 1 year older or younger for cases over 3 years old). The parents of cases and controls were interviewed about exposures and events prior to a common reference date: the case date of diagnosis. The household random-digit dialing screening response proportion was 74 percent (31). Among 703 eligible control mothers, 504 (72 percent) completed interviews.

Data collection

Mothers of cases and controls were contacted after signed consent forms were received from responsible physicians. After initial contact, parents were sent packets that contained consent forms and interview guides to facilitate recall and increase interview efficiency. Parents' telephone interviews were conducted by trained interviewers. Parents of cases and controls were asked about demographic characteristics, occupational history, pregnancy history and birth characteristics, medication use, children's illnesses and conditions, lifestyle, and other factors. Data related to infections and factors potentially promoting infections included history of day-care attendance, birth order of index children, history of selected childhood infections, history of ear infections, history of other infections. History of children's illnesses and conditions also were collected by maternal self-report. Day-care variables included day care (ever/never), age at starting day care, age at ending day care, and number of hours per week. Selected childhood infections included chickenpox, mumps, red measles, and German measles. Mothers were asked to report conditions diagnosed by physicians. Other conditions of interest were disorders such as asthma, hay fever, eczema, and other allergies (ear throat nose allergy as rinitis and sinusitis, dermatological allergy as urticaria, contact dermatitis, food dermatitis and hypersensitivity to drugs).

Statistical analysis

All analyses were performed using the SAS computer software (version 8.1, Cary, North Carolina). The odds ratio (OR) and 95% confidence interval (CI) were estimated using unconditional logistic regression. The original matching factor, reference age at diagnosis, was taken into account in the unmatched analyses using a six-level categorical variable (< 1 year, 1–2 years, 3–4 years, 5–6 years, 7–10 years, ≥ 11 years). Mothers' demographic characteristics such as educational level (<high school, high school, college), maternal race/ethnicity (white, black, Hispanic, other) and mothers' report of annual total household income in birth year (<$10,000, $10–20,000, $21–30,000, $31–40000, $41–50,000 >$50,000) also were included in analyses as potential confounders. Conditional logistic regression using the 504 matched pairs did not differ materially from the unconditional logistic regression analyses. Day care was defined as day care attendance outside the home. We used four different variables: a dichotomous variable (ever/never), age child started day care, day-care duration, and total hours day care exposure which combined day care duration and number of hours attended per week. We analyzed the day care measures excluding the year before diagnosis to eliminate the potential of the disease to affect day care utilization. The year before diagnosis has been excluded for both cases and controls, the year before diagnosis for controls is the year before the reference date. Childhood infections and allergies were analyzed in children older than 1 year. We included 538 cases and 504 controls in the analysis.

RESULTS

Among case children, 38 percent were less than 1 year old at diagnosis, 35 percent were 1 to 2 years of age, 17 percent 3 to 4 years, and 10 percent were 5 years old or more. Slight case-control differences were found for gender, maternal race, and maternal age at birth (table 1). More case mothers than control mothers had low educations (OR < high school vs. college= 1.4; CI = 0.9–2.2). The proportion of cases from lower-income households (< $ 10,000 annually) and higher-income households (> $ 50,000 annually) were higher than among controls.

Twenty-two percent of cases and 28 percent of controls ever attended day care (OR = 0.81; 95 percent CI = 0.56–1.17) (table 2). Day care duration of 6 months or more and total hours day care exposure of 500 hours or more suggested a decreased risk for neuroblastoma (OR = 0.75; 95 percent CI = 0.52–1.10; OR = 0.74, 95 percent CI = 0.51–1.09; respectively). Our results were more pronounced when the year before diagnosis was not excluded: OR = 0.74; 95 percent CI = 0.55–0.99 for day care ever/never, OR = 0.66; 95 percent CI = 0.48–0.90 for day care duration of 6 months or more, and OR = 0.65; 95 percent CI = 0.47–0.89 for total hours day care exposure of 500 hours or more. The analyses were adjusted for child's diagnosis reference age, household income and mother's education, all results remained unchanged after adjustment.

We found strong inverse association in children who were breast-fed and ever attended day care, with an OR of 0.46 (95 percent CI = 0.28–0.74) while ORs were 0.71 (95 percent CI = 0.48–1.04) and 0.85 (95 percent CI = 0.48–1.52), respectively for children who were breast-fed only and children who ever attended day care only. Moreover, an OR of 0.36 (95 percent CI = 0.16–0.81) for children who attended day care 6 months or more and children who were breast-fed more than 6 months was observed (table 4). We did not find any association between birth order and neuroblastoma (OR for three or more siblings compared with one sibling = 0.94, 95 percent CI = 0.67–1.31).

We found an inverse association between any selected childhood infections (chickenpox, mumps, German measles, and red measles) and neuroblastoma (OR = 0.60; 95 percent CI = 0.39–0.93) (table 3). The association was stronger for children who had two or more infectious diseases (OR = 0.13; 95 percent CI = 0.02–0.65), although the result is based on small numbers. Ear infections were associated with elevated odds ratios (OR = 1.76; 95 percent CI = 1.20–2.58). Decreased risk was found for history of hay fever, asthma, or any allergy (OR = 0.43; 95 percent CI = 0.18–1.04; OR = 0.69; 95 percent CI = 0.36–1.34; OR = 0.68; 95 percent CI = 0.44–1.07, respectively). There was a general pattern of lower risks for day care and breast-feeding with ear infections and other infections but not with infantile disease (table 4).

DISCUSSION

Our results suggest that day care attendance, selected childhood infections, and certain allergic disorders were associated with a reduced risk of neuroblastoma, although odds ratios for ear infection and other infections were elevated. The strengths of our study included a large sample, a detailed interview-administered questionnaire, and extensive collection of covariate information. However, our results should be considered in light of potential study limitations.

Response proportions in case and control groups were below 75 percent, which might indicate selection bias. We did not have direct information to characterize nonrespondents. Potential differences in the response proportions among mothers of cases and controls can result in socioeconomic-related differences. Day care attendance is more common among children of women with higher educations and incomes. Control mothers who participated in this study had slightly higher educations and household incomes than cases. The results remained unchanged after adjustment for these socioeconomic factors, but we cannot rule out the possibility that residual confounding by socioeconomic status or other unmeasured characteristics associated with participation among controls influenced our results.

Another concern is maternal recall, especially differential recall patterns. Maternal recall bias related to day care information seems unlikely, recall of childhood diseases and infections may have led to misclassification. Ten years ago, a British study investigated mother's reports of childhood infections and their concordance with general practitioner records. Questions of two types were asked about infections: closed-ended questions were used for specific childhood infections as chickenpox, mumps, red measles, and German

measles, and open-ended questions were asked for other infections. Specific childhood infections were systematically reported more often by mothers compared with general practitioners' records. Mother's reports might be considered the preferred data source for these specific infections that often do not require consultation with a physician. However, for report of other infections obtained by open-ended questions, the accuracy of mothers' recall was poor. In our study, questions about infections were asked with closed-ended questions for specific infections (chickenpox, mumps, red and German measles, and ear infections) and open-ended questions for other infections. Thus, with respect to misclassification, we could consider our results concerning specific infections as more valid than results for other infections. Another potential bias is that the cases' diseases might have reduced their day care attendance. We excluded the year before diagnosis to minimize potential for this bias.

To our knowledge this was the first study to evaluate the effect of markers of childhood infections and immune responses on risk of neuroblastoma. A recent analysis of data from the present neuroblastoma study found reduced ORs for children who breast-fed (27) and encouraged us to investigate the leukemia "infectious hypothesis" for neuroblastoma. Interestingly, we found a decreased risk of neuroblastoma for children who attended day care. Some recent studies of childhood acute leukemia found similar inverse association with breast-feeding (6, 13, 15–19) and day care (12–14). Day care and breastfeeding together further reduced the risk of neuroblastoma. Some of the infection and breastfeeding results indicated a reduced risk but were based on a small number of subjects. Our previous analysis of breastfeeding and neuroblastoma found a pattern of reduced risk with breastfeeding (27). The results of this study and the earlier report suggest that breastfeeding in combination with other factors deserves further investigation.

We observed reduced ORs for the usual childhood infectious diseases (chickenpox, mumps, German and red measles), that have never been investigated before in relation to neuroblastoma. Results on association between conditions such as chickenpox, measles, rubella, mumps, and childhood leukemia have been mixed (14, 15, 20, 21, 32, 33). Allergic disorders also were of interest because they involve challenges to the immune system. We observed reduced OR with hay fever and asthma. An inverse association between allergies and neuroblastoma also was found by Schuz et al. (34).

The biologic mechanisms that explain our findings are unclear at present. An infectious etiology or immunologic modifiers for neuroblastoma development have not been prominent hypotheses. Nonetheless, there are several lines of laboratory research that provide some clues. There has been significant interest in the mechanisms responsible for high spontaneous regression rate of neuroblastoma (the second highest of any human cancer). One possible mechanism involves immunologic factors and recent studies have reported that the presence of natural immunoglobulin (Ig)M antibodies was cytotoxic for human neuroblastoma cells *in vitro* and *in vivo* (35, 36). Another relevant research area involves investigation of viral etiology. A recent study suggested that the BK polyomavirus was associated with neuroblastoma. The virus is a relatively common childhood infection without symptoms, but latent or persistent infections may become reactivated. The study found BK virus DNA in the tumor cells of 17 of 18 neuroblastomas, but not in any of five normal adrenal medullas (37). Another common early childhood polyomavirus, the human neurotrophic JC virus, has been associated with pediatric medulloblastomas (38, 39). Although far from definitive, these disparate findings suggest that infectious agents and immune response may influence the risk of pediatric solid tumors.

Future epidemiologic studies should incorporate more direct measures of infection. Additional laboratory studies that evaluate immunologic influences on the development, progression, and regression of neuroblastoma also are warranted.

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Figures and Tables

TABLE 1

Demographic characteristics

	Cases		Controls		OR*	95% CI†
	N°	%	N°	%		
Gender						
Male	301	56	251	50	1.0	reference
Female	237	44	253	50	0.8	0.6–1.0
Mother's age at birth (years)						
< 20	48	9	35	7	1.3	0.8–2.1
20–24	119	22	110	22	1.1	0.8–1.5
25–30	212	39	206	41	1.0	reference
31–39	148	28	146	29	1.0	0.7–1.3
40 +	11	2	7	1	1.5	0.6–3.9
Mother's race						
White	429	80	396	79	1.0	reference
Black	42	8	39	8	1.0	0.6–1.6
Hispanic	49	9	54	11	0.8	0.5–1.2
Other	18	3	15	3	1.1	0.5–2.2
Mother's education						
< High school	60	11	51	10	1.4	0.9–2.2
High school	366	68	318	63	1.4	1.0–1.9
College	112	21	135	27	1.0	reference
Household income in birth year‡						
< $ 10 k	89	18	54	11	2.2	1.4–3.4
$ 10–20 k	92	18	91	19	1.3	0.9–2.0
$ 21–30 k	87	17	114	23	1.0	reference
$ 31–40 k	79	16	86	18	1.2	0.8–1.9
$ 41–50 k	54	11	52	11	1.4	0.9–2.2
> $ 50 k	107	21	89	18	1.6	1.1–2.4

*Unmatched odds ratio (OR) adjusted for child's diagnosis reference age.

†CI: confidence interval.

‡A total of 30 case and 18 control subjects had missing income data.

TABLE 2

Day care attendance and risk of neuroblastoma (year before diagnosis excluded)

	Cases		Controls		OR*	95% CI†
	N°	%	N°	%		
Day care attendance						
No	340	78	269	72	1.00	reference
Yes	97	22	103	28	0.81	0.56–1.17
Age at starting day care						
No day care	340	78	269	72	1.00	reference
< 6 months	55	13	52	14	0.90	0.57–1.41
≥ 6 months	36	8	42	12	0.72	0.43–1.22
Day care duration						
No day care	340	78	269	72	1.00	reference
< 6 months	9	2	7	2	1.01	0.36–2.83
≥ 6 months	88	20	99	26	0.75	0.52–1.10
Total hours day care exposure‡						
No day-care	340	78	269	72	1.00	reference
< 500 hours	15	3	12	3	0.99	0.44–2.22
≥ 500 hours	82	19	94	25	0.74	0.51–1.09

*Unmatched odds ratios (OR) adjusted for child's diagnosis reference age, mother's race, mother's education and household income at birth year.

†CI: confidence interval.

‡Total hours day care exposure took into account both day-care duration and number of day care hours attended per week.

TABLE 3

Infectious diseases, allergies and risk of neuroblastoma (children older than one year)

	Cases		Controls		OR*	95% CI*
	N°	%	N°	%		
Infectious diseases						
Selected childhood infections†						
Yes *vs* no	57	17	72	24	0.60	0.39–0.93
0	273	83	231	76	1.00	reference
1	55	17	64	21	0.66	0.42–1.02
2+	2	0.6	9	3	0.13	0.02–0.65
Ear infections						
Yes *vs* no	254	80	210	69	1.76	1.20–2.58
0	65	20	95	31	1.00	reference
< 1 per month	190	61	167	55	1.62	1.09–2.41
≥ 1 per month	55	18	39	13	2.13	1.24–3.66
Other infections‡						
Yes *vs* no	39	12	29	9	1.26	0.74–2.11
Allergies						
Asthma	18	5	25	8	0.69	0.36–1.34
Hay fever	8	2	17	6	0.43	0.18–1.04
Eczema	19	6	21	7	0.82	0.41–1.62
Any allergy§	45	14	58	19	0.68	0.44–1.07

*Unmatched odds ratios (OR) adjusted for child's diagnosis reference age, mother's race, mother's education and household income at birth year, CI: confidence interval.

†Selected childhood infections included chickenpox, mumps, German measles and red measles.

‡Other infection included upper and lower respiratory tract, digestive and kidney infection (ear infection excluded).

§Any allergy included asthma, hay fever, other ear throat nose allergiy as rhinitis and sinusitis, eczema, and other dermatological allergy as urticaria, contact dermatitis, food dermatitis and hypersensitivity to drugs.

TABLE 4

Day care attendance, Breastfeeding, Infectious diseases and risk of neuroblastoma

	OR*	95% CI†
Day care attendance (DC) and Breastfeeding (BF) ‡		
No DC, no BF	1.0	reference
DC, no BF	0.73	0.44–1.20
No DC, BF	0.63	0.41–0.96
DC, BF	0.46	0.28–0.74
No DC, BF ≤ 6 months	1.0	reference
DC, BF ≤ 6 months	0.82	0.46–1.47
No DC, BF > 6 months	0.90	0.52–1.55
DC, BF > 6 months	0.43	0.20–0.92
Day care duration (DCD) and Breastfeeding (BF)‡		
No DC, BF ≤ 6 months	1.0	reference
DCD < 6 months, BF ≤ 6 months	1.68	0.61–4.63
DCD ≥ 6 months, BF ≤ 6 months	0.65	0.35–1.21
No DC, BF > 6 months	0.89	0.52–1.53
DCD < 6 months, BF > 6 months	0.99	0.19–5.33
DCD ≥ 6 months, BF > 6 months	0.36	0.16–0.81
Infantile disease (ID) and Day care (DC)§		
ID, no DC	0.52	0.28–0.95
ID, DC	0.73	0.37–1.41
Ear infections (EI) and Day care (DC)§		
EI, no DC	1.99	0.69–3.24
EI, DC	1.43	0.46–2.81
Other infections (OI) and Day care (DC)§		
OI, no DC	1.43	1.23–2.94
OI, DC	1.04	0.73–2.34
Infantile disease (ID) and Breastfeeding (BF)§		
ID, no BF	0.37	0.18–0.76
ID, BF	0.93	0.49–1.76
Ear infections (EI) and Breastfeeding (BF)§		
EI, no BF	2.29	1.17–4.50

*Unmatched odds ratios (OR) adjusted for child's diagnosis reference age, mother's race, mother's education and household income at birth year.

†Confidence Interval

‡DC & BF: Analyses conducted in children older than 6 months

§Infection & DC/Infection & BF: Analyses conducted in children older than 1 year

Multicenter Study Leuk Res. 2004 Jul;28(7):713-24. doi: 10.1016/j.leukres.2003.11.010.

Population-based study of lymphoma in Germany: rationale, study design and first results

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PMID: 15158093 DOI: 10.1016/j.leukres.2003.11.010

Abstract

A multi-centre, population-based case-control study of lymphoma among adults was conducted in Germany from 1999-2003. The study comprised 700 incident cases (Hodgkin lymphomas and non Hodgkin's lymphoma, NHL) in the age range 18-80 years and 700 age-, sex- and area-matched controls obtained from population registries. Diagnosis was based on the REAL/WHO classification. Information on demographic characteristics, lifestyle, medical history and occupation was obtained by in-person interviews. Each participant was asked for a 24 ml blood sample. First results are focused on basic demographic characteristics, contact to animals, childhood diseases and vaccinations. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using conditional logistic regression. The ORs for lymphoma were decreased for exposure to sheep and goats (OR = 0.7; 95% CI = 0.5-0.9), for rabbits and hare (OR = 0.7; 95% CI = 0.5-0.9), measles infection (OR = 0.6; 95% CI = 0.5-0.9), Bordetella pertussis infection (OR = 0.7; 95% CI = 0.6-0.95), and tetanus vaccination (OR = 0.5; 95% CI = 0.3-0.9). Increased risk of lymphoma was associated with exposure to cattle (OR = 1.3; 95% CI = 1.03-1.7) and immunization for tuberculosis (OR = 1.5; 95% CI = 0.997-2.4). The results of this study are partly consistent with the hygiene hypothesis. The inconsistencies of some of the findings with an explanation by the Th1/Th2 paradigm, however, warrant further research and may indicate that broader explanatory concepts are needed.

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Exposure to childhood infections and risk of Epstein-Barr virus--defined Hodgkin's lymphoma in women

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Free article

Abstract

The role of Epstein-Barr virus (EBV) in Hodgkin's lymphoma (HL) etiology remains unresolved as EBV is detected in only some HL tumors and few studies have tried to reconcile its presence with factors suggesting viral etiology (e.g., childhood social class, infection history). In a population-based case-control study of San Francisco Bay area women, we analyzed interview data by tumor EBV status. Among 211 young adult cases, EBV-positive HL (11%) was associated with a single vs. shared bedroom at age 11 (OR = 4.0, 95% CI 1.1-14.4); risk was decreased for common childhood infections (OR = 0.3, 95% CI 0.1-1.0), including measles before age 10, but not with prior infectious mononucleosis (IM), which is delayed EBV infection. No study factors affected risk of young adult EBV-negative HL. Among 57 older adult cases, EBV-positive HL (23%) was unrelated to study factors; EBV-negative HL was associated with a single bedroom at age 11 (OR = 3.6, 95% CI 1.5-9.1) and IM in family members (OR = 3.1, 95% CI 1.1-9.0). Thus, delayed exposure to infection may increase risk of EBV-positive HL in young adults, but risk patterns differ in younger and older women for both EBV-positive and -negative HL. Late EBV infection does not appear relevant to risk, suggesting that other pathogens impact HL etiology in affluent female populations. Inconsistency of findings with prior studies may reflect failure of study risk factors to proxy meaningful exposures, risk differences by gender, or selection or misclassification bias. Null findings for EBV-negative HL indicate that etiologic models should be reconsidered for this common form.

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Acute infections as a means of cancer prevention: opposing effects to chronic infections?

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PMID: 16490323 DOI: 10.1016/j.cdp.2005.11.001

Abstract

Purpose: Epidemiological studies have found an inverse association between acute infections and cancer development. In this paper, we review the evidence examining this potentially antagonistic relationship.

Methods: In addition to a review of the historical literature, we examined the recent epidemiological evidence on the relationship between acute infections and subsequent cancer development in adult life. We also discuss the impact of chronic infections on tumor development and the influence of the immune system in this process.

Results: Exposures to febrile infectious childhood diseases were associated with subsequently reduced risks for melanoma, ovary, and multiple cancers combined, significant in the latter two groups. Epidemiological studies on common acute infections in adults and subsequent cancer development found these infections to be associated with reduced risks for meningioma, glioma, melanoma and multiple cancers combined, significantly for the latter three groups. Overall, risk reduction increased with the frequency of infections, with febrile infections affording the greatest protection. In contrast to acute infections, chronic infections can be viewed as resulting from a failed immune response and an increasing number have been associated with an elevated cancer risk.

Conclusion: Infections may play a paradoxical role in cancer development with chronic infections often being tumorigenic and acute infections being antagonistic to cancer.

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Infectious diseases and risk of leukemia and non-Hodgkin's lymphoma: a case-control study

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PMID: 22940258 DOI: 10.1016/j.leukres.2012.08.016

Abstract

The aim of this investigation was to evaluate the association between common infectious diseases and the risk of hematological malignancies in an adult population. Data were drawn from a population based case-control study that included 165 cases (125 lymphoid and 40 myeloid neoplasms) and 233 controls. Occurrence of childhood diseases (measles, rubella, chickenpox, mumps, pertussis and scarlet fever) was slightly inversely associated with the risk of both malignancies, but statistical significance was not reached. The data of infections occurring after 14 years of age indicated an increasing risk of lymphoid malignancies (OR=2.9, p<0.05).

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Childhood infectious disease and premature death from cancer: a prospective cohort study

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Abstract

Studies of the association between early life infections and cancer have produced inconsistent findings, possibly due to limited adjustment for confounding and retrospective designs. This study utilised data from the Newcastle Thousand Families Study, a prospective cohort of 1,142 individuals born in Newcastle-upon-Tyne in 1947, to assess the impact of various childhood infectious diseases on cancer mortality during ages 15-60 years. Detailed information was collected prospectively on a number of early life factors. Deaths from cancer during ages 15-60 years were analysed in relation to childhood infections, adjusting for potential early-life confounders, using Cox proportional-hazards regression. In a subsample who returned questionnaires at aged 49-51 years, additional adjustment was made for adult factors to predict death from cancer during ages 50-60 years. Childhood history of measles and influenza, were both independently associated with lower cancer mortality during ages 15-60 years (adjusted hazard ratios = 0.39, 95% CI 0.17-0.88 and 0.49, 95% CI 0.24-0.98 respectively). In contrast, childhood pertussis was associated with higher cancer mortality during ages 15-60 years (adjusted hazard ratio = 4.88, 95% CI 2.29-10.38). In the subsample with additional adjustment for adult variables, measles and pertussis remained significantly associated with cancer mortality during ages 50-60 years. In this pre-vaccination cohort, childhood infection with measles and influenza were associated with a reduced risk of death from cancer in adulthood, while pertussis was associated with an increased risk. While these results suggest some disease-specific associations between early-life infections and cancer, further studies are required to confirm the specific associations identified.

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Childhood infectious diseases and risk of leukaemia in an adult population

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Our study is aimed at investigating the association between common childhood infectious diseases (measles, chickenpox, rubella, mumps and pertussis) and the risk of developing leukaemia in an adult population. A reanalysis of a large population-based case–control study was carried out. Original data included 1,771 controls and 649 leukaemia cases from 11 Italian areas. To contain recall bias, the analysis was restricted to subjects directly interviewed and with a good quality interview (1,165 controls and 312 cases). Odds ratios (ORs) and their related 95% confidence intervals (95% CIs) were estimated by unconditional polychotomous logistic regression model adjusting for age, gender and occupational and lifestyle exposures. A protective effect of at least one infection (OR = 0.66, 95% CI: 0.45–0.97), measles (OR = 0.57, 95% CI: 0.39–0.82) and pertussis (OR = 0.66, 95% CI: 0.45–0.98) was observed for chronic lymphoid leukaemia (CLL). The number of infections was strongly inversely associated with the risk of CLL (p = 0.002, test for trend). With regard to the other types of leukaemia, only a protective effect of pertussis was observed for AML (OR = 0.52, 95% CI: 0.32–0.87). Our results pointed out a protective role of childhood infectious diseases on the risk of CLL in adults. Although a specific antioncogenic effect of some infectious disease, especially measles, cannot be ruled out, the observed decrease of risk with increasing number of infections suggests that a more general "hygiene hypothesis" could be the most likely explanation of the detected association. The protective role of pertussis remains to be elucidated.

Epidemiology

Aetiology of adult leukaemia is largely unknown. Most studies have been focused on occupational and lifestyle exposures indicating an association with the following risk factors: prolonged exposure to ionizing radiations, chemotherapeutic agents, benzene and other hydrocarbons, whereas other investigations have pointed out a possible role of smoking habits, obesity and exposure to electromagnetic fields and pesticides, but with a less consistent evidence.[1–8]

A possible role of infectious diseases has been intensively investigated for childhood leukaemia, mainly based on consistent evidence of clustering of cases following population mixing.[9] Furthermore, a protective effect of multiple infections during childhood has also been reported.[10]

In adult populations, a causal association between HTLV-1 retrovirus and T-cell leukaemia has been demonstrated, accounting for less than 10% of the total disease burden.[10] Studies on a possible role of other infections remain scarce.[11,12] Among them, a large multicentre case–control investigation in Italy[12] reported a protective effect of childhood infections on the risk of adult leukaemia, but a detailed analysis by specific infectious agent was not carried out. A recent investigation based on a small case–control study in Northern Italy found a similar protective effect both on lymphoid and on myeloid malignancies. However, no role of a single viral or bacterial agent was identified and the small sample size prevented statistical significance from been reached.[13]

Key words: leukaemia, measles, pertussis, infectious diseases, case–control study
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What's new?

We know that the retrovirus HTLV-1 is associated with adult leukemia, but little is known about the impact of other infections. For example, do childhood infections affect the risk of developing leukemia in adulthood? To begin to address this question, the authors analyzed a large multi-centre, case-control study, and found that childhood infections may actually lower the risk of developing chronic lymphocytic leukaemia (CLL) as an adult. This risk also decreased further as the number of infections increased.

Our study was aimed at investigating the specific association between the risk of adult leukaemia and childhood infectious diseases by means of a reanalysis of a large multicentre population-based case–control study.

Material and Methods

Subjects recruitment and interview

The present reanalysis includes 11 areas in Italy (*i.e.*, the provinces of Varese, Forlì, Siena, Latina, Ragusa, Imperia, Florence, Novara, Vercelli and Verona and the town of Turin).[12,14]

All new leukaemia cases, diagnosed between 1990 and 1993, aged 20–74 years at diagnosis, and resident in one of the 11 study areas were considered as eligible. Leukaemia cases were classified according to the International Classification of Diseases, 9th revision (ICD-9): 204–208. The following subtypes were analysed separately: acute lymphoid leukaemia (ALL, ICD-9: 204.0), chronic lymphoid leukaemia (CLL, ICD-9: 204.1), acute myeloid leukaemia (AML, ICD-9: 205.0), chronic myeloid leukaemia (CML, ICD-9: 205.1) and other acute leukaemia (OL, ICD-9: 206.0, 207.0 and 208.0). Data for CLL were available in all areas, while the other types of leukaemia were not collected in three areas (Novara, Varese and Vercelli).

Cases were identified by systematic searches in departments of haematology, general medicine, surgery and pathology in all hospitals within the above-mentioned areas. Furthermore, specialised hospitals outside these areas, where such patients could be admitted, were also considered. Diagnoses were based on histological analyses for CLL, and on morphologic, cytochemical and immunological analyses for the other types of leukaemia. All diagnoses of CLL were homogeneously classified by an experienced pathologist. Case ascertainment was complete, as indicated by a comparison with historical data of Cancer Registries in the same areas. Moreover, the level of case ascertainment did not vary across centres. Interview took place, on average, 266 days from the leukaemia diagnosis (standard deviation: 275 days).

Controls were randomly selected from the residents living in the study areas and frequency matched to cases by age ($\pm$5 years) and gender. Control selection was carried out through record linkage with population-computerised files in all areas.

Information about the known or alleged risk factors was collected through person-to-person interview. A standardised questionnaire was administered to cases and controls by trained interviewers to obtain detailed information about sociodemographic characteristics, residential history, lifestyle and occupational exposures and medical history. The following infectious diseases, typically occurring during childhood (named "childhood diseases" throughout this article, independently from the age at the infection), were included in the questionnaire: measles, chickenpox, rubella, mumps and pertussis. All subjects gave their informed consent to participate in the study, and the interview lasted, on average, about 1 hr.

More details about the study design and the questionnaire structure have been published elsewhere.[12,14,15]

To reduce the possible effect of recall bias, statistical analysis was restricted to subjects who were directly interviewed, declared to recall the age at the infectious disease onset, if any, and had a high-quality interview, defined as an interview that lasted for at least 40 min. Finally, a sensitivity analysis was also performed comparing the estimates obtained by the restricted analysis to those from models including the whole unrestricted group of interviewed cases and controls.

Statistical analysis

The association between leukaemia risk and the previous onset of infectious diseases was assessed by unconditional polychotomous logistic regression.[16] The polychotomous model was chosen because a single set of controls was used for multiple subgroups of leukaemia cases, while unconditional regression was preferred to the conditional one because of the small sample size of the leukaemia subgroups, in order to prevent a loss of statistical power as a consequence of the exclusion of matched controls. Odds ratio (OR) estimates and the corresponding 95% confidence intervals (95% CIs) were adjusted for the following putative confounders: age, gender, educational level, tobacco smoking, radiotherapy and chemotherapy treatment and professional exposures to pesticides, aromatic hydrocarbons, radiations and electromagnetic fields. Age at recruitment was modelled as a continuous variable, using both a linear and a quadratic term to take into account departures from linearity of the effects (if any). Radiotherapy and chemotherapy related to the disease used for the selection of cases were not considered. Tobacco smoking was categorised into two levels (*i.e.*, never/ever smokers), as were professional exposures (*i.e.*, absent/present). A subject was considered as exposed if he/she performed a job entailing

a specific exposure for at least 5 years during his/her life. The type of occupational exposure was indirectly assessed by using the first two digits of the International Standard Classification of Occupations code of economic activity.[17] Up to 12 lifetime different occupations were analysed. Because only six subjects (one case and five controls) declared to have been professionally exposed to radiations, they were aggregated to the therapeutic exposures. Gender and the linear term of age were included in each regression model, while confounders were included only when they significantly contributed to the model within a forward selection procedure.[16] Furthermore, the impact of confounding was quantify as the change percent in the ORs between models containing the putative confounders and those without them, and confounders whose impact was considered as negligible (*i.e.*, $< 10\%$) were excluded from the analysis to reduce the variance of the OR estimates.

Childhood infectious diseases were analysed both separately and grouped together using an indicator of presence/absence of at least one disease. The lifetime number of infections was also considered. Finally, an analysis by age at the infection was also carried out where study subjects were stratified into the following three groups: 0–5, 6–14 and >14 years at diagnosis of each infection.

All the analyses were performed by using the statistical package Stata for Windows (release 11.1, Stata Corporation, College Station, TX).

Results

Overall, 1,771 controls (81%) and 649 leukaemia cases (88%) were interviewed.[14] Direct interviews were available for 80% of cases and 96% of controls and, among them, a high-quality interview was performed on 1,453 controls and 418 cases. After a further exclusion of subjects who were not able to remember the dates of childhood infections, 80% of controls ($n = 1{,}165$) and 75% of leukaemia cases ($n = 312$) were considered as eligible for the analysis. Leukaemia cases included 22 ALL, 137 CLL, 84 AML, 51 CML and 18 OL. Subjects excluded from the analysis were slightly younger (mean age at the recruitment 53.8 *vs*. 56.2 years), more frequently females (49.5 *vs*. 44.6%) and more educated (the proportion of low educated persons was 49.1 *vs*. 56.8%). Such differences were observed both in cases and in controls.

Table 1 shows the main characteristics of the study subjects. Females were more represented among controls, ALL and OL. Controls, AML and CML cases had a similar age distribution, whereas CLL tended to be older and ALL and OL younger. Educational level was similar in controls and AML, whereas CLL and CML showed a higher proportion of low educated persons. Smoking habit was slightly less common among controls (54%) than among cases, with the exception of CML (41%). Radiation exposure was rare and almost homogeneously distributed across the groups (0–5%). Some of 25% of all subjects were exposed to pesticides with slightly higher proportion observed in CLL and CML (31 and 39%, respectively). Nearly one-third of subjects were exposed to aromatic hydrocarbons with the lowest proportion in the CML group (26%) and the highest among CLL and OL (40 and 44%, respectively). Exposure to electromagnetic fields and to chemotherapeutic treatments, not related to the case definition, was very rare in all groups.

Table 2 reports the distribution of infectious diseases among controls and the subgroups of cases, stratified by age at diagnosis. About 77% of controls and 60–80% of cases reported at least one childhood infectious disease during their life. Among controls, measles was the most commonly reported disease, accounting for about 60%, followed by mumps (46%), pertussis (41%), chickenpox (39%) and rubella (27%). Infectious diseases were very rare after 14 years of age, ranging between 0 and 5% among cases and 3% in controls, thus preventing multivariable statistical analysis from been performed. Moreover, only 13 cases and 33 controls reported the first infection after 14 years of age. The number of infections was higher among controls, ALL and OL, where about 40% of subjects reported at least three diseases, than among the other leukaemia subgroups (about 35% in AML and CML, and 23% in CLL).

Table 3 shows the estimated associations between childhood infectious diseases and the risks of developing different types of leukaemia. With regard to ALL, a protective effect was observed for measles (OR = 0.41, 95% CI: 0.17–1.0) and, consistently, although to a lower extent, for chickenpox, mumps, pertussis and any infection. Conversely, a higher risk was observed for rubella. The ORs decreased with increasing the number of infections, but failed to reach statistical significance. CLL risk was lower among subjects who declared an infection by measles (OR = 0.57, 95% CI: 0.39–0.82), by pertussis (OR = 0.66, 95% CI: 0.45–0.98) and by any infectious agent (OR = 0.66, 95% CI: 0.45–0.97). ORs below the unit were detected also for chickenpox, rubella and mumps infections, but statistical significance was not reached. Furthermore, the risk was inversely associated with the number of infectious diseases; the corresponding ORs were 0.84 (95% CI: 0.55–1.3) for one to two infections and 0.47 (95% CI: 0.29–0.77) for at least three infections ($p = 0.002$, test for trend). ORs for AML were below the unit for all considered diseases, with a significance reduction detected only for pertussis (OR = 0.52, 95% CI: 0.32–0.87). ORs tended to decrease slightly with increasing the number of infections, without reaching statistical significance. Concerning CML, no clear association emerged with any disease. Finally, OL risk estimates were below the unit, without reaching statistical significance.

In each model, confounding effect had a slight or negligible effect. Education was the only confounder with statistically significant ORs, with a high educational level negatively associated with CLL and CML and positively associated with AML, ALL and OL. The reintroduction into the models of the excluded subjects had a negligible effect on the inverse association observed between CLL and the number of childhood infections, whereas it caused a strong apparent inverse trend for ALL (OR = 0.71 for one to two infections and

Table 1. Selected characteristics and exposures of 1,165 controls and 312 leukaemia cases, Italy 1990–1993

	Controls (n = 1,165)		ALL (n = 22)		CLL (n = 137)		AML (n = 84)		CML (n = 51)		OL (n = 18)	
Subjects characteristics	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**
Age (years)												
20–34	116	10.0	6	27.3	1	0.73	7	8.3	2	3.9	1	5.6
35–44	133	11.4	7	31.8	8	5.8	12	14.3	5	9.8	2	11.1
45–54	210	18.0	1	4.6	15	11.0	11	13.1	5	9.8	9	50.0
55–64	332	28.5	6	27.3	53	38.7	27	32.1	19	37.3	4	22.2
>65	374	32.1	2	9.1	60	43.8	27	32.1	20	39.2	2	11.1
Gender												
Males	625	53.7	11	50.0	91	66.4	51	60.7	32	62.8	9	50.0
Females	540	46.4	11	50.0	46	33.6	33	39.3	19	37.2	9	50.0
Area												
Florence	254	21.8	10	45.5	30	21.9	29	34.5	12	23.5	6	33.3
Forlì	100	8.6	5	22.7	15	11.0	3	3.6	9	17.7	0	0.0
Imperia	73	6.3	2	9.1	12	9.1	8	9.5	6	11.8	2	11.1
Novara	71	6.1	0	0.0	3	2.2	0	0.0	0	0.0	0	0.0
Ragusa	75	6.4	0	0.0	9	6.6	1	1.2	1	2.0	0	0.0
Siena	10	0.9	0	0.0	2	1.5	4	4.8	2	3.9	0	0.0
Turin	131	11.2	0	0.0	29	21.2	27	32.1	10	19.6	10	55.6
Varese	223	19.1	0	0.0	25	18.3	0	0.0	0	0.0	0	0.0
Vercelli	45	3.9	0	0.0	3	2.2	0	0.0	0	0.0	0	0.0
Verona	131	11.2	3	13.6	0	0.0	7	8.3	9	17.7	0	0.0
Latina	52	4.5	2	9.1	7	5.1	5	6.0	2	3.9	0	0.0
Educational level												
Illiterate	48	4.1	2	9.1	8	5.8	2	2.4	4	7.8	0	0.0
Primary school	609	52.3	4	18.2	85	62.0	38	45.2	34	66.7	5	27.8
Middle school	262	22.5	9	40.9	30	21.9	24	28.6	8	15.7	5	27.8
High school	188	16.1	4	18.2	8	5.8	12	14.3	3	5.9	5	27.8
University	58	5.0	3	13.6	6	4.4	8	9.5	2	3.9	3	16.7
Tobacco smoking												
Ever smokers	633	54.3	15	68.2	80	58.4	52	61.9	21	41.2	12	66.7
Never smokers	532	45.7	7	31.8	57	41.6	32	38.1	30	58.8	6	33.3
Radiations												
Exposed	58	5.0	1	4.6	6	4.4	0	0.0	3	5.9	0	0.0
Unexposed	1,107	95.0	21	95.4	131	95.6	84	100	48	94.1	18	100
Pesticides												
Exposed	284	24.4	5	22.7	43	31.4	21	25.0	20	39.2	4	22.2
Unexposed	881	75.6	17	77.3	94	68.6	63	75.0	31	60.8	14	77.8
Aromatic hydrocarbons												
Exposed	413	35.4	7	31.8	55	40.2	30	35.7	13	25.5	8	44.4
Unexposed	752	64.6	15	68.2	82	59.9	54	64.3	38	74.5	10	55.6
EMF												
Exposed	42	3.6	0	0.0	9	6.6	3	3.6	3	5.9	2	11.1
Unexposed	1,126	96.4	22	100	128	93.4	81	96.4	48	94.1	16	88.9
Chemotherapy												
Exposed	10	0.9	0	0.0	4	2.9	1	1.2	1	2.0	1	5.6
Unexposed	1,155	99.1	22	100	133	97.1	83	98.8	50	98.0	17	94.4

Abbreviations: ALL: acute lymphoid leukaemia; CLL: chronic lymphoid leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; OL: other leukaemias; EMF: electromagnetic fields.

Table 2. Childhood infectious diseases and age at infection in 312 leukaemia cases and 1,165 controls, Italy 1990–1993

	Controls (n = 1,165)		ALL (n = 22)		CLL (n = 137)		AML (n = 84)		CML (n = 51)		OL (n = 18)	
Infectious disease	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**	***N***	**%**
Measles												
0–5 years	278	23.9	4	18.2	27	19.7	11	13.1	7	13.7	4	22.2
6–14 years	386	33.1	7	31.8	24	17.5	31	36.9	19	37.3	6	33.3
>14 years	24	2.1	0	0.0	2	1.5	2	2.4	1	2.0	0	0.0
All age	688	59.1	11	50.0	53	38.7	44	52.4	27	52.9	10	55.6
Chickenpox												
0–5 years	119	10.2	1	4.6	12	8.8	6	7.1	2	3.9	4	22.2
6–14 years	306	26.3	9	40.9	20	14.6	22	26.2	11	21.6	2	11.1
>14 years	28	2.4	0	0.0	3	2.2	3	2.2	1	2.0	0	0.0
All age	454	39.0	10	45.5	35	25.6	30	35.7	14	27.5	6	33.3
Rubella												
0–5 years	94	8.1	2	9.1	10	7.3	5	6.0	4	7.8	3	16.7
6–14 years	203	17.4	7	31.8	16	11.7	16	19.1	9	17.7	0	0.0
>14 years	20	1.7	1	4.6	1	0.73	1	1.2	0	0.0	0	0.0
All age	317	27.2	10	45.5	27	19.7	22	26.2	13	25.5	3	16.7
Mumps												
0–5 years	95	8.2	2	9.1	8	5.8	4	4.8	6	11.8	5	27.8
6–14 years	382	32.8	7	31.8	38	27.7	21	25.0	15	29.4	2	11.1
>14 years	61	5.2	2	9.1	7	5.1	6	7.1	3	5.9	0	0.0
All age	538	46.2	11	50.0	53	38.7	31	36.9	24	47.1	7	38.9
Pertussis												
0–5 years	143	12.3	1	4.6	15	11.0	10	11.9	4	7.8	4	22.2
6–14 years	299	25.7	5	22.7	22	16.1	11	13.1	11	21.6	3	16.7
>14 years	32	2.8	1	4.6	3	2.2	1	1.2	2	3.9	0	0.0
All age	474	40.7	7	31.8	40	29.2	22	26.2	17	33.3	7	38.9
Any infection												
0–5 years[1]	431	37.0	7	31.8	34	24.8	20	23.8	18	35.3	8	44.4
6–14 years[1]	430	36.9	10	45.5	43	31.4	35	41.7	16	31.4	4	22.2
>14 years[1]	33	2.8	1	4.6	7	5.1	3	3.6	2	3.9	0	0.0
All age	894	76.7	18	81.8	84	61.3	58	69.1	36	70.6	12	66.7
Number of infections												
None	271	23.3	4	18.2	53	38.7	26	31.0	15	29.4	6	33.3
1–2	407	34.9	9	40.9	53	38.7	28	33.3	19	37.3	5	27.8
≥3	487	41.8	9	40.9	31	22.6	30	35.7	17	33.3	7	38.9

[1]Age at the first infection.
Abbreviations: ALL: acute lymphoid leukaemia; CLL: chronic lymphoid leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; OL: other leukaemia.

OR = 0.31 for three infections or more, respectively) and for CML (OR = 0.48 and 0.33, respectively).

Table 4 shows the estimated associations between age at infection and the risk of leukaemia. ALL and OL were excluded from the analyses because of the low number of cases. A protective effect of infection in the second age class (6–14 years) on the risk of CLL was observed for measles (OR = 0.45, 95% CI: 0.28–0.73), chickenpox (OR = 0.61, 95% CI: 0.37–1.0) and pertussis (OR = 0.57, 95% CI: 0.35–0.92), while a protective effect of measles infection was observed for AML in the first age class (0–5 years; OR = 0.45, 95% CI: 0.22–0.90) and of pertussis in the second age class (6–14 years; OR = 0.40, 95% CI: 0.21–0.78). No statistically significant association emerged for CML. A protective

Table 3. Association between childhood infectious diseases and risk of leukaemia in 1,165 controls and 312 cases, Italy 1990–1993

	ALL (n = 22)		CLL (n = 137)		AML (n = 84)		CML (n = 51)		OL (n = 18)	
Disease	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**	**OR**	**95% CI**
Measles	0.41	0.17–1.0	0.57	0.39–0.82	0.75	0.48–1.2	0.97	0.54–1.7	0.68	0.26–1.8
Chickenpox	0.74	0.30–2.8	0.73	0.48–1.1	0.86	0.53–1.4	0.74	0.39–1.4	0.56	0.20–1.5
Rubella	1.8	0.77–4.4	0.79	0.50–1.2	0.96	0.58–1.6	1.0	0.53–1.9	0.46	0.13–1.6
Mumps	0.86	0.36–2.1	0.88	0.61–1.3	0.67	0.42–1.1	1.2	0.65–2.1	0.64	0.24–1.7
Pertussis	0.65	0.26–1.6	0.66	0.45–0.98	0.52	0.32–0.87	0.77	0.43–1.4	0.83	0.32–2.2
Any infection	0.74	0.23–2.4	0.66	0.45–0.97	0.66	0.40–1.1	0.95	0.50–1.8	0.41	0.14–1.1
No. of infections										
None	ref	–	ref[1]	–	ref	–	ref	–	ref	–
1–2	0.92	0.27–3.2	0.84	0.55–1.3	0.71	0.40–1.2	1.0	0.51–2.1	0.41	0.12–1.4
≥3	0.60	0.17–2.1	0.47	0.29–0.77	0.62	0.35–1.1	0.86	0.41–1.8	0.40	0.13–1.3

[1] p = 0.002, test for trend.
Abbreviations: ALL: acute lymphoid leukaemia; CLL: chronic lymphoid leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; OL: other leukaemia; OR: odds ratios adjusted for age, gender, education, tobacco smoking, chemotherapy, radiations and occupational exposures to pesticides, aromatic hydrocarbons and electromagnetic fields.

effect of any infection in the first two age classes was observed for CLL (OR = 0.57, 95% CI: 0.35–0.92 and OR = 0.67, 95% CI: 0.43–1.0) and for the age group 0–5 years for AML (OR = 0.45, 95% CI: 0.24–0.84). Finally, an analysis stratified for age at the infection, not shown in Table 4, highlighted a clear association between CLL risk and the number of infections between 6 and 14 years of age. In more details, selecting the group without any infection as the referent, the corresponding ORs were 0.66 (95% CI: 0.42–1.0) for one to two infections and 0.46 (95% CI: 0.25–0.83) for more than three infections (p = 0.005, test for trend).

A strong effect of any putative confounder did not emerge. However, ORs for educational level were statistically significant in each model with the only exception of rubella. Moreover, the smoking habit effect was statistically significant in the models for measles, rubella and pertussis with a negative association between smoke and CML.

Discussion

A protective effect of some childhood infections, especially measles and pertussis, on CLL risk was pointed out by this investigation. Interestingly, a statistically significant inverse trend was observed with increasing number of infections. A similar result was also observed for ALL, but the small sample size prevented to draw definitive conclusions. With regard to AML and CML, no significant associations were found, except for a protective effect of pertussis among AML.

Results about CLL are partly consistent with a previous hospital-based case–control investigation by Montella *et al.*[18] who reported a protective effect of measles on the risk of developing non-Hodgkin's lymphoma in an adult population in Italy diagnosed between 1999 and 2002. Cases included the small cell lymphoma subtype that, according to the adopted classification system (Working Formulation[19]), also included CLL. However, the authors did not report any analysis for this specific subgroup. Moreover, a clear association with the number of infections was not found. A recent pooled analysis of more than 12,000 cases and 15,000 controls from 17 datasets in Europe and Northern America (including that of Montella *et al.*[18] and part of the present database) has confirmed the protective role of childhood infectious diseases on the risk of developing non-Hodgkin's lymphomas in subjects aged 16 years or more.[20]

Previous studies that specifically addressed the risk of adult AML in association with childhood infectious diseases are rare and generally reported a higher risk for subjects with some infection, but with conflicting results. A large population-based case–control study, carried out in the USA and in Canada at the end of the 1980s, reported an excess risk of AML in association with childhood infections, especially measles.[21] The risk increased when the analyses were restricted to infections occurred in early childhood (*i.e.*, <6 years). However, only viral diseases were considered, and the association with pertussis was not evaluated. A case–control study carried out in Shanghai (People's Republic of China), including 236 patients with AML and 79 with CML, diagnosed between 1987 and 1989, did not find any association with a previous history of infections, except for a small excess risk for AML in subjects with tuberculosis.[22] More recently, a very large register-based case–control investigation in Sweden including 9,129 cases with AML, diagnosed between 1965 and 2004, 1,662 with myelodisplastic syndromes, diagnosed between 1993 and 2004, and 42,878 population-based controls reported a small statistically significant association between the risk of both diseases and a previous history of infections (OR = 1.3 for both malignancies).[23] These results are suggestive of a potential role of immunostimulation by infectious agent in the aetiology of myeloid leukaemia. Unfortunately, childhood diseases were not included among the considered diseases. Conversely, Larfors *et al.*[24] in

Table 4. Association between age at infection of childhood disease and risk of leukaemia in 1,165 controls and 272, Italy 1990–1993

	CLL (n = 137)		AML (n = 84)		CML (n = 51)	
Disease	OR	95% CI	OR	95% CI	OR	95% CI
Measles						
0–5 years	0.73	0.45–1.2	0.45	0.22–0.90	0.64	0.27–1.5
6–14 years	0.45	0.28–0.73	0.95	0.58–1.6	1.2	0.63–2.2
>14 years	0.47[1]	0.11–2.0	0.99[1]	0.22–4.4	0.83[1]	0.11–6.4
Chickenpox						
0–5 years	0.92	0.48–1.7	0.67	0.28–1.6	0.37	0.09–1.6
6–14 years	0.61	0.37–1.0	0.97	0.57–1.7	0.81	0.40–1.6
>14 years	0.75[1]	0.22–2.5	0.94[1]	0.22–4.1	0.69[1]	0.09–5.2
Rubella						
0–5 years	1.1	0.54–2.2	0.74	0.29–1.9	1.1	0.38–3.2
6–14 years	0.70	0.40–1.2	1.1	0.61–1.9	1.1	0.54–2.4
>14 years	0.39[1]	0.05–2.9	0.68[1]	0.09–5.2	0.0[1]	n.e.
Mumps						
0–5 years	0.81	0.38–1.8	0.48	0.17–1.4	1.7	0.69–4.4
6–14 years	0.91	0.60–1.4	0.62	0.37–1.1	1.0	0.54–2.0
>14 years	0.92	0.41–2.1	1.2	0.49–2.9	1.1[1]	0.34–3.9
Pertussis						
0–5 years	0.91	0.50–1.6	0.77	0.38–1.5	0.57[1]	0.20–1.6
6–14 years	0.57	0.35–0.92	0.40	0.21–0.78	0.84	0.42–1.7
>14 years	0.67[1]	0.20–2.2	0.35[1]	0.05–2.6	1.3[1]	0.29–5.5
First infection						
0–5 years	0.57	0.35–0.92	0.45	0.24–0.84	1.1	0.51–2.2
6–14 years	0.67	0.43–1.0	0.83	0.48–1.4	0.85	0.41–1.8
>14 years	1.1[1]	0.46–2.6	0.95[1]	0.27–3.3	1.1[1]	0.24–5.0

[1]Unadjusted odds ratios.
Abbreviations: CLL: chronic lymphoid leukaemia; AML: acute myeloid leukaemia; CML: chronic myeloid leukaemia; OR: odds ratios adjusted for age, gender, education, tobacco smoking, chemotherapy, radiations and occupational exposures to pesticides, aromatic hydrocarbons and electromagnetic fields; n.e.: not evaluable.

another large register-based investigation in Sweden reported a negative association between the risk of adult AML, diagnosed between 1962 and 2008, and the number of siblings, which could represent an indirect measure of exposure to infectious agents.

Among the limits potentially affecting our finding there is the anamnestic source of information about infectious diseases, without any diagnostic confirmation from medical records. The restriction to those subjects who were able to remember the time of infection does not guarantee a complete control for recall bias. However, the possibility of a protective effect of childhood infections on leukaemia risk in adults is not a common knowledge, then cases and controls should equally recall their previous infections. Nonetheless, the probability to correctly remember the occurrence of a disease during childhood and the time of its onset is likely to decrease with decreasing age at diagnosis. Results of the analysis stratified by age at infection suggest that the putative protective effect of infectious diseases on the CLL risk was higher when infections occurred between 6 and 14 years of age, but the lack of a similar association in early infancy could be attributable to recall bias. Nevertheless, the possibility of a selection bias as a consequence of the above-mentioned restriction cannot be completely ruled out, considering that the excluded subjects slightly differed by distribution of gender and educational level and that cases with very aggressive disease were excluded from the study. In Italy, studies about prevalence for adult population of the considered childhood infections for the period under study are few and probably prone to underreporting bias.[25] However, our estimates are consistent with those reported by another case–control investigation carried out in Italy at the end of 1990s.[18] Moreover, cases and controls showed a rather similar distribution of educational level, thus suggesting that selection bias, if any, could have been nondifferential. As a consequence, it should not be responsible for the finding of spurious associations. We have observed an apparent protective effect of the number of childhood infections for ALL and

CML after the reintroduction into the model of the excluded subjects, pointing out the occurrence of a strong information bias that the restriction has avoided (or at least reduced). In fact, as expected, such a bias was more evident among the two groups of cases with the highest number of interviews addressed to a next-of-kin (25% for ALL and 33% for AML). Among the unavoidable limits of our analysis there is also the adoption of an old classification of disease groups based on ICD-9 categories, which now are recognised to include some heterogeneous diseases (*e.g.*, B- and T-cell subtypes for ALL and CLL). Because different diseases probably have different aetiological factors, this limit could have induced a misclassification bias, thus contributing to lower statistical power, especially among the two smallest subgroups (*i.e.*, ALL and OL). Finally, the questionnaire did not include any information about vaccination. However, the proportion of vaccinated subjects is expected to be negligible: vaccination for the considered diseases in Italy is still not mandatory and it was introduced later than in other developed countries (rubella in 1972, measles in the 1976 and the other ones after 1980). The studied subjects were all aged 20 years or more when recruited between 1990 and 1993. Moreover, a large survey, carried out in 1985, showed that the prevalence of Italian population vaccinated for measles, rubella or pertussis was very low.[26]

In conclusion, results from our investigation are consistent with those from other recent studies and point out a protective role of childhood infectious diseases on the risk of developing CLL in adult populations. The related biological mechanism remains to be elucidated and it could involve a specific antioncogenic effect of some infectious agents, especially measles.[18] However, the decreasing trend in risk with increasing number of infections suggests a role of a "hygiene hypothesis," namely a protective effect of multiple infections during childhood on the risk of developing adult malignancies. Finally, the protective effect of pertussis on the risk of CLL and AML remains to be clarified.

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SHORT REPORT

History of chickenpox in glioma risk: a report from the glioma international case–control study (GICC)

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Abstract

Varicella zoster virus (VZV) is a neurotropic α-herpesvirus that causes chickenpox and establishes life-long latency in the cranial nerve and dorsal root ganglia of the host. To date, VZV is the only virus consistently reported to have an inverse association with glioma. The Glioma International Case-Control Study (GICC) is a large, multisite consortium with data on 4533 cases and 4171 controls collected across five countries. Here, we utilized the GICC data to confirm the previously reported associations between history of chickenpox and glioma risk in one of the largest studies to date on this topic. Using two-stage random-effects restricted maximum likelihood modeling, we found that a positive history of chickenpox was associated with a 21% lower glioma risk, adjusting for age and sex (95% confidence intervals (CI): 0.65–0.96). Furthermore, the protective effect of chickenpox was stronger for high-grade gliomas. Our study provides additional evidence that the observed protective effect of chickenpox against glioma is unlikely to be coincidental. Future studies, including meta-analyses of the literature and investigations of the potential biological mechanism, are warranted.

Introduction

Varicella zoster virus (VZV) is a neurotropic α-herpesvirus that causes chickenpox by initially infecting the respiratory mucosa and then progressing into viremia, during which the virus is transported to and replicates in the skin [1]. Prior to the licensing of the live attenuated VZV vaccine in the 1990s, chickenpox was an extremely common childhood illness, affecting over 90% of individuals [2, 3]. After acute infection, the virus establishes life-long latency in the cranial nerve and dorsal root ganglia of the host, and may later reactivate in about 10–20% of VZV-infected individuals, causing shingles. Viral reactivation can also result in other neurological complications, such as encephalitis and myelitis [2, 4].

Because of its neurotropism and its ability to establish decades-long latency across the neuraxis, [4] VZV is particularly interesting to investigate in relation to gliomagenesis. In fact, of the many viruses previously suspected to be involved in glioma susceptibility (i.e., simian virus 40, BK virus, JC virus, human cytomegalovirus, human herpesvirus-6) [5–7], VZV is the only virus consistently reported to have an inverse association with glioma [3]. The observed inverse relationship between VZV infection and glioma risk has remained relatively consistent across studies with different VZV exposure assessment methods, such as self-reported history of chickenpox [8, 9], total anti-VZV Immunoglobulin G (IgG) levels [9–12], and levels of antibodies against specific VZV proteins [13]. Furthermore, because of its ability to replicate rapidly and lyse malignant glioma cells in vitro, VZV has even been proposed as a novel candidate for glioma virotherapy [14].

The Glioma International Case-Control Study (GICC) is a large, multisite consortium with data on 4533 cases and 4171 controls collected across five countries [15]. The GICC provides an unparalleled opportunity to confirm the previously reported associations between history of chickenpox and glioma in the largest study to date on this topic.

Materials and Methods

Study population

Details on the GICC study population and recruitment methods are available elsewhere [15]. Briefly, the GICC is an international consortium with 14 recruitment sites: Brigham and Women's Hospital (Boston, MA, USA), Case Western Reserve University (Cleveland, Ohio, USA), Columbia University (New York, NY, USA), Danish Cancer Society Research Centre (Copenhagen, Denmark), The Gertner Institute (Tel Hashomer, Israel), Duke University (Durham, NC), University of Texas MD Anderson Cancer Center (Houston, TX, USA), Memorial Sloan Kettering Cancer Center (New York, NY, USA), Mayo Clinic (Rochester, MN, USA), NorthShore HealthSystem (Chicago, IL, USA), Umeå University (Umeå, Sweden), University of California, San Francisco (San Francisco, CA, USA), University of Southern California (Los Angeles, CA, USA), and The Institute of Cancer Research (London, United Kingdom). All participating institutions received Institutional Review Board (IRB) or ethical board approval for the study, and informed consent was obtained from participants.

Cases were defined as individuals within 18–80 years of age (at diagnosis) who had one of the following types of histologically confirmed, supratentorial, intracranial gliomas: fibrillary astrocytoma (9420/3), protoplasmic astrocytoma (9410/3), gemistocytic astrocytoma (9411/3), oligodendroglioma (9450/3), oligoastrocytoma (9382/3), anaplastic astrocytoma (9401/3), anaplastic oligodendroglioma (9451/3), anaplastic oligoastrocytoma (9382/3), gliosarcoma (9442/3), and glioblastoma (9440/3). All cases were recruited within a year of diagnosis and consented at their clinic visits. All sites started recruiting participants in April 2010.

Controls were 18–80 years of age. Because not all sites were able to recruit controls using the same methods (due to issues related to existing infrastructure and resources), four sites recruited clinic-based controls, three sites recruited population-based controls, and seven sites recruited visitors of cancer patients as controls [15].

Data collection

All 14 sites used a common study protocol and the same risk factor questionnaire. Study coordinators were trained to ensure site-to-site homogeneity in data collection practices. Data were stored in a centralized database, and were managed by the lead statistician. More details on our data collection methods have previously been published [15].

The GICC risk factor questionnaire included demographic characteristics, past medical history, and occupational exposure history. Questionnaires were administered through phone and/or in-person interviews, or through mailed self-administered forms. Specifically with regard to VZV-related conditions, participants were asked whether they had ever had any of a list of viral infections, which included chickenpox and shingles. If they answered yes, they were asked their age or what year it was when they had chickenpox or shingles.

Statistical analysis

The overall GICC analysis plan, as well as details of key sensitivity analyses, are available elsewhere [15]. Here, we

Table 1. Population characteristics by case-control status and tumor grade: The Glioma International Case-Control Study (GICC).

	Case No.(%)	Control No.(%)	High-Grade Cases[1] No.(%)	Lower Grade Cases[1] No.(%)
Sex				
Male	2679 (59.1)	2351 (56.37)	1728 (62.29)	916 (54.3)
Female	1854 (40.9)	1820 (43.63)	1046 (37.71)	771 (45.7)
Diagnosis/enrollment age				
18–29 years	308 (6.79)	294 (7.05)	62 (2.24)	228 (13.52)
30–39 years	521 (11.49)	473 (11.34)	108 (3.89)	398 (23.59)
40–49 years	813 (17.94)	680 (16.3)	417 (15.03)	384 (22.76)
50–59 years	1150 (25.37)	1079 (25.87)	796 (28.7)	338 (20.04)
60–69 years	1239 (27.33)	1098 (26.32)	993 (35.8)	238 (14.11)
70–80 years	502 (11.07)	547 (13.11)	398 (14.35)	101 (5.99)
Education[2]				
Less than high school	1127 (27.53)	912 (22.45)	717 (28.55)	392 (25.82)
Some college	1107 (27.05)	1295 (31.88)	653 (26.01)	434 (28.59)
Bachelor's degree	1031 (25.19)	958 (23.58)	600 (23.89)	415 (27.34)
Advanced degree	816 (19.94)	893 (21.98)	535 (21.31)	271 (17.85)
Missing	12 (0.29)	4 (0.1)	6 (0.24)	6 (0.4)
Race/ethnicity				
Non-Hispanic white	4163 (91.84)	3691 (88.49)	2577 (92.9)	1522 (90.22)
Non-Hispanic black	71 (1.57)	139 (3.33)	41 (1.48)	26 (1.54)
Asian	84 (1.85)	87 (2.09)	35 (1.26)	48 (2.85)
Hispanic	162 (3.57)	224 (5.37)	93 (3.35)	67 (3.97)
Other	38 (0.84)	26 (0.62)	22 (0.79)	15 (0.89)
Missing	15 (0.33)	4 (0.1)	6 (0.22)	9 (0.53)
Total	4533 (100)	4171 (100)	2774 (100)	1687 (100)

[1]The sum of the high-grade and lower grade cases is not equal to the total number of cases because of unclassified cases.
[2]One site (UK) did not collect education information.

compared cases and controls on selected characteristics, overall, by study site [not shown], and by tumor grade (high-grade: WHO Grade IV; lower grade: Grade II and III) among cases. Self-reported history of chickenpox and self-reported history of shingles were the exposures of interest.

Site-specific unadjusted and adjusted odds ratios (ORs), and their corresponding 95% Wald confidence intervals (CIs), were calculated, using unconditional logistic regression. Sites with less than five cases or controls in the exposed or unexposed groups were excluded from the meta-analyses. To calculate the meta-analysis ORs (mOR), we utilized both two-stage random-effects maximum likelihood and two-stage random-effects restricted maximum likelihood (REML) modeling [15]. Only final results from the two-stage REML are presented, as results were very similar using the other method. The I^2 statistic was used for each meta-regression model to evaluate the proportion of variability in the effect estimates due to heterogeneity, and the τ^2 statistic was calculated to assess the intersite variance. In some stratified analyses, the numbers became too sparse to calculate mORs, and thus pooled ORs (pORs) had to be provided instead.

Age and sex were considered potential confounders (determined a priori) and were adjusted for in all multivariable models, though adjustment for these factors did not meaningfully alter effect estimates. Education, race/ethnicity, allergy status, and cigarette smoking history were evaluated as potential data-based confounders and were not found to be such, based on a 10% change-in-estimate criterion. These variables were therefore not included in the final models. We also stratified our models by age at chickenpox development (<6, 6–9, and >9 years of age), and separately by glioma diagnosis/study enrollment age groups (<40, 40–59, and >59 years of age).

Sensitivity analyses were conducted in which we included and excluded proxy respondents in the final models and compared the results to ensure that there were no meaningful differences between the ORs. Possible patterns or discrepancies in effect estimates between sites with different control types (visitor, clinic-, or population-based) or different questionnaire administration methods (in-person, mailed, or telephone) were also evaluated [15]. All analyses were conducted in SAS 9.2 (SAS Institute, Cary, NC) or R version 3.1.2 (Vienna, Austria, http://www.R-project.org).

Results

Table 1 provides information on selected characteristics of the GICC study population (4533 total cases and 4171

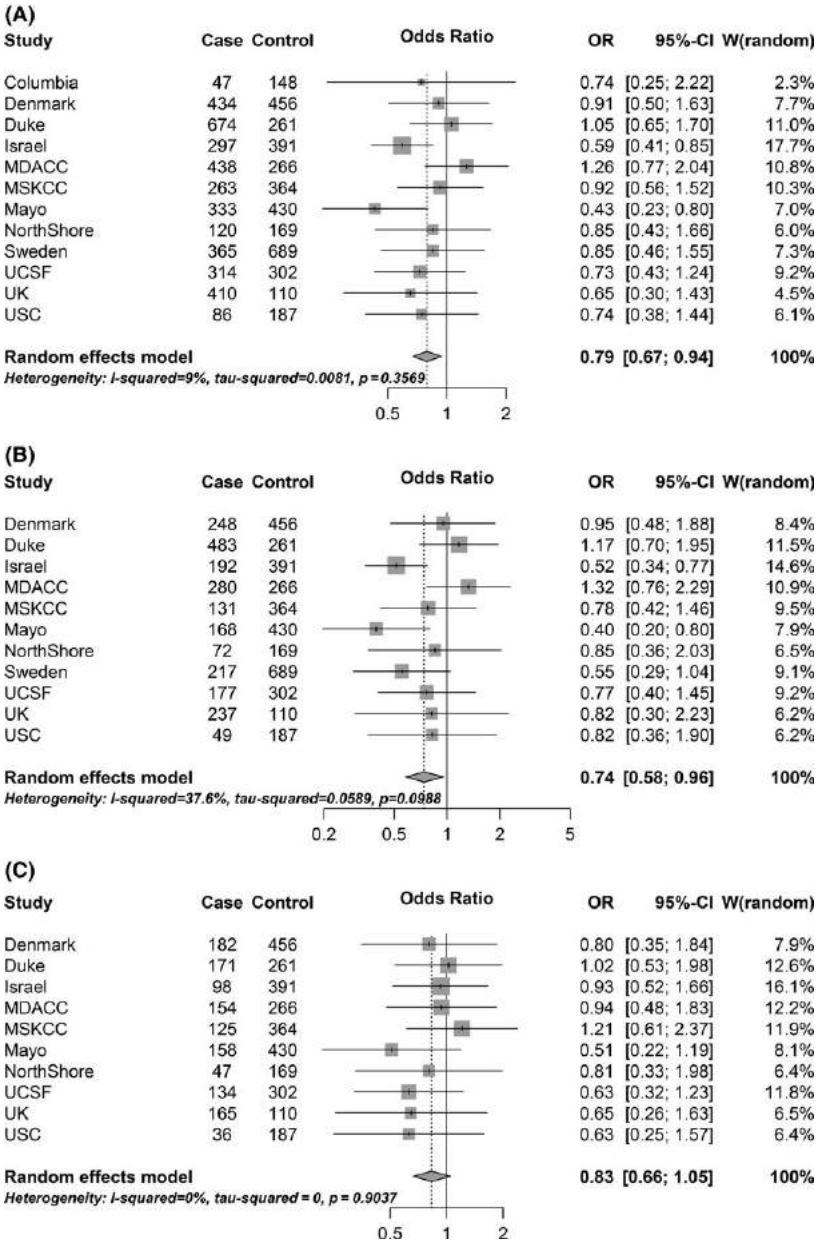

Figure 1. Forest plots for the associations between history of chickenpox and glioma: Findings from the Glioma International Case-Control Study (GICC). (A) In the overall study population. (B) Among high-grade glioma. (C) Among lower grade glioma.

total controls). A table of the study population demographics by site has previously been published [15]. The majority of the study population was non-Hispanic white, but there was a slightly higher preponderance of non-Hispanic black race/ethnicity among controls. The age distribution was similar among cases and controls, but as expected, high-grade glioma cases were slightly older.

Approximately, 79% of cases and 83% of controls reported a positive history of chickenpox. Overall, a positive history of chickenpox was associated with a 21% lower glioma risk, controlling for age and sex (Fig. 1A; mOR: 0.79, 95% CI: 0.65–0.96). A significant adverse OR was not observed at any site, and most site-specific ORs were in the protective direction, though many did not reach statistical significance (possibly due to small numbers/inadequate statistical power). Two sites, Case Western Reserve University and Brigham and Women's Hospital, were excluded due to having cell counts below five.

Restricting to high-grade gliomas, the mOR was slightly stronger and remained statistically significant (Fig. 1B;

mOR: 0.74, 95% CI: 0.58–0.96), whereas among lower grade gliomas, the effect was attenuated and no longer statistically significant (Fig. 1C; mOR: 0.83, 95% CI: 0.66–1.05). Further stratifying by glioma diagnosis/study enrollment age group, we found that the strongest inverse association of chickenpox with high-grade glioma risk was observed among the youngest age group (pOR: 0.53, 95% CI: 0.31–0.90, among participants <40 years of age at diagnosis/enrollment) [data not shown]. While the pORs among the older age groups remained similar to the overall estimate for high-grade glioma risk (pOR: 0.81 and pOR: 0.79, for 40–59 and >59 years of age, respectively), they did not attain statistical significance. No patterns were observed by glioma diagnosis/study enrollment age for lower grade glioma risk, and none of the age-stratified pORs were statistically significant.

The age at which participants developed chickenpox was also considered in our analyses [not shown]. A positive history of chickenpox was associated with an approximately 20–30% lower glioma risk, regardless of whether the participants developed chickenpox under age six (mOR: 0.70, 95% CI: 0.55–0.89), between the ages of six and nine (mOR: 0.74, 95% CI: 0.59–0.93), or above age nine (mOR: 0.76, 95% CI: 0.59–0.98).

In the overall study population, 10.3% of cases and 9.2% of controls reported having at least one episode of shingles. About 28% of participants reported having their first episodes of shingles before age 30 (overall median age: 44; median among cases: 44; median among controls: 43). A positive history of shingles was not significantly associated with glioma risk (mOR: 1.11, 95% CI: 0.89–1.38). The mORs were similar stratified by tumor grade.

Discussion

In our study, a positive history of chickenpox was associated with a 21% lower glioma risk, adjusting for age and sex. The protective effect of chickenpox was stronger for high-grade glioma, particularly among those under age 40. Our findings, which represent the results of the largest study to date on this topic, confirm the inverse associations previously reported in the literature on VZV and glioma.

The majority of published studies on VZV infection and glioma risk are from the San Francisco Bay Area Adult Glioma Study (SFBAGS) series [8–11, 13]. Using both self-reported and serologic (anti-VZV IgG) data to assess history of chickenpox, findings from this series have indicated that prior exposure to VZV is associated with an approximately 40% lower glioma risk [9–11]. Although the odds ratio presented here is not quite as strong as those reported from the SFBAGS series, our estimate is based a larger study population and may possibly be more precise. However, additional studies, including meta-analyses of all published findings, are necessary to estimate the true magnitude of effect.

Like our study, the SFBAGS analyses have implied that the inverse association with prior VZV infection may be stronger for high-grade glioma [9, 11]. For example, Wrensch et al. reported an OR of 0.6 for the association between anti-VZV IgG positivity and any glioma (95% CI: 0.3–1.3), whereas their effect estimate when restricting to glioblastoma was stronger and attained statistical significance (OR: 0.4, 95% CI: 0.1–0.9) [11]. Additionally, in a follow-up study, the SFBAGS investigators observed that mean log anti-VZV IgG levels were higher for controls than glioma cases, but were actually lowest for glioblastoma cases [9].

Besides the SFBAGS series, a few other epidemiologic studies have found similar associations between chickenpox and glioma risk [3, 12]. Sjostrom et al. utilized specimens from three Scandinavian cohorts to investigate the association between VZV antibodies and glioma risk [12]. Again, lower levels of anti-VZV IgG were more common in glioma cases than in controls, particularly 2 years before diagnosis (OR: 0.63; 95% CI: 0.37–1.08; inter-quartile P for trend = 0.03). Because of the use of prediagnostic specimens, this study provided further evidence that VZV antibody-glioma associations reported in the literature are unlikely to be a result of postdiagnostic or treatment-related factors (e.g., steroid use). Furthermore, such serologic studies also suggest that the associations observed between self-reported history of chickenpox and glioma risk are unlikely to be completely attributable to memory problems or cognitive deficits in glioma patients.

A particularly interesting finding of our study is that the protective effect of chickenpox against high-grade glioma was strongest among the youngest (<40) age group. Median age at glioma development is 55 years [16]. It is possible that high-grade gliomas that develop in younger individuals are etiologically heterogeneous from those that develop in older individuals. In fact, recent evidence indicates that potentially etiologically distinct glioma subtypes (defined by specific tumor molecular markers) have different ages at presentation [17]. Nevertheless, our finding needs to be confirmed in other studies before definitive conclusions can be drawn, especially given that this observation was made among the smallest sample size of the three age groups examined.

In our study, shingles was not associated with glioma risk. Some previous studies have found an inverse association with glioma risk, though shingles has not been studied as frequently as chickenpox and has often been combined with chickenpox, rather than examined separately [9]. In our study, the age at first shingles episode was skewed toward a younger distribution, compared to

previous reports [18–20]. In the U.S. and Europe, median age for shingles has been reported to be between 60 and 70 years. The median age in the GICC data was much younger (44 years). Although the incidence of shingles at younger ages may be increasing [21, 22], we believe that the age distribution reflected in our study is unlikely to be completely accurate. It is possible that some individuals are unsure of what shingles is or believed it to be synonymous with chickenpox or another viral rash. Unfortunately, there is no way to verify these data, and thus we must interpret our results on shingles cautiously.

The biological mechanism through which chickenpox may confer protection against glioma is currently unclear. One proposed mechanism is that VZV antibodies may demonstrate some cross reactivity to tumor cells (or other oncogenic viruses), and are thus capable of helping mount a protective immune response against existing tumor cells [9]. Conversely, it is also possible that individuals who are more likely to develop cancer may be unable to mount strong immune responses to infections such as VZV [23].

A limitation of our study is the amount of intersite heterogeneity between our 14 international sites. Accordingly, we have provided site-specific odds ratios and have used random-effects meta-regression in an effort to account for some of this heterogeneity. Because questionnaire administration methods and control types differed between sites, we have also conducted a number of sensitivity analyses (methods described in reference 15) to ensure that these differences did not detectably bias the results of our analyses.

Findings from the previous literature, bolstered by those of our study, provide strong epidemiologic rationale for continued investigation of the potential role of chickenpox (or other manifestations of VZV infection) in glioma development [3, 8–13]. Future studies will need to account for the potential impact of the VZV vaccine, which was licensed in 1995 in the U.S. for use among children [2] (and therefore cannot be evaluated in the older population of the GICC). Prior serologic analyses have demonstrated that antibody composition differs between children who experience a wild-type VZV infection versus those who were received the vaccine [24]. Some evidence indicates that antibodies against specific VZV-encoded proteins (i.e., VZV ORF2 and IE63) may be more important than others in conferring protection against glioma [13], but the vaccine does not contain antigens corresponding to all 70 VZV open reading frames [1, 3, 24]. Thus, future research ascertaining whether the vaccine confers similar protection against glioma as the wild-type VZV infection is of high importance and may lend insight into the biological mechanisms at play.

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Conflict of Interest

The authors declare no conflicts of interest.

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Researched Remedies

ASCORBIC ACID (VITAMIN C) TREATMENT OF WHOOPING COUGH*

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WHOOPING cough is an almost universal infectious disease, with its greatest infectivity in pre-school and school children. While some protection has been afforded against it by vaccination. treatment of the active disease has not progressed as has treatment of other infectious diseases such as scarlet fever and diphtheria. Madsen[1] reports that, of 1,842 vaccinated children, about 25 per cent escaped infection, while of 446 non-vaccinated children less than 2 per cent escaped. This decided improvement warrants the use of vaccines, but still leaves the infected child confronted with some weeks of unpleasantness and a not inconsiderable mortality rate. According to Tice,[2] in the registration area of the United States there were 7,518 deaths in 1934. In the years 1932-34 there were 45,755 cases of whooping cough reported to the Dominion Bureau of Vital Statistics, with 1,982 deaths. Of the fatal cases over 50 per cent occur in the first year of life. The non-fatal cases undergo a most disagreeable experience and lose considerable time from studies, in the case of the school-child. The disease is characterized by spasmodic coughing and vomiting, and this spasmodic or paroxysmal stage persists for weeks. How this paroxysmal stage originates, and why it should be so prolonged, has always intrigued investigators, and various hypotheses have been put forward. Among them is one suggested by Brown,[3] that a neurotropic toxin elaborated by the bacillus in the early catarrhal stage affects the vagus and respiratory centres and possibly the sensory nerve-endings in the upper respiratory mucosa. Fixation of this toxin in nervous tissue would explain the comparative failure of vaccines or convalescent serum to influence the course of the disease unless given in the incubation period or early in the catarrhal stage. Both exo- and endotoxins have been obtained from the Bordet-Gengou bacillus.

Ascorbic acid has been investigated by several workers from the standpoint of its detoxicating action. Grootton and Bezsonoff[4] record the results of mixing diphtheria toxin and ascorbic acid, incubating very briefly, and injecting the mixture into guinea-pigs. Unneutralized ascorbic acid completely destroyed the toxic action, but this effect was one of pH and not a specific effect. Ascorbic acid neutralized with soda and mixed with the toxin so altered its potency that, of four guinea-pigs receiving 4 M.L.D. of toxin each, one survived and the others died respectively on the 4th, 6th, and 9th day. Controls injected with 4 M.L.D. each of unaltered toxin all died on the 2nd day. These workers, in the same paper, tested the actual bactericidal action of ascorbic acid against various bacteria by adding varying amounts of the acid to the culture medium, bringing the mixture to a pH of 7.0, and inoculating with such organisms as staphylococcus, streptococcus, gonococcus, typhosus, Bordet-Gengou, etc. With 0.5 per cent ascorbic acid mixtures only the gonococcus and Bordet-Gengou bacillus were inhibited, as compared with controls. The gonococcus grew readily in a 0.2 per cent mixture. In a percentage of 0.008, ascorbic acid inhibited the growth of the Bordet-Gengou bacillus. Glacial acetic acid added to the culture medium in corresponding amounts, and then neutralized, failed to affect the growth of this bacillus.

Woringer and Sala[5] reported 4 cases of whooping cough complicated by scurvy occurring among a series of infants treated in their clinic. No scurvy appeared among the other children, although all were on exactly the same dietary regimen. They suggest that vitamin C is an essential part of the body's defence against the Bordet-Gengou bacillus, and that excessive demands made in the presence of such an infection may so deplete the vitamin stores of the tissues as to lead to the clinical condition of scurvy.

Gander and Niederberger[6] and Hochwald[7] report the use of ascorbic acid in the treatment of pneumonias. Pneumonia cases showed con-

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sistently a deficit in vitamin C. Administration of the vitamin produced an effect comparable with that of specific serum. The pulse and temperature subsided by crisis when the avitaminosis was completely relieved, as shown by beginning urinary excretion of the ascorbic acid. When small doses of ascorbic acid were given, the saturation point for the vitamin was reached slowly, and no clinical improvement was shown until this point was reached.

Various investigators[8, 9, 10] have shown that the tissues of normal children and young animals contain more vitamin C than those of normal older subjects, and that the saturation point, as judged by beginning urinary excretion, is attained in young subjects only by much larger doses than relative weights would indicate. This suggests a greater need of vitamin C by young animals, and so a greater storage of it in the presence of an ample supply.

From this evidence, ascorbic acid seemed to have possibilities in the treatment of whooping cough, and one of us (B.M.U.) has been using it in practice for the last two months or so. To date, we can report 9 cases, and 1 from another practitioner.* In each case, diagnosis was made from a history of contact with known cases together with personal observation of the typical cough, vomiting and nocturnal paroxysms. Cough plates or serological tests were not used in this preliminary investigation. Condensed case reports follow.

DISCUSSION

The short series of cases presented is too small to draw any statistical conclusions, but one fact stands out. Ascorbic acid has a definite effect in shortening the period of paroxysms from a matter of weeks to a matter of days. We have not checked by cough plates or otherwise in this preliminary work to see whether the infectivity subsides simultaneously with the spasmodic symptoms, but are continuing with a larger series of cases in which these and other tests will be employed.

* Case 4. We are indebted to Dr. C. H. A. Walton for details of this case.

TABLE

Case	*Age (years)*	*Sex*	*Contact*	*Duration of Symptoms*	*Treatment*	*Results*
1 R.T.	6	M	School	6 weeks—typical	150 mg. per day	7 days—cough reduced markedly 10 days—cough disappeared
2 C.H.	1½	M	Unknown Temperature 102 F. Bronchopneumonia when seen	3 weeks—typical 10 days "fever" at home	inhalations sinapisms expectorants } 3 days 175 mg. daily—11 dys.	No effect 7 days—temperature normal, cough reduced 14 days—cough disappeared
3 M.C.	12	M	School	10 days—typical	200 mg. daily	6 days—cough reduced 13 days—only occasional night coughs 15 days—all cough absent
4 J.P.	6	F	School	over 4 weeks—typical	200 mg. daily	3 days—cough less, no vomiting 7 days—occasional cough
5 B.O.	2½	M	Known case	2 weeks—typical	250 mg. daily	5 days—cough disappeared
6 H.F.	7	M	School	2 weeks—typical	375 mg. daily	4 days—cough less 9 days—night cough only 11 days—all cough absent
7 E.H.	22		Maid Child in house had whooping cough	4 dys., paroxysmal cough, vomited once, no whooping	500 mg. daily—3 days 125 mg. daily	4 days—cough less, no vomiting 6 days—coughed only once in 2 days 11 days—cough absent
8 B.P.	4	M	Known case	10 days—typical	500 mg. daily—4 days 250 mg. daily—4 days	5 days—cough disappeared
9 M.W.	6½	F	School	2 weeks—typical	500 mg. daily—4 days 250 mg. daily—5 days	4 days—cough reduced 7 days—coughed once in 24 hours 9 days—cough disappeared
10 W. C.	4½	F	Sister (Case 9)	1 week—typical	500 mg. daily—4 days 250 mg. daily—5 days	Same as for Case 9

The dosages used have been empirical, with a tendency to use larger doses early in the disease as our experience of its effects progressed. The acid is available at reasonable prices, and the danger of overdosage seems negligible. Animals have received 2,000 times their estimated requirements without any deleterious effects. Any excess is excreted by the kidneys.

CONCLUSIONS

1. A method has been described for the treatment of whooping cough by ascorbic acid (vitamin C).

2. Ascorbic acid definitely shortens the paroxysmal stage of the disease, particularly if relatively large doses are used early in the disease.

The ascorbic acid used by us was the Hoffmann-LaRoche product sold under the trade name of "Redoxon". Grootton and Bezsonoff[4] have shown that this product is identical chemically, physically and biologically with the original product prepared by Szent-Gyorgi.

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CHANGES IN CONDITIONED RESPONSES BROUGHT ABOUT BY ANÆSTHETICS AND SEDATIVES*

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Montreal

PAVLOV[7] (1927) and his co-workers first observed that conditioned salivary reflexes could be modified by drugs like alcohol, caffeine, chloral hydrate and bromide. Recently Wolff and Gantt[8] (1935) studied the effects of amytal upon conditioned salivary secretion. The object of the present research was to extend the work to conditioned alimentary-motor responses of dogs and cats. From this viewpoint we have re-investigated the influence of alcohol and of amytal, and tested several new drugs, namely nembutal, avertin, paraldehyde, bulbocapnine, carbon dioxide, ethylene, nitrous oxide, morphia, and hyoscine.

Two dogs and two cats served as subjects. The dogs received sodium amytal and nembutal intravenously, avertin per rectum, alcohol and paraldehyde by stomach tube, and morphia, hyoscine and bulbocapnine subcutaneously. The gaseous anæsthetics were administered to the cats under a bell jar. We naturally waited for full recovery from one drug before we administered a new drug or even a different dose of the same drug.

The general procedure for establishing conditioned reflexes is by now well known. The measured and recorded response may be salivary secretion or any other easily observed reaction (*cf.* Liddell, 1934).

In our work a lid-lifting response was used. This particular training procedure was described by Dworkin[2] (1935). The stimuli selected comprised auditory, visual and tactile signals. The successive tests were made at intervals of 2 to 6 minutes. During these intervals the animals had been trained not to touch the lid of the food container. Consistent absence of response between stimuli, eventually developed by training, may be called "interval inhibition" (Fig. 1A). The animals were also trained to make two discriminations, (1) between two different buzzers—"coarse" discrimination, (2) between a loud and a quiet musical tone of fixed frequency —"fine" discrimination. The time of incidence of the signals, as well as that of the animals' response, was recorded graphically. Thus we had information as to the latent period, presence or absence of conditioned response, duration of conditioned and unconditioned phases, and finally the amount of interval inhibition.

The latent period of the positive responses varied between 1 and 3 seconds. Often it was just as short for a visual as for a tactile or auditory stimulus. Nevertheless, a loud sound usually evoked a response sooner than a quiet sound; similarly, the latent period for a strong light was often shorter than for a weak light. When a negative stimulus was turned on for differentiation there was at times a slight turning of the head away from the food container, and other signs of general irritation, but no attempt to raise the lid (see Fig. 1B).

RESULTS

Our observations indicate that the eleven drugs tested may be classed into three main

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This important study was conducted in Bangladesh, where at the time of the research tetanus accounted for 26 percent of all infant deaths. Sixty-two tetanus patients aged 1 to 12 years and 55 patients aged 13 to 30 years received conventional antitetanus therapy. Additionally, 31 members of the younger group and 27 of the older group received injections of 1 gram of ascorbic acid daily as a supplement to conventional therapy. In the younger group receiving vitamin C, zero percent died, while 74 percent of those who did not get the injections failed to survive. In the older group, 37 percent of those who got the vitamin C died, while 68 percent of those who did not get the vitamin C succumbed. Injections of vitamin C were also found to protect two-day-old chicks from induced strychnine poisoning.—*R.D.M.**

Effect of Ascorbic Acid in the Treatment of Tetanus

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Bangladesh Med. Res. Counc. Bull., June 1984, pp. 24-28

Summary

The effect of daily intravenous administration of 1,000 mg ascorbic acid (AA) in tetanus patients aged 1-30 years was studied. In the age group of 1-12 years, 31 patients were treated with AA as additional to antitetanus serum, sedatives and antibiotics. It was found that none of the patients died who received AA along with the conventional antitetanus therapy. On the other hand, 74.2 per cent of the tetanus patients who received the conventional antitetanus therapy without AA (control group) were succumbed to the infection. In the other age group of 13-30 years, there were 27 and 38 patients in the treatment and control groups respectively. The mortality in the AA and control groups were 37 percent and 67.8 percent respectively.

*Editor's note: For those who insist on hard-nosed data before admitting the efficacy of injectable vitamin C, this study from Bangladesh should be an eye-opener. In 1937, Claus W. Jungeblut of Columbia University reported that vitamin C could neutralize tetanus toxin in guinea pigs ("Inactivation of Tetanus Toxin by Crystalline Vitamin C (l-Ascorbic Acid). [*Journal of Immunology* 1937. Vol. 33: 203-214]). By 1954, Dr. Fred Klenner was using injectable vitamin C to treat tetanus in his medical practice (see Klenner's 1954 article on pp.87-94 of this volume). In effect, Jungeblut's 1937 results with animals were confirmed by Dr. Klenner in his medical practice. This research from Bangladesh validates Jungblut and Klenner's finding in a rigorous medical trial using a control group.

Perhaps it is worth quoting from Jungeblut's 1937 report: "It must be concluded that vitamin C, as far as its in vitro action is concerned, cannot be regarded as a specific detoxicant for any given virus or toxin, but rather as a substance which acts indiscriminately against a variety of toxins and viruses" (p. 212). Obviously, the potential cost-benefit ratio of more research into and wider use of injectable vitamin C by mainstream medicine is too great to continue to ignore, especially in a world overflowing with medical need.

These results suggest that AA might play an important role in reducing the mortality of tetanus. This was supported by the fact that AA was found to mitigate the toxic effects of strychnine producing tetanus like condition in young chicks in the present study.

Introduction

While tetanus is no longer a problem in advanced countries as almost every body is immunized, it has remained a dreaded disease and a major killer in countries like Bangladesh. The neonatal death rate due to tetanus is estimated to be 24.05 per thousand live births and accounts for 26.20 per cent of all infant deaths (Islam, 1983). Considerable cases are also seen in older children and adults arising out of injuries while playing or working in the fields. There are incidents of the disease due to circumcision or surgical procedures where sterile condition is not maintained. However, the mortality of tetanus is high in rural areas where adequate treatment is hardly available. Even in the urban hospitals, mortality due to tetanus neonatorum is believed to be 80 to 90 per cent and in adult cases it is over 60 per cent (Infectious Disease Hospital Record, 1983). The conventional antitetanus therapy includes antitetanus serum, sedatives, antibiotics, muscle relaxant and sometimes steroids. But the role of AA in the therapy of tetanus has not been previously investigated.

It has been observed that ß-N-£ß oxalyl diaminopropionic acid (ODAP) isolated from lathyrus sativus (known as Khesari in local language) (Rao et al, 1964) was found responsible for neurolathyrism (Sarma and Padmaban, 1969). It was also observed that in both tetanus and in neurolathyrism some common characteristics such as spastic paralysis and neuroexcitation are seen. Both ODAP and tetanus toxin find their way to the central nervous sytem (CNS) to get themselves attached to the snyaptosomes (Lakshmanan and Padmanaban, 1977). Glutamic acid also under certain circumstances has been found to affect the CNS in the way ODAP does (Olney et al, 1976). Both ODAP and glutamate are considered to have common receptors (Lakshmanan and Padmanaban, 1977). It was found that biological effect of ODAP as well as glutamate was related to the serum level of AA and in fact both neurolathyrism and glutamate toxicity could be prevented by administration of AA (Ahmad and Jahan, 1983).

On the basis of the above fact, it was considered that AA might have some beneficial effects in the treatment of tetanus. The present study was therefore, undertaken to validate the above concept.

Materials and Methods

A total number of 117 tetanus patients addmitted into the Infectious Disease Hospital, Mohakhali, Dhaka were studied. They were divided into two different age groups. In the age group of 1-12 years, there were 31 patients in the treatment group who received 1000 mg. AA daily in addition to conventional antitetanus therapy which included antitetanus serum, sedatives, antibiotics and muscle relaxant etc. There were also 31 patients in the similar age groups who received only the conventional antitetanus therapy but no AA and this group served as control. In the other age group of 13-30

years, there were 27 and 28 patients in the treatment and control groups respectively and they were treated in a similar manner as in the age group of 1-12 years.

In view of the recognised similarity between the mode of action of tetanus toxin and strychnine, (Heyningen et al, 1971) an animal experiment was conducted in the Institute of Nutrition and Food Science, University of Dhaka to investigate whether AA could mitigate the toxicity induced by strychnine. Two-days old chicks weighing 32-35 gm were divided into four groups with l5 birds in each group. Birds of group I were received 5 µg of strychnine sulphate only and those of in group II received strychnine sulphate in the same dosage along with 30 mg AA 10 minutes before strychnine. Birds of group III were administered strychnine in a higher dosage of 10 µg only and the group-IV received both strychnine (10µg) and AA 30 mg. Both the drugs were administered intraperitoneally in aqueous solutions.

Results

The effect of AA in the treatment of tetanus was shown in Table I. In the age group of 1-12 years, there was no mortality in patients who received 1000 mg AA daily (i.v.) in addition to conventional antitetanus therapy. On the other hand, in the control group i.e. the patients who had not received AA along with antitetanus therapy, the mortality rate was 74.2 per cent (Table-I). In another age group of 13-30 years, addition of AA to the conventional antitetanus regimen caused a marked reduction in the morality [should be mortality per ed.]. In the treatment group (i.e. patients that received both AA and antitetanus therapy), the mortality was only 37 percent as opposed to 67.8 per cent in patients who had not received AA (Table-I).

The results of animal experiments are shown in Table-II. Administration of AA protected the chicks from strychnine toxicity and the chicks who received AA and strychnine did not develop the signs of strychnine toxicity indicating that AA mitigated the same.

Discussion

The results of the present study indicate that AA acts in some way to mitigate the toxicity of tetanus toxin so that in the age groups of 1-12 years none of the patient receiv-

TABLE I
Studies on the effect of daily administration of (i.v.) 1000 mg of AA as supplement to conventional treatment on the recovery of tetanus patients

	Patients receiving ascorbic acid			Patients not receiving ascorbic acid		
Age group (years)	No. of Patients	Patients who Recovered	Mortality	No. of Patients	Patients who Recovered	Mortality (percentage)
1-12	31	31	00%	31	8	74.2
12-30	27	17	37%	28	9	67.8

TABLE II
Effect of AA on the toxicity of strychnine sulphate (SS) in chicks

Groups	Dose of SS per chick (µg)	Dose of AA per chick (mg)	Observation
I	5	—	Wings of all birds stretched. Some walked on toes, others kept jumping and they could not walk.
II	5	30	No symptoms.
III	10	—	Extensor paralysis of legs, opisthotonus and severe convulsion. All but three died.
IV	10	30	Extensor paralysis in 3 chicks. No neurological symptoms in others. The affected birds recovered in about 30 minutes after the appearance of the symptoms.

The number of birds were 15 in each group.

ing AA succumbed to the toxinosis of tetanus as opposed to the corresponding control groups. In the other age group of 13-30 years, although some succumbed but there was substantial reduction in mortality due to the addition of AA. Even though several variable such as site injury, status of infection before start of treatment, nutritional status of the patients and exposure to risks of secondary infection must also have acted as determinants of mortality amongst tetanus patients, the beneficial effect of AA as seen in this study appears significant.

During the course of study, it was noticed that patients succumbed to tetanus even three to four weeks after admission. This is contrary to the literature report that death if it occurs follows relatively soon after the appearance of symptoms, the dictum of Hippocrates, such persons as are seized with tetanus die within four days or if they pass those they recover, still stands (cited by Burrows, 1968). In many instances it would appear that those patients had almost recovered when fresh wave of convulsions would overtake bringing the end.

The studies on human patients of tetanus and the studies on strychnine toxinosis in chicks indicate that AA interacts with tetanus toxin as well as strychnine to reduce their toxic effect although the mechanism of this interaction is yet to be understood.

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A RANDOMIZED, CONTROLLED TRIAL OF VITAMIN A IN CHILDREN WITH SEVERE MEASLES

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Abstract *Background.* Measles kills about 2 million children annually, and there is no specific therapy for the disease. It has been suggested that vitamin A may be of benefit in the treatment of measles.

Methods. We conducted a randomized, double-blind trial involving 189 children who were hospitalized at a regional center in South Africa because of measles complicated by pneumonia, diarrhea, or croup. The children (median age, 10 months) were assigned to receive either vitamin A (total dose, 400,000 IU of retinyl palmitate, given orally; n = 92) or placebo (n = 97), beginning within five days of the onset of the rash. At base line, the characteristics of the two groups were similar.

Results. Although clinically apparent vitamin A deficiency is rare in this population, the children's serum retinol levels were markedly depressed (mean [±SEM], 0.405±0.021 μmol per liter [11.6±0.6 μg per deciliter]), and 92 percent of them had hyporetinemia (serum retinol level <0.7 μmol per liter [20 μg per deciliter]). Serum concentrations of retinol-binding protein (mean, 30.1±2.0 mg per liter) and albumin (mean, 33.4±0.5 g per liter) were also low. As compared with the placebo group, the children who received vitamin A recovered more rapidly from pneumonia (mean, 6.3 vs. 12.4 days, respectively; P<0.001) and diarrhea (mean, 5.6 vs. 8.5 days; P<0.001), had less croup (13 vs. 27 cases; P = 0.03), and spent fewer days in the hospital (mean, 10.6 vs. 14.8 days; P = 0.01). Of the 12 children who died, 10 were among those given placebo (P = 0.05). For the group treated with vitamin A, the risk of death or a major complication during the hospital stay was half that of the control group (relative risk, 0.51; 95 percent confidence interval, 0.35 to 0.74).

Conclusions. Treatment with vitamin A reduces morbidity and mortality in measles, and all children with severe measles should be given vitamin A supplements, whether or not they are thought to have a nutritional deficiency. (N Engl J Med 1990; 323:160-4.)

MEASLES remains a devastating disease, for which specific therapy is lacking. Hopes for its control and eventual eradication rest on immunization, but measles kills about 2 million children each year[1] and cripples an untold number through blindness[2] and lung disease.[3,4] The idea that vitamin A may have a protective effect in measles was first suggested more than 50 years ago[5] but was ignored until Barclay et al.,[6] in a randomized clinical trial, found twice as many deaths in the control group (12 of 92) as among children given high doses of vitamin A (6 of 88).[6] Although the overall results did not reach statistical significance, vitamin A was significantly protective in the group under two years of age.[6]

That vitamin A should be of benefit in measles is biologically plausible.[7] Measles depresses serum levels of vitamin A,[8-11] and hyporetinemia (a serum retinol level below 0.7 μmol per liter [20 μg per deciliter]) is associated with increased mortality from the disease, particularly in children under two years of age.[11] In almost every known infectious disease, vitamin A deficiency is known to result in greater frequency, severity, or mortality.[12] Increased susceptibility to infection was one of the first features of nutritional vitamin A deficiency to be recognized,[13] and even mild deficiency appears to be associated with an increased risk of pneumonia, diarrhea, and death in childhood.[14-17] According to Scrimshaw et al., "no nutritional deficiency in the animal kingdom is more consistently synergistic with infection than that of vitamin A."[12] They list nearly 50 studies (including 8 in humans) of diseases of bacterial, viral, or protozoan origin in which vitamin A deficiency resulted in increased frequency, severity, or mortality.[12] In fact, vitamin A is sometimes referred to as the "anti-infective" vitamin.[18]

We embarked on this study because measles is a pressing problem in our part of the world[19] and because the results of Barclay et al.[6] and the circumstantial evidence appeared promising. Subsequently, acting on the same evidence, the World Health Organization recommended routine vitamin A supplementation for all children with measles in regions where vitamin A deficiency was a recognized problem and suggested that elsewhere "in countries where the fatality rate of measles is 1% or higher it would be sensible to provide vitamin A supplements to all children diagnosed with measles."[20] One difficulty with this advice is that in the communities in which measles poses the greatest problem, the mortality rate is often unknown. Another is that the recommendation is based on the less than conclusive evidence from the only two studies to have addressed the question of vitamin A therapy in measles.[5,6] These are some of the reasons why vitamin A supplementation is still not given routinely to children who are seriously ill with measles in South Africa, and presumably elsewhere.

METHODS

Children with acute measles who required hospital admission for the treatment of associated complications were entered in a randomized, double-blind, placebo-controlled trial to assess the effect of oral vitamin A on morbidity and mortality. The study was limited by a priori considerations to a fixed termination date, with a maximal enrollment of 200 cases. It was conducted from March to July 1987 at the City Hospital for Infectious Diseases, a regional center serving a population of about 2 million in Cape Town and

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surrounding areas. The Medical Faculty's ethics and research committee approved the study protocol.

Patient Selection and Randomization

All children under 13 years of age who were referred to the hospital for admission with measles were eligible for entry into the trial. The criteria for exclusion were vitamin A therapy before admission, xerophthalmia on admission or thereafter, rash for more than four days, or lack of parental consent.

Patients in the trial were randomly assigned to receive either 400,000 IU (120 mg) of water-miscible vitamin A (retinyl palmitate; Arovit drops, Roche, Basel, Switzerland) or an identical-appearing placebo from syringes coded according to a random-number table. The senior ward nurse gave half the dose on admission and the remainder a day later, either by mouth or by nasogastric tube. The children were cared for by the regular ward staff. Concurrent therapy included oxygen, intravenous fluids, and antibiotics as appropriate, but no additional vitamin supplements. One of the study investigators assessed the patients each day. The treatment-assignment codes were broken only at the completion of the trial.

Initial Investigations

The children's weight and height were recorded, and a venous-blood sample was drawn on entry into the trial. The weights and heights were evaluated against the standards of the National Center for Health Statistics.[21] Hemoglobin levels, white-cell counts (by Coulter model S5, Coulter Electronics, Hialeah, Fla.), and differential counts were estimated, and serum was stored at −70°C. Serum levels of total protein and albumin were measured by automated analysis (Astra-8, Beckman Instruments, Brea, Calif.). Serum concentrations of vitamin A (as retinol) were measured by high-performance liquid chromatography (Dupont Instruments, Wilmington, Del.), with concentrations of vitamin E (as alpha-tocopherol) obtained incidentally.[22] A programmable integrator was used to quantify the chromatographic results (Spectra-Physics, San Jose, Calif.). Retinol-binding protein was measured by radial immunodiffusion with a commercial kit (LC-Partigen, Behringwerke, Marburg, Federal Republic of Germany). Chest radiography and other investigations were performed when indicated.

Table 1. Base-Line Clinical Findings in 189 Children with Measles, According to Treatment Group.*

Characteristic	No. of Patients	Placebo (N = 97)	Vitamin A (N = 92)
Age (mo)		*15.06 (8, 10, 15)*	*15.89 (8, 10, 17)*
<6	7	3	4
6–12	117	64	53
13–23	37	18	19
≥24	28	12	16
Male/female		56/41	53/39
Mixed race/black	?	29/68	24/68
Weight for age†	189	*81.5 (74, 84, 92)*	*85.7 (77, 85, 96)*
<5th percentile	95	51	44
Height for age†	178	*96.0 (93, 97, 100)*	*97.1 (93, 96, 101)*
<5th percentile	52	25	27
Weight for height†	178	*89.0 (82, 88, 95)*	*90.3 (84, 91, 97)*
<5th percentile	70	41	29
Rash (days)‡		*1.91 (1, 2, 2)*	*1.72 (1, 1.5, 2)*
Diarrhea	152	75	77
No pneumonia	30	13	17
Pneumonia	146	74	72
No diarrhea	24	12	12
Pneumonia and diarrhea	122	62	60
Herpes stomatitis	4	1	3
Measles croup§	13	4	9

*Values in italics are means, followed in parentheses by 25th percentiles, medians, and 75th percentiles. All other values are numbers of patients.

†Expressed as a percentage of the 50th percentile of the standards of the National Center for Health Statistics.

‡$P<0.05$ for the comparison between groups.

§No patients with measles croup required airway interventions.

Assessment of Outcomes

Outcomes were assessed solely on the basis of clinical criteria. The outcome variables used were death and the severity of illness, as indicated by the duration of the hospital stay; the duration of pneumonia or diarrhea; the incidence of "postmeasles" croup or herpes stomatitis; and the need for a transfer to the Red Cross War Memorial Children's Hospital for intensive care. Pneumonia was defined as the presence of tachypnea (frequency of respiration >40 per minute) with retractions, crackles, or wheezes. Diarrhea was defined as the passage of four or more liquid stools a day. Measles croup was defined as croup presenting on or within a day of admission. Croup that developed subsequently was categorized as postmeasles.

Statistical Analysis

The data were analyzed by computer with the Epi-Info program (version 3, USD, Stone Mountain, Ga.). Categorical data[23] (e.g., the number of patients per group) were evaluated by the chi-square test, with Yates' correction for continuity applied routinely,[24] or by Fisher's exact test when the expected number in a cell was five or less.[24] Confidence intervals for the relative risks were calculated according to the method of Greenland and Robins.[25] Continuous data[23] (e.g., vitamin level) were compared by the nonparametric Kruskal–Wallis test.[23,24] All P values reported are two-tailed, with values of less than 0.05 considered statistically significant.

Results

Exclusion of Patients

Of 224 patients under 13 years of age who were admitted to the hospital with measles during the study, 35 were excluded from the trial. In 12 of these cases the rash was present for five or more days, in 2 vitamin A had previously been given, in 18 consent could not be obtained because the child was unaccompanied by a parent on admission, and in 3 the parents refused consent. Hence, 189 patients were entered in the trial. There were no exclusions for xerophthalmia or withdrawals after entry.

Base-Line Characteristics

The placebo and treatment groups were generally comparable (Tables 1 and 2), except that the patients in the vitamin A group were admitted about 12 hours earlier in terms of the duration of the rash and had lower serum levels of total protein and albumin than those in the placebo group. Two thirds of the children were 12 months old or younger (median, 10 months; range, 2 months to 5 years), and most were boys (58 percent). Blacks predominated (72 percent), and the remainder were of mixed race. The five white patients admitted with measles were excluded: consent was refused in the cases of two, and three were more than 13 years old. The hospital is open to all. Immunization and socioeconomic factors are thought to account for differences in racial makeup between the study population and the general population 14 years of age or

under in Cape Town (57 percent mixed race, 25 percent black, 18 percent white).[27] Heights were not measured for 11 patients. Height for age was below the fifth percentile in 52 children (29 percent) — a prevalence similar to that in the local reference population.[28] Weight for age (below the fifth percentile in 50 percent), and weight for height (below the fifth percentile in 39 percent) were considered to reflect short-term weight losses from measles[29,30] rather than preexisting acute protein-energy malnutrition, since that occurs in 1 percent or less of the local reference population.[28] A combination of pneumonia and diarrhea was the usual indication for hospital admission (64 percent). Diarrhea (16 percent), pneumonia (13 percent), or measles croup (7 percent) appearing as isolated symptoms precipitated the other admissions.

Table 2. Base-Line Blood and Serum Values, According to Treatment Group.

Characteristic*	No. of Patients	Placebo	Vitamin A
		mean (25th, 50th, and 75th percentile)	
Hemoglobin (g/dl)	177	10.73 (10, 10.6, 11.5)	10.78 (10, 10.5, 11.7)
Hematocrit (%)	177	32.4 (30, 32.5, 35)	32.8 (30, 32, 35)
Leukocytes ($\times 10^{-9}$/liter)	177	8.63 (6.3, 7.7, 10.2)	8.99 (6.2, 8.15, 10.25)
Lymphocytes ($\times 10^{-9}$/liter)	177	3.39 (2, 3.1, 4.2)	3.42 (1.8, 2.9, 4.3)
Total protein (g/liter)†	155	58.54 (55, 57, 62)	53.94 (51, 54, 58)
Albumin (g/liter)†		34.5 (32, 34, 37)	32.4 (29, 33, 35)
RBP (mg/liter)	156	29.6 (14, 18, 30)	30.48 (14, 17, 37)
Vitamin A (retinol) (μg/dl)	156	12.19 (7.7, 10.7, 14.4)	10.95 (6.7, 9.5, 12.6)
Age <2 yr	131	12.84 (11.4, 15.1, 46.5)	11.1 (6.7, 9.5, 12.4)
Age ≥2 yr‡	25	8.38 (7.1, 8.1, 10.5)	10.29 (6.4, 10.5, 13.6)
Hyporetinemia	143	68§	75§
Vitamin E (mg/liter)	156	7.94 (5.5, 7.8, 9.4)	6.84 (4.7, 6.8, 8.8)

*Reference values for the characteristics shown are as follows[26]: hemoglobin, 11.5 to 15.5 g per deciliter; hematocrit, 35 to 45 percent; leukocytes, 6 to 17×10^9 cells per liter; total protein, 62 to 80 g per liter; albumin, 35 to 50 g per liter; retinol-binding protein, 22 to 45 mg per liter; vitamin A (as retinol), 30 to 80 μg per deciliter; vitamin E (as alpha-tocopherol), 5.0 to 20 mg per liter. No reference values are given for lymphocytes because of considerable variation with age. RBP denotes retinol-binding protein. To convert grams of hemoglobin per deciliter to millimoles per liter, multiply by 0.6206; to convert micrograms of vitamin A per deciliter to micromoles per liter, multiply by 0.03491; and to convert milligrams of vitamin E per liter to micromoles per liter, multiply by 23.22.

†$P<0.05$ for the comparison between groups.

‡In the placebo group, the retinol level was significantly lower in children ≥2 years old than in those <2 years old ($P = 0.026$).

§Indicates the number of cases of hyporetinemia (serum retinol concentration <0.7 μmol per liter [20 μg per deciliter]).

No blood samples were obtained from 15 patients, and only partial results were available for another 19 (Table 2). Serum levels were low for total protein (mean [±SE], 56.2±0.7 g per liter), albumin (mean, 33.4±0.46 g per liter), retinol-binding protein (mean, 30.1±2.02 mg per liter), and vitamin A as retinol (mean, 0.405±0.021 μmol per liter [11.6±0.6 μg per deciliter]). Low levels of total protein principally reflect depressed serum albumin concentrations ($r^2 = 72.6$ percent, $P<0.001$). Serum retinol levels were below the lower limit of the normal range (0.7 μmol per liter [20 μg per deciliter]) in 92 percent of the children (143 of 156), and 46 percent (72) had levels below 0.35 μmol per liter (10 μg per deciliter), placing them at risk for xerophthalmia,[31] although no cases of this were observed. Vitamin E levels were in the normal range.

Outcome

The children who received vitamin A had markedly diminished mortality and morbidity (Table 3), with no clinically apparent adverse effects. Of the 12 children who died (6.3 percent), 10 were in the placebo group ($P = 0.046$). The children who died were 5 to 29 months of age, and seven were boys. Death occurred 3 to 32 days after admission (median, 10.5). Pneumonia[3,32] caused 10 deaths, and the two remaining children died after 15 and 32 days, respectively, of fulminant sepsis following chronic diarrhea and measles-induced kwashiorkor. Croup was present as an incidental finding in 5 of the 10 children who died of pneumonia.

Cases of pneumonia lasted almost twice as long in the placebo group as in the vitamin A group ($P<0.001$), and 66 percent of the children with chronic pneumonia (>10 days) were in the placebo group ($P = 0.008$). Similarly, diarrhea continued for a third longer in the placebo group ($P<0.001$), and 72 percent of the children with chronic diarrhea were in that group ($P = 0.023$). Postmeasles croup was more common in the placebo group ($P = 0.033$), as was herpes stomatitis ($P = 0.08$). Finally, the hospital stay of the survivors was shorter by a third in the vitamin A–treated group ($P = 0.004$).

Overall, 77 children had adverse outcomes (Table 3), of whom 52 were in the placebo group ($P = 0.004$). As compared with the children in the placebo group, the children treated with vitamin A were at lower relative risk for death (relative risk, 0.21; 95 percent confidence interval, 0.05 to 0.94), prolonged pneumonia ≥10 days (relative risk, 0.44; 95 percent confidence interval, 0.24 to 0.80), prolonged diarrhea ≥10 days (relative risk, 0.40; 95 percent confidence interval, 0.19 to 0.86), postmeasles croup (relative risk, 0.51; 95 percent confidence interval, 0.28 to 0.92), airway intervention (relative risk, 0.35; 95 percent confidence interval, 0.10 to 1.26), herpes stomatitis (relative risk, 0.23; 95 percent confidence interval, 0.05 to 1.06), and the need for intensive care (relative risk, 0.38; 95 percent confidence interval, 0.13 to 1.16). The overall risk for an adverse outcome in children treated with vitamin A was half that in the control group (relative risk, 0.51; 95 percent confidence interval, 0.35 to 0.74). Of the 77 children who had adverse outcomes, only 2 were ≥2 years of age ($P = 0.002$), and the risk in a child ≥2 years old was substantially lower than in

Table 3. Mortality and Morbidity in 189 Children with Measles, According to Treatment Group.*

Characteristic	Placebo (N = 97)	Vitamin A (N = 92)	Relative Risk (95% CI)†	P Value
Death	10	2	0.21 (0.05–0.94)	0.046
Age at death (mo)				
<6	1	0		
6–12	7	1		
13–23	1	1		
≥24	1	0		
Pneumonia (days)				
Duration	*12.37 (5, 8, 17)*	*6.53 (3, 5, 8.5)*		<0.001
≥10	29	12	0.44 (0.24–0.80)	0.008
Diarrhea (days)				
Duration	*8.45 (5, 7, 10)*	*5.61 (3, 5, 7)*		<0.001
≥10	21	8	0.40 (0.19–0.86)	0.023
Postmeasles croup	27	13	0.51 (0.28–0.92)	0.033
With airway intervention	9	3	0.35 (0.10–1.26)	0.16
Herpes stomatitis	9	2	0.23 (0.05–1.06)	0.08
Intensive care	11	4	0.38 (0.13–1.16)	0.13
Adverse outcome‡	52	25	0.51 (0.35–0.74)	<0.001
Hospital stay (days)§	*15.24 (8, 11, 19)*	*10.52 (7, 9, 13)*		0.004

*In the columns representing the treatment groups, the values in italics are means, followed in parentheses by 25th percentiles, medians, and 75th percentiles. All other values are numbers of patients.

†Relative risk denotes the ratio of the incidence of an event in the vitamin A group to the incidence of the event in the placebo group. CI denotes confidence interval.

‡Defined as death, pneumonia ≥10 days in duration, diarrhea ≥10 days in duration, postmeasles croup, or transfer for intensive care.

§Refers to children who survived.

younger children (relative risk, 0.15; 95 percent confidence interval, 0.02 to 0.91). No child with a serum retinol concentration ≥0.7 μmol per liter (20 μg per deciliter) died, but the smallness of this group (n = 14) leaves the significance of the finding in doubt.

Discussion

The results of our randomized, controlled trial indicate a remarkable protective effect of vitamin A in severe measles, notwithstanding the provision of good general medical care and the presence of complicated advanced disease. Vitamin A reduced the death rate by more than half and the duration of pneumonia, diarrhea, and hospitalization by about one third. Vitamin A also appeared to reduce the incidence of herpes stomatitis and the need for intensive care. The consistency of benefit with respect to all measures of outcome is noteworthy, since mortality is not a sensitive criterion. Because of their reliance on mortality rates, previous studies of measles[5,6] lacked the statistical power to establish the benefit of vitamin A therapy.

The favorable response to vitamin A therapy may be understood in terms of the very high incidence (92 percent) of hyporetinemia in our patients (Table 2). Hyporetinemia implies a state of vitamin A deficiency at the tissue level, since there are virtually no peripheral-tissue stores of vitamin A except in the retina.[33-36] Serum retinol levels below 0.7 μmol per liter (20 μg per deciliter) appear to be inadequate for the body's biologic needs.[33] Oral vitamin A is absorbed well even in patients with diarrhea,[37] so the observed effects of treatment may reasonably be ascribed to correction of the tissue deficit of vitamin A. We do not know, however, whether the deficit was rectified by increases in the serum retinol concentration or by some other mechanism, since serum retinol levels were not measured after therapy.

Hyporetinemia appears almost invariable in children with severe measles,[8-11] as in this study, and the reduction in the serum retinol level is associated with increasingly severe disease.[11] Since many of these data come from populations in which nutritional vitamin A deficiency is a known problem,[8-10] it has been inferred that hyporetinemia in measles represents the exhaustion of hepatic stores.[6,7,20] There is a possible alternative mechanism, however. Hyporetinemia may occur in the presence of adequate hepatic stores of vitamin A when the stores are not mobilized fast enough to meet demand.[36] This has been found in fever, pneumonia, rheumatoid arthritis, hepatitis, acute tonsillitis, and rheumatic fever[36]; in protein-energy malnutrition[38]; and now also in measles.[8] Inadequate mobilization of hepatic stores may therefore underlie the hyporetinemia in children with severe measles from Kinshasa, Zaire,[11] and Cape Town, where nutritional vitamin A deficiency is uncommon. A study 25 years ago showed vitamin A deficiency to be rare in Cape Town, even in children with severe protein-energy malnutrition,[38] and it still appears to be rare. A search of the computer data-base listing of inpatients at our children's hospital, which predominantly serves the local underprivileged community, found only three instances of clinical vitamin A deficiency among 161,381 children admitted over a 13-year period, with no cases since 1985.

In view of the evidence that hyporetinemia may occur in the presence of adequate hepatic stores of vitamin A[38] and in populations not known to be deficient in vitamin A,[11] it would seem prudent to proceed on the assumption that previous nutritional adequacy may not ensure against the development of hyporetinemia in severe measles. For all children seriously ill with measles, vitamin A replacement should thus be provided at the dose given by Barclay et al.[6] (400,000 IU), which proved effective and safe in our study. A lower dose (100,000 to 200,000 IU) is recommended by the World Health Organization,[20] but its efficacy in measles has yet to be established.

It may be asked whether it is cost effective to advocate treatment with vitamin A for all children with

severe measles. Clearly, children under two years of age are at highest risk of an adverse outcome and derive the most benefit from vitamin A. When resources are scarce, such children should be given priority. In our study, however, half the children over two years of age were at risk of xerophthalmia because of serum retinol levels below 0.35 μmol per liter (10 μg per deciliter),[31] and hence they should have vitamin A prophylaxis. Thus, when resources permit, all children with severe measles should be given supplemental vitamin A.

We are indebted to many colleagues for constructive criticism; to Mr. R. Sayed, statistician to the Department of Community Health of the University of Cape Town for advice on study design and randomization procedures; to Ms. G. Joubert of the South African Medical Research Council, for assistance with data collection and preliminary analyses; to Glaxo (South Africa), for the donation of our computer; to the nursing staff of the City Hospital for Infectious Diseases for invaluable assistance and to the hospital's medical superintendent, Dr. P.J.W. Roux, for providing facilities; to Mr. A.F. Rodriques for searching the data base of patients of the Red Cross War Memorial Children's Hospital; to the medical superintendent, Dr. R.O. Simpson, for giving access to the data base; to the McCaul Bell Bequest of the Institute of Child Health, University of Cape Town, for a grant to Professor H. de V. Heese that provided funding for the assays of vitamins and retinol-binding protein; and to Ms. Frances Pocock for performing these assays in the Institute laboratory.

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Review Nutr Rev. 1992 Oct;50(10):291-2. doi: 10.1111/j.1753-4887.1992.tb02467.x.

Low serum retinol is associated with increased severity of measles in New York City children

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Affiliations

PMID: 1436764 DOI: 10.1111/j.1753-4887.1992.tb02467.x

Abstract

Children with no known prior vitamin A deficiency exhibited a significant decline in their serum retinol levels during the acute phase of measles. This decline in circulating retinol was associated with increased duration of fever, higher hospitalization rates, and decreased antibody titers.

Pediatrics. 1993 Jun;91(6):1176-81.

Measles severity and serum retinol (vitamin A) concentration among children in the United States

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Abstract

Background: Studies in developing countries have shown that children with measles have low serum retinol concentrations and that lower retinol levels are associated with measles-related mortality. Vitamin A therapy has been shown to reduce mortality among African children with acute measles.

Objectives: To determine whether serum retinol concentration is low among children with measles in the United States and to determine whether retinol concentration is associated with illness severity.

Setting: Pediatric referral hospital and clinic in Milwaukee, WI, during the measles outbreak of 1989-1990.

Patients: One hundred fourteen patients < or = 5 years of age evaluated for serologically confirmed measles with serum obtained within 5 days following rash onset.

Methods: Serum retinol concentration was determined by high-performance liquid chromatography. Clinical data were collected by hospital record review. A modified Pediatric Risk of Mortality (PRISM) score was used to assess physiologic instability as a measure of illness severity.

Results: Retinol concentrations ranged from 0.25 to 1.18 mumol/L (median 0.58 mumol/L); 82 (72%) patients had low retinol concentration (< or = 0.70 mumol/L). Median retinol concentrations were lower among hospitalized patients (0.56 vs 0.70, P = .006) and patients with pneumonia (0.52 vs 0.64, P = .02) but higher among children with otitis media (0.63 vs 0.54, P = .01). Higher modified PRISM scores, reflecting greater physiologic instability, were associated with lower retinol concentration (beta coefficient -.0147, P = .025). In multivariate analysis, higher modified PRISM scores were associated with lower retinol concentration (beta coefficient -.0144, P = .025) even after controlling for hospitalization, presence of complications, race, age, receipt of Aid to Families With Dependent Children, gender, and interval from rash onset until serum was collected.

Conclusions: Among these children with measles in an urban United States community, retinol concentrations were depressed, and the degree of depression was associated with illness severity. Vitamin A therapy should be considered for children with measles in the United States who require hospitalization.

J Trop Pediatr. 1993 Dec;39(6):342-5. doi: 10.1093/tropej/39.6.342.

Routine high-dose vitamin A therapy for children hospitalized with measles

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Affiliations
PMID: 8133555 DOI: 10.1093/tropej/39.6.342

Abstract

Measles is without specific therapy and remains important globally as a cause of childhood death. In controlled studies, high-dose vitamin A therapy (Hi-VAT)--with 400,000 IU vitamin A--has been demonstrated to markedly reduce measles-associated morbidity and mortality. We performed a retrospective study of the hospital records of 1720 children < 15 years of age who were hospitalized for measles, to determine the extent to which these findings, in research settings, are applicable to the case management of measles under conditions of routine hospital practice. The outcomes were studied of children hospitalized during two non-consecutive 2 year periods (1985-6 and 1989-90). A policy of Hi-VAT for all children hospitalized with measles was started during the intervening period. As compared with the group of children on standard therapy (n = 1061), children receiving Hi-VAT (n = 651) had a shorter hospital stay (mean 10 versus 13 days; P < 0.001), a lower requirement for intensive care (4.3 versus 10.5 per cent; P < 0.001), and a lower death rate (1.6 versus 5 per cent; P < 0.001). No adverse effects of Hi-VAT therapy were observed. We conclude that a policy of high dose oral vitamin A (400,000 IU) supplementation in measles provides benefits which are equivalent to those previously observed only in controlled research trials, that it is highly cost effective, and that it should form part of the routine case management of all children hospitalized with measles.

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Vitamin A supplements for children could save 600,000 lives a year, experts predict

Date: August 25, 2011

Source: BMJ-British Medical Journal

Summary: Children in low and middle income countries should be given vitamin A supplements to prevent death and illness, a new study concludes.

FULL STORY

Children in low and middle income countries should be given vitamin A supplements to prevent death and illness, concludes a study published online in the *British Medical Journal*.

The researchers argue that the effectiveness of vitamin A supplementation is now so well-established that further trials would be unethical, and they urge policymakers to provide supplements for all children at risk of deficiency.

Vitamin A is an essential nutrient that must be obtained through diet. Vitamin A deficiency in children increases vulnerability to infections like diarrhea and measles and may also lead to blindness. Globally, the World Health Organisation estimates that 190 million children under the age of 5 may be vitamin A deficient. But, despite widespread efforts, vitamin A programmes do not reach all children who could benefit.

So a team of researchers based in the UK and Pakistan analysed the results of 43 trials of vitamin A supplementation involving over 200,000 children aged 6 months to 5 years. Differences in study design and quality were taken into account to minimise bias.

They found vitamin A supplements reduced child mortality by 24% in low and middle income countries. It may also reduce mortality and disability by preventing measles, diarrhea and vision problems, including night blindness.

The authors say that, if the risk of death for 190 million vitamin A deficient children were reduced by 24%, over 600,000 lives would be saved each year and 20 million disability-adjusted life years (a measure of quantity and quality of life) would be gained.

Based on these results, the authors strongly recommend supplementation for children under 5 in areas at risk of vitamin A deficiency. They conclude: "The evidence for vitamin A is compelling and clear. Further trials comparing vitamin A with placebo would be unethical."

This view is supported in an accompanying editorial by two experts at Harvard School of Public Health, who say "effort should now focus on finding ways to sustain this important child survival initiative and fine tune it to maximise the number of lives saved."

Story Source:

Materials provided by **BMJ-British Medical Journal**. *Note: Content may be edited for style and length.*

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Comp Immunol Microbiol Infect Dis. 2013 Mar;36(2):137-48. doi: 10.1016/j.cimid.2012.11.002. Epub 2012 Dec 27.

Resveratrol suppresses calcium-mediated microglial activation and rescues hippocampal neurons of adult rats following acute bacterial meningitis

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PMID: 23273676 DOI: 10.1016/j.cimid.2012.11.002

Abstract

Acute bacterial meningitis (ABM) is a serious disease with severe neurological sequelae. The intense calcium-mediated microglial activation and subsequently pro-inflammatory cytokine release plays an important role in eliciting ABM-related oxidative damage. Considering resveratrol possesses significant anti-inflammatory and anti-oxidative properties, the present study aims to determine whether resveratrol would exert beneficial effects on hippocampal neurons following ABM. ABM was induced by inoculating Klebsiella pneumoniae into adult rats intraventricularly. The time-of-flight secondary ion mass spectrometry (TOF-SIMS), Griffonia simplicifolia isolectin-B4 (GSA-IB4) and ionized calcium binding adaptor molecule 1 (Iba1) immunohistochemistry, enzyme-linked immunosorbent assay as well as malondialdehyde (MDA) measurement were used to examine the calcium expression, microglial activation, pro-inflammatory cytokine level, and extent of oxidative stress, respectively. In ABM rats, strong calcium signaling associated with enhanced microglial activation was observed in hippocampus. Increased microglial expression was coincided with intense production of pro-inflammatory cytokines and oxidative damage. However, in rats receiving resveratrol after ABM, the calcium intensity, microglial activation, pro-inflammatory cytokine and MDA levels were all significantly decreased. Quantitative data showed that much more hippocampal neurons were survived in resveratrol-treated rats following ABM. As resveratrol successfully rescues hippocampal neurons from ABM by suppressing the calcium-mediated microglial activation, therapeutic use of resveratrol may act as a promising strategy to counteract the ABM-induced neurological damage.

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Vitamin C for preventing and treating pneumonia

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Abstract

Background: Pneumonia is one of the most common serious infections, causing two million deaths annually among young children in low-income countries. In high-income countries pneumonia is most significantly a problem of the elderly.

Objectives: To assess the prophylactic and therapeutic effects of vitamin C on pneumonia.

Search methods: We searched CENTRAL 2013, Issue 3, MEDLINE (1950 to March week 4, 2013), EMBASE (1974 to April 2013) and Web of Science (1955 to April 2013).

Selection criteria: To assess the therapeutic effects of vitamin C, we selected placebo-controlled trials. To assess prophylactic effects, we selected controlled trials with or without a placebo.

Data collection and analysis: Two review authors independently read the trial reports and extracted data.

Main results: We identified three prophylactic trials which recorded 37 cases of community-acquired pneumonia in 2335 people. Only one was satisfactorily randomised, double-blind and placebo-controlled. Two trials examined military recruits and the third studied boys from "lower wage-earning classes" attending a boarding school in the UK during World War II. Each of these three trials found a statistically significant (80% or greater) reduction in pneumonia incidence in the vitamin C group. We identified two therapeutic trials involving 197 community-acquired pneumonia patients. Only one was satisfactorily randomised, double-blind and placebo-controlled. That trial studied elderly patients in the UK and found lower mortality and reduced severity in the vitamin C group; however, the benefit was restricted to the most ill patients. The other therapeutic trial studied adults with a wide age range in the former Soviet Union and found a dose-dependent reduction in the duration of pneumonia with two vitamin C doses. We identified one prophylactic trial recording 13 cases of hospital-acquired pneumonia in 37 severely burned patients; one-day administration of vitamin C had no effect on pneumonia incidence. The identified studies are clinically heterogeneous which limits their comparability. The included studies did not find adverse effects of vitamin C.

Authors' conclusions: The prophylactic use of vitamin C to prevent pneumonia should be further investigated in populations who have a high incidence of pneumonia, especially if dietary vitamin C intake is low. Similarly, the therapeutic effects of vitamin C should be studied, especially in patients with low plasma vitamin C levels. The current evidence is too weak to advocate prophylactic use of vitamin C to prevent pneumonia in the general population. Nevertheless, therapeutic vitamin C supplementation may be reasonable for pneumonia patients who have low vitamin C plasma levels because its cost and risks are low.

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http://www.virologyj.com/content/10/1/337

VIROLOGY JOURNAL

RESEARCH Open Access

In vitro inhibition of mumps virus by retinoids

Kaitlin J Soye[1,2], Claire Trottier[1,2], Thomas Z Di Lenardo[1,2], Katherine H Restori[1,2], Lee Reichman[1], Wilson H Miller Jr[2] and Brian J Ward[1,3*]

Abstract

Background: Mumps virus (MuV) is a highly infectious paramyxovirus closely related to measles virus (MeV). Despite the availability of a mumps vaccine, outbreaks continue to occur and no treatment options are available. Vitamin A and other naturally occurring retinoids inhibit the replication of MeV *in vitro*.

Methods: Anti-viral effects of retinoids were observed in cell culture using the myelomonocytic U937, NB4/R4, and Huh7/7.5 cells. Observations of anti-viral effect were quantified using TCID50 analysis. Molecular properties of the antiviral effect were analysed using quantitative RT-PCR and western blot.

Results: The current work demonstrates that retinoids inhibit MuV *in vitro* due to up-regulation of type I interferon (IFN) and IFN stimulated genes. This effect is mediated by nuclear retinoid receptor signalling and RIG-I is required. The antiviral retinoid-induced state makes cells less permissive to viral replication from subsequent challenge with either MuV or MeV for less than 12 hours.

Conclusions: These results demonstrate that retinoids inhibit MuV replication in uninfected bystander cells through a retinoid inducible gene I (RIG-I), retinoic acid receptor (RAR) and IFN dependent manner making them refractory to subsequent rounds of viral replication. These observations raise the possibility that pharmacological doses of retinoids might have clinical benefit in MuV infection.

Text

The *Paramyxoviridae* are single stranded, enveloped, negative sense RNA viruses. They are among the most important viral pathogens of humans and animals. Many of the *Paramyxoviridae* replicate only in the respiratory epithelium, but *Morbillivirus* and *Rubulavirus* members typically have wider tissue tropism and can cause severe, systemic disease [1]. *Paramyxovirdae* epidemics in virgin populations can be devastating [1]. Vaccines are available for only a small number of the *Paramyxoviridae* and antiviral drugs are not yet available for most of these agents.

Mumps virus (MuV) is a *Rubulavirus* in the *Paramyxoviridae* family. It is the causative agent of mumps [2]. MuV is a highly contagious infection of humans and was historically one of the most common childhood illnesses. The virus infects and replicates in the nasal mucosa and upper-respiratory tract [2]. A transient cell-associated viremia (of mononuclear cells) contributes to systemic viral spread [2]. In young children, MuV infection is typically a mild disease characterized by fever, headache and swelling of the salivary glands. Complications such as meningitis, encephalitis or orchitis may occur. Mumps is a leading cause of acquired sensorineural deafness among children. Rates of post-infectious meningoencephalitis can be 1-10% of clinical mumps cases. Although the fatality rate of mumps encephalitis is low (0.1-0.5% of clinical mumps cases), the risk of permanent neurologic sequelae in encephalitis cases is 25% [3]. Furthermore, MuV infection during the first trimester of pregnancy is associated with a 25% incidence of spontaneous abortion [3].

There is no current treatment for mumps other than supportive care [2]. Vaccination programs in developed countries have markedly increased the average age at which clinical mumps occurs and dramatically reduced the incidence of mumps infection [2]. Unfortunately, large outbreaks have recently occurred in Europe, North America, Australia and Israel [4–12].

In the last 2 decades, many studies have documented the beneficial effects of vitamin A supplements on general mortality and/or morbidity in young children in a wide

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range of developing countries. In 2000, a meta-analysis of eight studies demonstrated an overall 30% reduction in infant mortality attributable to vitamin A supplements [13–15]. A surprising spin-off from these vitamin A supplementation studies was the re-discovery that vitamin A 'treatment' can significantly decrease the morbidity and mortality associated with acute MeV infection [16–19]. The mechanism underlying the positive effects of vitamin A supplements and treatment in measles are not well understood [13]. Since the mid-1990s, the WHO and UNICEF have recommended vitamin A treatment for acute measles in regions of the developing world with high mortality rates [20].

Vitamin A (retinol) is a fat-soluble vitamin. Its natural and synthetic derivatives as well as metabolites are collectively referred to as retinoids [21,22]. Retinol is obtained from the diet as either retinyl esters or carotenoids. Retinoids are required for a wide-range of crucial biological processes including regulation of embryonic development, maintenance of the integrity of epithelial cell surfaces, vision and immunity [23]. The metabolite, all-*trans* retinoic acid (ATRA) is responsible for mediating many of the important biological functions of retinoids [22]. ATRA is the natural ligand for retinoic acid receptors (RAR), which form heterodimers with the retinoid X receptors (RXR) within the nucleus [24]. RAR-RXR heterodimers bind to retinoic acid response elements (RARE) on the promoters of target genes to activate transcription when bound by ligand [21,22,24]. The protein products of retinoid-responsive genes are responsible for exerting the effects of retinoids in the cell.

Retinoids have been shown to play a role in innate immune responses and to regulate the expression of a number of interferon stimulated genes [25–27]. Of particular interest among the retinoid-responsive genes is the type I interferon (IFN) pathway. A powerful trigger for type I IFN production is the recognition of virus-associated molecular patterns by pattern recognition receptors [28]. These cytokines trigger a rapid and strong innate defense against many viruses, leading to the transcription of several hundred ISGs controlled by the IFN-stimulated gene factor 3 (ISGF3) complex [29].

Of particular importance to the current work, retinoids have specifically been implicated in regulating expression of the ISG (Interferon Stimulated Gene) retinoid-inducible gene I (RIG-I) and IFN regulatory factor 1 (IRF-1) [30–39]. RIG-I is a pattern recognition receptor that was originally understood to detect 5'-triphosphorylated, single-stranded RNA [40–42] and is expressed at a basal level in many cell types. The current consensus is that the minimal requirement for RIG-I activation is a blunt-ended base paired RNA 10-20 bp long with a 5' triphosphate [43]. It can initiate the production of type I IFN and is itself an ISG [44]. IFN has been reported to induce RIG-I expression by causing the IRF-1 transcription factor to bind to the RIG-I promoter [45].

Anti-MeV effects of retinoids have been observed in a number of primary human cells and cell lines of diverse tissue origin [46–48], including the myelomonocytic U937 cells, which were an important model for our work with MuV presented herein. We hypothesize that ATRA treatment during other viral infections would also have an antiviral effect. We set out to test whether or not MuV replication could be inhibited by retinoids. Based on our previous studies, we hypothesize that retinoids would inhibit MuV replication *in vitro* and that this inhibition would depend upon RAR signalling, type I interferon and functional RIG-I.

Results

Mumps virus can be inhibited in vitro

U937 cells are neoplastic and histiocytic progenitors of monocytes that have been extensively used in immunological studies [49] including investigation of interferon pathways during MuV infection [50–52]. In these cells, increasing doses of retinol resulted in a significant inhibition of MuV replication as quantified by $TCID_{50}$ (Figure 1A). Significant inhibition was achieved at concentrations as low as 1 μM, a dose at which increased expression of the retinoid responsive gene RARβ is readily observed (Figure 1C) [53]. Treatment of U937 cells with increasing doses of ATRA was even more effective as an inhibitor of MuV output (Figure 1B) and in the induction of RARβ mRNA expression (Figure 1D) [53]. All subsequent investigations of the antiviral effect of retinoids on MuV were performed using ATRA at a dose of 1 μM.

Retinoid treatment enhances IFN signalling

The innate immune response is thought to be responsible for the initial control of infectious agents. It has long been known that up-regulation of the type I IFN response functions in an auto-response feedback loop that is critically important for antiviral responses. In the U937 model, MuV infection alone is able to induce the expression of IFNα1 mRNA (Figure 2A). However, ATRA treatment of MuV infected cells synergistically increases the expression of IFNα1 mRNA and supernatant protein levels (Figure 2A-B). IFNβ mRNA expression and protein levels are also synergistically increased by the combined treatment of ATRA and MuV infection (Figure 2C-D).

The increased type I IFN production leads to the expression of ISGs. In the U937 model, IRF-1 mRNA expression is significantly increased over control by ATRA treatment alone (Figure 2E), in agreement with our previous work [47] and the literature [30,38,39]. However, treatment of MuV infected cells with ATRA further increases the expression of IRF-1 mRNA (Figure 2E). This combined treatment (MuV + ATRA) resulted in a robust increase in RIG-I mRNA expression (Figure 2F). The mRNA levels of two other IFN-responsive

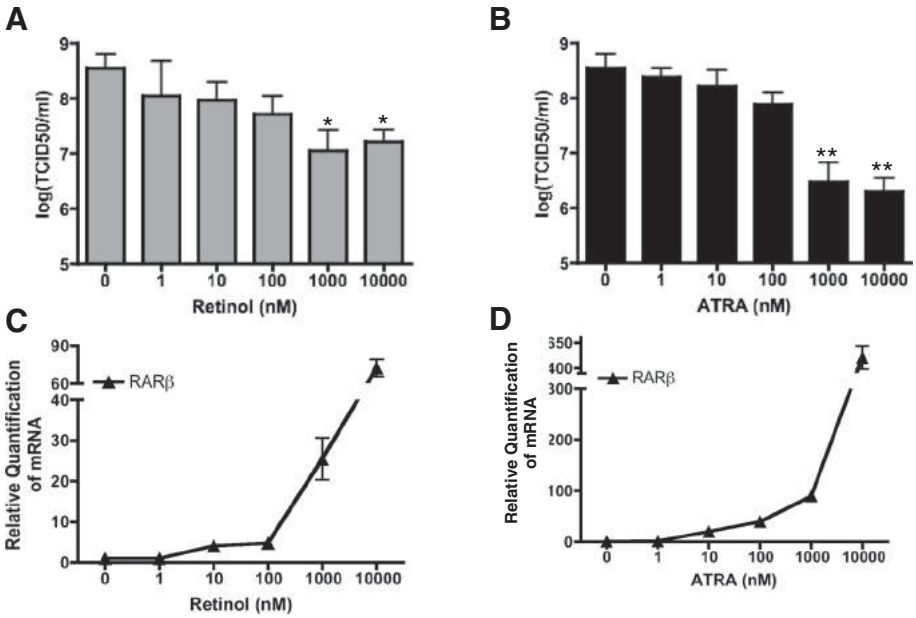

Figure 1 *In vitro* inhibition of mumps virus by retinoids. (A) (B) U937 cells were infected with MuV at an MOI of 0.01 and treated with increasing doses of retinol or all-*trans* retinoic acid (ATRA) as indicated. Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. **(C) (D)** RNA was extracted from parallel U937 cultures treated with increasing doses of retinol or ATRA and analyzed for RAR-β expression by qPCR. Data presented reflect three experiments performed in triplicate (N = 3). *$p < 0.05$, **$p < 0.01$.

genes, IRF-7 and MDA-5, also showed similar patterns of increased expression in response to MuV + ATRA (data not shown). In addition to the regulation of ISG expression, treatment of MuV infected U937 cells with ATRA also increased STAT1 activation as indicated by phosphorylation of tyrosine 701 (Figure 2G).

The increased expression of these ISGs can be attributed to the increased activation of the type I IFN pathway. When a monoclonal antibody specific to IFNα/β receptor 1 was used to prevent IFN signalling during MuV + ATRA treatment, ISG mRNA expression was blocked, as demonstrated by RIG-I mRNA (Figure 2H). This observation demonstrates that IFN signalling is required for the retinoid-MuV antiviral response.

Functional nuclear retinoid receptors mediate antiviral activity of retinoids

To determine whether the antiviral activity of retinoids requires nuclear receptor signalling, we utilized the well-characterized NB4/R4 cell model (retinoid responsive versus retinoid unresponsive) [54]. NB4 cells respond to ATRA at pharmacologic concentrations, while the NB4 subclone R4 is completely resistant, regardless of the concentration [54,55]. Both NB4 and R4 cells were readily infected with MuV. In NB4 cells, 1 μM of ATRA was able to inhibit MuV output but had no effect in R4 cells (Figure 3A). At this concentration, the level of inhibition observed was unlikely due to retinoid-driven differentiation of the NB4 cells [46,48]. Like the U937 cells, expression of the ISG, IRF-1, was also increased in NB4 cells exposed to ATRA alone but was higher in cells exposed to MuV + ATRA infection (Figure 3B). IRF-1 mRNA expression was very low during MuV infection alone in this model. In the retinoid-unresponsive R4 cells, IRF-1 expression was not seen either with ATRA treatment alone or in response to MuV + ATRA (Figure 3B). Exogenous IFNβ treatment alone was not able to induce the expression of IRF-1 in either cell line, suggesting the requirement of ATRA for IRF-1 expression.

RIG-I mRNA expression was also significantly increased by the combined treatment of MuV + ATRA in NB4 cells (Figure 3C). Both MuV alone and ATRA alone increased the expression of RIG-I over mock treatment, but the expression was greatly enhanced by combined treatment. Neither ATRA, nor MuV + ATRA induced the expression of RIG-I mRNA in R4 cells (Figure 3C). When treated with exogenous IFNβ, both NB4 and R4 cells increased the expression of RIG-I mRNA suggesting that IFN signalling is functional in both cell lines (Figure 3C). Expression of other ISGs, including IRF-7 and MDA-5, showed a similar pattern of up-regulation in NB4 cells and no response in R4 cells (data not shown).

As a further confirmation of the role of RARα mediated signalling in the retinoid-MuV antiviral response, treatment of U937 cells with RO 41–5253, a specific RARα antagonist, reversed the impact of ATRA on MuV replication and reduced the expression of the ISGs in response to MuV + ATRA (data not shown).

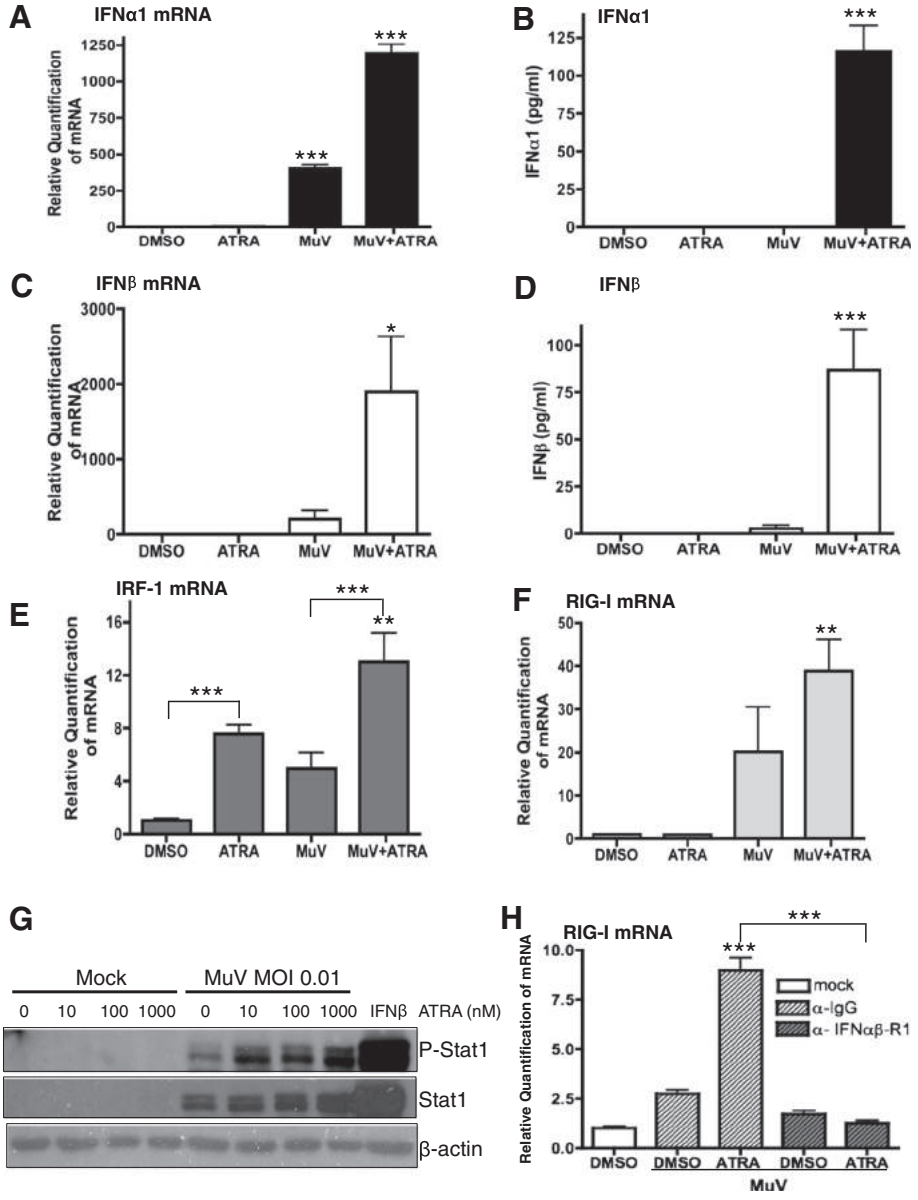

Figure 2 Type I interferon signaling is required for the induction of the retinoid anti-MuV response. U937 cells were infected with MuV at an MOI of 0.01 and treated with 1 μM ATRA or DMSO. 48 hr post-infection, RNA was extracted and analyzed for IFNα1 **(A)**, IFNβ (C), IRF-1 **(E)** and RIG-I **(F)** expression by qPCR. Supernatants were analyzed by ELISA for IFNα1 **(B)** or IFNβ **(D)** protein. **(G)** U937 cells were treated with increasing doses of ATRA (0-1000nM) and either mock infected or infected with MuV at an MOI 0.01. Protein was isolated from whole cell extracts and analyzed by western blot for phospho-STAT1 (Y701), total STAT1 or β-actin. **(H)** U937 cells were infected with MuV at an MOI of 0.01 and treated with 1 μM ATRA or DMSO and isotype control antibody or IFNAR2 antibody. 24 hr post-infection RNA was extracted and analyzed for RIG-I expression by qPCR. Data presented reflect three experiments performed in triplicate (N = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

RIG-I is required for the retinoid-induced antiviral response

RIG-I is both retinoid responsive and IFN stimulated. It was clearly up regulated in our *in vitro* model systems in response to MuV + ATRA (Figures 2F, 3C). To investigate the requirement of RIG-I signalling in the cellular response to combined MuV + ATRA exposure, we used the Huh7 cell line, which is derived from a human hepatocellular carcinoma and has been used extensively in hepatitis C virus (HCV) research [56,57]. Of particular interest for our studies, an Huh7 subclone (Huh7.5) has a point mutation in the first CARD domain of RIG-I, rendering the protein non-functional [57,58].

We turned to the Huh7/7.5 model to demonstrate the importance of RIG-I rather than using RNA interference (RNAi) after initial experiments demonstrated that both

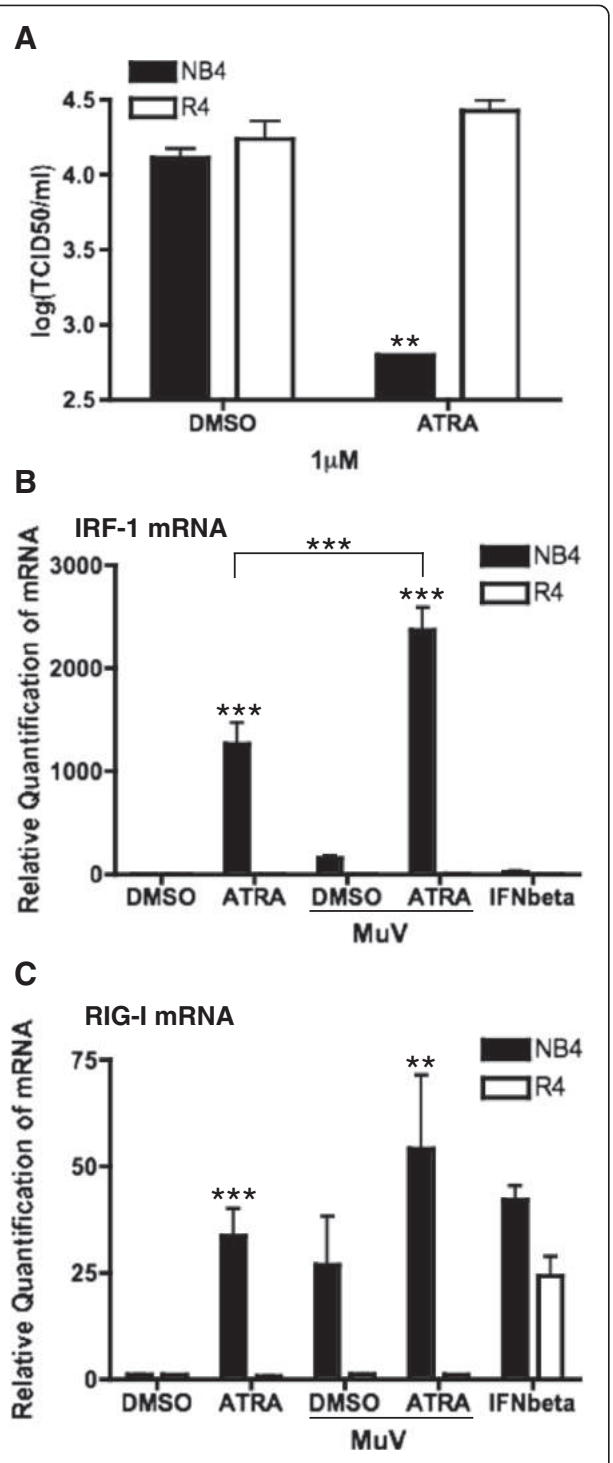

Figure 3 Retinoid signaling is required for the inhibition of MuV *in vitro*. NB4 and R4 cells were infected with MuV at an MOI of 0.01, treated with 1 μM ATRA or DMSO and/or treated with 1000 U/mL IFNβ (PBL Interferon Source, New Jersey) as indicated. **(A)** Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. RNA was extracted and analyzed for IRF-1 **(B)** and RIG-I **(C)** expression by qPCR. Data presented reflect three experiments performed in triplicate (N = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

control and RIG-I specific siRNA were sufficient to induce the expression of RIG-I and other interferon stimulated genes (data not shown, also demonstrated in [59,60]). In MuV infected Huh7 cells treated with ATRA, virus output was significantly reduced (Figure 4A) but ATRA had no effect on MuV replication in the Huh7.5, RIG-I non-functional cells (Figure 4A).

It has recently been demonstrated that RIG-I complementation in Huh7.5 cells can restore the IRF3 pathway, making these cells less permissive to Sendai virus (SeV) infection [58]. This observation suggests that the non-functional RIG-I encoded in the Huh7.5 cells can be complemented by exogenous expression of the protein. When RIG-I was transfected into the Huh7.5 cells, inhibition of MuV replication was restored (Figure 4B). These data demonstrate the requirement of RIG-I in the retinoid-MuV antiviral response.

Antiviral response is created in uninfected bystander cells

To determine whether or not a bystander effect was induced following MuV infection, we repeated key experiments using 0.02 μm-pore membrane transwell tissue culture inserts (depicted in [48] and [47]). In these experiments, the inner-chamber U937 cells could be exposed to the products of infection in the outer-chamber cells without direct contact with either MuV itself or the MuV-infected cells. We confirmed that MuV was not able to cross the membrane by $TCID_{50}$ assay of the inner-chamber cells in each experiment.

ATRA-stimulated ISG expression was just as strong in the inner-chamber (uninfected) as the outer-chamber (infected) cells despite the absence of active infection. Specifically, we found strong up-regulation of mRNA expression for IRF-1 (Figure 5A) and RIG-I (Figure 5B), as well as MDA-5 and IRF-7 (data not shown), in the inner-chamber cells.

When the supernatant (or conditioned media) from the inner-chamber bystander U937 cells was applied to fresh cells, we observed a striking induction in the expression of these same ISGs as shown for RIG-I (Figure 5C).

Bystander cells are protected from infection

To determine whether or not the uninfected inner-chamber, bystander cells would have reduced susceptibility to future infection these cells were harvested and challenged with MuV at an MOI of 0.1 immediately following incubation in the transwell. Compared with control cells not treated with ATRA and exposed to the products of MuV infection, the inner-chamber cells were relatively resistant to MuV replication (one log reduction in MuV titres produced, Figure 6A). This relatively refractory state persisted for up to 6 hours but was lost at 12 hours (Figure 6B).

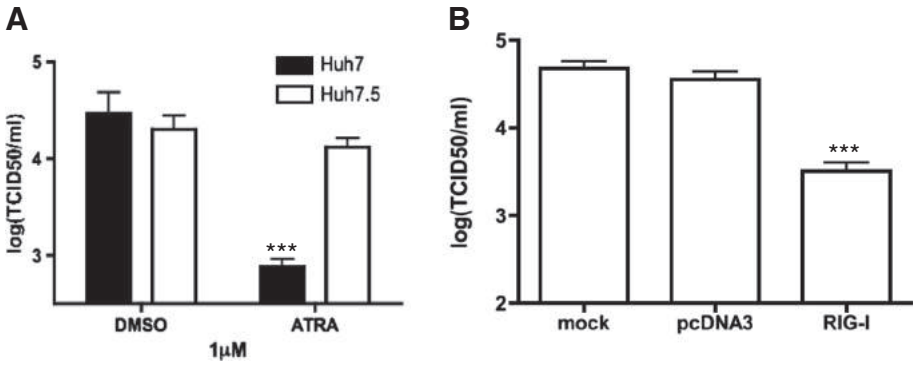

Figure 4 RIG-I is required for the inhibition of MuV by retinoids. (A) Huh7 and Huh7.5 cells were infected with MuV at an MOI of 0.01 and treated with 1 μM ATRA or DMSO. Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. **(B)** Huh7.5 cells were transfected with mock, pcDNA3.1 or pRIG-I-myc and incubated overnight. Following transfection, the cells were infected with MuV at an MOI of 0.01 and treated with 1 μM ATRA. Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. Western blotting was not performed since Huh7.5 cells produce a defective RIG-I protein that cannot be distinguished from the wild-type protein by commercially-available antibodies. Data presented reflect two experiments performed in triplicate (N = 2). ***$p < 0.001$.

These data suggest that the antiviral state created in the bystander U937 cells is short lived.

When inner-chamber bystander cells treated with ATRA and exposed to the products of MuV infection were challenged with MeV at an MOI 0.1 MeV replication was also reduced by at least 1 log compared to untreated controls or cells treated with only ATRA or exposed to the products of outer-chamber MuV infection (Figure 6C). The antiviral state induced in these cells was not virus-specific.

Discussion

The potential role of individual micronutrients in specific infectious diseases has been the subject of considerable interest for decades (reviewed in [61]). To our knowledge, retinol (Vitamin A) is currently the only micronutrient routinely used to 'treat' a viral disease. In fact, both vitamin A supplementation and therapy appear to have significant clinical benefit in natural MeV infection [16–19,21]. However, the effects of vitamin A on viral infections have been highly variable and at times, completely contradictory.

Although reduced mortality from diarrheal disease is associated with vitamin A supplements in children of the developing world this benefit appears to be due largely to milder bacterial infections [14,62,63]. In Mexican children receiving vitamin A supplements, the incidence of Norovirus diarrhea was reduced but gut viral titres and the period of virus shedding in these children were both significantly increased [64].

In human immunodeficiency virus (HIV) infection, pre-antiretroviral treatment (ART) studies suggested that low serum retinol levels were associated with rapid progression of acquired immunodeficiency syndrome (AIDS) but later studies showed little-to-no impact of supplements on disease progression or survival (reviewed in [65]). Perinatal vitamin A supplements in HIV-positive women can improve the survival of the seronegative children but can increase mother-to-child HIV transmission [65], possibly through increased viral loads in breast milk [66]. *In vitro*, retinoids have been found to both increase and decrease HIV replication in different model systems [67,68].

Patients infected with Hepatitis C virus (HCV) and treated with 9-*cis* retinoic acid or ATRA in combination with pegylated IFNα have lower viral loads [69,70]. In contrast, supplements do not increase viral clearance in human papilloma virus (HPV)-infected women [71].

Both vitamin A supplementation and treatment have either no or negative effects on respiratory tract infections including the common paramyxovirus, respiratory syncytial virus (RSV) [72–74]. Studies with another paramyxovirus have shown that vitamin A deficient chickens suffer increased morbidity from Newcastle disease virus (NDV) [75–77]. Using the paramyxovirus most closely related to measles, our group has demonstrated that canine distemper virus (CDV)-infected ferrets treated with vitamin A develop less severe disease [78]. In aggregate, these observations suggest that vitamin A and its derivatives may play an important role in antiviral responses but demonstrate clearly that mechanistic studies are essential to fully understand and exploit this potential.

Previously we have shown that retinoids can inhibit MeV replication *in vitro* via retinoid nuclear receptor activating type I IFN signalling [46,48]. We hypothesized that ATRA treatment during MuV infection may also inhibit MuV replication *in vitro*. We further sought to determine if the retinoid-MuV antiviral response would require type IFN signalling, RAR signalling and functional RIG-I. The current work demonstrates that ATRA similarly exerts anti-viral effects on MuV. We believe that

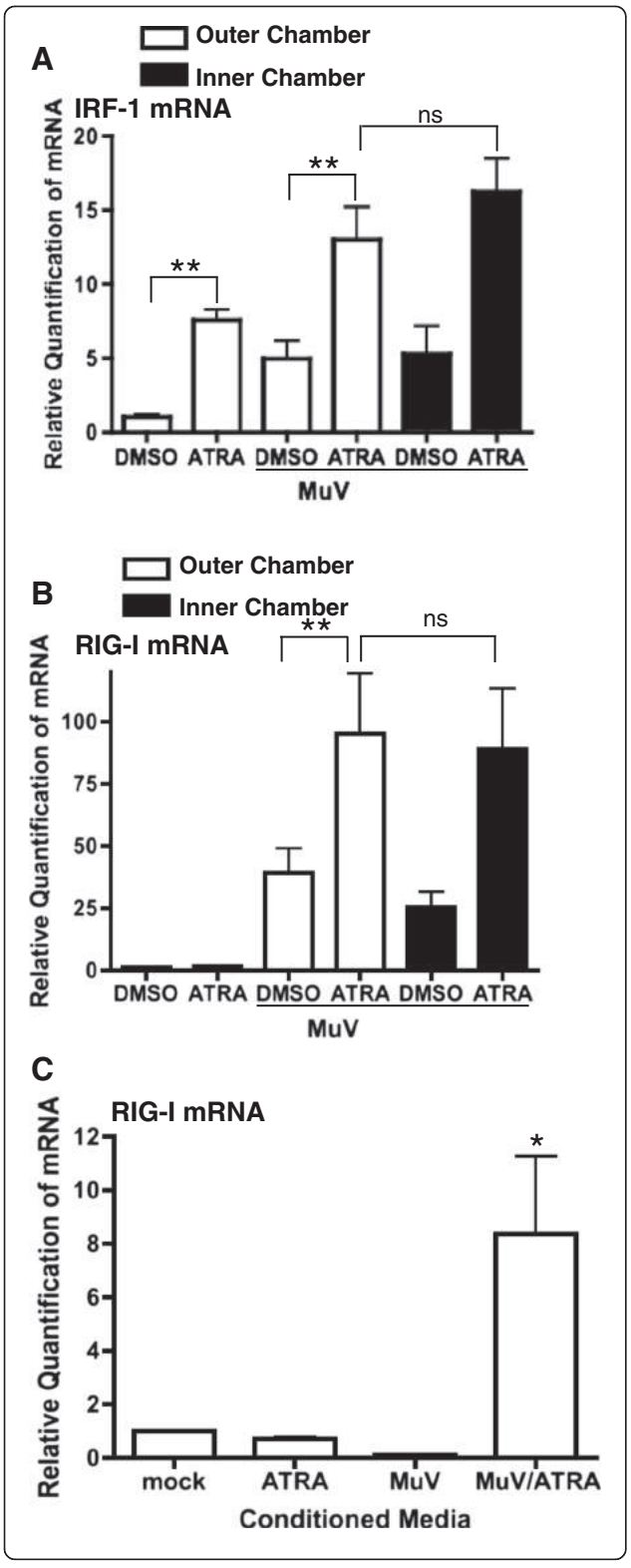

Figure 5 Retinoids induce an anti-MuV state in the uninfected, bystander cells. U937 cells were infected with MuV at an MOI of 0.01 in the presence of 1 μM ATRA or DMSO. Transwell membrane inserts with 0.02 μm pores were used to separate the infected cells in the outer chamber from the uninfected, bystander cells in the inner chamber [48]. Cells from control wells (no membrane insert), outer and inner chamber bystander cells were harvested after 48 hours and IRF-1 **(A)** and RIG-I **(B)** mRNA were measured by qPCR. As indicated on the Figure, outer chamber cells infected by MuV are represented by open bars and inner chamber (uninfected) cells are represented by the filled bars. **(C)** Conditioned media from the control and transwell inner chambers were applied to fresh U937 cells. After 24 hours of incubation with the conditioned media, RNA was extracted and RIG-I expression was analyzed. Data presented reflect three experiments performed in triplicate (N = 3). * $p < .02$,** $p < 0.01$.

these effects are not virus-specific, but rather extend to multiple members of the Paramyxovirus family or more broadly, to viruses that are detected by RIG-I. Figure 7 depicts our current understanding of retinoid action on Paramyxovirus infection. In Figure 7A, ATRA alone has no protective capacity on initially infected cells. These cells will produce the same amount of virus as untreated cells and ultimately, will die as a result of infection. However, the initially uninfected cells in the culture are primed for ISG expression by ATRA treatment through activation of the nuclear retinoid receptors. In Figure 7B, retinoid-primed cells effectively up-regulate ISG expression and type I IFN production upon viral infection. The combination of type I IFN and ATRA induces RIG-I expression in uninfected bystander cells, further improving the innate anti-viral response. ATRA is essential for initiating positive feedback through RIG-I activation and type I IFN pathways, which protects uninfected cells.

In the current work, we used a variety of *in vitro* models to extend our central observation of retinoid-induced anti-viral effects to MuV (Figures 1A, 1C, 3A, 4A). Although the cell lines used in this work varied in their overall sensitivity to retinoids (NB4 > U937 > Huh7>> R4), all supported the growth of MuV. Retinoid-induced suppression of MuV replication could be demonstrated in all but the R4 cells. Retinol (ROH) is the form of vitamin A found in the circulation at concentrations up to 2 μM [79]. The degree of inhibition of MuV replication was much greater using ATRA, a natural derivative of ROH and ligand that binds directly to nuclear receptors. ATRA is generally found in the intracellular space, but can be found in the serum in the 5–10 nM range [79]. As a result, we believe the mechanisms that we have documented *in vitro* to be potentially active *in vivo*. Indeed, the outcome of any infection is essentially a 'race' between pathogen replication and the developing immune response. In this context, it is plausible that the modest reduction in the rate of MuV replication that we observed with retinoid 'treatment'

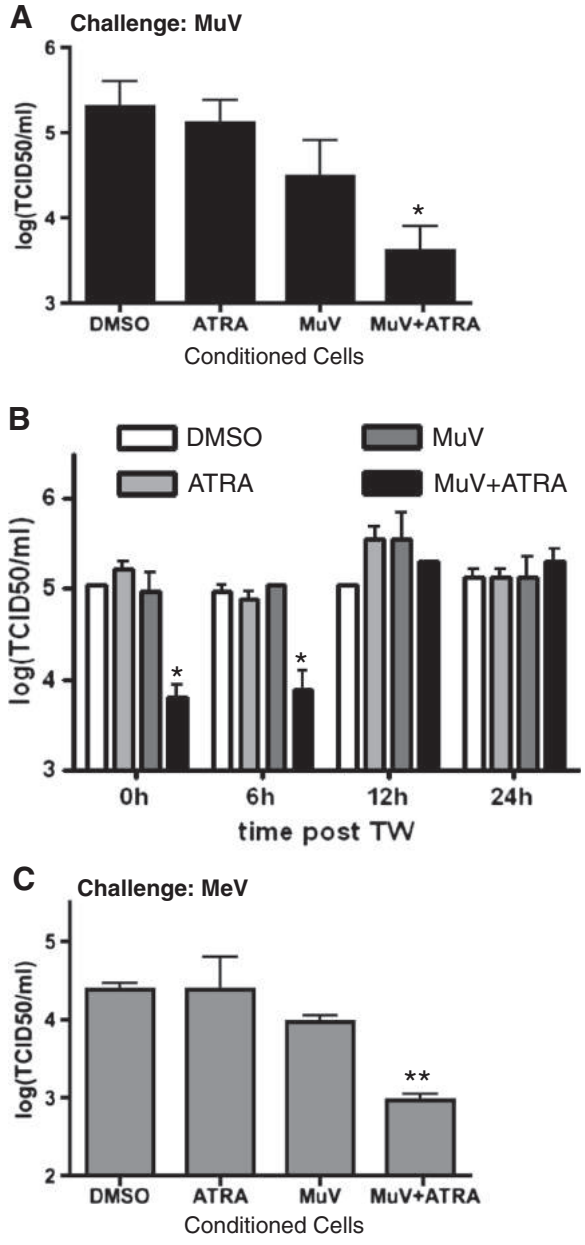

Figure 6 Antiviral state is short-lived and virus non-specific. (A) The inner chamber bystander cells were harvested and challenged with MuV at an MOI of 0.1. Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. **(B)** To assess the duration of the anti-viral state, U937 cells were suspended in TW inner-chamber conditioned media and incubated for the indicated time at 37°C then challenged with MuV at an MOI of 0.1. Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. **(C)** The inner chamber bystander cells were harvested and challenged with MuV at an MOI of 0.1. Whole cell lysates were harvested after 48 hours and viral titers were measured by $TCID_{50}$. Data presented are representative of two-three experiments performed in triplicate (N = 2-3). *$p < 0.05$, **$p < 0.01$.

in vitro could translate into clinical benefit during natural disease, as occurs with vitamin A treatment in natural MeV infection. To our knowledge, there has not yet been any attempt to use retinol (or other retinoids) to modulate the course of mumps infection. Unfortunately, there is no animal model for mumps in which this possibility can be directly tested.

The antiviral state created by the combination of MuV infection and ATRA treatment was ultimately generated by the expression of type I interferon. We have demonstrated that the combination of MuV + ATRA leads to transcription of IFN genes and at least additive increases in IFNα1 and IFNβ levels in culture supernatants, as well as enhanced transcription of ISGs (Figure 2A-F). Increasing doses of ATRA in the context of MuV infection led to marked increases in STAT1 activation (Figure 2G) and, when type I IFN signalling was blocked, the antiviral state is lost (Figure 2H). MuV normally escapes type I IFN control by targeting STAT1 for proteasomal degradation. Variations in the V protein sequence can decrease the efficiency of proteosomal targeting of STAT1, [80] resulting in differing sensitivity to type I IFNs and potentially the IFN dependent antiviral state produced by retinoid treatment. We are currently collecting wild-type MuV isolates to correlate retinoid sensitivity with V protein sequence to better understand this apparent paradox. At the current time, we also cannot fully explain differences in ATRA-induced up-regulation of RIG-I expression between the U937 and NB4 cells other that to postulate greater retinoid sensitivity in the RIG-I promoter of the former line. It is also possible that the timing of sample collection contributed to these results. Similarly, the timing of sampling may underlie the up-regulation of RIG-I mRNA in NB4 cells in response to IFNβ stimulation despite the apparent absence of IRF-1 induction (Figure 3B/C). Time course studies are currently underway to address these issues.

We further demonstrate that nuclear retinoid receptor signalling was also central to the antiviral effect of retinoids against MuV. Although it is possible that more than one nuclear receptor may be involved, our current data suggest that RARα plays an important role in mediating the antiviral effects against MuV. In our NB4/R4 model, RAR signalling was not only required for the antiviral effect (Figure 3A), it was essential for the expression of ISGs that contribute to the antiviral response (Figure 3B-C).

Finally, we demonstrate a similar retinoid signalling mechanism in response to MuV + ATRA (Figures 2E, 3C, Figure 4A). Most convincing, we have shown that overexpression of RIG-I in Huh7.5 cells with non-functional RIG-I signalling, can reinstate the retinoid-induced inhibition of MuV. The results in the Huh7.0/7.5 model are particularly interesting because MuV output does not differ greatly at 48 hours, suggesting that intact RIG-I

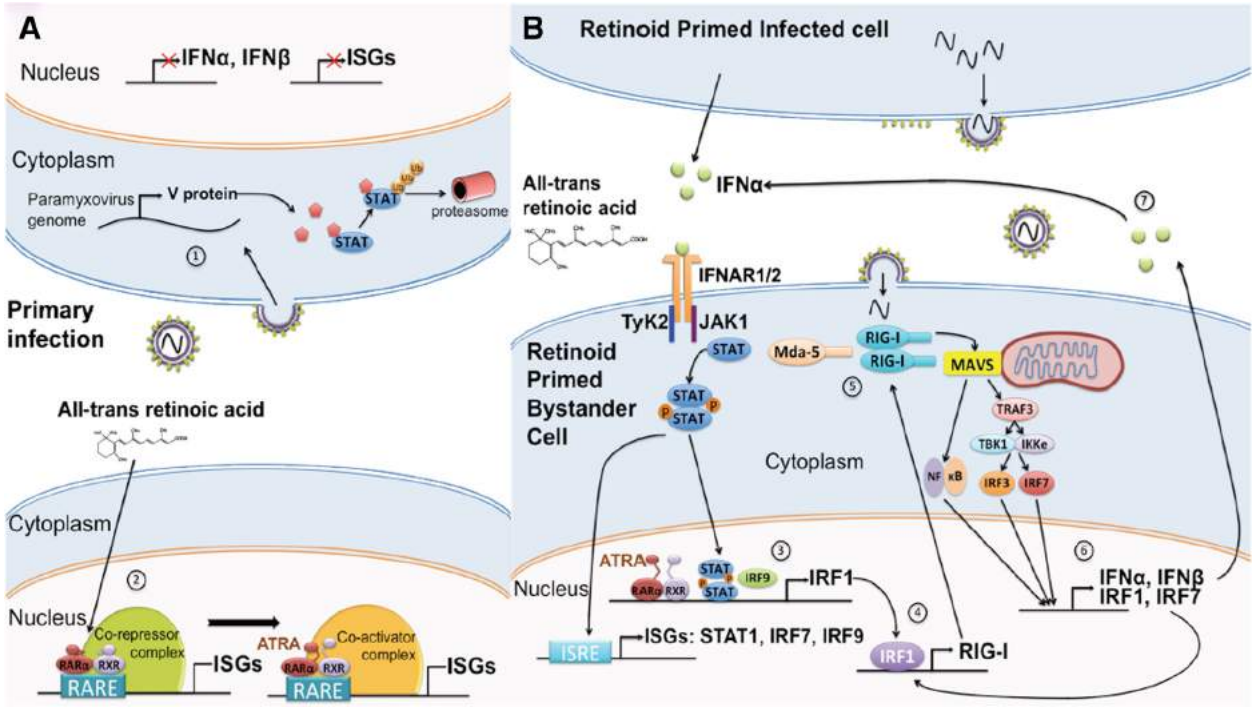

Figure 7 Retinoid action during paramyxovirus infection. (A) 1) Jak/STAT signaling is inhibited as a result of viral infection and ATRA treatment is unable to effectively promote unregulation of ISGs and type I IFN production. 2) Cells exposed to the products of the primary infection and primed with ATRA can more efficiently secrete type I IFN upon infection. **(B)** 3) Exposure to ATRA and type I IFN in the supernatant signals through the Jak/STAT pathway with the combination of nuclear localization of STAT1/2 heterodimer and activation of the RAR/RXR complex results in the transcription of multiple ISGs, importantly IRF1. For simplicity, only IFNα and its receptor IFNAR1/2 are shown to promote Jak/STAT signaling. 4) RIG-I expression is greatly up-regulated under these conditions, by IRF1 binding to the interferon response element (IRE) on its promoter. 5) Increased cytoplasmic quantities of RIG-I provide protection in the bystander cell upon virus infection. RIG-I detects viral RNA and associates with the adaptor protein MAVS, which initiates downstream activation of NFκB and IRF3/7. Nuclear translocation to their respective promoter elements induces transcription of antiviral genes, i.e., type I IFN and IRF1/7 production. It is not clear what role the second cytoplasmic RNA helicase (mda5) plays in this cascade although mda5 has been implicated in responses to several Paramyxoviridae. 6) These proteins feedback onto their respective pathways and promote RIG-I expression, further improving the antiviral state of the cell. 7) Increased production of type I IFN from the retinoid-primed bystander cell provides both an autocrine and paracrine signal to further protect the uninfected cells in culture.

signalling (by itself) does not play a major role in limiting viral replication. However. transfection of a functional RIG-I clearly restores retinoid responsiveness in this model. At least some of this paradox may be explained by the 48-hour time-point used for most experiments. Indeed, MuV output was lower in the Huh7.0 than Huh7.5 cells for the first 24–36 hours (data not shown). The 48 hour time-point was chosen for our experiments because retinoid effects were most obvious at this time. These findings are very similar to our observations with measles virus in the Huh 7.0/7.5 model where transfection of a dominant negative RIG-I eliminates the anti-viral activity of retinoids in the Huh 7.0 cells and transfection of a functional RIG-I gene into Huh 7.5 cells restores activity [47].

The Huh7.0/7.5 data are also intriguing because they suggest a larger role for RIG-I in defending against MuV than would have been predicted from the literature to date. It is widely thought that the double-stranded RNA sensor mda-5 is the primary target of the MuV V protein [81,82] and that RIG-I may respond primarily to Paramyxovirus defective interfering particles [83]. For several Paramyxoviruses, mda-5 signalling is inhibited by direct binding of the V protein and conserved residues in the helicase [82]. More recent data raises the possibility that Paramyxovirus V proteins may also target RIG-I indirectly by binding to laboratory of genetics and physiology 2 (LGP2) [84] Mutations in the carboxy-terminal domain of the V protein can result in a reduction or total loss of this interference [81]. In both NB4 and U937 cells, mda-5 expression is also increased by ATRA alone (data not shown). We are currently collecting wild-type (WT) MuV isolates to assess their susceptibility to retinoid-induced suppression and to correlate this suppression with V protein mutations. Our preliminary data (4 low-passage isolates to date) suggest that sensitivity to retinoid-induced suppression varies widely in WT MuV (50% suppressible) It is also interesting that retinoid sensitivity has been maintained in the two initially sensitive

WT isolates despite repeated *in vitro* passage in Vero cells in our laboratory.

The antiviral state created by MuV + ATRA was most profound in the initially uninfected bystander cells (Figure 5A-B) and could be transferred to fresh cells via the conditioned media leading to up-regulation of ISG expression (Figure 5C). Not surprisingly, since type I IFN responses are innate and non-specific, cells exposed to conditioned media from MuV + ATRA cells were relatively resistant to subsequent challenge with either MuV or MeV for less than 12 hours (Figure 6A-C). This last observation is consistent with the immediate and short-lived antiviral effects of type I IFNs [85].

The *Paramyxoviridae* including MeV, MuV, RSV, CDV, phocine distemper virus, Nipah virus and Hendra virus are among the most important human and animal pathogens. Commercial vaccines are not yet available for many of these viruses, and antiviral drugs are typically of little use [86]. Some of these viruses can have extraordinarily high mortality rates (for example, CDV in naïve seals and dogs, Nipah and Hendra viruses in man) [87]. The clinical evidence of benefit from retinoid therapy of MeV infection in children and CDV infection in ferrets is strong [17–19,78]. Our *in vitro* data suggest that ATRA may be far more potent that retinol in mediating antiviral effects. Our mechanistic studies in different tissue culture models of MuV infection suggest that common signalling pathways mediate these effects [46–48]. However, high doses of vitamin A in children with RSV infection have no benefit and may even cause harm [74,88]. In aggregate, these clinical and laboratory observations support further studies of the efficacy and mechanism of action of retinoids against a wider range of respiratory viruses in more sophisticated animal models, such as primates, or even clinical studies. It would be of particular interest to use retinoids other than retinol, ATRA in particular, in these latter studies to achieve more effective inhibition of viral replication. This conclusion is further supported by a recent study demonstrating that several synthetic retinoid analogues have much greater capacity to interfere with human herpes virus 8 (HHV8) replication *in vitro* than retinol [89].

Conclusions

In conclusion, this work has demonstrated that MuV can be inhibited *in vitro* by retinoids. This antiviral effect required RAR signalling, type I IFN signalling and functional RIG-I. The antiviral response was created in the initially uninfected bystander cells and was both short-lived and cross-protective against subsequent MuV or MeV challenge. This is the first work to demonstrate the antiviral effect of vitamin A on MuV and may contribute to better treatment options for MuV. We propose that IRF-1 is recruited to the RIG-I promoter under the influence of ATRA alone, and is required for the induction of RIG-I (47). In these models systems therefore, ATRA inhibits MuV replication through the RARα-dependent regulation of RIG-I and IRF-1 and via an IFN feedback loop.

Methods

Cells, reagents and viruses

All cell cultures were maintained at 37°C in a 5% CO_2 humidified incubator. U937 (ATCC, #CRL-1593.2), NB4 (M. Lanotte, INSERM UMR-S 1007, Paris, France) and R4, Huh7 and Huh7.5 (courtesy C. Richardson, Dalhousie University, Halifax, NS), Vero cells (ATCC, #CCL-81) were maintained as described in [47]. Retinol and All-trans retinoic acid (ATRA) (Sigma-Aldrich Fine Chemicals, Oakville, ON) stock solutions of 10^{-2} M were prepared in 100% DMSO and further dilutions were performed using RPMI. DMSO at equivalent final dilutions was used in all experiments as a control. All retinoids were stored in opaque eppendorf tubes at −80°C. The Jones MuV strain (ATCC, #VR-365) is a tissue culture-adapted virus that was, according to the supplier's web-site, extensively passaged in chicken embryos and Vero cells prior to purchase. Our MuV stock was initially plaque purified and then grown by infecting Vero cells with a maximum passage of three times from the original purchase (ATCC, #CCL-81) at a multiplicity of infection (MOI) of 0.001 at 33°C in a Cell-Stack 10 (Corning, Corning, NY). Harvested virus was concentrated by centrifugation at 15,752 x g for seven hours at 4°C in a fix-angle rotor, the pellet was resuspended in RPMI with gentle pipetting. The Chicago-1 MeV strain is a tissue culture-adapted genotype D3 virus (courtesy of W. Bellini, CDC, Atlanta, GA). MeV stock was grown as described in [47].

Cell culture infections

Cell lines were infected with MuV at the indicated MOIs. Media was removed and virus diluted in Hanks' Balanced Salt Solution with calcium and magnesium (Wisent, St-Bruno, QC). The virus was incubated with the cells for 1.5 hours, with gentle rocking at 15-minute intervals. The virus was removed and cells were resuspended in RPMI 1640 supplemented as previously described [46–48] using the specific MOIs and time points indicated in the figure legends and incubated at 37°C/5% CO_2.

Quantitative RT-PCR

RNA was extracted using Trizol (Invitrogen by Life Technologies, Burlington, ON) as per the manufacturer's instructions, and treated to remove possible genomic DNA contamination with Turbo DNAse (Ambion, Austin, TX). For experiments in which antibodies were used to block type I IFN signalling, an RNeasy Mini kit was used to extract RNA (Qiagen, Mississauga, ON). Equal quantities of RNA

were reverse-transcribed into cDNA for qPCR analysis using random primers. FAM-labelled TaqMan primer-probe assays for the following genes were obtained from ABI (Applied Biosystems by Life Technologies, Carlsbad CA): RIG-I, RARβ and IRF-1. The level of gene expression in untreated cells was used for calibration. Vic-labeled hGAPDH was used as the endogenous control.

Transwell

Transwell experiments (TW) were performed as previously described [47,48]. Briefly, TW membranes inserts with 0.02 μM pores served to separate infected cells in the outer chamber from the uninfected bystander cells of the inner chamber. Wells with no transwell inserts were used for control cultures. Preliminary experiments demonstrated that the presence/absence of the TW membrane had no impact on measured outcomes under control conditions.

Conditioned media

Supernatants were collected from TWs and used to treat fresh U937 cells. After 24 hours of incubation with the TW conditioned media, RNA was extracted and RT PCR performed. These samples were analyzed by qPCR for the expression of RIG-I.

Blocking antibody

Supernatants were collected from TWs and used to treat fresh U937 cells. These fresh cells were treated with anti-IFNAR2 blocking antibody (20 μg/μL, PBL Biomedical Laboratories, Piscataway, NJ) or isotype control antibody for one hour before infection and for the subsequent 24-hour incubation with the conditioned media. These samples were analyzed by qPCR for the expression of RIG-I.

Western blotting

Cells were infected with MuV and/or treated with ATRA at the indicated doses. 48 hours post infection, protein was harvested as previously described in [47]. The membranes were incubated in 5% non-fat milk or 5% BSA for 1 hour and incubated overnight at 4°C with primary antibody. Primary antibodies used were against phospho-STAT1 (Y701) (1/1000, BD Bioscience), Total STAT1 (1/1000, BD Bioscience) and β-actin (1/10000, Sigma). Following overnight incubation, membranes were washed three times for 10 minutes in TBS/0.1% Tween, incubated with secondary antibody (1/10000, GE Healthcare) at room temperature for 30 minutes, and washed three times for 10 minutes. The peroxidase-conjugated secondary antibodies were developed using a chemiluminescence kit according to the manufacturer's instructions (GE Healthcare).

Transfection

Huh 7.5 cells were seeded at 1.5×10^5 cell/mL, then were transfected with 3 μg of the RIG-I construct in a pcDNA3 plasmid (gift from J. Hiscott) or empty vector using a 3:1 ratio of FuGENE 6 (Roche, Toronto, ON) as per the manufacturer's instructions. At 18 hours post-transfection, cells were infected with MuV MOI 0.01 and at 48 hours post infection the cells and supernatants were quantified using plaque assay as previously described [46].

Viral challenge of bystander cells

Bystander cells from the TW inner chambers were pooled according to treatment and resuspended in Hanks' Balanced Salt Solution with calcium and magnesium (Wisent, St-Bruno, QC). Cells are infected with MuV or MeV at MOI 0.1 as described above and previously [46–48]. These cells were resuspended in RPMI 1640 (Wisent, St-Bruno, QC) supplemented with 10% heat-inactivated FBS (Wisent, St-Bruno, QC) and 0.1% gentamicin and incubated for the indicated time at 37°C/5% CO_2.

Tissue culture infectious dose$_{50}$ ($TCID_{50}$)

MuV concentrations were quantified by $TCID_{50}$. Briefly, Whole cells and supernatant were frozen at −80°C to lyse cells, samples were defrosted on ice, then serially diluted in Minimum Essential Medium Eagle (Wisent, St-Bruno, QC) supplemented with 3% heat-inactivated FBS (Wisent, St-Bruno, QC) and 0.1% gentamicin. Supernatants were not analysed separately in this series of experiments. Diluted virus was applied to Vero cells in 3% heat-inactivated FBS (Wisent, St-Bruno, QC) and 0.1% gentamicin in 96-well plates. The virus is incubated with the cells for 5 days at 37°C/5% CO_2. Syncytium formation was scored and $TCID_{50}$ was calculated using the Karber method [90,91].

Elisa

U937 cells were infected at an MOI of 0.01 with the indicated virus. At 48 hours post-infection, supernatant IFNα1 and IFNβ were measured by ELISA (PBL Interferon Source, Piscataway, NJ) as per the manufacturer's instructions.

Competing interests

The authors have no competing interest to declare.

Authors' contributions

KJS designed, completed, and analysed experiments, drafted and reviewed the manuscript; CT and LR designed and completed experiments; TZD and KHR drafted the model figure and revised the manuscript; WHM and BJW designed experiments and critically reviewed the manuscript. All authors read and approved the final manuscript.

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Vitamin B6 prevents cognitive impairment in experimental pneumococcal meningitis

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Abstract

Streptococcus pneumoniae is the relevant cause of bacterial meningitis, with a high-mortality rate and long-term neurological sequelae, affecting up to 50% of survivors. Pneumococcal compounds are pro-inflammatory mediators that induce an innate immune response and tryptophan degradation through the kynurenine pathway. Vitamin B6 acts as a cofactor at the active sites of enzymes that catalyze a great number of reactions involved in the metabolism of tryptophan, preventing the accumulation of neurotoxic intermediates. In the present study, we evaluated the effects of vitamin B6 on memory and on brain-derived neurotrophic factor (BDNF) expression in the brain of adult Wistar rats subjected to pneumococcal meningitis. The animals received either 10 µL of artificial cerebral spinal fluid (CSF) or an equivalent volume of S. pneumoniae suspension. The animals were divided into four groups: control, control treated with vitamin B6, meningitis, and meningitis treated with vitamin B6. Ten days after induction, the animals were subjected to behavioral tests: open-field task and step-down inhibitory avoidance task. In the open-field task, there was a significant reduction in both crossing and rearing in the control group, control/B6 group, and meningitis/B6 group compared with the training session, demonstrating habituation memory. However, the meningitis group showed no difference in motor and exploratory activity between training and test sessions, demonstrating memory impairment. In the step-down inhibitory avoidance task, there was a difference between training and test sessions in the control group, control/B6 group, and meningitis/B6 group, demonstrating aversive memory. In the meningitis group, there was no difference between training and test sessions, demonstrating impairment of aversive memory. In the hippocampus, BDNF expression decreased in the meningitis group when compared to the control group; however, adjuvant treatment with vitamin B6 increased BDNF expression in the meningitis group. Thus, vitamin B6 attenuated the memory impairment in animals subjected to pneumococcal meningitis.

Keywords: BDNF; Pneumococcal meningitis; memory; vitamin B6.

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Effect of Exclusive Breastfeeding on Rotavirus Infection among Children

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Abstract

Objectives: To assess, whether exclusive breastfeeding plays a protective role in Rotavirus infection among children under age of five and to estimate whether breastfeeding has an impact on reducing the severity and symptoms among children infected with Rotavirus.

Methods: A systematic search was performed in Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE/PubMEd (from 1980 to present), ScienceDirect (from 1980 to present), OVID (from 1980 to present) and regional database IndMED. All the studies along with the research publications with descriptive, case series, cross sectional, case control and cohort studies (prospective and retrospective) that provided effectiveness of exclusive breastfeeding were considered for this review. Two review authors independently scrutinized the studies and extracted the data. In case of disagreement, the senior reviewer was consulted.

Results: Total seven studies qualified for the systematic review in which 6 studies qualified for meta-analysis. Exclusive breastfeeding was found to be effective in prevention of Rotavirus infection and in reducing the risk of Rotavirus infection among children (OR = 0.62, 95 % CI = 0.48-0.81).

Conclusions: This systematic review suggests that there is significant benefit in prevention of Rotavirus diarrhea among children by practicing exclusive breastfeeding throughout first 6 mo of life. Thereby, this study provides next reason to promote exclusive breastfeeding practice among mothers.

Keywords: Breast feeding; Diarrhea; Rotavirus infection; Systematic review.

Safety and efficacy of N-acetyl-cysteine for prophylaxis of ventilator-associated pneumonia: a randomized, double blind, placebo-controlled clinical trial

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Abstract

Ventilator-associated-pneumonia (VAP) is characterized by morbidity, mortality, and prolonged length of stay in intensive care unit (ICU). The present study aimed to examine the effect of N-acetyl-cysteine (NAC) in preventing VAP in patients hospitalized in ICU. We performed a prospective, randomized, double-blind, placebo-controlled trial of 60 mechanically ventilated patients at high risk of developing VAP. NAC (600 mg/twice daily) and placebo (twice daily) were administered to NAC group (n = 30) and control group (n = 30), respectively, through the nasogastric tube in addition to routine care. The clinical response was considered as primary (incidence of VAP) and secondary outcomes. Twenty-two (36.6%) patients developed VAP. Patients treated with NAC were significantly less likely to develop clinically confirmed VAP compared with patients treated with placebo (26.6% *vs*. 46.6%; P = 0.032). Patients treated with NAC had significantly less ICU length of stay (14.36 ± 4.69 days *vs*. 17.81 ± 6.37 days, P = 0.028) and less hospital stay (19.23 ± 5.54 days *vs*. 24.61 ± 6.81 days; P = 0.03) than patients treated with placebo. Time to VAP was significantly longer in the NAC group (9.42 ± 1.9 days *vs*. 6.46 ± 2.53 days; P = 0.002). The incidence of complete recovery was significantly higher in the NAC group (56.6% *vs*. 30%; P = 0.006). No adverse events related to NAC were identified. NAC is safe and effective to prevent and delay VAP, and improve its complete recovery rate in a selected, high-risk ICU population.

Key words: acetylcysteine; pneumonia, ventilator-associated; prevention, control, prophylaxis; intratracheal intubation

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Introduction

Ventilator-associated-pneumonia (VAP) is a complication developed in about 30% of mechanically ventilated patients.[1-4] Patients with VAP have higher morbidity and mortality rates, and faced with prolonged intensive care unit (ICU) and hospital lengths of stay, and are consequently imposed greater hospital costs.[1,5-7] The pathogenesis of VAP is complex; however, bacterial colonization of respiratory and digestive tracts, biofilm formation, and micro aspiration of contaminated secretions are the most important pathogenic factors involved.[5,8] The current preventive strategies for VAP are mainly directed at colonization and aspiration modification. These strategies include elevation of the head of the bed, intensive oral care, subglottic secretion draining or silver-coated endotracheal tubes, and reducing the duration of mechanical ventilation using regular sedation vacations and weaning protocols.[3,8-12] Underlying disorders, prolonged hospital stay, and high prevalence of antibiotic-resistant pathogens obstruct the treatment of VAP.[13] Respiratory support dysfunctions such as mucociliary dysfunction and damage caused by oxidative stress are predisposing factors of VAP.[13] N-acetyl-cysteine (NAC) is a mucolytic drug with anti-inflammatory,[14] antioxidant[15,16] and immunomodulating[17] properties. Given the pathogenesis of VAP and the functions of NAC, it appears that NAC can be effective in preventing VAP as a non-antibiotic strategy. Therefore, we conducted a study aimed to examine the effect of NAC in preventing of VAP in patients hospitalized in ICU.

Subjects and Methods

Study design

This was a randomized, double blind, placebo-controlled clinical trial conducted from March 2014 until June 2016 in an academic infectious department of Vali-asr Hospital, Arak, Iran. Written informed consent was obtained from patients' legal guardians. The investigators were committed to the principles of the *Declaration of Helsinki* throughout the study. The protocol of the study was approved by the Ethical Committee of Arak University of Medical Sciences (approved No: 4-144-92).

Subjects

Adult ICU admitted patients undergoing endotracheal intubation and mechanical ventilation were eligible. All eligible patients who referred to ICU were selected for study and

recruitment after signing the informed consent. Our exclusion criteria during the follow-up duration were: 1) Less than 72-hour intubation, 2) death within 72 hours after intubation, 3) transference to other hospitals, and 4) termination of NAC administration: withdrawal with the consent of the patients' legal guardians; judgment if the physician in charge due to adverse events and safety concerns; and difficult administration due to GI problems and other reasons. Therefore, patients with pregnancy, recent gastrointestinal tract injury, oropharyngeal mucosal injury, tracheostomy, presence of pneumonia at the beginning of hospitalization, history of antibiotic consumption within the last 4 weeks prior to ICU hospitalization, and those disconnected from ventilator or died within 72 hours after intubation were excluded. Patients were also excluded if the investigators were unable to obtain informed written consent and administer the first dose of the study drug within 12 hours of intubation.

Randomization and blinding

Simple randomization using computer-assisted randomization table was considered for current study. NAC and placebo were provided by a pharmaceutical company (Avesina, Tehran, Iran). Two preparations were delivered to the nurses in glass containers with lid of similar shape and size, without a name and with a code. Investigators, primary care clinicians, and bedside nurses were blinded to group assignments.

Intervention

Demographic and baseline information collected using a checklist including questions regarding age, gender, VAP risk factors,[18] reason for ICU admission, Acute Physiology and Chronic Health Evaluation II (APACHE II) score (< 18, 18–24, > 24), oral prosthodontics, and oral hygiene condition (poor, good).

Patients were randomly assigned into NAC or placebo groups. NAC (600 mg; water-soluble tablets) was administered to the NAC group twice daily through the nasogastric tube. Placebo (water-soluble vitamin tablets) was administered to the control group through the nasogastric tube twice daily. Drug administration was started at the beginning of hospitalization within the first 12 hours of mechanical ventilation, and continued until performing extubation, tracheostomy, discharge, or death.

Patients received all routine care, including VAP-preventive measures as per hospital protocols and antibiotic therapy as deemed necessary, under the direction of their admitting physicians throughout the study.[18,19] Institutional VAP-prevention measures remained unchanged throughout the study period.

Outcome assessment

The clinical response was considered as primary and secondary outcomes. The primary outcome was the incidence of VAP. The secondary outcome included: 1) Time to VAP, 2) duration of mechanical ventilation, 3) ICU stay, 4) hospital stay, 5) VAP complications, and 6) recovery rate.

VAP was diagnosed based on clinical examinations and daily chest X-ray (CXR) results according to the American College of Chest Physicians (ACCP) criteria[18, 19] as follows: The presence of new and continuous infiltrations (for over 24 hours) in CXR results accompanied by 2–3 of the findings bellow: 1) Fever (< 38.5°C or < 35°C); 2) leukocytosis (WBC > 10,000/mm^3 or WBC < 3,000/mm^3); 3) purulent sputum.

The recovery rate was defined as follows: 1) Complete recovery: termination of fever after 48 hours, termination of initial physical-pulmonary examination results after 1 week, leukocytosis improvement after 4 days, and improved CXR results within 4–12 weeks. 2) Modest recovery: termination of fever after 4–7 days and improved examination results after over 10 days. 3) Lack of recovery: continuation of symptoms or the development of complications. 4) Mortality: death during hospitalization in ICU.

Patients who were excluded were replaced by the other eligible patients. Notably, the patients excluded were not analyzed and only the patients that completed the study were subjected to further analysis.

Statistical analysis

The sample size was determined based on alpha (0.05) and beta (0.2) values, *i.e.* type I and type II errors. The collected data were analyzed with SPSS 18.0 software (SPSS Inc, Chicago, IL, USA) and descriptive statistics methods for frequency determination. Groups were compared with *t*-test; continuous variables with abnormal distribution were compared by Mann-Whitney *U*-test and categorical variables with abnormal distribution were compared using chi-square test. *P*-values less than 0.05 were considered statistically significant.

Results

Demographic and baseline findings

A total of 174 patients admitted to the ICU required endotracheal intubation, but 114 were not enrolled because informed consent could not be obtained during the first 12 hours of mechanical ventilation, or patients were unlikely to require intubation for at least 72 hours. Finally, 60 patients were randomly assigned into NAC (n = 30) group and placebo group (n = 30). **Figure 1** illustrates the study recruitment process (CONSORT flowchart).

Patients were evenly distributed between groups based on demographic and other baseline characteristics (**Table 1**). The mean age was 49.43 ± 18.7 year and 71.6% were male. The mean APACHE II score was 22.2 ± 4.5. The most common VAP risk factor was smoking (NAC: 8 [26.7%], placebo: 11 [36.7%]; P = 0.29). The most common reason for ICU admission was trauma (NAC: 9 [30%], placebo: 8 [26.6%]; P = 0.89).

Primary outcome

Twenty-two (36.6%) patients developed VAP. The incidence of VAP was significantly lower in the NAC group (n = 8, 26.6%) than in the placebo group (n = 14, 46.6%) (P = 0.032).

Secondary outcomes

ICU stay was 16.08 ± 5.9 days. Hospital stay and time to VAP in all patients were 21.92 ± 6.3 days and 7.94 ± 3.01 days, respectively. Mechanical ventilation duration was 9.16 ± 8.2 days. Complete recovery and mortality were observed in 26 (43.3%) and 5 (8.3%) patients, respectively. Secondary outcome information is summarized in **Table 2**.

Table 1: Demographic and baseline characteristics of patients undergoing endotracheal intubation and mechanical ventilation

		NAC (n = 30)	Placebo (n = 30)	P-value
Age (mean±SD, year)		48.60±17.75	50.26±18.16	0.72
Male		22(73.3)	21(70)	0.82
APACHE II score (mean±SD)		21.7±7.5	22.7±8.0	0.45
VAP risk factors	Smoking	8(26.7)	11(36.7)	0.29
	COPD	7(23.3)	3(10)	0.52
	Chest trauma	4(13.3)	3(10)	0.82
	Nursing home resident	2(6.7)	3(10)	0.30
	Alcohol abuse	2(6.6)	1(3.3)	0.12
Reason for ICU admission	Trauma	9(30)	8(26.6)	0.89
	Respiratory failure	6(20)	7(23.3)	
	Cardiology	3(10)	4(13.3)	
	Neurology/neurosurgery	6(20)	5(16.6)	
	Gastrointestinal	2(6.6)	4(13.3)	
	Endocrine	1(3.31)	0	
	Infection	3(10)	2(6.6)	
Oral prosthodontics		4(13.3)	2(6.6)	0.64
Oral hygiene condition (poor)		27(90)	29(96.6)	0.30

Note: Data are expressed as number (percent), expect age and APACHE II score. APACHE: Acute Physiology and Chronic Health Evaluation; COPD: chronic obstructive pulmonary disease; VAP: ventilator-associated pneumonia; NAC: N-acetyl-cysteine; ICU: intensive care unit.

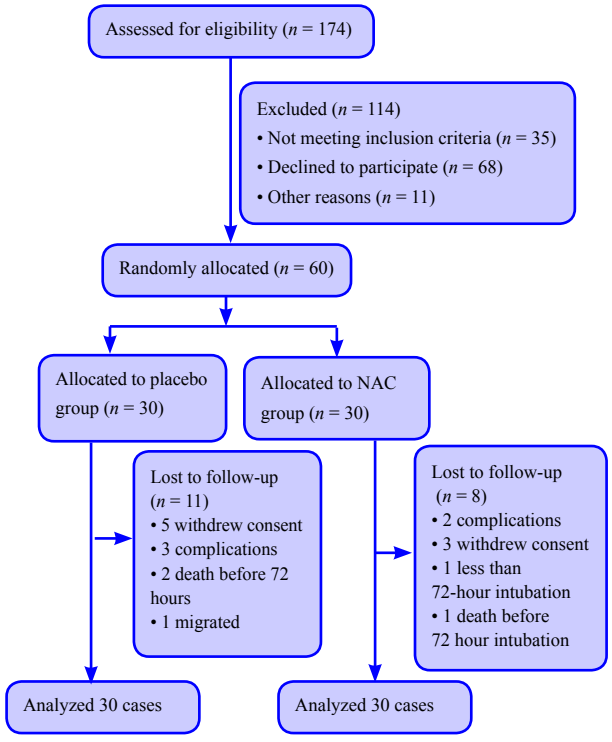

Figure 1: CONSORT flowchart of patients undergoing endotracheal intubation and mechanical ventilation.
Note: NAC: N-acetyl-cysteine.

Patients treated with NAC had significantly less ICU stay (14.36 ± 4.69 days vs. 17.81 ± 6.37 days, P = 0.028) and less hospital stay (19.23 ± 5.54 days *vs*. 24.61 ± 6.81 days; P = 0.03) than patients treated with placebo. Time to VAP was significantly longer in the NAC group (6.42 ± 1.9 days *vs*. 3.46 ± 2.53 days, P = 0.002). Mechanical ventilation duration was not significantly different between two groups (9.06 ± 4.81 days *vs*. 9.26 ± 6.05 days, P = 0.64).

Table 2: Secondary outcomes of patients undergoing endotracheal intubation and mechanical ventilation with NAC and placebo intervention

	NAC (n = 30)	Placebo (n = 30)	P-value
Duration of mechanical ventilation (days)	9.06±4.81	9.26±6.05	0.640
ICU stay (days)	14.36±4.69	17.81±6.37	0.028
Hospital stay (days)	19.23±5.54	24.61±6.81	0.030
time to VAP (days)	6.42±1.9	3.46±2.53	0.002
Recovery rate of VAP (number (percent))			
Complete	17(56.6)	9(30)	0.006
Modest	7(23.3)	11(36.6)	0.670
Lack	3(10)	8(26.6)	0.040
Death	3(10)	2(6.7)	0.120

Note: Data are expressed as the mean ± SD, expect recovery rate of VAP. NAC: N-acetyl-cysteine; ICU: intensive care unit; VAP: ventilator-associated pneumonia.

The incidence of complete recovery was significantly higher in the NAC group (56.6%, *vs*. 30%, P = 0.006). The incidence of lack of recovery was significantly lower in the NAC group (10% *vs*. 26.6%, P = 0.04). Mortality within 72 hours after intubation was not significantly different between two groups (2 [6.6%] in placebo group *vs*. 3 [10%] in NAC group).

Five (16.6%) of 30 patients in the NAC group and 7 (23.3%) patients in the control group had complications (P = 0.58). Two patients in NAC group and three patients in placebo had serious complications that who excluded from study. Complications were related to underlying diseases. No complications were thought to be drug-related.

Discussion

The current study suggests that NAC can be effective to prevent and delay VAP and improve its complete recovery rate

in a selected, high-risk ICU population.

The effectiveness of NAC has been shown in patients with some lung diseases such as acute respiratory distress syndrome (ARDS),[20,21] idiopathic pulmonary fibrosis (IPF),[22,23] chronic obstructive pulmonary disease (COPD),[14,24] influenza[17,25] specially influenza A (H5N1),[26] cystic fibrosis[27] and smoking-related damage.[28] However, studies on the efficacy of NAC on pneumonia, especially the VAP remain limited.

In 2010, Zhao et al.[29] showed that NAC has anti-bacterial properties against P. aeruginosa and may detach *P. aeruginosa* biofilms in chronic respiratory tract infections. Based on this study, NAC at 0.5 mg/mL could detach mature *P. aeruginosa* biofilms. Disruption was proportional to NAC concentrations, and biofilms were completely disrupted at 10 mg/mL NAC. Extracellular polysaccharides (EPS) production by *P. aeruginosa* were also decreased by 27.64% and 44.59% at NAC concentrations of 0.5 mg/mL and 1 mg/mL, respectively.

Qu et al.[30] in 2016, evaluated NAC inhalation on VAP caused by biofilm in endotracheal tubes. They selected 117 cases tracheally intubated and undergoing mechanical ventilation for ≥ 48 hours in ICU. All the cases were randomly divided into control group ($n = 60$) and study group ($n = 57$). The patients in the study group were treated with different doses of aerosolized NAC according to different ages, once every 8 hours, until stopping mechanical ventilation. Electron microscopy showed that biofilm had formed in the endotracheal tube inner wall in early period of mechanical ventilation. With prolonged mechanical ventilation, biofilm structure improved. During the mechanical ventilation, the thickness of biofilm in the study group decreased compared with control group. Biofilm culture positive rate and incidence of VAP decreased in the study group compared with the control group (65% [37/57] *vs*. 80% [48/60], $P < 0.05$; 11% [6/57] *vs*. 32% [19/60], $P < 0.01$). The most similar study to our study was a clinical trial reported by Qu et al.[30] Although studies method was notable differences, however, the results were consistent about the effectiveness of NAC in VAP.

In a systematic review, Chalumeau et al.[31] investigated 6 trials with 497 participants for the evaluation of the efficacy of NAC in the treatment of acute lower and upper respiratory tract infections in children without chronic pulmonary diseases. NAC seemed to have a moderate efficacy in children older than 2 years, and NAC administration was safe in these children.

The majority of studies on the efficacy of NAC in pulmonary diseases have been conducted based on functions defined for NAC, i.e. mucociliary, antioxidant and anti-inflammatory functions.[13,16,21,22] Our study was also designed based on these functions: 1) The mechanical mucociliary function is a defense mechanism of the respiratory system directed at preventing the deposition of its contaminated secretions in respiratory tracts and subsequent contaminations.[21] The mucociliary system consists of respiratory cilia that keep the respiratory system clear of secretions through their beating motion.[13] Diminished respiratory support functions such as mucociliary function against respiratory infections and stress induced by underlying diseases are among disorders that leave ICU patients susceptible to pneumonia.[13] NAC is a mucolytic drug that facilitates mucus flow and discharge through breaking disulfide bonds of thick bronchial system secretions, reducing the development and prolongation risks for infectious respiratory system diseases.[16] 2) The second significant function of NAC is its antioxidant role fulfilled *via* two pathways: a) the direct pathway (reaction with free radicals), and b) the indirect pathway (glutathione synthesis precursors as a major body antioxidant).[16,32] Dekhuijzen[15] has interpreted the NAC antioxidant effect well.

Oxidative agents play a significant destructive role in the course of VAP development. The prevention of oxidative degradation cycle through antioxidant agents may reduce the incidence or severity of VAP.[33,34] Duflo et al.[33] examined alveolar and serum oxidative stress in 78 and 10 patients with and without VAP, respectively. Serum oxidative stress markers and patients' bronchoalveolar lavage (BAL) samples were evaluated for oxidative activity. VAP group exhibited significantly greater oxidative activity compared with the non-VAP group. Manzanares et al.[34] examined the efficacy of selenium, as an antioxidative agent, in VAP patients through a clinical study. Results revealed that not only could selenium reduce the incidence of VAP in ICU patients, but also reduce its severity. In addition to the positive effects of NAC in preventing VAP and facilitating clinical recovery, the results of our study also demonstrated the lower ICU and hospital length of stay, in the NAC group compared with the placebo. Complications of VAP were not different between the two groups. No adverse events related to NAC administration were identified and NAC was well tolerated. NAC also was well tolerated in other studies in patients with[30] and without[14,15] endotracheal intubation.

Limitations

The small sample size in this study, compared with other clinical trials on VAP, stemmed from the insufficient number of ICU beds and lack of consent on the part of a number of patients' legal guardians for their participation in the study (due to their critical condition), with the latter resulting in the exclusion of 114 eligible patients from the onset of study.

The high mean of APACHE II score (22.2) in our patients revealed that the ICU patients in the study exhibited high risks for VAP. One of the inclusion criteria in the study included an extremely high prognosis for undergoing at least 72 hours of mechanical ventilation, meaning that the included patients were basically critically ill. Of course, this was inevitable, due to patients requiring less than 48-hour ventilation could not have been defined as cases of VAP.[35] This issue demonstrates that the results of this study do not encompass all ICU patients and can only be attributed to those with high risks for VAP.

We did not investigate the VAP microbiology and the effect of NAC on the VAP microbiological pattern. According to Morrow et al.,[18] evaluation of the effect of adjunct treatment on the VAP microbiology can help in the selection of its treatments.

We did not review hospital costs in the groups. The assessment of hospital costs along with the impact of adjunct treatment can be decisive in choosing the appropriate treatment for VAP.

Conclusion

These data suggest that NAC is safe and effective to prevent and delay VAP and improve its complete recovery rate in a

selected, high-risk ICU population. Future studies are needed to explore efficacy and safety of different doses of NAC in VAP patients with different clinical conditions in ICU.

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Author contributions
All the authors have contributed to drafting/revising the manuscript, study concept, or design, as well as data gathering and interpretation. All the authors approved the final version of the manuscript for publication.

Conflicts of interest
All authors declared that they had no conflict of interest.

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This study was financially supported by Arak University of Medical Sciences, Iran. Funders had no involvement in the study design; data collection, management, analysis, and interpretation; paper writing; or decision to submit the paper for publication.

Research ethics
The investigators were committed to the relevant principles and laws of the *Declaration of Helsinki* throughout the study. The study protocol was approved by the Ethical Committee of Arak University of Medical Sciences (4-144-92).

Declaration of patient consent
The authors certify that they have obtained all appropriate patients' legal guardians consent forms. In the form the patients' legal guardians have given their consent for the patients' images and other clinical information to be reported in the journal. The patients' legal guardians understand that the patients' names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Research Article

Intravenous Administration of Vitamin C in the Treatment of Herpes Zoster-Associated Pain: Two Case Reports and Literature Review

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Background. Herpes zoster (HZ) is an acute inflammatory neurocutaneous disease caused by the reactivation of varicella-zoster virus. It is estimated that the incidence of postherpetic neuralgia following HZ is 10–20%. The leading risk factors of the prognosis are aging and immunity dysfunction. Vitamin C plays a pivoted role in enhancing white blood cell function. Epidemiological evidence and clinical studies have indicated an association between pain and suboptimal vitamin C status. At present, vitamin C has been used as an additional option in the treatment of HZ-associated pain. Despite the current controversy, case reports and randomized controlled studies have indicated that both acute- and postherpetic neuralgia can be dramatically alleviated following intravenous vitamin C infusions. *Case Presentation*. Two patients (male aged 72 and female 78 years) with HZ did not respond well to antiviral therapy and analgesics. Skin lesions in the right groin and front thigh healed after early antiviral therapy, but the outbreak of pain persisted in the male patient. The female patient presented to our clinic with clusters of rashes in the right forehead with severe edema of her right upper eyelid. Because nerve blockade could not be conducted for both patients, intravenous infusion of vitamin C was applied and resulted in an immediate remission of the breakthrough pain in the male patient and cutaneous lesions in the female patient. *Conclusions*. The use of vitamin C appears to be an emerging treatment alternative for attenuating HZ and PHN pain. Hence, we recommend the addition of concomitant use of intravenously administered vitamin C into therapeutic strategies in the treatment of HZ-associated pain, especially for therapy-resistant cases. Furthermore, animal studies are required to determine analgesic mechanisms of vitamin C, and more randomized clinical trials are essential to further determine the optimal dose and timing of administration of vitamin C.

1. Background

Herpes zoster (HZ) is an acute inflammatory neurocutaneous disease caused by the reactivation of varicella-zoster virus (VZV) that remains latent in the dorsal root ganglia or cranial nerve ganglia after initial infection [1, 2]. Postherpetic neuralgia (PHN), which is the most frequent chronic complication of HZ and the most common peripheral neuropathic pain resulting from infection, is defined as pain persisting more than 30 days after the onset of the rash in the same affected dermatome [3, 4]. Patients with PHN may suffer from continuous or paroxysmal pain, which can be characterized by hyperaesthesia, hyperalgesia, and allodynia [5]. It is estimated that the annual incidence of acute HZ is 2.0–4.6 cases per 1000 persons in Europe [6], and the incidence of PHN following HZ is 10–20%, rising significantly from the age of 50 years [7], while in patients in their 80s, the incidence of PHN is over 30% [8].

Pharmacologic therapy is the first-line treatment for both HZ and PHN. Treatment for HZ is focused on inhibiting viral replication, alleviating pain, and preventing PHN [9–11]. Current medical treatments for patients with

PHN include calcium channel blocker, tricyclic antidepressants, and opioid analgesics [12, 13]. Other methods include nerve blockade or modulation, topical therapy, physical therapy, and alternative therapy [14]. Treatment with antivirals within 72 hours of onset of rash has shown a reduction in herpes zoster and its complications [15]. Famciclovir and valacyclovir were preferred to aciclovir for antiviral therapy, and a general preference towards pregabalin is shown for the treatment of increasing severity of pain [16]. Spinal cord stimulation is effective in reducing and preventing PHN but at an increasing cost [17]. Acute zoster pain can be reduced with epidural anesthetics and steroids [18]. Despite several therapeutic modalities for herpes zoster and its complications, the treatment remains a challenge.

It has been confirmed that aging and suppressed cellular immunity are the strongest risk factors for both HZ and PHN [19]. Therefore, enhancing the immune function of patients is an important therapeutic strategy, especially in the elderly frail patients. Vitamin C, also known as L-ascorbic acid, has been widely studied since its discovery and isolation by Szent-Gyorgyi in the 1930s [20, 21]. Vitamin C is an essential micronutrient in many metabolic pathways, acting as a water-soluble antioxidant, and plays a key role in enhancing white blood cell function and promoting protein metabolism and neurotransmitter production [22, 23]. Unlike animals, humans are unable to synthesize this essential vitamin due to a lack of L-gulonolactone oxidase activity, and therefore, it is taken from natural dietary sources or supplements [24].

Epidemiological evidence has indicated an association between several models of pain (musculoskeletal, virus-associated, cancer-related, and postsurgical pain) and suboptimal vitamin C status [25–28]. A community-based case-control study has revealed that lower vitamin C intake significantly increases HZ risk among daily micronutrient intakes [29]. Another study has shown that the concentration of vitamin C in the plasma of PHN patients is lower, and the high sensitivity of PHN patients to vitamin C deficiency may be a permanent factor in the formation of chronic neuropathic pain [26]. Although the current efficacy of vitamin C in the treatment of HZ-associated pain is still controversial, recent reports have shown that vitamin C can exhibit analgesic properties in the treatment of both acute herpetic neuralgia (AHN) and PHN [30, 31]. Herein, we report two patients with AHN who reported an immediate decrease in pain after intravenous administration of vitamin C and review articles about intravenous vitamin C treatment for herpetic neuralgia and PHN. We also analyze the pros and cons of high-dose vitamin C administration and highlight the need for an advanced understanding of the pharmacokinetics of intravenous vitamin C in future studies. Both the patients gave written informed consent for publication of this report.

2. Case Report 1

The first case was of a 72-year-old male whose somatic anamnesis was unremarkable and no prior intake of medications. He was hospitalized for localized zoster in the right groin and front thigh for 23 days. Eight days after tooth extraction, the patient developed a local rash with pinching pain and was diagnosed with extensive HZ of dermatomes T12 to L2 by a dermatologist. The oral medication was initially 200 mg celecoxib twice a day, 75 mg pregabalin, and 250 mg of famciclovir three times a day, for 7 days, respectively. The patient-reported visual analogue scale (VAS) score was controlled, no more than 6. On the 19th day after the appearance of the rash, the patient developed severe breakthrough pain, with more than ten attacks per day (about 5–6 outbreaks at night), each episode lasting 5–8 minutes, accompanied by a tremor of the right lower limb. The VAS pain score was 10 in the onset of breakthrough pain and 0 in the resting. The initial dermatologist tried to increase the dosage of pregabalin, but the patient reported intolerable dizzy and lethargy without any alleviation of his breakthrough pain. Therefore, the patient was admitted and prescribed 75 mg pregabalin three times a day, as well as 100 mg tramadol hydrochloride every 12 hours. Two days after hospitalization, the patient reported no reduction of the intensity and number of episodes of breakthrough pain, after which epidural blockade was performed to resolve the pain. However, during the process, breakthrough pain recurred and the patient was unable to keep his lateral position and switched to a supine position; therefore, the treatment was suspended. The patient received repetitive infusions of 4 g of vitamin C in 250 ml of physiological saline solution, without adjusting the dosage of pregabalin and tramadol hydrochloride. On the second night, there were still 5 episodes of breakthrough pain, but the duration of each attack did not exceed 3 minutes, and the VAS dropped to 8. On the fourth day, although the daily attack frequency did not decrease markedly, the duration had dropped to about 1 minute and the VAS was 6. On the seventh day, the second attempt of epidural blockade using 10 ml 1% lidocaine with 40 mg methylprednisolone was performed successfully. Although the patient suffered an attack of breakthrough pain after lying in the lateral position, the pain intensity was tolerable and lasted for merely 1.5 minutes. During the first 24 hours after the epidural blockade, there were 5 times of attacks, each time lasting for no more than 1 minute and the pain intensity remaining, with a VAS of 3-4. On the tenth day after intravenous administration of vitamin C, the patient had only one attack in the daytime, lasting for half a minute, with a VAS of 1-2. The next day, he was totally pain-free and was discharged from the hospital with an oral prescription for 75 mg pregabalin three times a day for two days. At 1-week and 3-month follow-up, there was no pain recurrence.

3. Case Report 2

A 78-year-old female patient, with a history of diabetes mellitus and rheumatoid arthritis (treated with metformin, Tripterygium, and total glucosides of paeony accordingly), presented to our clinic with clusters of rashes in the right forehead for 13 days with severe edema on her right upper eyelid (Figure 1). She was diagnosed with acute HZ in the first branch of the right trigeminal nerve. The patient suffered from constant burning pain after the cutaneous eruption, and the intensity on the VAS was 8, with sleep

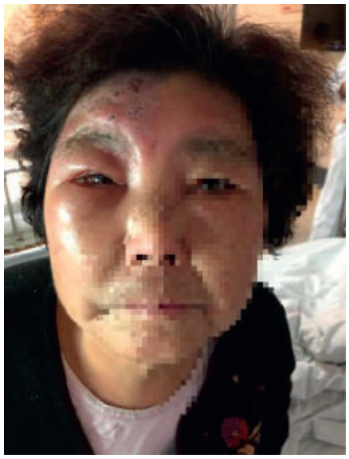

Figure 1: State of the right upper eyelid on the day of hospitalization (13 days after the onset of rash).

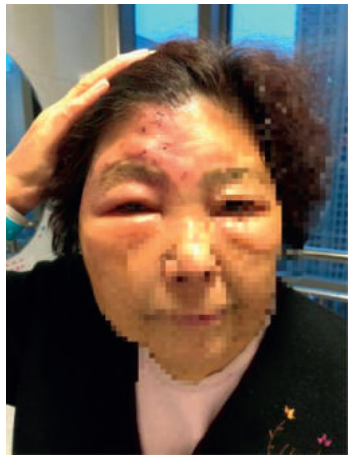

Figure 2: State of the right upper eyelid five days after intravenous administration of vitamin C.

quality seriously affected. She has prescribed 75 mg pregabalin along with paracetamol/tramadol (37.5 mg/325 mg) three times per day. There was no pain relief on the second day. Therefore, paracetamol/tramadol was replaced by 100 mg tramadol hydrochloride every 12 hours. Two days later, the VAS dropped to 6, but her duration of sleep at night still lasted for no more than 3 hours. It was unrealistic to perform supraorbital nerve blockade as her eyelid was severely edematous, and thus, the right stellate ganglion block (SGB) was recommended. However, the patient refused to take SGB. After confirming that there was no contraindication of using vitamin C, a mixture of 250 ml normal saline water and 4 g vitamin C was administered intravenously on the fourth day of hospitalization. However, it did not alleviate the pain, and therefore, vitamin C dose was added up to 8 g the next day. The VAS was reduced to 3 at night, and the patient slept for about 6 hours without pain disruption. After 5 days of treatment, the patient claimed that the pain was unperceivable, and there was a significant improvement in her eyelid edema (Figure 2). After discharge from the hospital, pregabalin and tramadol hydrochloride dose were reduced gradually and stopped within one week, after eyelid edema disappearance (Figure 3). At 3-month follow-up, she continued to be pain-free without any complications.

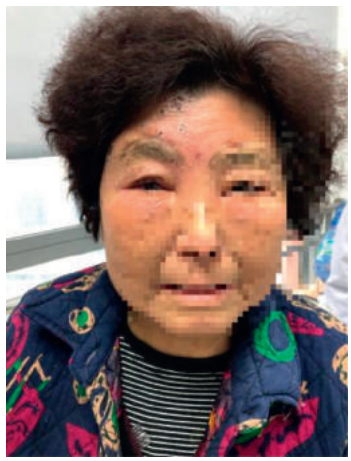

Figure 3: State of the right upper eyelid one week after discharge from hospital (28 days after the onset of rash).

4. Literature Review

4.1. Herpes Zoster-Associated Pain and Several Analgesic Mechanisms of Vitamin C. Studies have shown that the decline of cell-mediated immune function plays a critical role in the reactivation of VZV infection and the development of PHN; therefore, investigating the role of immune-relevant micronutrition from the therapeutic point of view is worthwhile [2–4, 32]. VZV remains dormant in the spinal or cranial sensory ganglia after primary infection earlier in life and becomes reactivated afterwards, traveling down the sensory root ganglia to cause damage to peripheral and central neurons, ultimately resulting in an inflammatory immune response [33].

Newly synthesized viral particles can be transported along the axons of all types of sensory neurons, resulting in neuronal necrosis in the affected ganglia and sensory nerves to the skin [1] and loss of the ability to inhibit the transmission of nociceptive pain after peripheral nerve injury, thereby reducing the threshold for nociceptive activation and producing spontaneous ectopic discharge. In addition, VZV-induced neuroinflammation impairs the central pain-suppressing pathway and leads to central sensitization, resulting in an enhanced central response from normal stimulation of peripheral nociceptors, which play an important role in the pathogenesis of PHN [3, 4].

Chen et al. [26] found that plasma vitamin C concentration was lower in PHN patients than in healthy volunteers. They subsequently conducted a randomized, double-blind,

placebo-controlled trial and found that short-term intravenous administration of large doses of vitamin C helped attenuate spontaneous pain. Currently, vitamin C has been postulated to alleviate HZ-associated pain through several possible mechanisms of action.

First, recent studies have revealed that VZV-induced peripheral inflammation sensitized nociceptors can produce excessive reactive oxygen species (ROS), which strongly reacts with noxious stimuli, causing the response to peripheral sensitization and thereafter inducing central sensitization in the spinal cord [21, 22, 34]. The high concentration of vitamin C around the immune cells and neurons may explain the result of Chen's study that vitamin C concentration is lower in PHN patients and intravenous administration of vitamin C as a ROS scavenger can exhibit analgesic properties [35]. For HZ, vitamin C can also reduce inflammation by the production of antiviral cytokine and interferon and thus has a direct antiviral effect [36].

Second, enhancing the spinal descending inhibitory pathway is another possible mechanism of vitamin C to reduce HZ-associated pain [37]. One critical mechanism of neuropathic pain in spontaneous pain is disinhibition, which is mediated by the spinal descending inhibitory pathway [38]. Spinal monoamines, including norepinephrine and serotonin, have been known to be involved in the descending inhibition of nociceptive transmission. Noradrenergic fibers from the brainstem terminate in the superficial dorsal horn and release norepinephrine to exert its antinociceptive actions [39, 40]. Vitamin C is a key cofactor of dopamine b-monooxygenase through which dopamine can be converted into norepinephrine. And the conversion is maximally efficient only in cells repleting with external vitamin C [41, 42].

Third, β-endorphin has been reported to be able to elevate the threshold of chronic neuropathic pain, and vitamin C can augment the production and release of β-endorphin by enhancing the adenylyl cyclase-cyclic adenosine monophosphate system. [43–45]. Besides, one recent study demonstrated elevated levels of tumor necrosis factor (TNF)-a, interleukin-1b (IL-1b), and IL-6 in an animal model of artificially induced neuropathic pain. Furthermore, due to the progression of PHN, elevated levels of IL-8, which is known to be secreted by VZV-infected cells, were verified as a marker and predictor of neuropathic pain [46, 47]. Another recent animal study concerning the influence of vitamin C on the production of TNF-a and IL-6 in ethyltoxic liver disease showed that, in vitamin C-treated rats, the serum concentration of TNF-a and IL-6 was significantly decreased [48]. Thus, it is proposed that vitamin C can also exhibit analgesic properties by modulating serum levels of the cytokine.

Carr and McCall [49] proposed a novel analgesic mechanism for vitamin C in a literature review. It is well established that vitamin C can act as a cofactor for peptidylglycine α-amidating monooxygenase, which is the only enzyme known to amidate the carboxy terminal residue of neuropeptides and peptide hormones [50]. Therefore, vitamin C participates in the amidation of peptides as a cofactor for the biosynthesis of amidated opioid peptides. The proposed mechanism was based on studies that showed a decreased requirement for opioid analgesics in surgical and cancer patients administered with high-dose vitamin C [51, 52].

4.2. Application Profile and Curative Effect Analysis of Intravenous Supplement of Vitamin C in the Treatment of HZ-Associated Pain. Nowadays, research on vitamin C in neuropathic pain mainly revolves around its preventive effects on complex regional pain syndrome (CRPS) after fracture [53, 54]. Inflammation after tissue trauma and neuroinflammation of the peripheral nervous system in rats has been shown to induce spontaneous pain [38]. It has been advocated that it may be beneficial to supply and increase plasma concentrations of vitamin C for wrist fracture patients at high risk for CRPS (type I) [55]. Zollinger and colleagues [56] performed a randomized controlled study on the effect of vitamin C on the frequency of reflex sympathetic dystrophy (RSD) in wrist fractures. The results showed that higher vitamin C intake was positively associated with fewer risks of RSD, and the authors assumed that vitamin C could have a similar beneficial effect in other forms of trauma. Since 2000, several scholars in different countries have conducted numerous trials to investigate the role of vitamin C in preventing CRPS after fractures [57]. The American Association of Orthopaedic Surgeons has recommended the use of vitamin C in patients with distal radius fractures to prevent CRPS [54].

Based on the mechanisms previously mentioned in this study, researchers have conducted several studies on the application of vitamin C in HZ-associated pain:

(1) Case report: an animal study conducted by Hanck and Weiser [58], published as early as 1985, reported a dose-dependent pain reduction by oral vitamin C in rats. Chen et al. [59] reported in 2006 that a patient with intractable PHN was treated intravenously with 2.5 g/d vitamin C. His spontaneous pain was completely resolved within 1 week, with his plasma vitamin C level increasing to 14.9 mg/L compared to the pretreatment baseline value of 4.9 mg/L. On follow-up examination after 3 months, the patient had no pain recurrence and the plasma vitamin C level was 11.6 mg/L.

Subsequently, Schencking et al. [60] in 2009 and Byun and Jeon [61] in 2011 reported 2 cases and 1 case, respectively, of intravenous administration of vitamin C in treating AHN, and both the results showed the positive analgesic effect of vitamin C. The former reported a patient that suffered from the acute phase of HZ with severe pain in the frontooccipital in the forehead area and was treated with intravenous administration of 15 g vitamin C every two days over a period of two weeks. The patient stated a reduction of pain from a VAS score of 8 to total pain-free on the fourth date of infusion. Complete remission of the rash was noted as well. The pain intensity was reduced rapidly and markedly without the application of strong analgesic drugs in

the whole course. The latter also reported a patient who suffered from constant aching pain with intermittent, spontaneous sore and shooting pain over the right occipital area. The pain intensity did not decrease after pregabalin and SGB administration, and therefore, intravenous administration of vitamin C was attempted. Immediate pain relief for about 12 hours was noted after the first administration of 4 g vitamin C, and the patient's pain intensity had been maintained at a VAS of 0-1. Five days after discharge, the patient reported a complete resolution of pain and stopped taking the prescription of pregabalin and vitamin C. There was neither relapse of pain at 3-month follow-up nor any complications.

(2) Randomized controlled trial: Chen et al. [26] identified a lower plasma vitamin C concentration in patients with PHN in a cross-sectional study of 39 healthy volunteers and 38 PHN patients in 2009. They also found a significant correlation of plasma vitamin C concentrations with the intensity of spontaneous pain but not with brush-evoked pain. Subsequently, they performed a randomized, double-blind, placebo-controlled trial, which revealed that seven days of treatment with vitamin C supplementation effectively increased plasma vitamin C concentrations in PHN patients, and spontaneous pain was decreased by 3.1 on a numerical rating scale (NRS) from baseline. However, this effect was not observed in brush-evoked pain. The authors attributed this difference to different mechanisms that spontaneous pain and brush-evoked pain involved in individual patients.

The latest randomized controlled study was conducted by Kim et al. [62] in 2016 to evaluate intravenously administrated vitamin C on AHN and its preventive effects on PHN. They found that compared with the control group (42 cases), there was no significant change in the acute pain score within 4 weeks of hospitalization in the vitamin C treatment group (45 cases). However, there were statistically significant differences after the eighth week, which continued thereafter, and the incidence of PHN was dramatically decreased in the vitamin C treatment group. They concluded that vitamin C supplement exerts no positive influence on acute zoster-associated pain, but it is effective in reducing the incidence of PHN. This brought more controversy to the clinical effect of vitamin C. Similarly, Schencking et al. [30] performed a multicenter, prospective cohort study in Germany to evaluate the safety and efficacy of intravenous vitamin C (7.5 g/d for approximately 2 weeks) in 67 participants with symptomatic HZ. A total of 59 patients (92.2%) improved in their VAS scores, and the mean VAS decreased significantly from baseline values in all visits; dermatologic symptoms of shingles between baseline and follow-up assessments were also statistically significant. The overall incidence of PHN in participants was 6.4% and significantly lower than that reported in previous studies (18%–33%). Nevertheless, the lack of a placebo-control group was the major limitation of this study, and the author proposed that more randomized, placebo-controlled clinical trials should be conducted to confirm these findings.

4.3. Dosage and Adverse Reactions of Intravenous Infusion of Vitamin C. Because humans cannot synthesize endogenous vitamin C like most animals, intake from dietary sources or supplements is necessary. Pharmacokinetic studies have shown that vitamin C concentrations are tightly regulated through renal resorption and higher oral intake (>100 mg/d) for an adult can barely result in higher absorptivity [63, 64]. Therefore, short-term therapeutic plasma concentration for HZ-associated pain can only be achieved by parenteral administration [65]. Currently, the administration of vitamin C via the intravenous route is widely used in clinical studies, with the daily dosage for treatment varying significantly in different studies. The optimal dose has to be determined through stronger evidence in the future.

There is no consensus on the metabolic process and transformation mode of intravenously administered vitamin C in vivo although it may be the least toxic of all vitamins. The adverse effects of the use of high-dose intravenous vitamin C reported from available data are mostly minor. Sebastian et al. [66] surveyed attendees at annual Complementary and Alternative Medicine (CAM) Conferences in 2006 and 2008, queried for side effects, compiled published cases, and analyzed FDA's Adverse Events Database. A total of 11233 patients in 2006 and 8876 patients in 2008 (20109 total) accepted intravenous vitamin C therapy. The average dose was 28 g every 4 days. Available data revealed that, out of 9328 patients, 101 had minor side effects, including lethargy, fatigue, vein irritation, and mental status change, and 2 patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency died of intravascular hemolysis.

Besides, the metabolic end-product of vitamin C metabolism is oxalate. Patients with renal impairment have been reported to develop oxalate nephropathy when given gram doses of intravenous vitamin C [67]. As for patients with renal failure, long-term high-dose intravenous use increases plasma oxalate concentrations and results in increased urinary oxalate in patients receiving total parenteral nutrition. A high dose of vitamin C has also been proved to elevate the excretion of calcium, iron, and manganese in the urine, potentially increasing the risk of urinary stone formation [68]. Nevertheless, most studies indicated that the administration of vitamin C in patients with normal renal function is unlikely to cause any severe damage. High-dose oral or intravenous vitamin C should be used cautiously in patients with preexisting renal insufficiency. Physicians should also be alert to potential interactions of high-dose

vitamin C with conventional medicine and alternative medicine. Importantly, physicians should be cognizant of potential adverse or other unexpected effects. We recommend starting with a low dose and slow intravenous infusion and detection of renal function and G6PD levels before treatment.

5. Conclusion

In summary, low plasma levels of vitamin C detected in PHN patients may be due to the excessive oxygen-free radicals caused by a varicella-zoster infection in the early stage. During this process, vitamin C utilization is increased as it functions physiologically as a water-soluble antioxidant by its high reducing power [69]. Vitamin C deficiency has been noted in patients with various painful diseases, such as orthopedic pain, virus-associated pain, and cancer-related pain [26, 70, 71]. Moreover, vitamin C plays a key role in the function of leukocytes, protein metabolism, and the production of neurotransmitters [72, 73]. High vitamin C concentration has been found around immune and nerve cells, indicating the possible positive role of vitamin C in HZ-associated pain. Based on the literature, patients with viral infections exhibit vitamin C deficiencies, which play a critical role in the pathogenesis of herpes infections and the development of PHN. Intravenous vitamin C therapy has not been widely used in patients with AHN or PHN because its beneficial effects on disease conditions are unproven. This review recommends vitamin C treatment as an option when patients do not respond well to conventional therapies.

So far, most studies have been performed in patients with orthopedic trauma and cancer pain [74, 75]. Possible mechanisms of action of vitamin C have been elucidated, making its therapeutic effects biologically plausible for the first time. We expect more rigorous studies to confirm that high-dose intravenous vitamin C may become a safe and effective adjunctive therapy for acute and chronic pain relief in diverse groups of patients, especially in the early stage of varicella-zoster virus infection. Future researches are necessary to ascertain the optimal dosage, interval, and periods of vitamin C administration to achieve the desired therapeutic or preventive effect on HZ-associated pain.

Data Availability

Data can be made available upon request to the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Yao Liu and Mi Wang contributed equally to this work.

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Review

The Potentials of Melatonin in the Prevention and Treatment of Bacterial Meningitis Disease

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Abstract: Bacterial meningitis (BM) is an acute infectious central nervous system (CNS) disease worldwide, occurring with 50% of the survivors left with a long-term serious sequela. Acute bacterial meningitis is more prevalent in resource-poor than resource-rich areas. The pathogenesis of BM involves complex mechanisms that are related to bacterial survival and multiplication in the bloodstream, increased permeability of blood–brain barrier (BBB), oxidative stress, and excessive inflammatory response in CNS. Considering drug-resistant bacteria increases the difficulty of meningitis treatment and the vaccine also has been limited to several serotypes, and the morbidity rate of BM still is very high. With recent development in neurology, there is promising progress for drug supplements of effectively preventing and treating BM. Several in vivo and in vitro studies have elaborated on understanding the significant mechanism of melatonin on BM. Melatonin is mainly secreted in the pineal gland and can cross the BBB. Melatonin and its metabolite have been reported as effective antioxidants and anti-inflammation, which are potentially useful as prevention and treatment therapy of BM. In bacterial meningitis, melatonin can play multiple protection effects in BM through various mechanisms, including immune response, antibacterial ability, the protection of BBB integrity, free radical scavenging, anti-inflammation, signaling pathways, and gut microbiome. This manuscript summarizes the major neuroprotective mechanisms of melatonin and explores the potential prevention and treatment approaches aimed at reducing morbidity and alleviating nerve injury of BM.

Keywords: bacterial meningitis; neuron injury; melatonin; neuroprotection

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1. Introduction

Bacterial meningitis (BM) is the major cause of the central nervous system (CNS) infectious diseases among infants, adults, and older people, which usually induce high mortality and 50% of the survivors left permanent neurological sequelae [1,2]. Bacterial meningitis can affect anyone of any age, including neonatal bacterial meningitis, adult bacterial meningitis, and senile bacterial meningitis. Simultaneously, environmental conditions and immunocompromised people are susceptible to bacterial meningitis. The pathogenesis of bacterial meningitis is as follows: firstly, meningitis bacteria can colonize the skin or different mucosal surfaces of healthy persons, then disseminated by blood and penetrated host barrier, finally resulting in systemic infection and neuronal injury [3,4]. The pathogenesis of BM mainly includes high-level bacteremia in the bloodstream, the destruction of the BBB integrity, and cerebrospinal fluid (CSF) pleocytosis, overwhelming inflammatory response in the CNS [3,5,6], which results in serious damage to the nervous system and even death. Up to now, lots of reports have shown the molecular mechanisms of BM resulting from bacterial ligand–receptor interactions, degradation of tight junction proteins, high matrix metalloproteinases (MMPs) expression, oxidative stress, and associated signaling pathways. Although antibiotics and vaccines have been able to significantly

reduce meningitis mortality for clearing bacteria, the emergence of drug-resistance bacteria and the limitations of vaccine serotype make BM still cause high morbidity and seriously neurological damage sequelae. Hence, new therapies of prevention or treatment need to improve the BM.

Melatonin is a hormone with various biological functions. It is first found to be secreted by the pineal gland and then melatonin can be found to be secreted by other various organs including skin, retina, kidneys, pancreas, ovaries, and gastrointestinal tract [7–9]. Melatonin with amphiphilicity can easily cross the BBB so that it enters the central nervous system (CNS) and the cerebrospinal fluid (CSF) [7]. This is particularly important for the effective prevention and treatment of CNS diseases after the supplement of exogenous melatonin. Initially, melatonin is well known for regulating circadian rhythms, sleep, and reproduction [10,11]. Subsequently, a number of studies have shown that melatonin has many other crucial functions, such as antibacterial, antioxidant, anti-inflammation, anti-apoptosis regulating the immune system, and gut microbiome [12–15]. At present, the beneficial effects of melatonin on protecting the BBB integrity, inhibiting neuronal and glial injury in various models of CNS disease have been well documented [16–19]. In addition, the levels of metabolism productions of melatonin in the CSF, N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK), and N^1-acetyl-5-methoxykynuramine (AMK) were elevated, and then it was found that they exerted neurocyte-protective properties in the conditions of inflammation and oxidative stress, which also play a critical role in anti-inflammatory and neuroprotection in the CNS [20].

Hence, this review mainly focuses on the neuroprotective effects of melatonin, which include antibacterial, blocking the interaction of bacteria and receptors, protection of the BBB integrity, resisting oxidative stress, anti-inflammatory activity, and major signaling pathway in both in vivo and in vitro models.

2. Bacterial Meningitis

2.1. Epidemiological Characteristics

Bacterial meningitis is one of the top ten causes of infectious-disease death, and there are approximately 1.2 million bacterial meningitis cases per year worldwide, 300,000 of which are estimated fatal in 2015 [21,22]. Meanwhile, permanent neurological sequelae occur in half of the survivors [1,23]. The occurrence of bacterial meningitis is affected by many elements, such as geographic location, socioeconomic status, seasonal variations, age, vaccination, and health status of the individual [24]. In developing countries, the morbidity of meningitis is significantly higher than in developed countries [2,25,26]. In sub-Saharan Africa, bacterial meningitis can reach 1000 cases per 100,000 people per year, whereas the incidence is 1–2 cases per 100,000 people per year in the UK [27]. Epidemiological surveys published in 2018 show that the incidence of bacterial meningitis in Western countries (Finland, Netherlands, the US, and Australia) gradually declined to 0.7–11 per 100,000 in the past 10–20 years, and in African countries (Burkina Faso and Malawi), bacterial meningitis can still reach 10–40 per 100,000 persons per year [22]. This finding demonstrates the bacterial meningitis is closely related to environmental and economic conditions.

Bacterial meningitis can affect anyone of any age, but different bacteria mainly infect the hosts of different ages. For example, Group B *Streptococcus* (GBS) and *Escherichia coli* K1 are mainly meningitis pathogens of the neonate and infant [3,28,29] and *Streptococcus pneumonia* and *Neisseria meningitides* mainly infect adults [3,30–32]. Simultaneously, immunocompromised neonate or infant and adult with digestive tract diseases, smoking, drinking, human immunodeficiency virus (HIV), or cancer are susceptible to bacterial meningitis. Most bacterial meningitis remain an acute and severe disease with a high risk of complications that lead to death or permanent sequelae. These complications include shock, respiratory failure, organ failure, intracranial complication stroke or seizures, etc. [33–35]. Among elderly patients with bacterial meningitis, septicemia and respiratory failure were the primary cause of death; the main complication among younger patients

was brain herniation [36]. If the host survives post-infection, it may leave pathogen-specific sequelae, such as deafness, blindness, or certain kinds of retardation.

2.2. Pathogenesis of Bacterial Meningitis

Most pathogens of bacterial meningitis firstly colonize oropharynx, nasopharynx, or digestive mucosal surfaces and cross the mucosal barrier, survive and disseminate in the bloodstream, then adhere and invade the BBB, eventually invade into the CNS (see Figure 1) [3]. Meningitis bacterium, including *Streptococcus pneumonia*, *Neisseria meningitis*, *Group B Streptococcus*, *Streptococcus aureus*, or *Escherichia coli K*1, can colonize mucosal surfaces of healthy people. Pathogens cross the mucosal barrier into the bloodstream, and bacterial survival and replication in the bloodstream are the prerequisites for reaching the BBB [3]. In bacterial meningitis, the complement system and Toll-like receptors (TLR) play an important role in clearing pathogens. For example, complement factors were induced to deposit on the surface of pathogens for promoting phagocytosis of phagocytes [37], and TLR activation prevents bacterial growth by inducing inflammation [38–40].

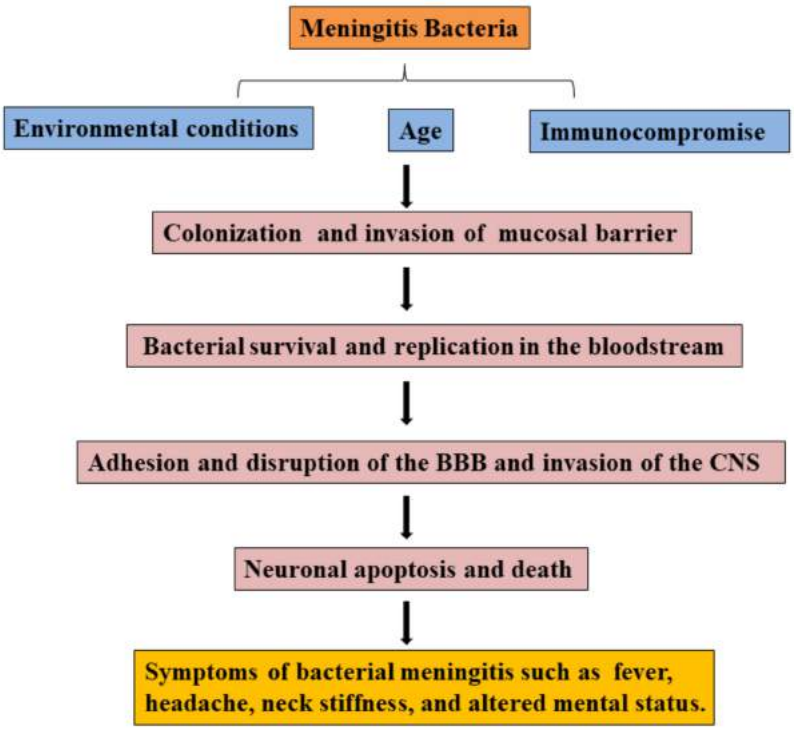

Figure 1. Overview of the pathogenic process in bacterial meningitis.

Bacteria initially adhere and invade the BBB via the interaction between bacterial components and host receptors, then degrade tight junction proteins of BBB depending on bacterial products or other components for invading into the CNS. Meanwhile, lots of pro-inflammatory cytokines, Matrix metalloproteinases (MMPs) expression, and free radicals contribute to the BBB disruption. Immune cells, including recruited neutrophils and resident cells in the CNS, can be activated by bacteria and express more pro-inflammatory cytokines, MMPs, and free radicals. Following invasion into the CNS, bacteria cause neurocyte and neural injury, which is usually caused by bacterial products, excessive inflammatory response, and major signaling pathways. In recent years, reports have found that the gut microbiome is one of the considerable factors in patients with bacterial meningitis, and gut disorders contribute to the development of meningitis.

In the early stages of bacterial meningitis, antibiotics are regarded as an important therapy to increase survival and reduce morbidity. The principal treatment strategy is to clear bacteria and reduce CNS damage. However, most of the antibiotics do not efficiently cross the BBB and play an antibacterial role in the CNS. Moreover, investigators

explore novel therapeutic approaches for improving the experimental meningitis models by modulating reactive nitrogen species (RNS), inhibiting caspase or inflammatory factors, coagulant, or complement cascades [41–43]. For vaccines, researchers are interested in developing the efficacy of polysaccharide conjugates without serotype replacement or with broad and ideally universal coverage for different bacterial meningitis. Table 1 gives an overview of treatments available to prevent meningitis bacteria and cure patients. Moreover, the released production of bacteria can still stimulate immune system response, promote neutrophil invasion, and activate resident immune cells in CNS, resulting in death or severe sequelae of nerve damage. Therefore, bacterial meningitis would benefit from new therapies and more effective drugs, which can prevent or cure the disease and alleviate nerve damage for reducing morbidity, mortality, and sequela.

Table 1. The treatments of bacterial meningitis.

Main Meningitis Bacteria.	Mainly Infected Age Group	Vaccine	Antibiotic	Adjunctive Treatment	Reference
Streptococcus pneumoniae	Children < 5 years; Adults > 50 years	Live attenuated vaccine (Whole-cell vaccine); Inactivated vaccine (Whole-cell vaccine); Subunit vaccine: Polysaccharide vaccine (PPV23), Conjugate vaccine (PCV7/10/13/15), Protein-based vaccine (PcsB, StkP, PsaA, PspA, PcpA, PhtD, PlyD1, Ply).	Penicillin; Macrolides.	Magnesium; Efflux pump inhibitors; C5 antibodies; Dexamethasone; Corticosteroids.	[44–47]
Neisseriameningitis	Children < 5 years Adolescents	Conjugate vaccine (MenACWY, Hib_MenCY-TT, Men A conjugate vaccine, Men C conjugate vaccine); Polysaccharide vaccine (MPSV4); Protein-based vaccine (Multicomponent Men B vaccine, Men B bivalent vaccine)	Penicillin; Ceftriaxone; Ciprofloxacin; Rifampicin.	BB-94 (MMP inhibitor); Doxycycline;	[48–50]
Group B *Streptococcus*	<3 months	CPS conjugate vaccines (CPS-CRM_{197} GBS conjugate vaccine); Protein-based GBS vaccines (Alpha-like protein, Rib, AlpC); Polysaccharide conjugates vaccine (serotypes Ia, Ib, and III)	Penicillin G; Clindamycin; Erythromycin; Fluoroquinolones; Ampicillin; First-, second-, and third-generation cephalosporins; Carbapenems; Vancomycin.	Gentamicin; Migration inhibitory factor inhibitor (ISO-1); Insulin; MAPK inhibitors; Brain-derived neurotrophic factors; Hypothermia.	[51–58]
Streptococcus suis	Adults	Autogenous bacterins; Subunit vaccine (muraminidase-released protein, suilysin, extracellular factor); 6-phosphogluconate-dehydrogenase; SsnA (the cell wall-associated DNase); Subtilisins; Glycoconjugates; Capsular material coupled with botulinum toxin.	Penicillin G; Ceftiofur; Amoxicillin; Gentamicin; Florfenicol; Fluoroquinolones	Aluminum hydroxide adjuvant; Imugen®; Rehydragel® and Emulsigen®.	[59–61]
Escherichia coli K1	<3 months	Mutation of aro A gene; Recombinant ISS gene; Outer membrane protein A ($OmpA_{TM}$, transmembrane domain; $OmpA_{per}$, periplasmic domain; OmpAVac); Capsular polysaccharides	Gentamicin; Ceftriaxone; Penicillin G; Ampicillin; Amoxicilline; Meropenem.	Pentoxifylline; Palmitoylethanolamide;	[62–67]

3. Melatonin

3.1. The Chemical and Physical Characteristic of Melatonin

Melatonin is a tryptophan derivative, belongs to indole heterocyclic compounds. Chemically, it is *N*-acetyl-5-methoxytryptamine, also called pineal hormone. The molecular formula of melatonin is $C_{13}H_{16}N_2O_2$ and its molecular weight is 232.28. Meanwhile, melatonin also has fat solubility and water solubility, which can effectively enter the cells and cross the BBB. In vertebrates, the secretion of melatonin has an obvious circadian rhythm, which is inhibited during the day (0–20 pg/mL) and active (60–200 pg/mL) at night. The secretion rate of melatonin is about 29 mg/day in humans.

3.2. The Synthesis and Metabolism of Melatonin

It was firstly found that melatonin was produced from pinealocytes in the pineal gland, and then it was later discovered that melatonin is also synthesized in other organs, in which the content of melatonin secreted from the gut is two orders of magnitude greater than that in the pineal gland. The processes of melatonin biosynthesis include hydroxylation, decarboxylation, acetylation, and methylation. Tryptophan as an initial precursor is turned into 5-hydrpxytryptophan by tryptophan hydroxylase. Then, 5-hydroxytryptophan decarboxylase decarboxylate 5-hydrotryptophan into 5-hydroxytryptamine (also called serotonin). Next, serotonin is acetylated into *N*-acetylserotonin via serotonin *N*-acetyl transferase. Finally, *N*-acetyserotonin is methylated to *N*-acetyl-5-methoxytryptamine [68].

Compared to melatonin synthesis, melatonin metabolism has multiple pathways which are complex with various enzymatic, pseudoenzymatic, and free radical interactive processes [69]. At present, the productions of melatonin metabolism mainly include 6-Hydroxymelatonin, 2-Hydroxymelatonin, cyclic 3-hydroxymelatonin, AFMK, and AMK, which play an important role under the condition of oxide stress [20]. During enzymatic processes, cytochrome P450 can catabolize melatonin to 6-hydroxymelatonin and then is conjugated to sulfate to form 6-hydroxymelatonin sulfate in the cerebral cortex, kidney, and heart of rats. In addition to enzymatic processes, melatonin can interact with $ONOO^-$, $^{\cdot}OH$ or under the condition of UV-B irradiation to form 6-hydroxymelatonin. 6-Hydroxymelatonin can inhibit lipoperoxidation and the production of ROS, resulting in decreasing neurotoxicity. 2-Hydroxymelatonin is the production of melatonin which interacts with ROS/RNS, and it was also found that UV-B irradiation can induce melatonin to form 2-hydroxymelatonin in the cells. Cyclic 3-hydroxymelatonin is an oxidative melatonin metabolite and a reliable biomarker of endogenous $^{\cdot}OH$ levels. It was also reported that the interaction of melatonin with $ONOO^-$ promoted Cyclic 3-hydroxymelatonin formation. Meanwhile, cyclic 3-hydroxymelatonin can form AFMK by scavenging radicals. The coexistence of cyclic 3-hydroxymelatonin and AFMK was usually found in the metabolic pathway of melatonin both in vitro or in vivo. For the past years, scholars were becoming more and more interested in AFMK [70]. It was found that AFMK was a pivotal molecule and original production in melatonin metabolism. It was initially found that AFMK formed via indoleamine 2,3-dioxygenase catalyzed melatonin, then it was found that AEMK is produced by an interaction of melatonin with H_2O_2. Subsequently, it was reported that UV irradiation can induce melatonin to form AFMK. In addition, AFMK can be further deformylated into AMK via arylamineformamidase, hemoperoxidases, or interacted with ROS/RNS. Many studies demonstrate that organisms can produce AFMK, including unicellular alga, metazoans, plants rodents, and humans. Meanwhile, AFMK and AMK may be exclusive metabolites of melatonin in tissues, especially in CNS. For example, the concentration of AFMK was greatly high (13,200 pg/mL) in the CSF of patients with meningitis, which was three orders of magnitude higher than healthy persons [71]. Leukocytes are another critical site for producing AFMK. The levels of AFMK were significantly increased in activated leukocytes. In cellular organelles, mitochondria are the major site for forming AFMK. Cytochrome C in mitochondria can catalyze melatonin into AFMK.

3.3. The Bioavailability of Melatonin

Melatonin as a health product is widely sold in the market. Over the past few decades, it was found that the bioavailability of melatonin in humans was significantly lower than that in rodents. Melatonin bioavailability is affected by various factors in humans, such as sexual difference, the heterogenic properties of cytochrome C P450 subtype gene expression, and the interactions with drugs. At present, the commercially available formula for melatonin is a 3 mg tablet. This dose is beneficial to promote sleep for some subjects, yet may not be effective to relieve insomnia and other related disorders in others. For example, the bioavailability of melatonin in females is 16.8 ± 12.7%, and that in males is 8.6 ± 3.9% following oral administration [72]. Fourtillan and colleagues have shown that the plasma level of melatonin was 165 pg/mL in males and 200 pg/mL in females after intravenous administration [72]. However, the level of melatonin dropped to 70 pg/mL in males and females after 1 h, which indicated over the physiological level and were eliminated by the liver. These results suggested that sex and route of administration affected the bioavailability of melatonin in the host. However, intranasal administration is not suitable for clinical application because of strong irritation. Mao et al. improved intranasal administration that developed melatonin starch microspheres [73]. The absorption of melatonin is increased and the bioavailability is markedly improved, but it disrupts the circadian rhythms of patients.

Later, researchers have used melatonin in combination with other drugs to increase the bioavailability of melatonin in humans. In healthy subjects, co-administration of melatonin with fluvoxamine (cytochrome P450 inhibitor) markedly increase the levels of melatonin in the blood. Furthermore, the bioavailability of melatonin is also significantly increased when taken with caffeine or vitamin E/C in human subjects. Hence, it is necessary to deeply understand the pharmacokinetics of melatonin in the serum and its interaction with other substances or adjusting the dose in different situations and individuals.

4. Neuroprotective Properties of Melatonin against Bacterial Meningitis

4.1. The Antibacterial Activity of Melatonin

At present, antibiotic treatment is related to mortality in the early stages of bacterial meningitis. However, antimicrobial resistance is occurring all over the world. In particular, the emergence of multiple antimicrobial resistance makes treatment more difficult. In addition, some antibiotics, such as vancomycin, poorly cross the BBB, which greatly reduces the antimicrobial efficiency. Meanwhile, antibiotics residue in animal products also threatens human health.

Melatonin as an endogenous molecule has been widely investigated in cells and organisms, but few studies are explored in antimicrobial activities of infectious diseases. In 2008, Tekbas and colleagues have shown that melatonin could inhibit the growth of gram-positive and gram-negative bacteria. In the study, it was reported that melatonin play bacteriostasis ability against methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *Pseudomonas aeruginosa*, *Staphylococcus aureus* ATCC 29123, and *Pseudomonas aeruginosa* ATCC 27853. The minimum inhibitory concentration(MIC)concentrations of melatonin was, respectively, 250 μg/mL, 125 μg/mL, 125 μg/mL, 250 μg/mL, and 125 μg/mL at 24 h of incubation. Melatonin's MIC values were, respectively, decreased to 250 μg/mL, 125 μg/mL, 125 μg/mL, 250 μg/mL, and 125 μg/mL after 48 h of incubation. Moreover, it was found that melatonin in lower doses has a potent antimicrobial function, which is possibly caused by the reduction of intracellular substrates, which makes bacteria enter the death phase earlier [74]. It is necessary to bind free iron for bacterial growth. Melatonin has a high metal-binding capacity, such as iron, and can resist bacterial growth by binding free iron in the cytoplasm. Konar et al. have demonstrated that melatonin at a concentration of 300 μg/mL could effectively inhibit *Candida albicans* by reducing lipid levels. Moreover, it was reported that melatonin can interact with receptors on the neutrophils, and promote neutrophil extracellular trap

(NET) formation to enhance the antibacterial ability of neutrophils, then contribute to the clearance of *E. coli* and *S. aureus* in mice to relieve sepsis caused by bacteria [75].

4.2. Melatonin and Immune Activation

Usually, the innate immune system is regarded as the first line of defense against invading pathogens. The complement system plays an important role in clearing pathogens, such as complement-mediated phagocytosis and opsonization of inflammation [37]. Similarly, outer membrane protein A of *E. coli* is able to bind to C4bp for resisting the serum bactericidal activity [76]. During bacterial infection and inflammation in CNS, brain resident cells can produce complement factors except for monocytes and macrophages, resulting in recruiting lots of leukocytes and causing the inflammatory storm. In some cases, it was demonstrated that there was a decreased numbers of leukocyte, reduced cytokines, and chemokines in the CSF of C1q and $CR3^{-/-}$ mice compared with WT mice [77]. Recently, numerous experiments in bacterial meningitis have shown that complement intervention, such as complement monoclonal antibody, was beneficial in the treatment of acute bacterial meningitis. Previous studies reported that the change of serum melatonin levels was related to the complement system. Pro-inflammatory factors and complement proteins are associated to promote Aβ deposits in Alzheimer's disease (AD). In AD mice, melatonin contributes to improve learning and memory by significantly inhibiting the expression of interleukin-1α (IL-1α) and complement 1q in the hippocampus [78]. Meanwhile, serum melatonin is closely related to complement 3 or complement 4 levels in patients with depression, but the detailed mechanism would be deeply explored. These results do demonstrate the proposed regulation of melatonin on complement proteins expression. Unfortunately, there are no associated studies on melatonin regulating complement resistance to bacterial meningitis and more mechanisms.

TLRs of the immune cells recognize different bacterial pathogen-associated molecular patterns (PAMPs), and TLR activation is a key step in the meningeal inflammatory response, prevents bacterial growth, and also participates in meningitis-induced tissue damage [38–40]. Among TLRs, TLR2, TLR4, TLR9 are involved in the pathogenesis of bacterial meningitis [79]. TLR2 is mainly activated by lipoteichoic acid and TLR4 interacts with lipopolysaccharides (LPS) or pneumolysin. TLR9 can interact with bacterial DNA. MyD88 signaling molecule is stimulated during activation of TLRs, which is necessary to induce an effective immune response. In clinical tests of blood samples from child patients with bacterial meningitis and healthy adult, Zhang found that TLR2 and TLR9 with polymorphism gene were markedly higher in Chinese children with bacterial meningitis (seizures), and it is suggested that they may be related with severity and prognosis [80]. For pneumococcal meningitis, TLR2 and 4 are central to resist pathogens invasion and regulate the host inflammation. For example, weakened immune response, increased *S. pneumoniae* burden, and low expression of antimicrobial peptides were found in TLR2/4 double knockout mice [81–83]. At present, many researchers focus on selecting effective adjuvant treatment with the drug to interfere with the TLR pathway; for instance, activin A can increase phagocytosis of *E. coli* k1 by microglial cells stimulated by TLR2, 4, and 9 agonists without inducing excessive inflammatory response [84]. However, there is no study on exploring the protective mechanisms of melatonin in bacterial meningitis focusing on TLR innate signaling. However, in hepatic ischemia/reperfusion study, mechanisms that melatonin effectively protect the liver by attenuating the increased level of MyD88, TLR3, and TLR4 protein expression have been intensively investigated, and it was also been found that the inhibitory effects of melatonin on the MyD88 signaling pathway of TLR system was related with suppression of activation of NF-κ B, mitogen-activation protein kinase s (MAPKs), which contribute to the pathogenesis of bacterial meningitis processes [85–87].

4.3. Melatonin and Pro-inflammatory Cytokine

After bacterial infection, there are many resident cells in CNS and invading immune cells from the bloodstream, which can produce pro-inflammatory cytokines to respond to bacterial components and replication. In a study of patients with bacterial meningitis, the levels of pro-inflammatory cytokines were detected in the CSF. IL-6, IL-1β, and tumor necrosis factor-α (TNF-α) are produced by brain microvascular endothelial cells (BMECs), astrocytes, and microglial cells at the early stages of bacterial infection [88]. These early-produced cytokines can increase the expression of some adhesion factors on the BMECs, which recruit a large number of neutrophils into the CSF. Moreover, massive inflammatory reactions induced by pathogens can contribute to functional and structural brain damages, as well as are major features of bacterial meningitis [89]. Lots of pathological reports have shown that the release of increased pro-inflammatory cytokines (e.g., IL-1β, IL-6, TNF-α) in activated microglial cells may promote neuronal apoptosis in the hippocampal regions [90,91]. The excessive release of pro-inflammatory factors, likewise, could break the integrity of BBB and interrupt the bioenergetics activity or the metabolic activity of injured neurons [92].

Within the past years, experts have well demonstrated the anti-inflammatory properties of melatonin in alleviating neuron damage and improving the recovery of injured neurons' functions [93–95]. Melatonin was found to inhibit inflammatory response by decreasing MMP-9 expression and vascular endothelial growth factor expression, thus preventing the disruption of tight junction proteins (Zonula occluding-1 (ZO-1); occluding; claudin-5) and attenuating brain edema following BBB dysfunctions [96]. In adult rats inoculated with acute *Klebsiella pneumonia* meningitis model, it is found that TNF-α, IL-1β, and IL-6 levels were significantly decreased following melatonin dose of 100 mg/kg administration [18]. Then, the study has clearly demonstrated that melatonin treatment can successfully block microglial activation and reduce inflammatory responses in the hippocampal and subsequently rescues hippocampal neurons from apoptotic damage [18,95,97]. However, when melatonin treatment was started after 12 h in a rabbit *Streptococcus pneumonia* or *Escherichia coli* meningitis model, melatonin exerted anti-inflammatory effects but did not alleviate neuronal injury [98]. The reason for such an issue may be associated with the time of melatonin treatment.

4.4. Melatonin and MMPs

Over the past years, lots of clinical and animal model reports demonstrated that MMPs play a central role during the development of bacterial meningitis. MMPs as an endopeptidase are involved in cleaving extracellular matrix proteins but also regulating signaling molecules and receptors [99–101]. During bacterial infection, resident activated-cells (microglia, astrocytes, and neurons) and blood-derived leukocytes (neutrophils, macrophages, and lymphocytes) can release the MMPs [102–106].

In bacterial meningitis, MMPs are major mediators of BBB damage and modulators of inflammation in the brain, which cleave extracellular matrix and nonmatrix proteins under pathophysiological conditions [107–109]. Over past years of clinical and experimental studies, upregulation of MMP-9 in human BM was reported in 19 patients [110]. MMP-8 also is up-regulated in CSF of children with BM [110]. MMP-9 can increase the permeability of the BBB by degrading collagen, proteoglycan, or basal laminin, resulting in pathogen invasion and leukocyte extravasation [111]. Moreover, MMPs can cleave inflammatory cytokines and chemokines and stimulate their production for hyperinflammatory reactions driving brain damage [112,113]. For example, a high level of MMP-9 has been described that can improve the risk for the development of neuronal damage, such as hearing impairment and secondary epilepsy in infected children [114,115]. To date, lots of adjuvants targeting MMPs are applied in clinical studies of bacterial meningitis. In infant rats with pneumococcal meningitis, Trocade as an adjuvant can inhibit collagenases and gelatinase activity, decrease pro-inflammatory factors and mortality, and alleviate CNS injury. Furthermore, antibiotic

treatment might increase the expression of MMP-9, and antibiotics with dexamethasone could inhibit the expression of MMP-9 in rats with *Streptococcus pneumonia* [116].

Treatment with melatonin protected the integrity of the BBB and against neuroinflammation by regulating MMP gene expression and activity [117,118]. Under physiological and pathological conditions, TIMP-1 (Tissue inhibitors of metalloproteinase-1) bind to the MMP catalytic domain for inhibiting MMP-9 activity [111]. Moreover, the administration of exogenous melatonin actually increases the TIMP-1 expression by inducing MAPK pathways, which reduces the MMP-9 translation and activity [111]. MMP-9 secretion induced by IL-1β in pericytes can disrupt VE-cadherin, occluding, claudin-5, and ZO-1, resulting in increasing BBB permeability [117]. The melatonin can downregulate MMP-9 and upregulate TIMP-1 gene expression through regulating the NOTCH3/ nuclear factor kappa B (NF-κB)/p65 signaling pathway in pericytes to protect the disruption of the BBB integrity induced by IL-1β [117,119].

In a mouse model of meningitis induced by LPS, melatonin (5 mg/kg) significantly attenuated cerebral MMP-9 activity following brain inflammation; and in the RAW264.7 and BV2 cells, the results showed that pretreatment or cotreatment with melatonin effectively inhibited LPS-induced MMP-9 activation [96]. It has been reported that melatonin control redox-dependent negative regulation of the MMP-2 gene and also can induce MMP-9 downregulation by inhibiting TNF-α [108,120]. Meanwhile, MMPs are involved in the apoptosis and death of neurons, and the injury of hippocampal neurons was alleviated in MMP-9-deficient mice with global ischemia [121]. Based on many reports, melatonin can play an important role in the neuroprotective effect by regulating MMP-9 activation, and melatonin may tightly bind to the active site of MMP-9 for inhibiting MMP-9 activation. Hence, MMP-9 may be a major target of melatonin in neuroprotection against brain injury.

4.5. Melatonin and Oxidative Stress

Oxidative stress is the primary cause of brain injury, which includes high lipid content, ROS, and RNS [41,42,122,123]. Under physiological conditions, free radical (ROS, RNS) generation and antioxidant response are usually balanced. However, oxidative stress can be induced if ROS or RNS formation is excessively increased or antioxidant levels are depleted under pathological conditions [124,125]. Studies in experimental animals and humans with bacterial meningitis have shown that neuronal injury and the BBB breakdown are regulated by ROS, RNS, nitric oxide, and peroxynitrite [126,127]. For example, oxidative stress facilitates the disruption of the BBB by reducing the expression of tight junction proteins (claudin-5, occludin, ZO-1, and junction adherensive molecular-1). Oxidative stress can also markedly activate MMPs and break the BBB integrity during pneumococcal meningitis. Leib and colleagues have found that ROS was produced from predominantly polymorphonuclear leukocytes in the subarachnoid and ventricular space, cortical vessels, endothelial cells in group B streptococci meningitis [128,129]. There is also a large generation of ROS from microglia, neurons, and astrocytes induced by *E. coli* lipopolysaccharides, cytokines (TNF-α and interleukin-1β [IL-1] β) [130–133]. Oxidative stress or free radicals increasingly became a vital event in promoting the development of neuronal injury during bacterial meningitis. For example, peroxynitrite can induce cytotoxicity through inhibition of mitochondrial function, leading to depletion of NAD^+ and ATP, resulting in neuronal cell death [134,135]. In vivo, phenyl *N*-*t*-butylnitrone (PBN), which is a radical scavenger, prevented CNS injury caused by group B streptococcal meningitis, and NAC as antioxidant can decrease neuronal death induced by pneumococcal meningitis [136]. Moreover, NAC has been applied in clinical treatment for several years with minor side effects.

Melatonin is a highly effective free radical scavenger and powerful antioxidant with direct or indirect effects. The massive production of ROS and RNS causes significant nerve injury. Melatonin has directly nonreceptor-mediated free radical scavenging activity, as well as it eliminates ROS including hydroxyl radical, peroxyl radicals, hydrogen peroxide, and hypochlorous acid [137,138]. Usually, lipid peroxidation is regarded as a marker of

oxidative stress [139–141]. The lipid peroxidation marker 4-hydroxynonenal and malondialdehyde contribute to superoxide anion (O_2^-) production and are elevated in the cerebrospinal fluid (CSF) of patients with pneumococcal meningitis [142,143]. Melatonin can significantly reduce 4-Hydroxynonenal (4-HNE) and malondialdehyde (MDA) concentration; therefore, inhibit lipid peroxidation and oxidative stress against acute tissue injury in a study [144]. Additionally, melatonin likewise inhibits nitric oxide synthase and lipoxygenase [144–147]. Bacterial meningitis stimulates inducible Nitric Oxide Synthase (iNOS), resulting in significantly increasing NO levels in the brain and induce neurotoxicity [3,148]. Lipid A treatment enhances iNOS expression by activating NF-κB signaling cascades in the choroid plexus epithelium that is a part of the blood–CSF barrier against microbial pathogens and plays a crucial role in brain inflammatory processes in bacterial meningitis [149]. Meanwhile, the inhibition of iNOS expression completely prevented brain damage induced by *E. coli* K1. NO as a major inflammatory mediator is also responsible for the enhanced invasion of *E. coli* K1 into human brain microvascular endothelial cells (HBMECs), which is an in vitro model of the BBB [150]. Melatonin directly reduces nitrite concentration that represents the level of iNOS expression in microglial cells and the CSF of rabbits infected with *Streptococcus pneumonia*, resulting in reducing neuronal injury [151,152].

Furthermore, melatonin can play an indirect role in antioxidants by inducing antioxidative enzyme expression [153,154]. Within the past years, several animal studies on melatonin have shown that melatonin can stimulate lots of antioxidative enzymes including superoxide dismutase (SOD), MnSOD, CuZnSOD, glutathione peroxidase, glutathione reductase, and catalase [145,155]. For example, SOD can catalyze the breaking down of superoxide in H_2O_2 and oxygen molecule, and CAT is able to exert detoxification H_2O_2 [145]. The activity of SOD in meningitis patients is related to ROS formation due to lipid peroxidation for neutralizing the free radical [41]. SOD mimetics pre-treatment could alleviate brain edema and decrease intracranial pressure and CSF leukocyte count in a bacterial meningitis rat model [156]. In melatonin-treated rabbits infected with *Streptococcus pneumonia* study, melatonin significantly increases the activity of SOD and reduces the nitrite concentrations for resisting oxidative stress [98]. The levels of Glutathione in CSF of patients with meningitis were significantly reduced, which enhance the risk of oxidative stress and lead to severe neurological dysfunction [98,157,158]. Melatonin can increase intracellular GSH levels by stimulating γ-glutamylcysteine synthase to protect the nervous system from oxidative damage [159].

4.6. Melatonin and Mitochondrial Dysfunction

It was demonstrated in several studies that mitochondrial dysfunction has a related function in the pathogenesis of bacterial meningitis [127,160,161]. Normally, the mitochondrion plays an important role in aerobic metabolism for providing energy and maintaining cellular homeostasis [162,163]. It is well known that neurons of the brain have a high metabolic rate and contain a large number of mitochondria, therefore, more susceptible to the reduction of energy metabolism [126]. Lots of brain biological processes are regulated by mitochondria, including ATP production, oxidative stress, calcium balance, and apoptosis [161]. Approximately 50% of patients with bacterial meningitis in the epidemiological investigation have demonstrated that cerebral oxidative metabolism was affected, indicating mitochondrial dysfunction [127]. For example, in patients with severe streptococcus meningitis, the study recorded the data that can reflect cerebral cytoplasmic redox state by evaluating cerebral interstitial lactate/pyruvate (LP) ratio, and increase in LP ratio indicates impaired cerebral oxidative metabolism, which is determined by mitochondrial dysfunction [126]. Then, the cell energy is obtained by oxidative phosphorylation that depends on various respiratory enzyme complexes located in the inner mitochondrial membrane [126]. The experimental studies have demonstrated that pneumococcal meningitis can inhibit mitochondrial chain complex I in the brain, causing impairment of energy metabolism for facilitating the development of pathogenesis [164]. In fact, excess ROS

production in bacterial meningitis can induce defects in the mitochondrial chain, causing impairment in oxidative phosphorylation that promotes ATP generation and more ROS [165]. More and more ROS can result in mitochondrial dysfunction, then cause the release of apoptosis-inducing factors into the cytosol; these factors have been found that execute the caspase-independent pathway [166]. Furthermore, numbers of polymorphonuclear leukocytes in animal models of pneumococcal meningitis can also increase the release of pro-apoptotic factors such as cytochrome c from mitochondria into the cytosol, which leads to caspase-3 cleavage, resulting in neuronal apoptosis [167,168]. Hence, compounds or drugs that can maintain the mitochondrial function and inhibit associated apoptotic signaling pathways will be effectively used in combating bacterial meningitis.

Under pathological conditions, mitochondria are regarded as an important target of melatonin due to the accumulation of melatonin in high concentrations on mitochondria [169]. Melatonin can relieve mitochondrial dysfunction by scavenging free radicals, regulating the electron transport chain, and increasing antioxidase activities. In in vivo or in vitro experiments, it has been found that melatonin can attenuate mitochondrial dysfunction in sepsis and protects mitochondria from oxidative damage by scavenging free radical [170]. Melatonin also plays a critical role in protecting mitochondria by increasing the activity electron transport chain, improving ATP production, attenuating calcium overload, inhibiting ER stress, regulating mitochondrial gene expression, and preventing mitochondrial apoptosis [171]. The study has been shown that melatonin can interact with complexes I and IV of the mitochondrial electron transport chain to promote electron flux under the normal physiological conditions for increasing ATP production for maintaining mitochondrial homeostasis [172–174]. In addition, melatonin increases GSH synthesis for improving the mitochondrial defense mechanism [175]. At the same time, melatonin can increase the activity of NADH dehydrogenase in brain mitochondria against neurotoxicants [176,177]. The protective function of melatonin against apoptosis has been demonstrated in a number of neural injury studies [166]. It has been reported that melatonin diminishes apoptosis by increasing anti-apoptotic proteins, such as B-cell lymphoma-2 (Bcl-2), or inhibiting pro-apoptotic proteins, such as Bax [178]. For instance, pre-treatment of melatonin can induce the overexpression of Bcl-2 and inhibit caspase 3 or Cyt c release under oxidative stress [178]. Melatonin also facilitates Bax to translocate into the mitochondria, leading to reducing the apoptotic tendency [178]. However, there are few studies on melatonin in alleviating mitochondrial damage in bacterial meningitis. Thus, according to multiple mechanisms or functions of melatonin, it is worth exploring to protect mitochondria from dysfunction induced by oxidative stress or other virulence.

4.7. Melatonin and Signaling Pathways

Many studies have shown that there were some major intracellular signaling pathways involved in the process of bacterial meningitis, such as nuclear factor kappa B (NF-κB) pathway, phosphoinositide 3-kinase (PI3K)/Akt pathway, mitogen-activation protein kinase (MAPK) pathway. These signaling pathways contribute to developing the process of bacterial meningitis. For instance, once the pathogens invade the BBB, most bacteria are able to activate the NF-κB pathway by phosphorylation of serine residues on the IκB proteins, resulting in increasing inflammatory factors, chemokines, bacterial invasion of BMECs, and polymorphonuclear (PMN) migration across the BBB. For example, IbeA protein of *E. coli* K1 interacted with vimentin of BMEC and stimulates NF-κB and extracellular signal-related kinases 1/2 (ERK1/2) activation, resulting in promoting bacterial invasion and PMN transmigration across the BBB [179,180]. Meanwhile, NF-κB pathway and PI3K/Akt/mammalian target of rapamycin (mTOR) signaling pathway were reported that involved in inhibiting autophagy for increasing intracellular bacterial survival rate in *E. coli* K1 meningitis [181]. *Streptococcus suis* serotype 2 (SS2) can interact with epidermal growth factor receptor to initiate MAPK-ERK1/2 and NF-κB pathway in hBMEC that facilitate the proinflammatory cytokines and chemokines expression [182].

This evidence expands our ideas on finding a drug to prevent or treat bacterial meningitis. In past years, brain-derived neurotrophic factor (BDNF) was demonstrated that played an important role in anti-inflammatory and anti-apoptotic in CNS diseases. In the rat model of pneumococcal meningitis, BDNF supplement can effectively reduce inflammation and hippocampal apoptosis by regulating NF-κB pathway and PI3K/Akt/mTOR signaling pathway [181]. Signaling pathway inhibitors have been used in bacterial meningitis, such as U0126 (MAPK inhibitor), CAY10657, or BAY-11072 (NF-κB inhibitor), which could effectively inhibit neuroinflammation in vitro.

In previous reports, the MyD88/NF-κ B signaling pathway could cause neurological injury in bacterial meningitis and melatonin inhibits NF-κ B-driven signaling for protective and anti-inflammatory action in the LPS-stimulated RAW 264.7 and BV2 cells [96]. In addition, the inhibitory effect of melatonin on post-inflammatory NF-κ B translocation and proMMP9 activation is effective following LPS-induced meningitis [183]. Similarly, some studies have demonstrated that the PI3K/Akt signaling pathway is important in alleviating neuronal apoptosis and promoting neuronal survival [184]. Additionally, Melatonin can inhibit neuron apoptosis and increase cellular survival. In mice experiments, melatonin treatment reduced p53 phosphorylation by the PI3K/Akt pathway for decreasing apoptosis in the brain [85]. Meanwhile, melatonin regulates the expression of brain and muscle Arnt-like protein 1 (Bmal 1) by PI3K/Akt pathway and increases cellular survival via survival kinases in vivo and in vitro [185]. Hence, melatonin can be regarded as a novel strategy targeting the major signaling pathway, for the prevention and treatment of bacterial meningitis.

4.8. Other Functions of Melatonin in Bacterial Meningitis

Bacterial adhesion is a prerequisite for the development of infection and usually interacts with host-specific surface adhesion receptors for nutrient intake, promoting bacterial invasion and immune evasion. There are two natural barriers to defense against meningitis bacteria before they invade the CNS, respectively, the mucosal barrier and the BBB. Meningitis bacteria can use adhesion or other bacterial virulence factors to bind to surface receptors of barriers for invading into the CNS. For example, type IV pili can contribute *N. meningitides* to adhere to the BBB by targeting CD147 receptors on BMEC [186]. In our study, we have been found OmpA and IbeA in APEC TW-XM (isolated from duck) could, respectively, induce gp96 and caspr1 receptor expression, as well as contribute to bacterial adhesion and disrupt the integrity of the BBB via activating the focal adhesion kinase (FAK) pathway. Then, we found that melatonin can decrease the expression of OmpA and IbeA, resulting in reducing the adhesion and invasion of APEC TW-XM (unpublished data). Lots of scholars focus on exploring the mechanisms of bacteria-binding to host receptors during bacterial infection. However, few studies have explored the mechanisms of how melatonin affects the interaction of meningitis bacteria and host receptors. Hence, it is might be a new and useful target with a broad spectrum for the prevention or treatment of meningitis bacteria.

The gut microbiome as a line provides resistance against foreign pathogens. Commensal microbes in the gut can release bacteriocins, utilize nutrient depletion mechanisms, regulate metabolism and immunity to resist pathogens. In infectious diseases, many studies have shown that pathogens and induced cytokines caused gut dysbiosis, resulting in the promotion of colonization of pathogens. Moreover, antibiotic treatment of immunocompromised host would enhance susceptibility to bacterial meningitis, and it also have been demonstrated to mediate microbiota damage. It was reported that commensal bacteria can decrease the bacterial adhesion in *Listeria monocytogenes* meningitis. Particularly, the clostridiales of commensal bacteria exerted antibacterial activity in vitro and conferred into germ-free mice to increase resistance against *L. monocytogenes*. These studies indicated that the intestinal microbiome is closely related to the disease process [187].

Several works suggest that melatonin can regulate gut microbiome balance and relieve some diseases. Ren and colleague found that melatonin supplementation could alleviate

weanling stress and decrease intestinal ETEC infection by shaping the composition of intestinal microbiota in weanling mice. Meanwhile, this study also demonstrated that melatonin failed to alleviate weanling stress and defense ETEC infection both in antibiotic-treated weanling mice and germ-free weanling mice [14]. It is suggested that melatonin could regulate the gut microbiome to alleviate disease. In the spinal cord injury (SCI) mice model, gastrointestinal system dysfunction is a typical symptom, and alteration of the gut microbiome may affect disease progression. It has been demonstrated that melatonin treatment can not only improve some main pathology of SCI but also regulate the composition of intestinal microbiota (including increase in abundance of *Lactobacillus* and *Lactobacillales* and decrease in the abundance of *Clostiddiales*). The neuroprotective effect of melatonin on SCI was significantly reduced in gut dysbiosis mice model induced by antibiotics treatment [188]. At present, there are few studies to explore the function of melatonin on the prevention and treatment of bacterial meningitis by alteration of intestinal microbes. In our study, we have found that APEC TW-XM can induce gut dysbiosis and melatonin could prevent APEC TW-XM-induced bacterial meningitis by maintaining gut microbiome in Institute of Cancer Research (ICR) mice. We applied melatonin by intraperitoneal injection and found that melatonin can maintain gut microbiome homeostasis by increasing abundance of *Alistipes*, *Parabacteroides,* and *Lactobacillus*, as well as decreasing in the abundance of *Strenotrophomonas*, yet lost the function of prevention in antibiotic-treatment ICR mice (unpublished data). Hence, intestinal microorganisms can be regarded as a target of melatonin. Melatonin could regulate host metabolism by improving gut dysbiosis, so as to enhance the resistance to pathogens or alleviate nerve injury in bacterial meningitis.

5. Conclusions and Future Perspective

The outcome of bacterial meningitis is related to the destruction of the BBB integrity, excessive inflammatory responses, and nerve cell apoptosis. Although advances in antibiotic therapy and vaccine development, bacterial meningitis still remarkably causes high morbidity and mortality among children, infants, elders, and immunocompromised patients. The most difficult to prevent and treat bacterial meningitis is the diversity of pathogens and severe nerve injury. However, the limitation of vaccine and antibiotic resistance increases more difficulty in preventing and treating bacterial meningitis, and cannot timely and effectively prevent neural tissue from injury. Another major hurdle for bacterial meningitis treatment is the inefficient delivery of some antibiotic or macromolecular drugs into the brain due to the BBB.

It is clearly demonstrated that melatonin plays a beneficial role in neurological diseases. These functions mainly depend on the chemical and biological characteristics of melatonin. In recent years, most of the studies pointed out that melatonin with high solubility, which mainly releases from the pineal gland and across the BBB, is a functionally diverse molecule involved in the regulation of physiology, modulation of the immune system, and neuroprotection function [189,190]. In this review, we have shown that melatonin plays an important role as antibacterial, antioxidant, free radical scavenger, and immune system regulator, and so on. Furthermore, there are many clinical studies of melatonin on neuroprotection in different neurological diseases. For example, it was demonstrated that a 20 mg dose of melatonin supplement could reduce inflammation in serum and increase survival of newborns with sepsis [191]. In addition, the application of melatonin at a dose of 10 mg/kg, 5 times a day, could reduce newborns' new epilepsy and brain anomalies. In multiple sclerosis patients, supplement melatonin at 5 mg/day could improve the life quality of 102 patients by reducing MDA. Hence, these studies provide a positive effect on the widespread use of melatonin [192].

Until now, there is no clinical trial of melatonin on the treatment or prevention of human meningitis. The findings on CSF of patients with meningitis have shown that N^1-acetyl-N^2-formyl-5-methoxykynuramine (AFMK) levels in the presence of the melatonin metabolite were in a remarkably high concentration, aimed to control the intensity of the

inflammatory process by scavenging ROS [71]. Thus, it is well considered that the increment in AFMK concentration from melatonin metabolite in meningitis may be a physiological response to protect the brain tissue damage [71]. In in vivo or in vitro experiments, the ability of melatonin and its metabolites to cross the BBB into CNS has been identified for protecting nerve cells from injury and inducing neuritogenesis [98]. Even if there are no harmful effects of melatonin at different doses on the rodent meningitis model at present, the effective dose of melatonin for neuroprotection may be different due to bacterial meningitis caused by different pathogens. In a rabbit *Streptococcus pneumoniae* meningitis model, melatonin as an adjunctive treatment at a dose of 1.67 mg/kg 12 h after infection had anti-inflammatory effects but did not alleviate neuronal injury. Moreover, in the rat *Klebsiella pneumonia* meningitis model, melatonin effectively reduced inflammatory response and decreased microglial activation and the number of apoptotic neurons at dose 100 mg/kg [18]. According to the patient's age, autoimmunity, and bacterial type, we need to consider the effective dose of melatonin supplement, which stage to supplement, route of melatonin, melatonin supplement time, and the safety of melatonin in future clinic trials of bacterial meningitis. The aim is to make melatonin play a greater role in bacterial meningitis. Thus, the safety and effective treatment methods of melatonin for preventing or treating bacterial meningitis patients need more clinical studies.

In conclusion, melatonin has been found to have various mechanisms against bacterial meningitis (see Figure 2). Lots of reports identified that melatonin seems to be very promising, but there are still more studies on discussing and establishing guidelines to the clinical application of melatonin for preventing or treating bacterial meningitis patients.

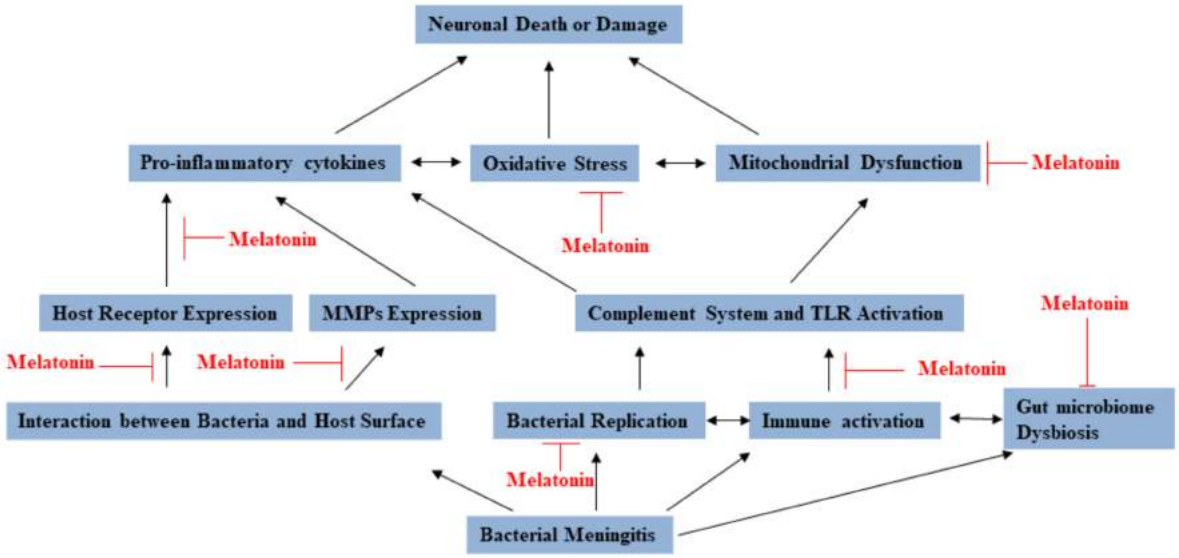

Figure 2. The multiple functions of melatonin that provide prevention and protection against bacterial meningitis.

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Abbreviations

BM	Bacterial meningitis
CNS	Central nervous system
BBB	Blood-brain barrier
GBS	Group B Streptococcus
HIV	Human immunodeficiency virus
CM	Cryptococcal meningitis
HiB	Haemophilus influenzae
MIC	Minimum inhibitory concentration
TNF-α	Tumor necrosis factor-α
TLR	Toll-like receptors
IL-1α	Interleukin-1α
NLRs	Nod-like receptors
LPS	Lipopolysaccharides
PAMPs	Pathogen-associated molecular patterns
NF-κ B	Nuclear factor kappa B
MAPK	Mitogen-activation protein kinase
PBN	Phenyl *N*-*t*-butylnitrone
4-HNE	4-Hydroxynonenal
MDA	Malondialdehyde
iNOS	inducible Nitric Oxide Synthase
HBMEC	Human Brain Microvascular Endothelial Cell
IL-6	Interleukin-6
ROS	Reactive oxygen species
RNS	Reactive nitrogen species
MMPs	Matrix metalloproteinases
CSF	Cerebrospinal fluid
BMECs	Brain microvascular endothelial cells
AJs	Adherens junctions
TJs	Tight junctions
ZO-1	Zonula occluding-1
CSF	Cerebrospinal fluid
SOD	Superoxide dismutase
PMN	Polymorphonuclear
mTOR	Mammalian target of rapamycin
Bmal 1	Brain and muscle Arnt-like protein 1
FAK	Focal Adhesion Kinase
LP	Lactate/pyruvate
MyD88	Myeloid differentiation factor 88
ERK1/2	Extracellular signal-related kinases 1/2
EGFR	Epidermal growth factor receptor
AFMK	N^1-acetyl-N^2-formyl-5-methoxykynuramine
PCV	Pneumococcal conjugate vaccines
PPV	Pneumococcal polysaccharide vaccine
PspA	Pneumococcal surface protein
Ply	Pneumolysin

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Potential benefit of high-dose intravenous vitamin C for coronavirus disease 2019 pneumonia

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The coronavirus disease 2019 (COVID-19) has been declared as a pandemic by the World Health Organization.[1] Most COVID-19 patients exhibit mild to moderate symptoms, while approximately 15% progress rapidly to severe pneumonia, and about 5% eventually develop acute respiratory distress syndrome (ARDS),[2] which requires mechanical ventilation (MV) and even extracorporeal membrane oxygenation. The mortality of COVID-19 patients who received MV was reported to be as high as 66%.[3] Therefore, the treatments aiming to improve mortality should focus on two aspects: first, prevention of the aggravation of the disease in mild and moderate COVID-19 patients; second, the rescue therapy for patients in serious conditions.

We have been applying high-dose intravenous vitamin C (HDIVC) in the treatment of critical illnesses for almost 10 years in our center. Our previous *in vivo* research showed that HDIVC protected hemorrhagic shock-related multiple organ failure (MOF) by inhibiting inflammatory cytokines and oxidative indicators through activating Sirtuin1 pathway.[4] Two randomized controlled trials are now being conducted to evaluate the efficiency and safety of HDIVC in sepsis (ChiCTR1800017633) and severe acute pancreatitis (ChiCTR1900022022). Based on that, we started to apply HDIVC in COVID-19 pneumonia since February 2, 2020 in Shanghai Public Health Clinical Center. By summarizing the experience with these patients, a HDIVC protocol [Figure 1] was proposed by the Shanghai COVID-19 Clinical Treatment Expert Group. The application of HDIVC protocol varied according to the disease severity which was classified as mild, moderate, severe, and critical.[5] The mild type did not require HDIVC treatment. The HDIVC protocol for moderate, severe, and critical type mainly consisted of two parts: the routine usage of HDIVC at admission and then for seven consecutive days, which might be beneficial for the prevention of disease aggravation. The other part is about the rescue therapy, which might be essential for live saving when disease aggravation occurs. Our studies showed the inflammatory response, immune and organ function improved after HDIVC application in a retrospective case series study,[6] and the number of moderate COVID-19 patients transferring to severe type was reduced after HDIVC protocol application.[7]

The rationale for HDIVC in the treatment of COVID-19 is based on the following aspects: (1) Rapid scavenging of reactive oxygen species (ROS) and relieving ROS related inflammatory response, endothelial dysfunction, coagulopathy, ARDS, and MOF. Under the condition of hypoxemia induced by COVID-19, ROS are produced in mitochondria in a huge amount.[8] ROS induce release of cytokines and chemokines via certain mechanisms such as nuclear factor kappa B (NF-κB) signaling pathway,[8]

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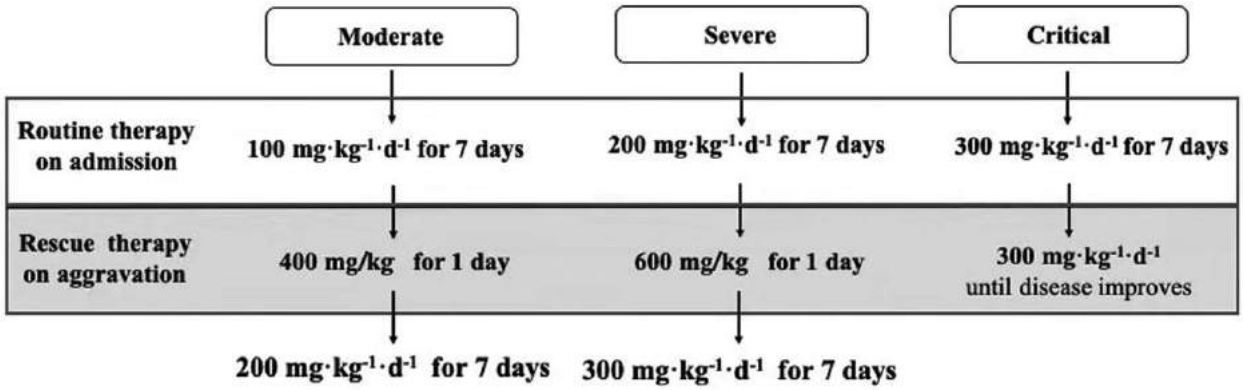

Figure 1: Flowchart of HDIVC protocol. Aggravation means disease severity transfers to the next level within 24 to 48 hours. HDIVC: High-dose intravenous vitamin C.

resulting in so-called "cytokine storm." ROS directly damages the vascular endothelial cells and causes the pulmonary interstitial edema, which represents the main pathophysiology of ARDS. Furthermore, ROS can induce the occurrence of coagulopathy by triggering platelet aggregation and activating the coagulation system. Coagulopathy is a common feature of COVID-19 characterized by an increase in D-dimer, fibrin degradation product levels and longer activated partial thromboplastin time.[9] The extensive microvascular clot formation further augments tissue hypoxia due to increased diffusion distance for oxygen, thereby leading to MOF. Vitamin C, eliminating ROS in direct and indirect way, has been shown to be beneficial for severe sepsis patient. The vitamin C infusion for treatment in sepsis induced acute lung injury trial,[10] the largest randomized clinical trial on HDIVC, reported that the 28-day all-cause mortality (29.8% *vs*. 46.3%) was reduced by HDIVC in a cohort of septic patients with ARDS. (2) Potential improving effect on lymphopenia. Lymphopenia was correlated with the development of ARDS and disease severity.[3] The mechanisms of lymphopenia mainly include growth inhibition and apoptosis of hematopoietic cells and T lymphocytes induced by the severe acute respiratory coronavirus 2 through the promotion of autoimmune antibody and production of certain cytokine.[11] Vitamin C is essential for the development, maturation, and proliferation of functional T-lymphocytes.[12] Although there are limited studies on the effect of HDIVC on lymphocytes in sepsis, it is speculated that HDIVC might be beneficial for the lymphopenia occurring in COVID-19 patients as we found the number of CD4+ T lymphocyte increased after HDIVC application.[6,7] (3) Maintaining circulation function stability. Vitamin C is an important co-factor for the synthesis of the endogenous hormone, including catecholamine, corticosteroid, and vasopressin.[13] A previous clinical study[14] showed less need for vasopressor and lower 28-day mortality in septic shock patients after HDIVC application. For COVID-19 patients with septic shock, HDIVC, especially a high bolus dose over a short time, might help the recovery of circulation failure. (4) Attenuating the COVID-19 related scurvy rapidly. According to the latest report,[15] the level of vitamin C is almost undetectable in severe COVID-19 patients. The "scurvy" state is correlated with MOF in critically ill patients.[16] Giving vitamin C intravenously can quickly increase the serum levels of vitamin C from scurvy (10–20 μmol/L) to mmol/L level.[17] Therefore, patients might draw benefits from HDIVC because of its quick supplemental effect compared to oral pathway. (5) Safety of HDIVC. One major concern of HIDVC is its potential adverse effects including oxalate nephropathy and formation of urine stone. No confirmed evidence supported the high-dose vitamin C related adverse events mentioned above in critically illness.[18] Similarly, we did not observe any potential adverse events either.

In conclusion, HDIVC is an efficient and safe treatment for patients with COVID-19. It might be applied in prevention of disease aggravation in moderate types, as well as rescue therapy for the severe and critical type. Anyway, high-quality randomized clinical trials are warranted.

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Conflicts of interest

None.

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ADVERSE REACTIONS & EVENTS

Autism

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A positive association found between autism prevalence and childhood vaccination uptake across the U.S. population

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Abstract

The reason for the rapid rise of autism in the United States that began in the 1990s is a mystery. Although individuals probably have a genetic predisposition to develop autism, researchers suspect that one or more environmental triggers are also needed. One of those triggers might be the battery of vaccinations that young children receive. Using regression analysis and controlling for family income and ethnicity, the relationship between the proportion of children who received the recommended vaccines by age 2 years and the prevalence of autism (AUT) or speech or language impairment (SLI) in each U.S. state from 2001 and 2007 was determined. A positive and statistically significant relationship was found: The higher the proportion of children receiving recommended vaccinations, the higher was the prevalence of AUT or SLI. A 1% increase in vaccination was associated with an additional 680 children having AUT or SLI. Neither parental behavior nor access to care affected the results, since vaccination proportions were not significantly related (statistically) to any other disability or to the number of pediatricians in a U.S. state. The results suggest that although mercury has been removed from many vaccines, other culprits may link vaccines to autism. Further study into the relationship between vaccines and autism is warranted.

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Do aluminum vaccine adjuvants contribute to the rising prevalence of autism?

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Abstract

Autism spectrum disorders (ASD) are serious multisystem developmental disorders and an urgent global public health concern. Dysfunctional immunity and impaired brain function are core deficits in ASD. Aluminum (Al), the most commonly used vaccine adjuvant, is a demonstrated neurotoxin and a strong immune stimulator. Hence, adjuvant Al has the potential to induce neuroimmune disorders. When assessing adjuvant toxicity in children, two key points ought to be considered: (i) children should not be viewed as "small adults" as their unique physiology makes them much more vulnerable to toxic insults; and (ii) if exposure to Al from only few vaccines can lead to cognitive impairment and autoimmunity in adults, is it unreasonable to question whether the current pediatric schedules, often containing 18 Al adjuvanted vaccines, are safe for children? By applying Hill's criteria for establishing causality between exposure and outcome we investigated whether exposure to Al from vaccines could be contributing to the rise in ASD prevalence in the Western world. Our results show that: (i) children from countries with the highest ASD prevalence appear to have the highest exposure to Al from vaccines; (ii) the increase in exposure to Al adjuvants significantly correlates with the increase in ASD prevalence in the United States observed over the last two decades (Pearson r=0.92, p<0.0001); and (iii) a significant correlation exists between the amounts of Al administered to preschool children and the current prevalence of ASD in seven Western countries, particularly at 3-4 months of age (Pearson r=0.89-0.94, p=0.0018-0.0248). The application of the Hill's criteria to these data indicates that the correlation between Al in vaccines and ASD may be causal. Because children represent a fraction of the population most at risk for complications following exposure to Al, a more rigorous evaluation of Al adjuvant safety seems warranted.

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A possible central mechanism in autism spectrum disorders, part 1

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Abstract

The autism spectrum disorders (ASD) are a group of related neurodevelopmental disorders that have been increasing in incidence since the 1980s. Despite a considerable amount of data being collected from cases, a central mechanism has not been offered. A careful review of ASD cases discloses a number of events that adhere to an immunoexcitotoxic mechanism. This mechanism explains the link between excessive vaccination, use of aluminum and ethylmercury as vaccine adjuvants, food allergies, gut dysbiosis, and abnormal formation of the developing brain. It has now been shown that chronic microglial activation is present in autistic brains from age 5 years to age 44 years. A considerable amount of evidence, both experimental and clinical, indicates that repeated microglial activation can initiate priming of the microglia and that subsequent stimulation can produce an exaggerated microglial response that can be prolonged. It is also known that one phenotypic form of microglia activation can result in an outpouring of neurotoxic levels of the excitotoxins, glutamate and quinolinic acid. Studies have shown that careful control of brain glutamate levels is essential to brain pathway development and that excesses can result in arrest of neural migration, as well as dendritic and synaptic loss. It has also been shown that certain cytokines, such as TNF-alpha, can, via its receptor, interact with glutamate receptors to enhance the neurotoxic reaction. To describe this interaction I have coined the term immunoexcitotoxicity, which is described in this article.

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Hepatitis B vaccination of male neonates and autism diagnosis, NHIS 1997-2002

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Abstract

Universal hepatitis B vaccination was recommended for U.S. newborns in 1991; however, safety findings are mixed. The association between hepatitis B vaccination of male neonates and parental report of autism diagnosis was determined. This cross-sectional study used weighted probability samples obtained from National Health Interview Survey 1997-2002 data sets. Vaccination status was determined from the vaccination record. Logistic regression was used to estimate the odds for autism diagnosis associated with neonatal hepatitis B vaccination among boys age 3-17 years, born before 1999, adjusted for race, maternal education, and two-parent household. Boys vaccinated as neonates had threefold greater odds for autism diagnosis compared to boys never vaccinated or vaccinated after the first month of life. Non-Hispanic white boys were 64% less likely to have autism diagnosis relative to nonwhite boys. Findings suggest that U.S. male neonates vaccinated with the hepatitis B vaccine prior to 1999 (from vaccination record) had a threefold higher risk for parental report of autism diagnosis compared to boys not vaccinated as neonates during that same time period. Nonwhite boys bore a greater risk.

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Aluminium in brain tissue in autism

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ABSTRACT

Autism spectrum disorder is a neurodevelopmental disorder of unknown aetiology. It is suggested to involve both genetic susceptibility and environmental factors including in the latter environmental toxins. Human exposure to the environmental toxin aluminium has been linked, if tentatively, to autism spectrum disorder. Herein we have used transversely heated graphite furnace atomic absorption spectrometry to measure, for the first time, the aluminium content of brain tissue from donors with a diagnosis of autism. We have also used an aluminium-selective fluor to identify aluminium in brain tissue using fluorescence microscopy. The aluminium content of brain tissue in autism was consistently high. The mean (standard deviation) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. These are some of the highest values for aluminium in human brain tissue yet recorded and one has to question why, for example, the aluminium content of the occipital lobe of a 15 year old boy would be 8.74 (11.59) μg/g dry wt.? Aluminium-selective fluorescence microscopy was used to identify aluminium in brain tissue in 10 donors. While aluminium was imaged associated with neurones it appeared to be present intracellularly in microglia-like cells and other inflammatory non-neuronal cells in the meninges, vasculature, grey and white matter. The pre-eminence of intracellular aluminium associated with non-neuronal cells was a standout observation in autism brain tissue and may offer clues as to both the origin of the brain aluminium as well as a putative role in autism spectrum disorder.

1. Introduction

Autism spectrum disorder (ASD) is a group of neurodevelopmental conditions of unknown cause. It is highly likely that both genetic [1] and environmental [2] factors are associated with the onset and progress of ASD while the mechanisms underlying its aetiology are expected to be multifactorial [3–6]. Human exposure to aluminium has been implicated in ASD with conclusions being equivocal [7–10]. To-date the majority of studies have used hair as their indicator of human exposure to aluminium while aluminium in blood and urine have also been used to a much more limited extent. Paediatric vaccines that include an aluminium adjuvant are an indirect measure of infant exposure to aluminium and their burgeoning use has been directly correlated with increasing prevalence of ASD [11]. Animal models of ASD continue to support a connection with aluminium and to aluminium adjuvants used in human vaccinations in particular [12]. Hitherto there are no previous reports of aluminium in brain tissue from donors who died with a diagnosis of ASD. We have measured aluminium in brain tissue in autism and identified the location of aluminium in these tissues.

2. Materials and methods

2.1. Measurement of aluminium in brain tissues

Ethical approval was obtained along with tissues from the Oxford Brain Bank (15/SC/0639). Samples of cortex of approximately 1 g frozen weight from temporal, frontal, parietal and occipital lobes and hippocampus (0.3 g only) were obtained from 5 individuals with ADI-R-confirmed (Autism Diagnostic Interview-Revised) ASD, 4 males and 1 female, aged 15–50 years old (Table 1).

The aluminium content of these tissues was measured by an established and fully validated method [13] that herein is described only briefly. Thawed tissues were cut using a stainless steel blade to give individual samples of *ca* 0.3 g (3 sample replicates for each lobe except for hippocampus where the tissue was used as supplied) wet weight and dried to a constant weight at 37 °C. Dried and weighed tissues were digested in a microwave (MARS Xpress CEM Microwave Technology Ltd.) in a mixture of 1 mL 15.8 M HNO_3 (Fisher Analytical Grade) and 1 mL 30% *w/v* H_2O_2 (BDH Aristar). Digests were clear with no fatty residues and, upon cooling, were made up to 5 mL volume using

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Table 1
Aluminium content of occipital (O), frontal (F), temporal (T) and parietal (P) lobes and hippocampus (H) of brain tissue from 5 donors with a diagnosis of autism spectrum disorder.

Donor ID	Gender	Age	Lobe	Replicate	[Al] μg/g
A1	F	44	O	1	0.49
				2	4.26
				3	0.33
				Mean (SD)	1.69 (2.22)
			F	1	0.98
				2	1.10
				3	0.95
				Mean (SD)	1.01 (0.08)
			T	1	1.13
				2	1.16
				3	1.12
				Mean (SD)	1.14 (0.02)
			P	1	0.54
				2	1.18
				3	NA
				Mean (SD)	0.86 (0.45)
			All	Mean (SD)	1.20 (1.06)
A2	M	50	O	1	3.73
				2	7.87
				3	3.49
				Mean (SD)	5.03 (2.46)
			F	1	0.86
				2	0.88
				3	1.65
				Mean (SD)	1.13 (0.45)
			T	1	1.31
				2	1.02
				3	2.73
				Mean (SD)	1.69 (0.92)
			P	1	18.57
				2	0.01
				3	0.64
				Mean (SD)	6.41 (10.54)
			Hip.	1	1.42
			All	Mean (SD)	3.40 (5.00)
A3	M	22	O	1	0.64
				2	2.01
				3	0.66
				Mean (SD)	1.10 (0.79)
			F	1	1.72
				2	4.14
				3	2.73
				Mean (SD)	2.86 (1.22)
			T	1	1.62
				2	4.25
				3	2.57
				Mean (SD)	2.81 (1.33)
			P	1	0.13
				2	3.12
				3	5.18
				Mean (SD)	2.82 (1.81)
			All	Mean (SD)	2.40 (1.58)
A4	M	15	O	1	2.44
				2	1.66
				3	22.11
				Mean (SD)	8.74 (11.59)
			F	1	1.11
				2	3.23
				3	1.66
				Mean (SD)	2.00 (1.10)
			T	1	1.10
				2	1.83
				3	1.54
				Mean (SD)	1.49 (0.37)
			P	1	1.38
				2	6.71
				3	NA
				Mean (SD)	4.05 (3.77)
			Hip.	1	0.02
			All	Mean (SD)	3.73 (6.02)
A5	M	33	O	1	3.13
				2	2.78
				3	1.71
				Mean (SD)	2.54 (0.74)
			F	1	2.97
				2	8.27
				3	NA
				Mean (SD)	5.62 (3.75)
			T	1	1.71
				2	1.64
				3	17.10
				Mean (SD)	6.82 (8.91)
			P	1	5.53
				2	2.89
				3	NA
				Mean (SD)	4.21 (1.87)
			All	Mean (SD)	4.77 (4.79)

ultrapure water (cond. < 0.067 μS/cm). Total aluminium was measured in each sample by transversely heated graphite furnace atomic absorption spectrometry (TH GFAAS) using matrix-matched standards and an established analytical programme alongside previously validated quality assurance data [13].

2.2. Fluorescence microscopy

All chemicals were from Sigma Aldrich (UK) unless otherwise stated. Where available frontal, parietal, occipital, temporal and hippocampal tissue from 10 donors (3 females and 7 males) with a diagnosis of ASD was supplied by the Oxford Brain Bank as three 5 μm thick serial paraffin-embedded brain tissue sections per lobe for each donor (Table S1). Tissue sections mounted on glass slides were placed in a slide rack and de-waxed and rehydrated via transfer through 250 mL of the following reagents: 3 min in Histo-Clear (National Diagnostics, US), 1 min in fresh Histo-Clear, 2 min in 100% *v/v* ethanol (HPLC grade) and 1 min in 95, 70, 50 & 30% *v/v* ethanol followed by rehydration in ultrapure water (cond. < 0.067 μS/cm) for 35 s. Slides were agitated every 20 s in each solvent and blotted on tissue paper between transfers to minimise solvent carry-over. Rehydrated brain tissue sections were carefully outlined with a PAP pen for staining, in order to form a hydrophobic barrier around the periphery of tissue sections. In between staining, tissue sections were kept hydrated with ultrapure water and stored in moisture chambers, to prevent sections from drying out. Staining was staggered to allow for accurate incubation times of brain tissue sections. We have developed and optimised the fluor lumogallion as a selective stain for aluminium in cells [14] and human tissues [15]. Lumogallion (4-chloro-3-(2,4-dihydroxyphenylazo)-2-hydroxybenzene-1-sulphonic acid, TCI Europe N.V. Belgium) was prepared at *ca* 1 mM via dilution in a 50 mM PIPES (1,4-Piperazinediethanesulphonic acid) buffer, adjusted to pH 7.4 with NaOH. Lumogallion staining was performed via the addition of 200 μL of the staining solution to rehydrated brain tissue sections that were subsequently incubated at ambient temperature away from light for 45 min. Sections for autofluorescence analyses were incubated for 45 min in 200 μL 50 mM PIPES buffer only, pH 7.4. Following staining, glass slides containing tissue sections were washed six times with 200 μL aliquots of 50 mM PIPES buffer, pH 7.4, prior to rinsing for 30 s in ultrapure water. Serial sections numbered 1 and 2 for each lobe were incubated in 50 mM PIPES buffer, pH 7.4 or stained with 1 mM lumogallion in the same buffer, respectively, to ensure consistency across donor tissues. All tissue sections were subsequently mounted under glass coverslips using the aqueous mounting medium, Fluoromount™. Slides were stored horizontally for 24 h at 4 °C away from light, prior to analysis via fluorescence microscopy.

Stained and mounted human brain tissue sections were analysed via

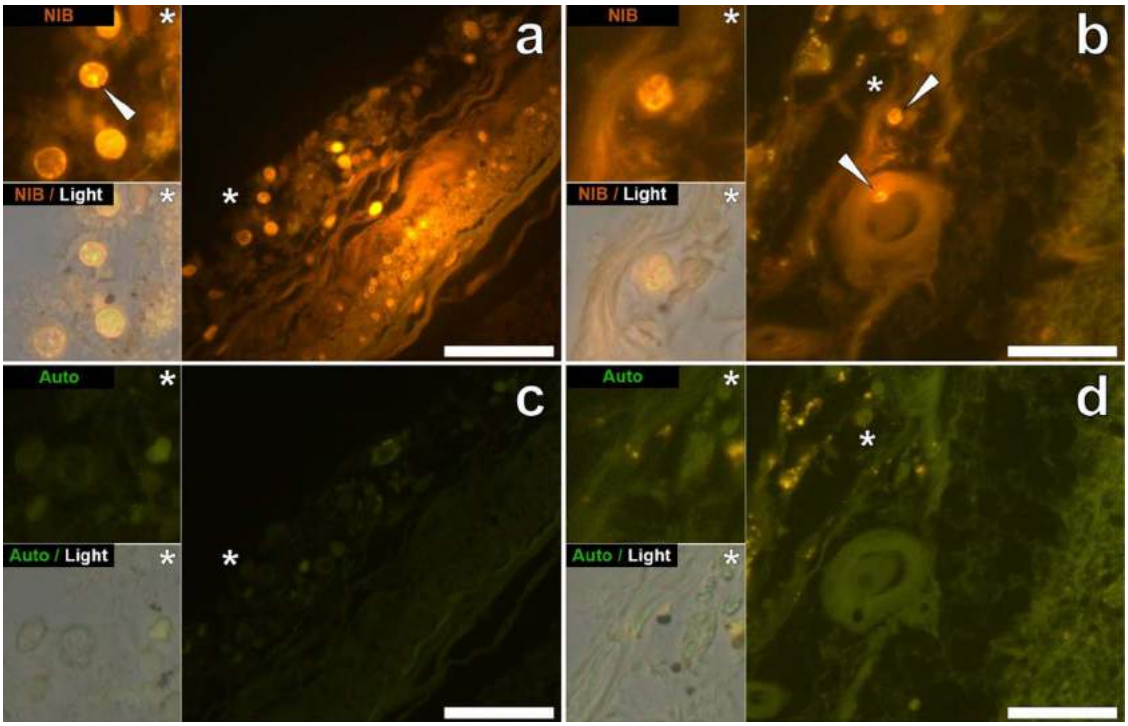

Fig. 1. Mononuclear inflammatory cells (probably lymphocytes) in leptomeningeal membranes in the hippocampus and frontal lobe of a 50-year-old male donor (A2), diagnosed with autism. Intracellular lumogallion-reactive aluminium was noted via punctate orange fluorescence emission (white arrows) in the hippocampus **(a)** and frontal lobe **(b)**. A green autofluorescence emission was detected in the adjacent non-stained (5 μm) serial section **(c & d)**. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the use of an Olympus BX50 fluorescence microscope, equipped with a vertical illuminator and BX-FLA reflected light fluorescence attachment (mercury source). Micrographs were obtained at X 400 magnification by use of a X 40 Plan-Fluorite objective (Olympus, UK). Lumogallion-reactive aluminium and related autofluorescence micrographs were obtained via use of a U-MNIB3 fluorescence filter cube (excitation: 470–495 nm, dichromatic mirror: 505 nm, longpass emission: 510 nm, Olympus, UK). Light exposure and transmission values were fixed across respective staining treatment conditions and images were obtained using the CellD software suite (Olympus, Soft Imaging Solutions, SiS, GmbH). Lumogallion-reactive regions identified through sequential screening of stained human brain tissue sections were additionally imaged on autofluorescence serial sections, to assess the contribution of the fluorophore. The subsequent merging of fluorescence and bright-field channels was achieved using Photoshop (Adobe Systems Inc. US). When determining intracellular staining the type of cells stained were estimated by their size and shape in the context of the brain area sampled and their surrounding cellular environment.

3. Results

3.1. Aluminium content of brain tissues

The aluminium content of all tissues ranged from 0.01 (the limit of quantitation) to 22.11 μg/g dry wt. (Table 1). The aluminium content for whole brains (n = 4 or 5 depending upon the availability of hippocampus tissue) ranged from 1.20 (1.06) μg/g dry wt. for the 44 year old female donor (A1) to 4.77 (4.79) μg/g dry wt. for a 33 year old male donor (A5). Previous measurements of brain aluminium, including our 60 brain study [13], have allowed us to define loose categories of brain aluminium content beginning with ≤1.00 μg/g dry wt. as pathologically benign (as opposed to 'normal'). Approximately 40% of tissues (24/59) had an aluminium content considered as pathologically-concerning (≥2.00 μg/g dry wt.) while approximately 67% of these tissues had an aluminium content considered as pathologically-significant (≥3.00 μg/g dry wt.). The brains of all 5 individuals had at least one tissue with a pathologically-significant content of aluminium. The brains of 4 individuals had at least one tissue with an aluminium content ≥5.00 μg/g dry wt. while 3 of these had at least one tissue with an aluminium content ≥10.00 μg/g dry wt. (Table 1). The mean (SD) aluminium content across all 5 individuals for each lobe were 3.82(5.42), 2.30(2.00), 2.79(4.05) and 3.82(5.17) μg/g dry wt. for the occipital, frontal, temporal and parietal lobes respectively. There were no statistically significant differences in aluminium content between any of the 4 lobes.

3.2. Aluminium fluorescence in brain tissues

We examined serial brain sections from 10 individuals (3 females and 7 males) who died with a diagnosis of ASD and recorded the presence of aluminium in these tissues (Table S1). Excitation of the complex of aluminium and lumogallion emits characteristic orange fluorescence that appears increasingly bright yellow at higher fluorescence intensities. Aluminium, identified as lumogallion-reactive deposits, was recorded in at least one tissue in all 10 individuals. Autofluorescence of immediately adjacent serial sections confirmed lumogallion fluorescence as indicative of aluminium. Deposits of aluminium were significantly more prevalent in males (129 in 7 individuals) than females (21 in 3 individuals). Aluminium was found in both white (62 deposits) and grey (88 deposits) matter. In females the majority of aluminium deposits were identified as extracellular (15/21) whereas in males the opposite was the case with 80 out of 129 deposits being intracellular. We were only supplied with 3 serial sections of each tissue and so we were not able to do any staining for general morphology which meant that it was not always possible to determine which subtype of cell was showing aluminium fluorescence.

Aluminium-loaded mononuclear white blood cells, probably lymphocytes, were identified in the meninges and possibly in the process of

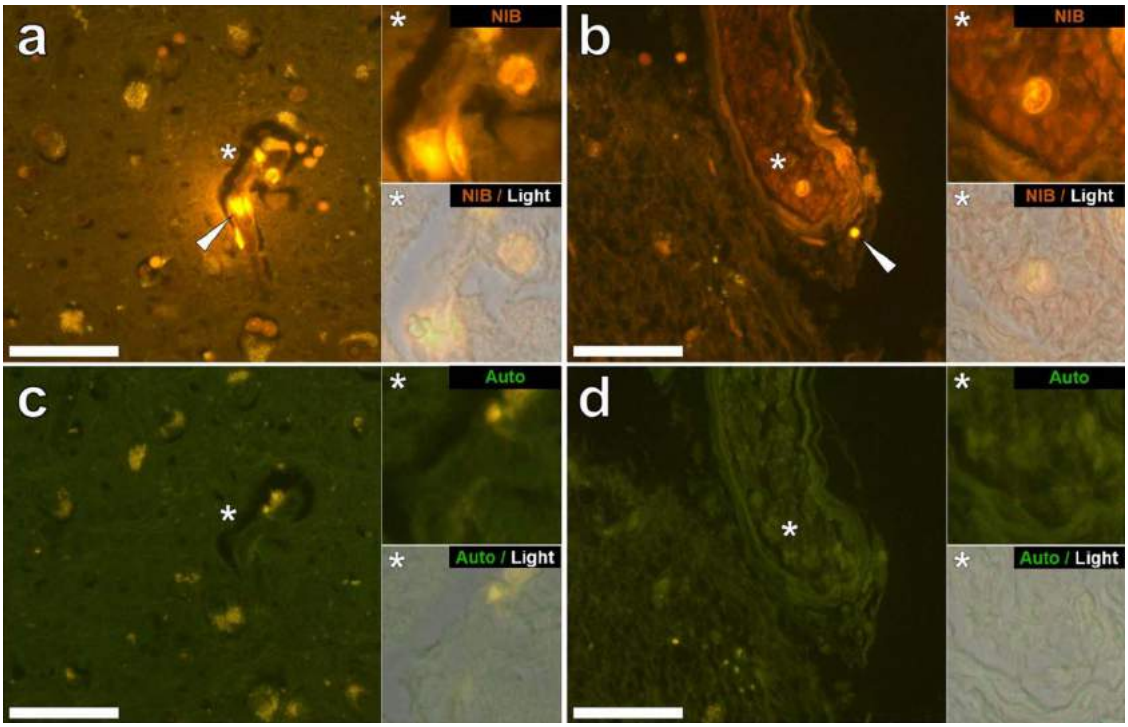

Fig. 2. Intracellular lumogallion-reactive aluminium in the vasculature of the hippocampus of a 50-year-old male donor (A2), diagnosed with autism. Aluminium-loaded inflammatory cells noted in the hippocampus in the vessel wall (white arrow) **(a)** and depicting punctate orange fluorescence in the lumen **(b)** are highlighted. An inflammatory cell in the vessel adventitia was also noted (white arrow) **(b)**. Lumogallion-reactive aluminium was identified via an orange fluorescence emission **(a & b)** versus a green autofluorescence emission **(c & d)** of the adjacent non-stained (5 μm) serial section. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

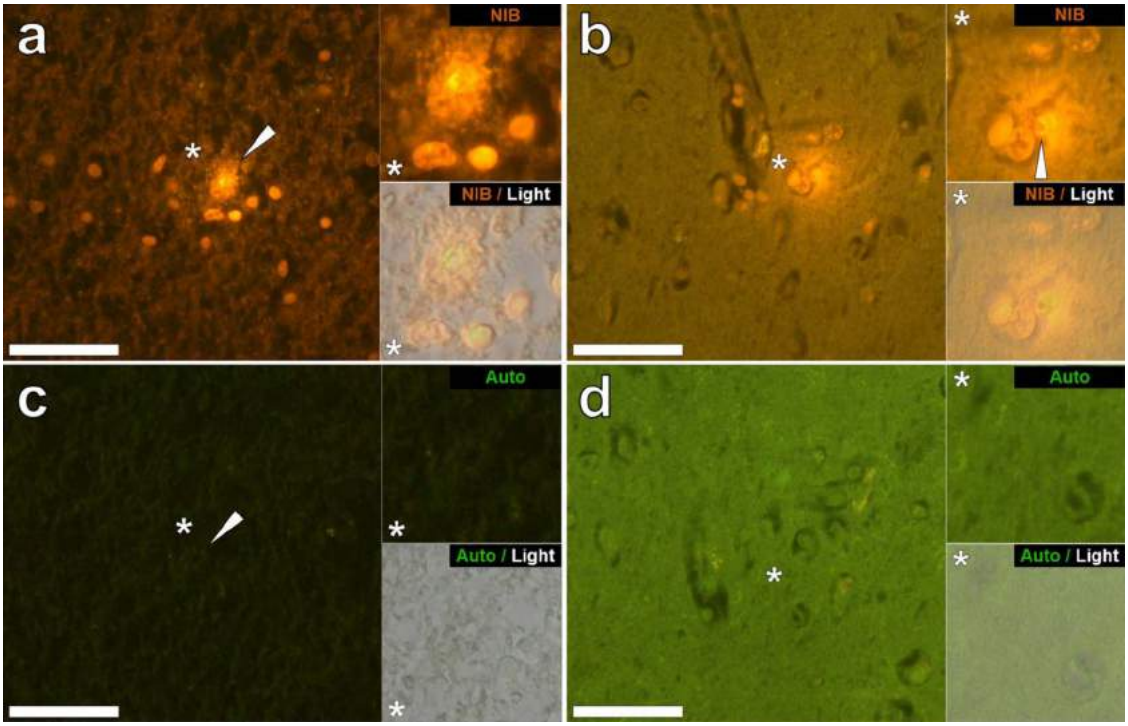

Fig. 3. Intracellular aluminium in cells morphologically compatible with glia and neurones in the hippocampus of a 15-year-old male donor (A4), diagnosed with autism. Lumogallion reactive cellular aluminium identified within glial-like cells in the hippocampus **(a)** and producing a punctate orange fluorescence in glia surrounding a likely neuronal cell within the parietal lobe **(b)** are highlighted (white arrows). Lumogallion-reactive aluminium was identified via an orange fluorescence emission **(a & b)** versus a green autofluorescence emission **(c & d)** of the subsequent non-stained (5 μm) serial section (white arrow/asterisk). Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

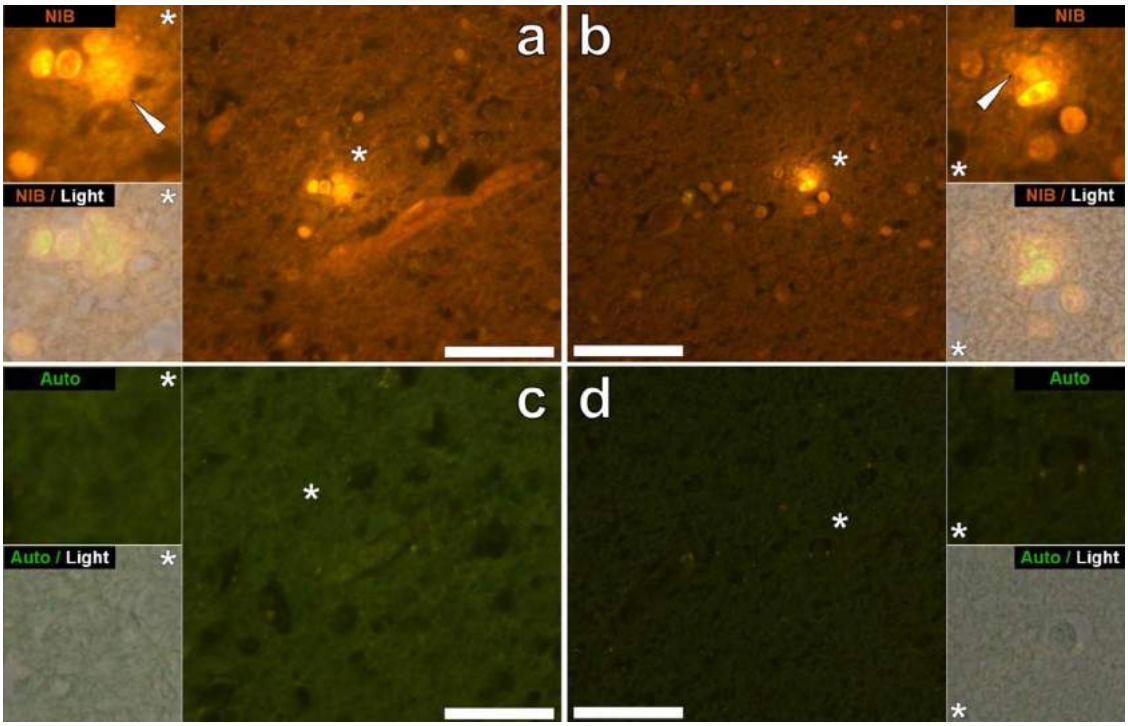

Fig. 4. Intracellular aluminium in cells morphologically compatible with microglia within the parietal and temporal lobes of 29-year-old (A8) and 15-year-old (A4) male donors, diagnosed with autism. Lumogallion-reactive extracellular aluminium (white arrows) producing an orange fluorescence emission was noted around likely microglial cells in the parietal **(a)** and temporal lobes **(b)** of donors A8 and A4 respectively. Non-stained adjacent (5 μm) serial sections, produced a weak green autofluorescence emission of the identical area imaged in white **(c)** and grey matter **(d)** of the respective lobes. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

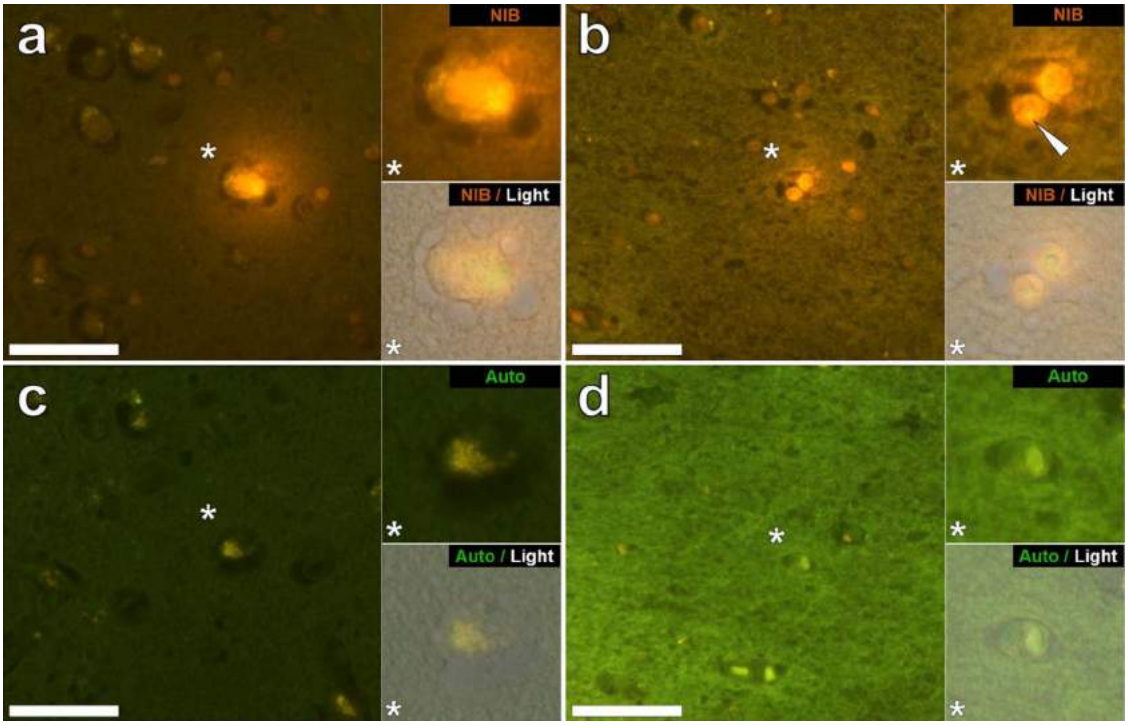

Fig. 5. Lumogallion-reactive aluminium in likely neuronal and glial cells in the temporal lobe and hippocampus of a 14-year-old male donor (A10), diagnosed with autism. Intraneuronal aluminium in the temporal lobe **(a)** was identified via an orange fluorescence emission, co-deposited with lipofuscin as revealed by a yellow fluorescence in the non-stained auto-fluorescence serial (5 μm) section **(c)**. Intracellular punctate orange fluorescence (white arrow) was observed in glia in the hippocampus **(b)** producing a green autofluorescence emission on the non-stained section **(d)**. Upper and lower panels depict magnified inserts marked by asterisks, of the fluorescence channel and bright field overlay. Magnification ×400, scale bars: 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

entering brain tissue from the lymphatic system (Fig. 1). Aluminium could be clearly seen inside cells as either discrete punctate deposits or as bright yellow fluorescence. Aluminium was located in inflammatory cells associated with the vasculature (Fig. 2). In one case what looks like an aluminium-loaded lymphocyte or monocyte was noted within a blood vessel lumen surrounded by red blood cells while another probable lymphocyte showing intense yellow fluorescence was noted in the adventitia (Fig. 2b). Glial cells including microglia-like cells that showed positive aluminium fluorescence were often observed in brain tissue in the vicinity of aluminium-stained extracellular deposits (Figs. 3 and 4). Discrete deposits of aluminium approximately 1 μm in diameter were clearly visible in both round and amoeboid glial cell bodies (e.g. Fig. 3b). Intracellular aluminium was identified in likely neurones and glia-like cells and often in the vicinity of or co-localised with lipofuscin (Fig. 5). Aluminium-selective fluorescence microscopy was successful in identifying aluminium in extracellular and intracellular locations in neurones and non-neuronal cells and across all brain tissues studied (Figs. 1–5). The method only identifies aluminium as evidenced by large areas of brain tissue without any characteristic aluminium-positive fluorescence (Fig. S1).

4. Discussion

The aluminium content of brain tissues from donors with a diagnosis of ASD was extremely high (Table 1). While there was significant inter-tissue, inter-lobe and inter-subject variability the mean aluminium content for each lobe across all 5 individuals was towards the higher end of all previous (historical) measurements of brain aluminium content, including iatrogenic disorders such as dialysis encephalopathy [13,15,16–19]. All 4 male donors had significantly higher concentrations of brain aluminium than the single female donor. We recorded some of the highest values for brain aluminium content ever measured in healthy or diseased tissues in these male ASD donors including values of 17.10, 18.57 and 22.11 μg/g dry wt. (Table 1). What discriminates these data from other analyses of brain aluminium in other diseases is the age of the ASD donors. Why, for example would a 15 year old boy have such a high content of aluminium in their brain tissues? There are no comparative data in the scientific literature, the closest being similarly high data for a 42 year old male with familial Alzheimer's disease (fAD) [19].

Aluminium-selective fluorescence microscopy has provided indications as to the location of aluminium in these ASD brain tissues (Figs. 1–5). Aluminium was found in both white and grey matter and in both extra- and intracellular locations. The latter were particularly pre-eminent in these ASD tissues. Cells that morphologically appeared non-neuronal and heavily loaded with aluminium were identified associated with the meninges (Fig. 1), the vasculature (Fig. 2) and within grey and white matter (Figs. 3–5). Some of these cells appeared to be glial (probably astrocytic) whilst others had elongated nuclei giving the appearance of microglia [5]. The latter were sometimes seen in the environment of extracellular aluminium deposition. This implies that aluminium somehow had crossed the blood-brain barrier and was taken up by a native cell namely the microglial cell. Interestingly, the presence of occasional aluminium-laden inflammatory cells in the vasculature and the leptomeninges opens the possibility of a separate mode of entry of aluminium into the brain i.e. intracellularly. However, to allow this second scenario to be of significance one would expect some type of intracerebral insult to occur to allow egress of lymphocytes and monocytes from the vasculature [20]. The identification herein of non-neuronal cells including inflammatory cells, glial cells and microglia loaded with aluminium is a standout observation for ASD. For example, the majority of aluminium deposits identified in brain tissue in fAD were extracellular and nearly always associated with grey matter [19]. Aluminium is cytotoxic [21] and its association herein with inflammatory cells in the vasculature, meninges and central nervous system is unlikely to be benign. Microglia heavily loaded with aluminium while potentially remaining viable, at least for some time, will inevitably be compromised and dysfunctional microglia are thought to be involved in the aetiology of ASD [22], for example in disrupting synaptic pruning [23]. In addition the suggestion from the data herein that aluminium entry into the brain via immune cells circulating in the blood and lymph is expedited in ASD might begin to explain the earlier posed question of why there was so much aluminium in the brain of a 15 year old boy with an ASD.

A limitation of our study is the small number of cases that were available to study and the limited availability of tissue. Regarding the latter, having access to only 1 g of frozen tissue and just 3 serial sections of fixed tissue per lobe would normally be perceived as a significant limitation. Certainly if we had not identified any significant deposits of aluminium in such a small (the average brain weighs between 1500 and 2000 g) sample of brain tissue then such a finding would be equivocal. However, the fact that we found aluminium in every sample of brain tissue, frozen or fixed, does suggest very strongly that individuals with a diagnosis of ASD have extraordinarily high levels of aluminium in their brain tissue and that this aluminium is pre-eminently associated with non-neuronal cells including microglia and other inflammatory monocytes.

5. Conclusions

We have made the first measurements of aluminium in brain tissue in ASD and we have shown that the brain aluminium content is extraordinarily high. We have identified aluminium in brain tissue as both extracellular and intracellular with the latter involving both neurones and non-neuronal cells. The presence of aluminium in inflammatory cells in the meninges, vasculature, grey and white matter is a standout observation and could implicate aluminium in the aetiology of ASD.

Competing interests

The authors declare that they have no competing interests.

Author contributions

CE designed the study, carried out tissue digests and TH GFAAS. DU carried out tissue digests and TH GFAAS. AK carried out brain neuropathology on sections prepared by MM. MM carried out all microscopy and with CE wrote the manuscript. All authors read and approved the manuscript.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.jtemb.2017.11.012.

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Impact of environmental factors on the prevalence of autistic disorder after 1979

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Abstract

The aim of this study was to investigate a previously overlooked, universally introduced environmental factor, fetal and retroviral contaminants in childhood vaccines, absent prior to change points (CPs) in autistic disorder (AD) prevalence with subsequent dose-effect evidence and known pathologic mechanisms of action. Worldwide population based cohort study was used for the design of this study. The United States, Western Australia, United Kingdom and Denmark settings were used. All live born infants who later developed autistic disorder delivered after 1 January 1970, whose redacted vaccination and autistic disorder diagnosis information is publicly available in databases maintained by the US Federal Government, Western Australia, UK, and Denmark. The live births, grouped by father's age, were from the US and Australia. The children vaccinated with MMRII, Varicella and Hepatitis A vaccines varied from 19 to 35 months of age at the time of vaccination. Autistic disorder birth year change points were identified as 1980.9, 1988.4 and 1996 for the US, 1987 for UK, 1990.4 for Western Australia, and 1987.5 for Denmark. Change points in these countries corresponded to introduction of or increased doses of human fetal cell line-manufactured vaccines, while no relationship was found between paternal age or Diagnostic and Statistical Manual (DSM) revisions and autistic disorder diagnosis. Further, linear regression revealed that Varicella and Hepatitis A immunization coverage was significantly correlated to autistic disorder cases. R software was used to calculate change points. Autistic disorder change points years are coincident with introduction of vaccines manufactured using human fetal cell lines, containing fetal and retroviral contaminants, into childhood vaccine regimens. This pattern was repeated in the US, UK, Western Australia and Denmark. Thus, rising autistic disorder prevalence is directly related to vaccines manufactured utilizing human fetal cells. Increased paternal age and DSM revisions were not related to rising autistic disorder prevalence.

Key words: Autism disorder, change point, vaccine, paternal age.

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Epidemiologic and Molecular Relationship Between Vaccine Manufacture and Autism Spectrum Disorder Prevalence

Theresa A Deisher, Ngoc V Doan, Kumiko Koyama, Sarah Bwabye

PMID: 26103708

Abstract

Objectives: To assess the public health consequences of fetal cell line manufactured vaccines that contain residual human fetal DNA fragments utilizing laboratory and ecological approaches including statistics, molecular biology and genomics.

Method: MMR coverage and autism disorder or autism spectrum disorder prevalence data for Norway, Sweden and the UK were obtained from public and government websites as well as peer reviewed published articles. Biologically, the size and quantity of the contaminating fetal DNA in Meruvax II and Havrix as well as the propensity of various cell lines for cellular and nuclear uptake of primitive human DNA fragments were measured and quantified using gel electrophoresis, fluorescence microscopy and fluorometry. Lastly, genomic analysis identified the specific sites where fetal DNA fragment integration into a child's genome is most likely to occur.

Results: The average MMR coverage for the three countries fell below 90% after Dr. Wakefield's infamous 1998 publication but started to recover slowly after 2001 until reaching over 90% coverage again by 2004. During the same time period, the average autism spectrum disorder prevalence in the United Kingdom, Norway and Sweden dropped substantially after birth year 1998 and gradually increased again after birth year 2000. Average single stranded DNA and double stranded DNA in Meruvax II were 142.05 ng/vial and 35.00 ng/vial, respectively, and 276.00 ng/vial and 35.74 ng/vial in Havrix respectively. The size of the fetal DNA fragments in Meruvax II was approximately 215 base pairs. There was spontaneous cellular and nuclear DNA uptake in HFF1 and NCCIT cells. Genes that have been linked to autism (autism associated genes; AAGs) have a more concentrated susceptibility for insults to genomic stability in comparison to the group of all genes contained within the human genome. Of the X chromosome AAGs, 15 of 19 have double strand break motifs less than 100 kilobases away from the center of a meiotic recombination hotspot located within an exon.

Conclusion: Vaccines manufactured in human fetal cell lines contain unacceptably high levels of fetal DNA fragment contaminants. The human genome naturally contains regions that are susceptible to double strand break formation and DNA insertional mutagenesis. The "Wakefield Scare" created a natural experiment that may demonstrate a causal relationship between fetal cell-line manufactured vaccines and ASD prevalence.

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Newborn immune activation may have long-term negative impact on brain function

New research shows that triggering the immune system in infant mice produces symptoms often seen in autism spectrum disorder and other developmental conditions

Date: January 12, 2018

Source: McLean Hospital

Summary: Neuroscientists have found that even a brief episode of immune system activation within days of birth can cause persistent changes in sleep patterns concurrent with increases in epilepsy-like brain activity -- a combination of symptoms common in autism spectrum disorder (ASD) and other developmental conditions.

FULL STORY ***Note: Vaccination is intended to cause immune activation. The Hepatitis B vaccine is given on the day of birth and contains an aluminum adjuvant. The purpose of an adjuvant is to activate the immune system.***

McLean Hospital neuroscientists have found that even a brief episode of immune system activation within days of birth can cause persistent changes in sleep patterns concurrent with increases in epilepsy-like brain activity -- a combination of symptoms common in autism spectrum disorder (ASD) and other developmental conditions. The detailed findings are available in the January 12, 2018, issue of *Neuropsychopharmacology*.

"A growing body of evidence suggests that immune system activation, such as that caused by bacterial and viral infections, can play important roles in many brain disorders," explained William Carlezon, PhD, chief of the Division of Basic Neuroscience at McLean Hospital, and senior author of the paper. "While previous research in laboratory animals has established that immune activation during critical prenatal (before birth) developmental periods can later produce the core features of ASD, including decreased social interaction, aberrant communication, and increased repetitive behavior, we wanted to evaluate whether postnatal (during infancy) immune activation could also produce other symptom clusters that are often seen in ASD and related conditions."

In humans, ASD is also frequently associated with certain co-occurring medical conditions, such as sleep disorders and seizures. To determine whether early postnatal immune system activation can produce these types of effects, McLean researchers treated mice with a lipopolysaccharide (LPS), a chemical that simulates a bacterial infection and causes a temporary (1-3 day) activation of the immune system. The LPS was given at a time point in mice (9 days after birth) that approximates the stage of brain development in humans at birth after full-term pregnancy. The mice were then implanted with micro-transmitters that enabled the researchers to collect an uninterrupted stream of data on sleep, muscle movement, and activity levels. Data collection continued through 12 weeks of age, a time point considered to represent adulthood in mice.

Carlezon, who is a professor of psychiatry at Harvard Medical School, and his team discovered that temporary immune system activation shortly after birth produced two main findings in the adult mice. First, immune-activated mice spent more time in slow-wave sleep, a sleep phase often associated with systemic inflammation. Second, the mice also showed dramatic increases in brief (lasting 2-3 seconds) bouts of abnormal brain wave activity. These events had the hallmark characteristics of spike-wave discharges (SWDs), a type of epilepsy-like brain activity that

is not accompanied by full-body seizures. Although the SWDs occurred throughout the day, they were much more prevalent during periods when the mice were sleeping. When they occurred during wakefulness, they were accompanied by complete behavioral arrest -- a period of no movement throughout the body -- and immediately followed by recovery of normal brain activity and movement. Collectively, these findings demonstrate that even a brief period of immune system activation during critical periods of early development can leave a long-term signature upon the brain.

"The fact that immune system activation can produce these effects on its own, without any type of accompanying injury or trauma, provides new insight on the many paths that can lead to abnormal brain function" said Carlezon. "While there are clearly other factors that can cause these types of abnormalities, including genetic vulnerabilities, demonstrating that immune activation alone can produce these effects offers new hope for treatments that might reduce their severity, or prevent them altogether, in certain individuals."

While Carlezon's research focuses on animal models, his findings have implications for humans. The researchers believe that studying early developmental immune activation in mice may be valuable for diagnosing certain human illnesses and understanding how they develop. Persistent alterations in slow-wave sleep may represent a biomarker that could help differentiate immune-related neuropsychiatric conditions from those with other causes. Meanwhile, understanding epilepsy-like brain activity during both sleep and wakefulness may be useful in developing improved models of ASD. Studies in humans have shown that up to 60% of individuals with ASD experience SWDs during sleep, despite no diagnosis of clinical epilepsy, suggesting accuracy of the mouse model. The SWDs during wakefulness may resemble conditions such as "absence seizures" in humans, which are characterized by a brief loss of consciousness, a blank stare, and cessation of movement, and are often confused with inattention or intellectual disability.

"While more research needs to be conducted, these findings are a significant step forward in unlocking the mystery of ASD and other developmental disorders," said Carlezon.

Story Source:

Materials provided by **McLean Hospital**. *Note: Content may be edited for style and length.*

Journal Reference:

1. Galen Missig, Emery L Mokler, James O Robbins, Abigail J Alexander, Christopher J McDougle, William A Carlezon. **Perinatal Immune Activation Produces Persistent Sleep Alterations and Epileptiform Activity in Male Mice**. *Neuropsychopharmacology*, 2017; 43 (3): 482 DOI: 10.1038/npp.2017.243

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CBS NEWS INVESTIGATES ›

Family to Receive $1.5M+ in First-Ever Vaccine-Autism Court Award

BY SHARYL ATTKISSON
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The first court award in a vaccine-autism claim is a big one. CBS News has learned the family of Hannah Poling will receive more than $1.5 million dollars for her life care; lost earnings; and pain and suffering for the first year alone.

In addition to the first year, the family will receive more than $500,000 per year to pay for Hannah's care. Those familiar with the case believe the compensation could easily amount to $20 million over the child's lifetime.

Hannah was described as normal, happy and precocious in her first 18 months.

Then, in July 2000, she was vaccinated against nine diseases in one doctor's visit: measles, mumps, rubella, polio, varicella, diphtheria, pertussis, tetanus, and Haemophilus influenzae.

Afterward, her health declined rapidly. She developed high fevers, stopped eating, didn't respond when spoken to, began showing signs of autism, and began having screaming fits. In 2002, Hannah's parents filed an autism claim in federal vaccine court. Five years later, the government settled the case before trial and had it sealed. It's taken more than two years for both sides to agree on how much Hannah will be compensated for her injuries.

In acknowledging Hannah's injuries, the government said vaccines aggravated an unknown mitochondrial disorder Hannah had which didn't "cause" her autism, but "resulted" in it. It's unknown how many other children have similar undiagnosed mitochondrial disorder. All other autism "test cases" have been defeated at trial. Approximately 4,800 are awaiting disposition in federal vaccine court.

On July 20, 2010, respondent filed a Proffer on Award of Compensation (Proffer). On July 20, 2010, petitioners orally accepted respondent's Proffer. Based on the record as a whole, the undersigned finds that petitioners are entitled to an award as stated in the Proffer. Pursuant to the terms stated in the attached Proffer, the court awards petitioners:

1. A lump sum payment of $1,507,284.67, representing compensation for life care expenses expected to be incurred during the first year after judgment ($624,713.32), lost future earnings ($674,410.67) and pain and suffering ($208,160.68), in the form of a check payable to petitioners, as the court appointed guardian(s)/conservator(s) of the estate of Child Doe/77, for the benefit of Child Doe/77. No payments shall be made until petitioners provide respondent with documentation establishing that they have been appointed as the guardian(s)/conservator(s) of Child Doe/77's estate;

Time Magazine summed up the relevance of the Poling case in 2008: ...(T)here's no denying that the court's decision to award damages to the Poling family puts a chink -- a question mark -- in what had been an unqualified defense of vaccine safety with regard to autism. If Hannah Poling had an underlying condition that made her vulnerable to being harmed by vaccines, it stands to reason that other children might also have such vulnerabilities."

Then-director of the Centers for Disease Control Julie Gerberding (who is now President of Merck Vaccines) stated: "The government has made absolutely no statement indicating that vaccines are a cause of autism. This does not represent anything other than a very specific situation and a very sad situation as far as the family of the affected child."

Sharyl Attkisson is a CBS News investigative correspondent based in Washington.

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THE BLOG

High Rates of Autism Found in Federal Vaccine Injury Program: Study Says More Answers Needed

05/10/2011 12:41 pm ET | **Updated** Jul 10, 2011

By David Kirby

On Tuesday in Washington, members of the Elizabeth Birt Center for Autism Law and Advocacy (EBCALA), along with parents and children who received federal vaccine injury compensation, are having a press conference "to unveil an investigation linking vaccine injury to autism."

For the past two decades, according to the group, "the federal government has publicly denied a vaccine-autism link, while at the same time its Vaccine Injury Compensation Program (VICP) has been awarding damages for vaccine injury to children with brain damage, seizures and autism."

Their investigation, "based on public, verifiable government data, breaks new ground in the controversial vaccine-autism debate," and reports that "a substantial number of children compensated for vaccine injury also have autism — the evidence suggests that autism is at least three times more prevalent among vaccine-injured children than among children in the general population."

The following is a written Q&A conducted with EBCALA Directors:

Q) What is the Elizabeth Birt center, and who are the principal investigators on this project?

A) The Elizabeth Birt Center for Autism Law and Advocacy (EBCALA) is a nonprofit organization founded in 2008 to educate lawyers, advocates and parents about the legal challenges of autism (www.ebcala.org). The authors of this study are EBCALA board members. Mary Holland, Robert Krakow and Lisa Colin are attorneys and Louis Conte is a law enforcement officer who served as lead investigator.

Q) What were the main findings of this investigation?

A) The investigation found 83 cases of autism associated with compensated cases of vaccine-induced brain injury. It found that autism is at least three times more prevalent among vaccine injured children than among children in the general U.S. population today.

Q) How were the data compiled and analyzed?

We began collecting data over two years ago. We asked the federal government to provide us with this data through a Freedom Of Information Act request. We were told that our request would take four to five years, would cost $750,000, and would afford us incomplete information. We then assembled data about VICP decisions from legal databases and settlement information from publicly available docket reports. We found 21 published cases detailing autism spectrum disorders by name or description, which the study includes. We then interviewed families that we located through the docket reports. We trained interviewers to use a structured interview form for gathering information about the compensated cases. We also asked parents to complete standardized screening questionnaires for autism and to provide additional documentation. In these interviews, 62 families reported autism associated with vaccine injury.

Q) What evidence do you have that all these children actually received an ASD diagnosis?

A) In almost half the 83 cases, we have confirmation of autism beyond parental report, including medical and education records and completed standard autism screening questionnaires which are have a high degree of accuracy. The study calls for the complete medical review of all compensated cases of vaccine injury, including formal autism diagnosis, where appropriate.

Q) The government has conceded that vaccine injury can lead to brain disease (encephalopathy) and seizure disorders, but what scientific evidence is there to show that these injuries can result in autism symptoms?

A) This study is a review of decided and settled cases of vaccine-induced brain injury; it does not purport to be a scientific study. HHS or the Vaccine Injury Compensation Program compensated these cases based on the best available scientific information. Through interviewing the families of compensated claims, we have uncovered an association between vaccine-induced brain injury and autism. The article suggests that there is significant overlap between the definition of autism and the VICP's definitions of encephalopathy, seizure and sequela (resulting events).

Q) Don't these results simply suggest that children with ASD are more susceptible to vaccine injury than typical children? In others words, wasn't the injury an effect, rather than a cause of the ASD?

A) The parents interviewed in this study report that vaccines caused their children's autism as well as brain damage and seizures. The study notes a clear association between vaccine injury and autism in 83 compensated cases. The government has not previously brought this association to public attention. Whether this association between vaccine injury and autism is causal is one of the critical unanswered questions to which the study seeks answers. That is why the study calls on Congress to investigate further and to require full medical and scientific evaluation of all compensated claims of vaccine injury.

Q) Many critics say that it is easy to win a case in the VICP ("Vaccine Court") and that the legal standards of proof are much lower than in civil court. Dr. Paul Offit called it a "Kangaroo Court" after Hannah Poling won compensation for her autism and epilepsy (though he praised the court when it ruled against children with autism) — what is your response?

A) The VICP and HHS rely on the best science available in making compensation decisions. Proceedings are based largely on scientific and medical evidence in a field that the Court of Appeals for the Federal Circuit has described as "bereft of complete and direct proof of how vaccines affect the human body." Less than one in five claims in the VICP have received compensation. There is little question that those cases that have received compensation, including the 83 noted in our study, were the result of vaccine injury. Yet despite having received compensation, most of the families we interviewed were highly critical of the VICP, finding it to be exceptionally slow, parsimonious and hostile to petitioners.

Q) Critics also charge that these are merely legal decisions made by administrative judges, and not scientific conclusions based on rigorous analysis of all the existing data, and as such, they have no bearing on the debate about the causes of autism. Your reply?

A) We disagree. These compensation decisions are based on the best medical and scientific information available to the VICP and HHS. Many peer-reviewed scientific studies have used these compensated cases to elucidate the nature of vaccine injury. We have uncovered an association between vaccine injury and autism. Because we were only able to reach a fraction of the more than 2,500 individuals compensated for vaccine injury, we believe that we have identified the tip of the iceberg of this association. The study calls on Congress to investigate further and to ensure rigorous scientific analysis of all cases of compensated vaccine injury.

Q) Others contend that most of the seizure disorders reported in your paper were compensated following DTP vaccination, and that the government removed seizure disorders as an outcome of the DTP vaccine years ago after new evidence showed there was no association.

They contend that these cases should not have been compensated and do not provide evidence of an association between vaccines and autism, especially since many ASD children also suffer from seizure disorders. Your response?

A) Residual seizure disorder was removed as a presumption for vaccine injury from the VICP; that certainly does not mean that vaccines no longer cause seizure disorders in some children. In practice, the removal has meant that compensation for vaccine-induced seizure disorder is more difficult, but there have many compensated cases since that removal nonetheless. The study details compensation decisions before and after the removal of residual seizure disorder as a presumption for causation.

Q) How many of the cases you listed were for DTP vaccine, and what other vaccines were involved?

A) Diphtheria-petussis-tetanus (DPT) is the stated cause for 62 of the 83 cases; measles-mumps-rubella (MMR) makes up the next largest group, followed by cases caused by the diphtheria-acellular pertussis-tetanus (DpaT) vaccine.

Q) Many people will dismiss this paper as the act of desperate parents who willfully ignore all of the epidemiological studies done to date that show no link between vaccines and autism. Do they have a point? Again, why should anyone care about the legal proceedings of some obscure court when so much published science says otherwise?

A) Government officials in HHS or the VICP decided that the children in this study suffered vaccine injury based on science. We uncovered that these children also have autism. How can the government then continue to assert that there is no link between vaccines and autism? If in fact there is no link, why would there be even one case of vaccine-associated autism, let alone 83? The government itself is now calling for more research on vaccines and autism, including the VICP itself. Congress should investigate the vaccine-autism association in the VICP.

Q) In addition to being legal professionals and EBCALA board members, the authors are parents of children with an ASD diagnosis, and two of them have claims before the VICP. Does this bias the reporting and analysis of the study?

A) The two authors' pending claims on behalf of family members are disclosed; those claims are not the subject of this study in any way. The authors' experiences fuel their motivation in undertaking this investigation; they do not bias the study results which are based exclusively on government compensation decisions and structured interviews conducted by trained researchers. No attorney-authors conducted interviews with parents or caregivers; only trained non-lawyer researchers conducted interviews to avoid any possible conflict of interest.

Q) Where and when will this paper be published?

A) The article will be published on Tuesday, May 10 in the Pace Environmental Law Review at digitalcommons.pace.edu.

Q) What impact do you hope it will have?

The article calls on Congress to investigate the Vaccine Injury Compensation Program and to ensure that there is a medical review of all compensated cases of vaccine injury. We hope that the article leads to these results.

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High Rates of Autism Found in Federal Vaccine Injury Program: Study Says More Answers Needed | HuffPost

https://www.huffingtonpost.com/entry/high-rates-of-autism-foun_b_859234

Screen captured: 7/10/2018

Review Article

What is regressive autism and why does it occur? Is it the consequence of multi-systemic dysfunction affecting the elimination of heavy metals and the ability to regulate neural temperature?

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There is a compelling argument that the occurrence of regressive autism is attributable to genetic and chromosomal abnormalities, arising from the overuse of vaccines, which subsequently affects the stability and function of the autonomic nervous system and physiological systems. That sense perception is linked to the autonomic nervous system and the function of the physiological systems enables us to examine the significance of autistic symptoms from a systemic perspective. Failure of the excretory system influences elimination of heavy metals and facilitates their accumulation and subsequent manifestation as neurotoxins: the long-term consequences of which would lead to neurodegeneration, cognitive and developmental problems. It may also influence regulation of neural hyperthermia. This article explores the issues and concludes that sensory dysfunction and systemic failure, manifested as autism, is the inevitable consequence arising from subtle DNA alteration and consequently from the overuse of vaccines. (Ewing G.W. What is regressive autism and why does it occur? Is it the consequence of multi-systemic dysfunction affecting the elimination of heavy metals and the ability to regulate neural temperature? **North Am J Med Sci** 2009; 1: 28-47).

Key words: autism, physiological systems, autonomic nervous system

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Introduction

That the occurrence of autism has risen steadily in the last decades is not in dispute. Prior to the 1930's and the introduction of vaccinations autism was unknown. By 1968 in the UK, when Polio and DPT vaccines were given at 6 and 7 months autism was very rare. In 1988, when Polio and DPT was given at 3 months, DPT at 5 months and MMR at c13 months autism rates were still low. In 1996, when Polio and DPT/HIB injections were given at 2, 3 and 4 months, followed by MMR at c13 months autism rates began rising rapidly. By 2006 the occurrence of autism had reached pandemic proportions. In the period shortly before the 1980's the occurrence of autism was estimated to be circa 3-5 per 10,000; the majority having autism from birth [1]. Since the introduction of multiple vaccines the prevalence of autism has increased to an estimated 1 in 166 i.e. 60 per 10,000. Furthermore the trend is that of a continued increase. Some British teachers are claiming to see ASD in one in every 86 children [2]. This is supported by research which suggests that one in 100 British children may have some form of autism [3] and that ASDs are more prevalent than hitherto imagined [4] i.e. only severe cases of autism are recorded in the statistics. Such claims have been dismissed as mere speculation on the basis that there is not yet definitive proof of such claims however the perceived lack of evidence does not indicate that proof does not exist [5,6]. It may indicate that the understanding of the condition remains 'beyond the prevailing level of knowledge' (Table 1) [7].

Table 1

~1980	3-5 per 10,000
By 1985	6-10 per 10,000
By 1997	30-35 per 10,000 [8]
By 2001	60 per 10,000 (1 in 166) [266]
By 2002	Less than 1 in 100 [3]
In 2009	no sign of plateauing [9]

By 1985 the incidence of regressive autism had equalled that from birth. By 1997 both types had increased although the regressive form was now >75% of the total occurrence. This suggests that an acquired condition was overtaking birth defects or purely genetic conditions. Autism affects four boys to every girl [10]. By contrast Autism appears not

to occur in communities which do not use vaccines [11]. It occurs in immigrants from tropical climates who appear to have greater familial predisposition to autism [12] e.g. among Somali students in Minneapolis there was a rate of 1 in 28 (which compares with the local average of 1 in 56). This is more than five times the national rate of 1 in 150. Since the 1960's the number of vaccines given to a child before entering school has risen to c33. In children born to military families the occurrence of autism may now be as low as 1 in 67. In the vast majority of cases, the emergence of autistic indications appears to happen in children who had developed normally [10,13,14], and before three years [15, 16]. The development of normal immune function appears to cease in the second year and is linked to the schedule of vaccines [17] and/or the MMR vaccine [18,19]. The consequences to society are estimated at c£2.4M in an autistic child's lifetime [20] which, if it continues to increase as many predict, will impose an unsustainable financial burden upon healthcare, education and social welfare systems.

The Systemic Nature of Physiology and Function

The body is a bio-dynamic, wholistic and systemic organism. It responds to sensory input which enables the autonomic nervous system thereby influencing behavior, the regulation of physiological systems, and function of the visceral organs (Fig. 1). The established association between visual perception, the autonomic nervous system, physiological systems, and biochemistry [21] raises issues which may be relevant to autism research.

- Different diseases are associated with differing colour perception [22] e.g. a yellow-blue deficit in diabetes [23], etc
- Different drugs are associated with altered color perception [24].
- Enzymes/Proteins are active in the visual spectrum [25,26].
- Suppressed immune function affects cognition [27]. In particular, t-cell deficiency (a common indicator of stress) is linked to cognitive dysfunction.

Any form of biochemical variation must therefore influence sense perception, sensory coordination and cognitive function. The existence of the physiological systems is not in doubt although there is not universal agreement on their structure. There is wide recognition that they regulate the function of organs (in each system), and that there are higher and lower levels for each system (homeostatic limits), however such systems remain an elusive and under-researched area of medicine. The Russian researcher I.G.Grakov [28,29] has mathematically modelled the consequences of cognition upon the autonomic nervous system and physiological systems. This included identifying and mapping the nature and structure of the physiological systems (Table 2).

Physiological Systems

Sleeping, Breathing, Digestion, Excretion, Osmotic Pressure, Blood Pressure, Blood Cell Content, Blood Volume, Blood Glucose**,** Sexual Function, pH, Temperature, Posture and Locomotion. See Table 2.

Such an explanation is highly inclusive and complete by comparison to the currently accepted but exclusive and limited explanation. The essential functions of temperature, sleeping and pH are now included; excretion is not limited to urination; whilst blood cell content (and other related systems) comprise what has hitherto been regarded as the immune system. Absorption of nutrients is influenced by system function including (but not limited to) blood pressure, blood volume, blood cell content, pH, temperature, etc. Elimination of toxins is similarly influenced by the complexities of system function.

The brain manages the autonomic nervous system and the function of the physiological systems. In addition, the brain waves are in a dynamic relationship with molecular biochemistry illustrating how drugs can be used to influence the body's biochemistry in order to act upon the symptoms of disease and how brain wave technologies such as neurofeedback can be used to alter the brain waves, physiological systems, organs, cells and molecular bio-chemistry.

Such systems regulate the function of the body's biochemistry e.g. (1) Most enzymatic reactions in the body are temperature dependent and catalysed by Magnesium. (2) The body requires maintenance of pH within a narrow operating range, and also the supply of minerals and vitamins/cofactors, to catalyse protein-substrate reactions in the body. (3) Appropriate blood volume, blood pressure, blood cell content and pH are required to ensure optimal absorption of minerals, vitamins, fatty acids from the intestines.

It is increasingly accepted that the synchronised activity of groups of neurons [30] in functionally coherent structures (the physiological systems), which exist in the brain *and the body*, synchronise their electrical impulses [31]. This may be evident when noting the evoked visual potentials, indicative of neural synchronisation, which are atypical in autism [32] and which may be part of the processes influencing sense perception (figure 1), sense coordination, memory [33], learning, etc. If so, this indicates that sensory input through the neurovisual pathways is integrated into actions, behaviour and movement and that learning requires synchronised activity between the brain, sensory organs [34-36], and visceral organs. This is severely disrupted in the autistic [37]. Autism affects the function of all of the brain [38-40]. It is a neurobiologic, multi-systemic disorder i.e. affecting the function of every organ but not necessarily its structures [41]. It affects all aspects of the autonomic nervous system and hence influences all aspects of brain's function including that of neural networks involved in learning, memory, the function of the senses and the visceral organs.

Table 2 Typical Physiological Systems

1. Posture Organs and Functional Systems monitored: Brain, Pituitary Gland; Thyroid Gland; Adrenal Glands; Spinal Cord; Peripheral Nervous System; Skeletal and Muscular System; Blood and Peripheral Blood Vessels.
2. Sleeping Pattern Organs and Functional Systems monitored: Brain; Pituitary Gland; Spinal Chord; Peripheral Nervous System; Ear and Nose.
3. Blood Pressure Organs and Functional Systems monitored: Brain; Pituitary Gland; Thyroid Gland; Adrenal Gland; Liver; Heart; Spleen; Blood and Peripheral Blood Vessels.
4. Digestion Organs and Functional Systems monitored: Brain; Pituitary Gland; Thyroid Gland; Adrenal Gland; Liver; Gall Bladder; Pancreas; Blood and Peripheral Blood Vessels; Oesophagus; Stomach; Duodenum; Small Intestine; Large Intestine.

(Origin: I.G.Grakov)

The cerebellum, considered to be implicated in autistic spectrum disorders [42] comprises an estimated 50% of the brain's total processing capacity yet its role is not clear or understood [43]. It is involved in the accumulation of sensory data from the internal environment, including the organs in the body and those in the brain (including the sensory organs), thus distinguishing between sensory input from the external environment (a significant function of the cerebrum) and that of the biochemistry affecting the function of every organ (a significant function of the cerebellum), including the cerebellum. Such a role includes the processing, regulation and distribution of this data, through structures such as the Purkinje cells in the cerebellum which are attached by nervous structures to every part of the body. This includes the receipt of biosignals involved in the processes of movement, coordination and balance. Impaired flow of data to the brain via the cerebellum (and brainstem) may lead to functional problems affecting the body's fine control of e.g. balance, coordination, etc. Movement and balance involve the coordinated function of all body systems and organs and are coordinated by (1) sensory feedback from the external and internal environments and (2) the allocation of energy resources to and from each organ. They are dependent upon the precise nature, and timing, of data about each organ being provided to and by the cerebrum *and* cerebellum. This illustrates how the brain determines behaviour and actions appropriate to developing situations. It illustrates how changes at the organ, cell or molecular level influence brain function and vice-versa.

There are indications of cerebellar dysfunction in autism [44]. Inhibited flow of data to the cerebellum may be followed by developmental decay, cerebellar dysfunction [45,46], and reduced size of brain-stem. This is equivalent to the 'use it or lose it' phenomena affecting muscle tone and function.

Without cognitive input the brain cannot and does not function. Disease and drugs create cognitive dysfunction, altered sense perception, in particular affects visual perception. Accordingly, vaccines must also influence sense perception and coordination. Vaccines have a long-term influence and hence may have a more pervasive influence upon sense perception.

Our cognitive function depends upon the extent and coordination of sense perception i.e. between the eyes, ears, nose, mouth and skin. Genetic and/or environmental influences affect sense perception, the degree of sensory coordination and ultimately our connectedness with the surrounding world. Visual function is linked to the primary mechanism (rods, cones and pigments) but is also influenced at the biochemical level – noted by how pathology and drugs alter color perception [22,47] and affect the magnocellular and parvocellular neurovisual pathways which alter color perception and visual contrast. This influences the stability and function of the autonomic nervous system [48] and alters the processes of memory fixation, concentration, and behavior [49].

Anyone contracting disease e.g. measles, mumps, rubella, tetanus, etc; experiences altered visual perception therefore a weakened strain of the disease e.g. in vaccines, must also influence visual perception/cognition. Chronic disease is also accompanied by significant cognitive dysfunction and influences the coordination and processing of sense signals by the brain. The greater the number of illnesses, drugs or vaccines [50] the greater the alteration to the body's biochemistry therefore the greater its influence upon sense function and the degree of sensory distortion. It influences the autonomic nervous system and physiological systems and hence the coordination and function of every organ – visceral and sensory. This is a significant feature of autism [51,52].

Almost all diseases are linked to cognitive and behavioral disorders. Conversely, behavioral traits are influenced by biochemistry e.g. testosterone, oestrogen, cortisol, oxytocin, adrenaline, etc. Oxytocin influences the formation of social bonds influencing social engagement and attachment - which are dysfunctional in the autistic child [53-57].

Autonomic nervous system dysfunction?

In general problems with the stability of the autonomic nervous system [21,58] can be expected to be manifest as follows:

• Loss of Sense perception and Sensory Coordination
• System dysfunction (e.g. influencing breathing, blood pressure, heart rate, etc)
• Behavioural dysfunction (including learning problems, information feedback)
• Problems with Diet and Elimination (of toxins and wastes)
• Impaired and/or Delayed Neural Development
• Atypical brain waves

These are prevalent in autism.

Evidence of Systemic Dysfunction in Autism

Multi-systemic dysfunction is associated with a wide range of physiological disorders e.g. diabetes and obesity [59], cancer, cardiovascular disorders, pre-eclampsia, dyslexia [60], depression, etc. It affects the central [39] and autonomic nervous system in autistic children [61]. Systemic dysfunction in Autism includes that of temperature, blood cell content and immune function [62], blood pressure [63,64], digestion, excretion, posture and locomotion, sleep [65-67], pH, breathing; respiration rates, lower skin temperature. Each influences metabolic rate [68]. Autonomic dysfunction has also been linked to problems with appetite, swallowing food, nausea, recurrent vomiting, and abdominal bloating; constipation or diarrhoea; dry eyes, dilated pupils; dry skin, flushed skin following a meal, abnormal sweating, and unexplained high fevers; sleep apnoea, insomnia; bed-wetting, difficulty urinating, difficulty potty-training; altered perception of pain, sensory defensiveness, poor socialisation skills, anxiety, phobias, tics, emotional instability; and light intolerance. That autistic seizures are often linked to neural blood flow [69-71] is supported by fact that medications used to raise or lower blood pressure can alter the occurrence of seizures and improve sleep in the autistic child.

Autism affects sensory processing and sensory coordination [72] which is manifest in various ways e.g. tactile perception [73], vision [74], hearing [75], and smell. Autistic children may also display synaesthesia in which sensations become confused with one another [76]. Sounds may be experienced as touch or as visual stimulation e.g. autistic children may cover their eyes when they hear a loud sound. That autistic children have such sensory synaesthesia and sensitivity may indicate that their brains have extreme problems with sensory processing, regulation and coordination [77,78,60].

Vaccines and Vaccine Side-effects

Background

The introduction of modified live viruses as vaccines enable the virus to attach its genetic material into the cell which replicates i.e. the host cell continues to function whilst producing the viral protein. This stimulates the production of antibodies. Under normal circumstances exposure to a viral disease would be countered (in vivo) at various levels enabling the body to steadily increase its immune response. By contrast, the injection of vaccines directly into the blood system overpowers the normal immune response leading to its rapid depletion. It is now suspected that long-term persistence of viruses and other proteins may produce chronic disease i.e. instead of producing a genuine immunity the vaccines are altering the body's systemic and biochemical stability, suppressing the production of differing types of white blood cells and hence immune function. Furthermore the introduction of many vaccines (up to 30 in a typical vaccination schedule) introduces a large number of foreign proteins which may be sufficient to ensure that immune function never returns to baseline and/or that immune biochemistry is fundamentally altered [62]. Consequently there now exists a growing concern which links immunizations to the huge increase in recent decades of auto-immune diseases [79] e.g., rheumatoid arthritis [80,81], multiple sclerosis, lupus erythematosus, lymphoma, leukemia, autoimmune demyelinative optic neuritis, diabetes mellitus, etc.

Vaccinations influence the balance of viral scavengers [82,83]. They suppress the production of b-cells, t-cells, etc. The synergistic action of these cells impairs antibody formation and becomes less effective in phagocytosis. This influences recognition of viral pathogens, leads to the progressive failure of immune function and hence to the increased incidence of auto-immune disease which we note as allergies [84-86] and immunodeficiency [87].

Some vaccinations have a greater effect than others e.g. Hib vaccine, pertussis vaccine [88-90], measles vaccine [91], etc. Indeed some articles indicate that the use of such vaccines can reliably induce asthma [92] by moderating adrenergic function [93].

Modified live viruses alter the structure and function of DNA. Each virus is a large molecule therefore its spatial arrangement must be influenced by its biochemistry which influences cross-helical structures and linkages within the DNA helix. Accordingly it is inevitable that the steady accumulation of such foreign proteins arising from an intensive vaccine programme will reach the stage where it significantly weakens DNA, gene, and chromosome structure and function. The prevailing reaction conditions - the consequence of protein expression which has been influenced by previous vaccines - will also affect the introduction of each modified live virus. Each will depress immune function. The greater the number of viruses and foreign proteins (1) the greater the influence upon immune function and the time required for recovery from each vaccination; (2) the greater their influence upon DNA, gene and chromosome structure and function, the greater will be the risk of protein inhibition, system dysfunction, reproduction, etc.

The greater the amount of vaccines, introduction of foreign

proteins and hence of alterations to the body's biochemistry the greater the risk that the body's immune function no longer recognizes or responds to existing vaccines or diseases [94] and/or that its immune response has been altered [95] and/or that sugar chains attached to an antibody alters its ability to bind to its receptors [96]. This may lead to mutated forms of disease [97-104] e.g. the reemergence of whooping cough [105], and a differentiated disease profile e.g. up to 30 per cent of individuals with a persistent cough are infected with B. pertussis [106]. Furthermore enhanced susceptibility to virus infection by vaccines is documented [107]. This could enable tougher strains to flourish [108].

Vaccines are not entirely safe. The currently used vaccines are merely less unsafe than previous vaccines [109,110] e.g.

• The Urabe strain of mumps vaccine in the MMR vaccine was replaced by the Jeryl Lynn mumps strain in response to reports from Japan linking the Urabe strain used, in the MMR vaccine, with high levels of meningoencephalitis.

• The Pluserix-MMR and Immramax-MMR vaccines were withdrawn because of reports of mild transient meningitis. The withdrawal of the smallpox vaccination led to a reduction in the incidence of TB.

• The Rubini vaccine continues to be used in some European territories although discredited [111].

• Leningrad-Zagreb strain is commonly used in developing countries, and may have superior efficacy when used during epidemics [112,113].

• Different strains of disease have different safety profiles [114]

• Different strengths of vaccine [115] carry risks which affect age groups or sexes differently.

• There are concerns over the use of whole-cell vaccines [116,117] although some argue that acellular vaccines are less effective [118].

• Sudden Infant Death Syndrome has been largely eradicated following withdrawal of the pertussis vaccine in Sweden and Japan.

• Side-effects arising from vaccination are associated with the onset of autoimmune disease [79,119], arthritis, diabetes mellitus, autoimmune demyelinative optic neuritis, etc.

• Sensory defects are a c ommon side-effect of vaccines [120-122] e.g. sensori-neural hearing loss induced by the MMR vaccine.

• Drugs inhibit the effectiveness of vaccines (see 3.3.2). Systemic glucocorticoids (steroids) suppress the immune system and create risk of disseminated infection from live virus vaccines [123]. Vaccines may also be influenced by levels of immune function, dietary factors, and stress [124]. Many parents of autistic children and a number of medical experts believe the MMR vaccine is the culprit behind autism. In c15-20% of children it causes fever 7-12 days following immunization.

What are the risks from the diseases against which a vaccine is meant to protect?

Diphtheria, Polio, Tetanus, Meningitis, Pertussis

Diptheria [125], Polio and Pertussis have largely been eradicated in the developed world although there may now be mutated forms of disease, a differentiated disease profile and/or an altered immune profile, which may be responsible for further outbreaks in vaccinated children and adults. Diphtheria is an upper respiratory tract infection characterized by sore throat and minor fever. It affects the central and peripheral nervous systems leading to deterioration of myelin sheaths, loss of motor control and sensation. Fatality rates are 5-10% although the rate of mortality may be higher for those under 5 years and over 40 years. It can be treated by antibiotics which prevent its transmission e.g. using erythromycin, procaine penicillin G, rifampin or clindamycin. Other minor complications including neck swelling, nausea, vomiting, listlessness, pallor, and a racing heart beat; lead to long term effects e.g low blood pressure, cardiac myopathy and peripheral neuropathy. Poliomyelitis is an infectious viral disease. Although c90% of polio infections are symptom-free, if the virus enters blood circulation this may lead to further complications. In c1% of cases, where the virus enters the central nervous system, it in fects and/or destroys motor neurons thereby leading to muscle weakness and paralysis, usually involving the legs. Tetanus infection occurs through open wounds. It occurs commonly in hot, damp climates with soil rich in organic matter. It creates muscle spasms in the jaw, difficulty in swallowing, muscle stiffness and spasms throughout the body. The neonatal form of the disease is a significant public health problem in the developing and/or agricultural economies. There are about one million cases of tetanus reported each year, mainly in the developing world, causing an estimated 300,000 to 500,000 deaths. In the United States, there are about five deaths from tetanus each year. Tetanus is the only disease that is infectious but not contagious. Pertussis is a highly contagious disease. There are 10–90 million pertussis cases and about 600,000 deaths per year. Sixty percent of all cases occur in the developing world. In children it is characterized initially by mild respiratory infection symptoms before developing into the characteristic 'whooping' cough. Other complications may include encephalitis, pneumonia, and secondary bacterial infections. Naturally-acquired disease caused by Hib (H. influenza) appears only to occur in humans with low natural immunity [126]. In infants and young children, H. influenza type b may cause pneumonia, and acute bacterial meningitis. Both

H. influenza and S. pneumonia can be found in the upper respiratory system of humans i.e. both reside naturally in the body. Alterations in the immune response; attributed to poor nutrition, stress or transmission; enable their proliferation with potentially serious outcomes.

Measles, Mumps and Rubella

Measles is largely a co nsequence of compromised immunity arising from poor diet and is linked to high levels of mortality [127] in the developing world. In developed countries, most children are immunized against measles by the age of 18 months, generally as part of the triple vaccine treating measles, mumps and rubella (children younger than 18 months usually retain measles antibodies (Immunoglobulins (Ig)) transmitted from the mother during pregnancy). The rate of mortality from measles is typically 0.3% however in the developing world this may be as high as 28%. The classical symptoms of measles are typically fever (up to 40C), cough, coryza and conjunctivitis. Complications include mild diarrhoea, pneumonia, encephalitis, SSPC, and corneal ulceration or scarring. They are usually more severe amongst adults. Permanent hearing loss or damage to vision is recognized complications of measles. Measles has been known to occur in children with congenital rubella syndrome, and has been implicated in the etiology of inflammatory bowel diseases (IBDs). The more common symptoms of mumps are parotitis, fever (typically 38.3C), headache and orchitis [128]. Other symptoms of mumps include sore face and/or ears, and loss of voice. Known complications of mumps include infection of other organ systems, sterility in older men, mild forms of meningitis, encephalitis, sensorineural hearing loss, pancreatitis, inflammation of the ovaries, and risk of spontaneous abortion during pregnancy. Rubella is a mild disease which often passes unnoticed [129]. The primary reason for the introduction of a v accine is to prevent infection during pregnancy. The common symptoms of rubella are the appearance of a rash on the face, trunk and limbs (after an incubation period of 14-21 days) which usually fades after several days. Other symptoms include fever (typically 38C), swollen glands (post cervical lymphadenopathy), joint pains, headache and conjunctivitis. Rubella is generally a mild disease, rare in infants or those over the age of 40. The older the person the more severe the symptoms e.g. some women experience arthritis type symptoms. Children exposed to rubella in the womb may show developmental delay, inhibited growth, hearing disabilities, diabetes, glaucoma, schizophrenia, etc. If infected during the first 12 week period of pregnancy this may lead to congenital rubella syndrome (CRS), which is manifest as a series of complications including spontaneous abortion and, in the neonate: cardiac, cerebral, ophthalmic and auditory side-effects. Known complications include prematurity, low birth weight, and neonatal thrombocytopenia, anemia and hepatitis. CRS is the main reason a vaccine for rubella was developed. It increases the risk of miscarriage or still birth in mothers who contract rubella shortly before or early in pregnancy. If the baby survives, it may have heart disorders, blindness, deafness, etc. CRS is manifest as sensorineural deafness, eye problems, heart disease. Other complications include low birth weight, mental retardation, problems with the spleen, liver and bone marrow, etc. Hepatitis B is difficult to catch and comes from blood or sexual contact with an infected carrier. Further, vaccine-derived immunity is thought to be short-lived. Hpv**,** an infection transmitted during sexual intercourse, clears naturally after several months/years. Mumps and Rubella may occur without the patient being aware that they have the disease.

Some diseases may confer natural immunity e.g. the mumps virus may confer a degree of immunity against ovarian cancer [130-133].

In summary, disease side-effects reflect the effect of the disease upon the body's functional systems i.e. upon temperature, digestion, excretion, etc. Typical viral fevers are circa 1-2C above the body's normal body temperature. Measles is particularly noteworthy because fever may reach 40C (or higher), some 3-4C above normal body temperature and just 1C below the point where proteins denature and at which brain death commences.

What are the risks from the Vaccine? Typical vaccine side-effects

There is evidence that BCG and measles vaccinations administered singly reduce child mortality [134] but that this is unrelated to the incidence of measles or measles deaths [135,136]. By contrast the pertussis vaccine is associated with a negative effect [137].

Dtap: Recorded common side-effects with the DtaP vaccine include *fever*, tiredness, poor appetite, vomiting and inflammation. Less common and more severe side-effects include distress (crying), seizures, lowered consciousness or *coma*, brain damage.

MMR: Recorded common side-effects with the MMR vaccine include *fever*, *swelling of the lymph glands*, tiredness, poor appetite, and *abhorrence of bright lights*. More severe problems include *low platelet count*, pain and stiffness in the joints/inflammation. Less common and more severe side-effects include distress (crying), seizures, *deafness*, lowered consciousness or *coma*, brain damage.

Tdap: Recorded common side-effects with the Tdap vaccine include pain, chills, *fever*, headache, tiredness, poor appetite, stomach ache, *vomiting, diarrhoea* and inflammation

The above listed vaccine side-effects are indicative of systemic instability affecting most physiological systems – temperature (chills and fever), excretion (inflammation of the lymph glands), blood cell content (low platelet count), excretion (diarrhoea), digestion (poor appetite, vomiting), sleep (coma), and metabolic rate (tiredness, lowered levels of consciousness). In addition there is evidence of altered sense perception, indicative of problems with the autonomic nervous system, which affects hearing, visual perception (abhorrence of bright lights), smell and touch.

Significant vaccine side-effects have been linked to swine flu vaccine (Guillain-Barre paralysis); in RSV vaccine [138]; in the measles, mumps and MMR vaccines [139]; hepatitis A and B vaccine [140]; tetanus vaccine; smallpox vaccine; polio vaccine; pertussis vaccine [141], etc. The incidence of vaccine side-effects may now be sufficiently great to question the claims that the risks from the disease exceed that of vaccines [109].

The MMR vaccine has been linked to autism, Crohn's disease, inflammatory bowel disease [142,143] and other serious chronic stomach problems [144], epilepsy, brain damage including meningitis [145,146], cerebral palsy, pancreatitis [147] and diabetes mellitus [148-150], encephalopathy, encephalitis [151,152], hearing and vision problems, arthritis, behavioural and learning problems, chronic fatigue syndrome, diabetes, Guillain-Barre syndrome, idiopathic thrombocytopaenic purpura, subacute sclerosing panencephalitis (SSPE), leukaemia, multiple sclerosis, and death.

There is evidence that in cases of immune deficiency that viruses continue to persist in the body [143,153-155]. The measles virus is known to persist in patients with subacute sclerosing panencephalitis (SSPE), measles inclusion body encephalitis (MIBE) [156] and multiple sclerosis [157]. Since the introduction of measles vaccines, vaccine-associated SSPE has increased in the USA. Furthermore patients with B or T-cell immunodeficiencies have cognitive side-effects [27] and are advised against vaccination due to the risk of severe and/or fatal infection (Merck). That viruses persist in the body and are linked to autoimmune disorders is a feature of rubella virus [158-160], anthrax vaccination [161], hepatitis B [162], etc. There is a reported increased risk of death with combined vaccination DPT and polio [134].

In summary, vaccine's side-effects reflect the vaccine's influence upon the body's functional systems i.e. upon temperature, digestion, excretion, blood cell content, etc.

The Cumulative Effect of Vaccines

There is concern that the cumulative effect of vaccines upon the body's function has not been properly assessed [137]. Unvaccinated children appear to have less exposure to disease [84,85], delaying vaccination reduces exposure to disease [163], contracting the disease naturally leads to less disease in future [164], and that excessive vaccination is considered ineffective and dangerous [165].

Vaccine-vaccine and Vaccine-drug interactions

In general, vaccines may be influenced by antibiotics [166], immunoglobulins, immunosuppressants, monoclonal antibodies, anticoagulants and corticosteroids. The interaction between a vaccine and a drug has been reported only with influenza vaccine and four drugs (aminopyrine, phenytoin sodium, theophylline, and warfarin sodium), and with BCG vaccine and theophylline. The clinical significance of vaccine-drug interactions is not fully determined [167]. There is further evidence of interactions involving most vaccines e.g. HPV Vaccine: (http://hpv.emedtv.com/hpv-vaccine/drug-interactions-with-the-hpv-vaccine.html); Shingles Vaccine: An Introduction: (http://senior-health.emedtv.com/shingles-vaccine/drug-interactions-with-the-shingles-vaccine.html); yellow fever vaccine; polio vaccine (neomycin, streptomycin, phenoxy ethanol, formaldehyde), rotavirus vaccine, etc.

Vaccines are not subject to double blind clinical trials despite the evidence of vaccine-drug interactions and perhaps also of vaccine-vaccine interactions.

Effectiveness of Vaccines/Vaccines are not 100% effective

Whooping cough is becoming increasingly prevalent [168-170]. Although claimed to be 88 per cent effective among children of 7-18 months, during a nationwide epidemic of whooping cough in 1993, a group of researchers discovered that 82 per cent had completed their full complement of DPT vaccines [171]. Others have commented that the whooping cough vaccine is only to be 36% effective [109].

Many studies show that the measles vaccine isn't completely effective [172-175] and that a significant proportion of those infected in measles outbreaks (>60%) had been vaccinated. There is also a lack of consensus concerning the effectiveness of whole or acellular vaccines, each having their own side-effects and effectiveness [176] e.g. vaccine efficacy was estimated at 75.4% for an acellular 5 component vaccine, 42.4% for an acellular two component vaccine and 28% for a whole cell DTP vaccine [177]. The whole-cell vaccine was associated with different levels of side-effects including significantly higher rates of crying, cyanosis, fever, and local reactions than the other three vaccines.

There is evidence of declining vaccine immunity [178] illustrated by transmission of mumps [179], measles [180,181], rubella [182], polio [183], Hib [184,185], Hepatitis B [186,187], smallpox, diphtheria, varicella [188], whooping cough [189], etc.

Effect upon Learning

One in 14 children i.e. up to half of all children starting school, have problems with speech, language and communication [190]. Is this significant bearing in mind [4] that the occurrence of autism may be more widely spread than has hitherto been considered possible i.e. that only the most severe and chronic cases of autism are recorded? Learning problems are a significant problem in autism [191]. It affects the body's processing of data from the external and internal environments. This affects, in the autistic, the ability of the autonomic nervous system to regulate organ function and hence influences their ability to make sense of the external world. The problem may be part of a spectrum of biochemical disorders [60] influencing all aspects of the learning process e.g. including memory, concentration, sense perception and sense coordination.

Biochemical Evidence

Biochemical Instability

Indications of almost complete physiological instability are manifest in the autistic as a proliferation of biochemical deficiencies e.g. (1) Fatty acid deficiency [192]; (2) a distinctly different immune response [62] including reduced natural killer cell activity [193], decreased immunoglobulins and T cells and altered lymphocyte functions [194-197], (3) Vitamin D deficiency [198]. Vitamin D regulates the levels of glutathione which may explain the link between heavy metals and autism. Depleted levels of glutathione increase oxidative stress, suppress the detoxifying effect of liver enzymes e.g. P450, reduce the elimination of heavy metals, and increase the neurodegenerative effects of heavy metals. Mercury inhibits the enzyme methionine synthase which converts homocysteine into methionine. Accordingly, levels of cysteine, glutathione and metallothionine are low. This illustrates that the methionine pathway may be faulty in many with autism and supports earlier suggestions that redox imbalances [199-200] and detoxification are impaired. (4) Vitamin A deficiency [201-202] is a commonly observed symptom of measles. The severity of complications have been linked to the degree of Vitamin A deficiency; (5) Carnitine deficiency [203]; (6) increased norepinephrine levels and decreased dopamine-hydroxylase activity [204]; (7) demonstration of inter- and intra- species differences in serotonin binding sites by antibodies from an autistic child [205]; (8) the levels of gut flora [206]; (9) Enterocolitis in Children with Developmental Disorders [207]; (10) Adenosine Deaminase Activity Decreased in Autism [208,209]; (11) Small intestinal enteropathy with epithelial, IgG and complement deposition in children with regressive autism [210]; (12) Mitochondrial disorder [211]. Findings suggest that mitochondrial dysfunction, including abnormal enzyme function, mitochondrial structure, and mitochondrial DNA integrity, may be present in children with autism [212].

Other biochemical deficiencies/chromosomal abnormalities include:

Phosphoribosylpyrophosphate (PRPP) synthetase superactivity, Adenylosuccinate lyase deficiency, Histidinemia, Lesch-Nyhan disease, Fragile X syndrome, Rett Syndrome, Dihydropyrimidine dehydrogenase (DPD) deficiency, Tuberous sclerosis, Superactivity of pyrimidine 5'-nucleotidase (P5N), etc.

The use of Drugs

Biochemical instability is a feature of autism. Accordingly, drugs are used to mitigate autistic symptoms e.g. (1) Lofexidine [213] has been shown to improve prefrontal cortical function in nonhuman primates. This is consistent with the view that the prefrontal cortex regulates executive/system function. (2) An open trial [214] suggested that methylphenidate use in autistic hyperactive children may ameliorate hyperactivity, and impulsivity in autistic children. (3) Neuroleptics e.g. haloperidol, are mildly effective in reducing hyperactivity, impulsivity, and inattention in children with autistic disorder [215]; clonidine is used in the treatment of tic disorders and ADHD [216]. Other drugs used include Tianeptine [217]; Galanthamine [218]; Immunoglobulins [219]; melatonin [220]; and beta-blockers [221].

The Cause of Autism

The occurrence of autism is due to a significant genetic insult [222] but it is not considered to be an inheritable condition. How and when this occurs can be debated however, for a young child with a developing immune system, there are few factors which could be held responsible other than vaccines and/or the related and damaging effect of exposure to high levels of mercury. No other factor or explanation has been offered as a viable alternative explanation for the occurrence of regressive autism. The evidence indicates there is alteration to chromosome structure and/or function. It indicates the influence of external stressor(s) influencing mitochondrial structure and DNA, chromosomal instability and translocation, which ultimately influences protein expression. The combined effect influences system stability, organ function, the prevailing levels of biochemistry, sense perception, behavior, etc. It influences protein expression and the rate and completeness of subsequent protein-substrate reactions leading to lowered immune function, reduced absorption of nutrients, slowed metabolism, impaired development [262], etc; i.e. the body's biochemical processes do not proceed as they should.

Is this an indication of chromosomal damage?

Viruses are able to infiltrate cells, inserting their genetic material into them. As outlined earlier (see 4.1) there are biochemical markers of vaccine damage. That it affects four boys to every girl [10] illustrates that the condition is largely due to a defect with the X-chromosome and leads to consideration of the factors which could influence at the genetic/chromosomal level. In general, chromosomal damage is linked to radiation e.g. due to adverse nuclear events which leads ultimately to birth defects. The prevailing evidence appears to suggest the influence of e.g. proteolytic enzymes or temperature [223,224] which may alter chromosome structure. Little evidence has been offered for the 1 in 5 occurrence experienced by girls although this appears likely to be the consequence of a chromosomal stressor.

It is widely recognised that genetic predisposition and protein expression can be influenced by environment influences [7], and that genetic damage can be the result of exposure to radiation, however the evidence being offered appears to suggest a subtle form of genetic alteration - associated with the wider use of vaccines [17] - which may not necessarily be inherited but is responsible for altered system stability and function and consequently of altered biochemistry and function. There is evidence that system

function is intact but dysfunctional i.e. that homeostasis is severely compromised. Such findings are supported by research into Gulf-War Syndrome (GWS) in which [225] untypical RNA was found in the blood of sick GW veterans. This illustrates that the viral encephalopathies originated from RNA-viruses and hence from vaccines. That immunosuppression, shown to be a factor in GWS [226] and autism, is associated with the concentrated use of vaccines [227] is further supported by the fact that French soldiers who were not vaccinated yet who served in the gulf war did not get GWS however American and British soldiers [228], irrespective of whether they served in Iraq or not, reported a s ignificantly greater incidence of autistic-spectrum disorders and GWS.

The Effect of Heavy Metals

Heavy Metals and Mercury in particular, affects the function of the CNS and are extensively documented and associated with autism [229]. Amongst a variety of side-effects mercury decreases lymphocyte viability, and in the brain: dysfunction in the amygdala, hippocampus, basal ganglia, and cerebral cortex; destruction of neurons in the cerebellum; and brainstem abnormalities. Demyelination is evident in such conditions. The brain's electrical patterns are similarly abnormal.

The most significant contributors to the increased mercury burden are: Mercury in vaccines (e.g. DTP (at typically 25 micrograms of mercury per dose), Tetanus, Hepatitis B & (most) influenza vaccines), contamination of fish [230], wild/bush fires; and emissions from power stations [231] and industrial chimneys including incinerators, waste-burning cement works, crematoria, etc. The characteristics of autism and mercury poisoning are extremely similar which suggests that autism arises from mercury poisoning [232,233]. Children with autism have greater amounts of mercury and other heavy metals in their system [234]. For these children the exposure route is considered to be predominately via childhood vaccines, most of which* contain thimerosal. Vaccinated children of circa 10-20 kgs are exposed to an adult overdose of mercury, over 62.5 micrograms of mercury within the first three months, which significantly increases a child's risk of developing some form of neuro-developmental disorder such as impaired development, speech and language, autism, stuttering and attention deficit disorder.

*used to

Children living downstream of coal-fired power stations have a g reater incidence of autistic spectrum disorders [231]. This indicates that the innate physiological processes, which the body uses to eliminate heavy metals, are being overcome by overexposure.

Mercury poisoning is an insidious process. In general the symptoms do not appear immediately upon exposure, although they may in especially sensitive individuals or in cases of excessive exposure. The initial preclinical stage is followed by the development of symptoms of mercury poisoning over a period which may last from weeks, months, and years [235-237]. Consequently, mercury given in vaccines to very young children would not be expected to lead to a r ecognizable disorder, except for subtle signs, before age 6-12 months, and might not emerge for several years [233].

In autistic children, the initial signs occur shortly after the first injections, and consist of abnormalities in motor behavior and in the sensory systems, particularly touch sensitivity, vision, and numbness in the mouth [15,238]. These signs are followed by parental reports of speech and hearing abnormalities appearing before the child's second birthday [10]. Finally, there is the development of autistic-like traits and a continuing regression or lack of development in subsequent years. These symptoms change [239] depending upon the circumstances surrounding each child.

Most autistic children have impaired liver detoxification. Many have low levels of metallothionine, conceivably the consequence of a deficiency of Zinc, which is indicative of a lowered capacity to chelate mercury and other heavy metals. Mercury is a p owerful oxidant which depletes cellular antioxidants, especially glutathione. The P450 detoxifying enzymes of the liver rely heavily on adequate availability of glutathione. EthylMercury the active component in thimerosal causes apoptosis of the t-cells [240-242].

Although the withdrawal of mercury from vaccines has not resulted in an overall decline in the occurrence of autism this does not mean that the problem does not lie with thimerosal [243,263]. It may indicate that the problem is associated with the elimination of mercury [244] i.e. affecting function of the lymphatic system and excretion [245]. This is supported by noting evidence of urea cycle dysfunction. Problems with the urea cycle, conceivably the consequence of mercury poisoning, have been linked to autism. A child with ornithine transcarbamylase (OTC) deficiency is likely to be lacking in energy, have appetite problems, poorly-controlled breathing rate and/or body temperature, and slow development. Significantly, OTC deficiency is an X-linked recessive disorder (http://www.merck.com/mmpe/sec13/ch164/ch164a.html) one of a number of primary immunodeficiencies associated with vaccine use.

As in autism, onset of Hg toxicity symptoms is gradual in some cases, sudden in others [232,233]. In the case of poisoning, the first signs to emerge are abnormal sensation and motor disturbances. As exposure increases, these signs are followed by speech problems, and hearing deficits [246]. Upon removal of the mercury the symptoms tend to recede except in instances of severe poisoning, which may lead to death [232]. As in autism, epilepsy arising from Hg exposure is also associated with a poor prognosis [247]. Mercury acts upon the catecholamines and influences the function of the autonomic nervous system [245]. This affects cognitive performance [248], spatial vision [249], etc.

Other metals have been implicated in adverse neurodevelopmental outcomes in children e.g. lead and mercury [250,251], with exposure to cadmium, arsenic, antimony and chromium also a concern. Studies have found adverse effects of prenatal lead exposure on growth and development, but little research has examined an association with autism. Whilst Mercury is of concern, because of evidence for neurotoxic effects and the fact that it has become so prevalent in the wider environment [250], Aluminum also shares common mechanisms with mercury e.g. it interferes with cellular and metabolic processes in the nervous system. Children given the recommended vaccinations are injected with nearly 5 mg of aluminum by the time they are just 1.5 years old, almost 6 times the safe level. Furthermore the nature of the Aluminium affects the prevailing blood levels and is also increasingly implicated, through their use as vaccine adjuvants, in autism [252].

Current Therapeutic Approaches used to Treat Autism

There is evidence that autism is a treatable disease and that some therapies can mitigate the effects of autism [253,254]. Although there is no recognised method of treatment, or of significant and/or proven outcomes, autistic children appear to respond to therapies which enhance the function of the breathing**,** to enhance oxygen levels [255], and excretory system e.g. by osteopathy [256]. Moreover a commonly observed side-effect with autistic children is that when a child has an elevated temperature, perhaps resulting from a fever, the autistic symptoms appear to recede and the child behaves normally [41]. Autistic children suffer from adverse sleep patterns. In the US autistic children are often treated by chelation therapy and biofeedback [257-259].

Dysfunction of the Excretory or lymphatic system leads to long-term exposure to mercury which under normal circumstances would have been rapidly eliminated from the body. This may also lead to higher neural temperatures which will inevitably influence brain function.

Further evidence of biochemical deficits [260] and of the benefit of biochemical based supplements e.g. vitamin B6 and magnesium; melatonin; methylcobalamin; vitamin A, C & D supplements; dimethylglycine (DMG) and trimethylglycine (TMG). DMG provides building blocks that are required for purine nucleotide synthesis. DMG comes from TMG when TMG methylates homocysteine. Significantly, absorption of Vitamin A Palmitate requires an intact gut mucosa at the appropriate pH and in the presence of bile for metabolism. Many autistic children have damaged mucosal surfaces therefore they have impaired capacity to absorb vitamin A [261].

That some children can become normal when their temperature increases above normal levels e.g. due to a viral infection, [41] may illustrate that the levels of the homeostatic mechanism affecting the physiological systems have been reset at what can be considered to be abnormal levels [47]. This may indicate that autism is treatable - perhaps to a greater degree than has hitherto been considered possible.

Discussion

The mass of scientific evidence compiled by researchers clearly indicates that the incidence of autism occurs following vaccination and is most closely associated with the schedule of vaccines culminating in the MMR vaccine. That vaccines suppress natural immune function is not in dispute e.g. those with naturally low levels of immune function (immigrants from tropical climates) show greater predisposition to autistic spectrum disorders.

The immediate effect arising from vaccination influences gene function and protein expression. This leads to lower levels of white blood cells including e.g. lymphocytes, immunoglobulins, t-cells, b-cells and/or neutrophils, and disturbs their synergistic action and hence their ability to memorize and respond to immune responses when challenged. This impairs the ability to kill pathogens thereby predisposing to further infections. The short and long-term outcome is to the neural mechanisms regulating system function affecting e.g. pH, the excretory system, temperature, and the elimination of toxins and heavy metals. This explains why the discontinuation of thimerosal in vaccines was followed by a steady increase in the incidence of autism and hence that researchers did not find a correlation between the incidence of autism and the use of thimerosal-containing vaccines [263]. This may also explain the effect of multiple vaccines, in particular the MMR vaccine, and the greater predisposition to autistic spectrum disorders in military families.

In most autistic children brain structures are initially unaffected but become steadily underdeveloped as a consequence of exposure to mercury and other heavy metals. This evolves into a neurodevelopmental problem leading to chromosomal abnormalities, affecting myelination, the subsequent degeneration of the cerebellum, etc.

The MMR triple vaccine may inhibit normal immune function which, directly or indirectly, ultimately leads to chromosomal and/or genetic damage and/or dysfunction. The occurrence of GWS in adults, a condition with many features which are common with autism, indicates the problem may be due to the number and/or intense schedule of vaccinations however this does not excuse the measles or MMR vaccine from suspicion. The combined vaccine raises body temperature whilst lowering immune and system function. This may make a mild measles vaccine more virulent which may increase fever to an abnormally high level. It suggests (1) single vaccines may pose less risk than triple vaccines; (2) some vaccines pose a greater risk than others e.g. pertussis and measles; and (3) the way in which vaccines are administered will be accompanied by different side-effects e.g. if pertussis is followed by measles or vice-versa, if BCG gives a b eneficial effect to be followed by pertussis, if vaccines are given in combination, etc. Increased disease loading is the inevitable consequence

of multiple vaccine or lots of single vaccines or triple vaccines e.g. of asthma, autoimmune disease, etc. It suggests that adherence to the vaccine schedule is the problem – too many vaccines, too quickly.

Vaccines cause an inflammatory response in some e.g. for those with an inadequately developed or artificially lowered immune system, for those genetically predisposed, or perhaps due to viral or bacterial infection. This creates genetic damage and/or dysfunction and hence influences the brain's ability to regulate the physiological systems, and especially to the lymphatic system and its ability to excrete mercury and heavy metals, would lead to long-term damage and problems processing sensory/cognitive input. This would inevitably affect the brain's ability to maintain a regulated temperature below that which affects brain damage (41° C). This inevitably influences the autonomic nervous system and the stability of all related physiological systems including temperature, blood pressure, blood cell content, blood glucose, digestion, excretion, sleeping, etc.

Further evidence of multi-level dysfunction is evident from unusual brain-wave stability, aberrant sleep patterns, loss of sense perception and coordination, mirror neuron dysfunction, lower pain thresholds, mental and physical deterioration, short periods of concentration, etc. That it is a problem of systemic dysfunction is further supported by noting how it can be treated using sensory therapies which may facilitate the re-establishment of some degree of physiological stability.

Where is the proof that vaccines are safe? The argument has never been that they are completely safe but that the consequences are less than having the disease. Now it is illustrated that the consequences of intensive vaccination schedules pose a greater risk than could ever have been imagined. This leads to the evolution of new viral strains, an unsurprising development when the environment to which it is exposed is being altered by new proteins, structural variants and altered DNA.

Vaccines are an essential component of preventative healthcare however it may be necessary to review the ways in which vaccines are used, administered and regulated [141,264] i.e.

- As drugs are tested in the clinical environment to assess their interaction with other drugs, the cumulative use of vaccines including that of multiple vaccines should be researched and shown, through double-blind placebo controlled clinical trials, to be free from any such interactions i.e. of one single vaccine with another single or multiple vaccine or drug. It has been considered unethical to select a control group of children which would otherwise not be vaccinated yet such is the levels of conscientious objectors in the industrialized world and through circumstances of impoverishment in the underdeveloped countries that such statistics must currently exist.
- Measures to assess the suitability of children for vaccination i.e. how to assess whether a child has a greater predisposition to an adverse vaccine reaction and the subsequent development of autism? [265]
- The time when vaccinations should be given and the time between vaccinations e.g. giving mumps and rubella vaccinations later in childhood.
- Are some vaccines necessary in the industrialized world e.g. mumps, rubella, Hib, Hpv, etc? With more than 200 other vaccines under development this must be an issue of review.

The risks from disease and vaccinations differ upon location. In the developed world, there is an estimated 0.1-0.3% risk of mortality from measles which compares with a 0.6% risk and rising (with some estimates at 1-2%) of autism. This excludes the cost of treating the wide range of side-effects which must clearly be attributed to the use of vaccines. The cost of treating vaccine-related side-effects may now be far greater than the diseases against which the vaccine(s) were designed to protect. Furthermore, in the developed world there is a highly developed social structure which is able to assist parents to deal with the condition. By comparison, what are the implications for an autistic child in the developing world where there is absence of resources to deal with the condition?

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Statement of Interest

Graham and Elena Ewing (Dr) are Directors of Montague Healthcare a company devoted to the commercialisation of Virtual Scanning and hence to the diagnostic and therapeutic use of Virtual Scanning. They are co-authors of the book 'Virtual Scanning – a new generation of healthcare – beyond biomedicine?' ISBN 978-0-9556213-0-7 published by Montague Healthcare books.

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The development of rheumatoid arthritis after recombinant hepatitis B vaccination

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Abstract

Objective: Hepatitis B vaccination has been associated with reactive arthritis and rarely rheumatoid arthritis (RA). We defined the clinical, serologic, and immunogenetic background of patients developing RA, soon after recombinant hepatitis B vaccination.

Methods: The clinical, serologic, and HLA antigens of a cluster of firefighters who developed arthritis after prophylactic recombinant hepatitis B vaccination (5 subjects), as well as a second group of sporadic cases of arthritis (6 patients) after hepatitis B vaccination are described.

Results: Ten of 11 patients fulfilled revised American College of Rheumatology criteria for RA. All cases had persistent arthritis for more than 6 months; at 48 months followup 2 cases no longer had inflammatory arthritis. Nine patients required disease modifying antirheumatic drugs. Five subjects were HLA-DR4 positive. HLA class II genes expressing the RA shared motif were identified in 9/11 patients genotyped for HLA-DRbeta1 and DQbeta1 alleles (0401, 0101, or 0404). All the firefighters shared the HLA-DRbeta1 allele 0301 and the DQbeta1 allele 0201, with which it is in linkage disequilibrium.

Conclusion: These polymorphic residues in the binding site of the MHC class II molecules of the affected patients appear capable of binding some peptide sequences of the recombinant vaccine peptides they received and may be responsible for hepatitis B vaccine triggering development of RA in these cases. Recombinant hepatitis B vaccine may trigger the development of RA in MHC class II genetically susceptible individuals.

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Aluminum phagocytosis in quadriceps muscle following vaccination in children: relationship to macrophagic myofasciitis

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Abstract

Macrophagic myofasciitis (MMF) is a rare, seemingly emerging entity among adult patients in France. We encountered two children with the first two cases of MMF in North America. A 5-year-old male with chronic intestinal pseudo-obstruction required nighttime parenteral nutrition. Abnormal pupillary reflexes and urinary retention suggested a diffuse dysautonomia, which prompted a neurological diagnostic work-up. A 3-year-old child had developmental delay and hypotonia. Both children received age-appropriate immunizations. Quadriceps muscle biopsy from each child showed the typical patchy, cohesive centripetal infiltration of alpha-1-antitrypsin+, alpha-1-antichymotrypsin+, CD68+, PAS+, CD1a-, S-100-, factor XIII- granular macrophages with adjacent myofiber atrophy, dilated blood vessels, and mild endomysial and perimysial fibrosis. No myonecrosis was observed and no discrete granulomas were seen. A single aluminum peak was demonstrated on energy dispersive X-ray microanalysis. The etiology of the clinical symptoms in these cases and in cases reported as MMF remains intriguing. Despite numerous stains to demonstrate organisms, most infectious causes leading to macrophage activation were ruled out. These cases are being reported to increase awareness of this condition and to encourage a systematic epidemiologic and clinicopathologic study in North America.

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CASE REPORT

Identical Twins With Macrophagic Myofasciitis: Genetic Susceptibility and Triggering by Aluminic Vaccine Adjuvants?

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Introduction

Macrophagic myofasciitis (MMF) is an inflammatory myopathy, recently described (1,2). Clinical symptoms include myalgias, arthralgias, muscle weakness, asthenia, and fever. Diagnosis is based on deltoid muscle biopsy that usually shows specific histologic abnormalities including infiltration of connective tissue structures by densely packed large and grossly rounded CD68+ histiocytes. These cells are characterized by central, round, and often nucleolated nuclei, and clear, slightly basophilic cytoplasms with fine PAS-positive granules (1). Aluminic vaccine adjuvants (3) as well as *Tropheryma whippelii* infection (4,5) have been recently suggested as possible etiopathogenic agents of MMF. We report 2 cases of MMF observed after hepatitis B vaccination in twin sisters. This observation illustrates the importance of the genetic background in MMF, and its possible triggering by aluminic vaccines.

Case report

Two 64-year-old identical twin sisters were referred to our department for possible rheumatoid arthritis. They had no family history of inflammatory disease. The first sister's medical history was unremarkable until December 1993, 6 months after she received a third and final injection of hepatitis B vaccine (Engerix B vaccination, containing aluminic hydroxide, vaccination in April, May, and July, 1993) in the left deltoid. At this time, she began complaining of arthritis. On physical examination, arthritis involving both wrists and the proximal and distal interphalangeal joints of the hands and feet were noted. Arthritis was associated with myalgias and upper limb muscle weakness. She complained of dry eyes and mouth and of oral aphthae. Treatment with oral prednisone (5 mg/day) and methotrexate (20 mg weekly) proved ineffective. Neurologic examination revealed distal paresthesias and cramps affecting the 4 limbs, slight weakness of hand muscles, no pyramidal syndrome, and partial visual acuity loss. Sicca syndrome was confirmed by Schirmer's test. Salivary gland biopsy showed Chisholm 3 grade, consistent with the diagnosis of Gougerot-Sjögren's syndrome. Erythrocyte sedimentation rate (ESR) was 20 mm/hour, C-reactive protein was 30 mg/l. Serum creatine kinase and cerebrospinal fluid were normal. Antinuclear antibodies (ANA) were weakly positive (200 UI/ml) with spotted pattern. Anti-DNA, anti-SSA, anti-SSB, rheumatoid factor, and anticardiolipin antibodies were not detected. Serologic tests for hepatitis A, B, C, and parvovirus B 19 were negative. The patient typed as HLA–A01, A02, B13, B35, DRB1*01, DRB1*07. Electromyography showed a slight myogenic aspect. Radiographs of the hands, shoulders, wrists, ankles, and feet were normal. Technetium bone scan showed symmetric increased uptake of the main joints. Upper right limb muscle magnetic resonance imaging (MRI) showed general muscle atrophy. The morphology and chronology of the visual-, auditory-, motor-, and somaesthesic-evoked responses were normal, and cerebral MRI was normal. Esophago-gastro-duodenoscopy was normal with no evidence of PAS+ cells on duodenal biopsies, the culture for *Tropheryma whippelii* in duodenal biopsies was negative. Diagnosis of MMF was obtained by histologic examination of biopsy sample of the left deltoid muscle, which showed stereotypical epi-, peri- and endomysial infiltrates of densely packed CD68+ macrophages twice associated T and B cells (Figure 1). Electron microscopic examination was not performed on this biopsy.

The twin sister had a similar clinical presentation with bilateral arthritis of wrists, shoulders, temporomandibular joints, hands, and ankles. She received prednisone (15

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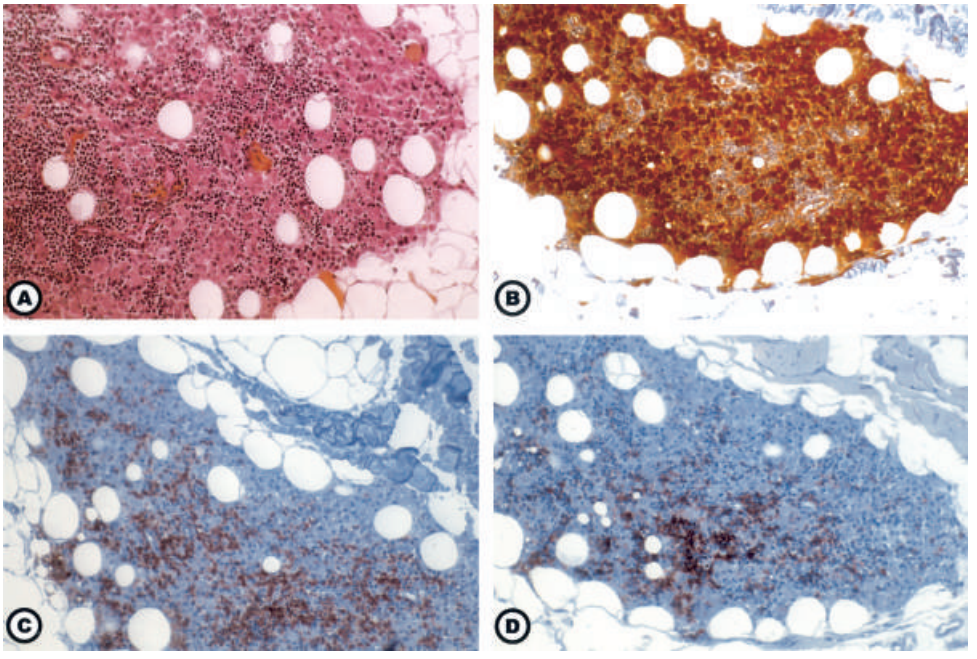

Figure 1. Deltoid muscle biopsy (light microscopy, serial sections, original magnification × 125) demonstrating macrophagic myofasciitis in patient 1. **A,** inflammatory infiltrates of adipose tissue of fascia (fasciitis) by macrophages notably large in size and lymphocytes (Hematoxylin and eosin stained). **B,** immunoreactivity of CD68+ showing large predominance of macrophages. **C,** immunoreactivity of CD3+ mononucleated cells corresponding to T cells. **D,** immunoreactivity of DBB42 antibody showing B cells.

mg/day), hydroxychloroquine (400 mg/day), and methotrexate (20 mg/week). Her medical history was unremarkable except for mild asthma and high blood pressure. On admission, she reported peripheral arthritis with myalgias. Neurologic examination showed distal paresthesias and cramps of the 4 limbs, severe weakness of the hand muscles, hyperreflexia of the right upper limb, right Hoffmann's sign, and normal plantar reflexes urgency of micturition. The symptoms had started 7 months after intramuscular hepatitis B vaccination in the left deltoid (Engerix B containing aluminic hydroxide, vaccination in December 1995, February, and July 1997). ESR was 30 mm at one hour. Serum creatine kinase was normal. ANA, anti-DNA, anti-SSA, anti-SSB, rheumatoid factor, and anticardiolipin antibodies were negative. Serologic tests for hepatitis A, C and parvovirus B 19 were negative. Hepatitis B serology confirmed the vaccination status, specifically the absence of hepatitis B surface, anti-hepatitis B core and anti-hepatitis B e antigens, and the presence of anti-hepatitis B surface antibodies. The patient typed as HLA–A01, A02, B13, B35, DRB1*01, DRB1*07. Schirmer's test was positive. Electromyography showed myogenic aspect. Radiographs of the hands, shoulders, wrists, ankles, and feet were normal. MRI of the superior right limb muscles showed a diffuse muscle atrophy. Evoked potentials and brain MRI were normal. Esophago-gastro-duodenoscopy was normal and cultures for *Tropheryma whippelii* in duodenal biopsies were negative. Once again, muscle biopsy of the left deltoid muscle confirmed the diagnosis of MMF.

Discussion

We report cases of MMF affecting twin sisters, which occurred 6–7 months after hepatitis B vaccination. In both cases, clinical symptoms were remarkable because of the importance of arthritis associated with muscle involvement. Laboratory tests showed slight inflammation, and normal muscle enzyme levels. Remarkably, although both twin sisters had had complete hepatitis B vaccination, only the second had developed antibodies to the hepatitis B surface antigen. Indeed, the HLA–DRB1*07 allele, which both twins expressed, is associated with poor humoral responses to hepatitis B vaccination (6). Thus, antibody responses to hepatitis B surface antigen may not be crucial to the development of MMF.

Conversely, the role of vaccines containing aluminum hydroxide in the pathogenesis of MMF has been recently suggested (3). Despite the fact that histologic abnormalities are present only at the site of vaccination, systemic symptoms are generally observed. However, there is a discrepancy between the wide usage of aluminum hydroxide-containing vaccines (especially anti hepatitis B vaccines) and the very limited number of MMF cases reported so far. We did not perform electron microscopy on muscle biopsies to check for aluminium inclusions in macrophages. This report suggests that additional factors, perhaps genetic, may influence the occurrence of MMF. Aluminum hydroxyde may trigger unusual muscle inflammatory infiltrates in patients with increased susceptibility to inflammatory disease or decrease macrophages' capacity for aluminum hydroxyde digestion. The nature of the predis-

posing genetic factor is unknown. HLA–DRB1*01, which was found in both sisters (identical twins) could be a potential candidate. Thus, our observation suggests that aluminic vaccinations may trigger MMF on the HLA–DRB1*01 genetic background.

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CONCISE COMMUNICATIONS

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Chronic fatigue syndrome in patients with macrophagic myofasciitis

Macrophagic myofasciitis (MMF), a condition first reported in France in 1998, is defined by the presence of a stereotyped and immunologically active lesion at deltoid muscle biopsy (1,2). It was recently demonstrated that this lesion is an indicator of long-term persistence of the immunologic adjuvant aluminum hydroxide within the cytoplasm of macrophages at the site of previous intramuscular (IM) injection (2). MMF is typically detected in patients with diffuse arthromyalgias that have appeared subsequent to aluminum hydroxide administration in the absence of a clearly defined anatomic substratum (2). Patients also report unexplained chronic fatigue (1). These manifestations are reminiscent of the so-called chronic fatigue syndrome (CFS), a poorly understood condition manifesting as disabling fatigue, musculoskeletal pain, sleep disturbance, impaired concentration, and headaches (3). The present study was conducted to determine the proportion of MMF patients fulfilling international criteria for CFS.

Thirty unselected consecutive patients with biopsy-proven MMF identified in Créteil and Bordeaux were retrospectively included, regardless of symptoms that led to indication of muscle biopsy. As previously described (2), MMF was assessed by 1) well-circumscribed sheets of densely-packed, large, nonepithelioid macrophages with a finely granular, periodic acid–Schiff–positive content, in the connective structures of deltoid muscle; 2) lymphocytic infiltrates intermingled with macrophages and forming microvascular cuffs; and 3) absence of significant muscle fiber injury (see Figure 1). In each patient, we determined, through both chart review and either direct patient questioning or telephone interview, 1) the presence of chronic fatigue of >6 months' duration, 2) the alleged severity of fatigue, and 3) the presence of CFS according to Centers for Disease Control and Prevention (CDC) criteria (1994) (4) or Oxford criteria (1991) (5). In addition, in 20 patients, we retrospectively evaluated history of immunization as well as prevalence of fever and neurologic features suggestive of central nervous system demyelinating disease; laboratory findings, including erythrocyte sedimentation rate, creatine kinase levels, and ^{67}Ga scintigraphy; and responsiveness to steroids.

The male:female ratio was 1:2. The mean age of patients was 52 years (range 12–78 years). Chronic fatigue was found in 28 of 30 patients (93%) and was considered disabling in 26 of 30 patients (87%). Sixteen patients (53%) fulfilled CFS criteria from either the CDC (14 of 30 patients, 47%) or Oxford (12 of 30 patients, 40%), 11 of 30 patients (37%) fulfilled both CDC and Oxford criteria. Other symptoms, laboratory findings, and steroid responsiveness are detailed in Table 1. ^{67}Ga scintigraphy was performed in 5 patients and showed increased levels of ^{67}Ga uptake in muscle and paraarticular areas, mainly in lower limbs. A history of vaccination was available for 19 of 20 patients. All 19 patients had received IM administration of aluminum-containing vaccine prior to the onset of CFS symptoms, and the delay from the last vaccination to the first manifestations ranged from 1 month to 72 months (median 12 months).

We have previously determined that myalgias are a major symptom in patients with MMF. The prevalence of myalgias was much higher in such patients than in other patients who had undergone deltoid muscle biopsies at the same time in the same centers (85% versus 45%; $P < 0.0001$ by Fisher's exact test) (2). We show now that chronic disabling fatigue is a symptom as frequent as diffuse myalgias in patients with MMF (87%), a finding also noted in the French Institut de Veille Sanitaire exploratory investigation report (6). More than half of the patients also reported other manifestations of CFS. Therefore, MMF should be alternatively considered as a cause of CFS or as an additional exclusion criterion, along with rheumatoid arthritis, lupus, and other diseases, for the diagnosis of idiopathic CFS (4). Consequently, we suggest that patients with CFS should be carefully checked for a history of IM administration of aluminum hydroxide, and, if there is consistent chronology, a muscle biopsy to search for MMF at the site of injection should be considered, even many years after onset of symptoms.

Pathophysiology of CFS is still fiercely debated by psychologists, neuroendocrinologists, and immunologists. Chronic immune stimulation that fails to switch off has been previously reported as a possible cause of CFS (7–9), and such a situation may very well result from persistence of the immunologic adjuvant aluminum hydroxide within antigen-presenting cells (2,10). Therefore, MMF may well represent a paradigm for CFS of immunologic origin. We believe that clarification of MMF pathophysiology would significantly contribute to the understanding of the whole spectrum of chronic fatigue and its syndromes.

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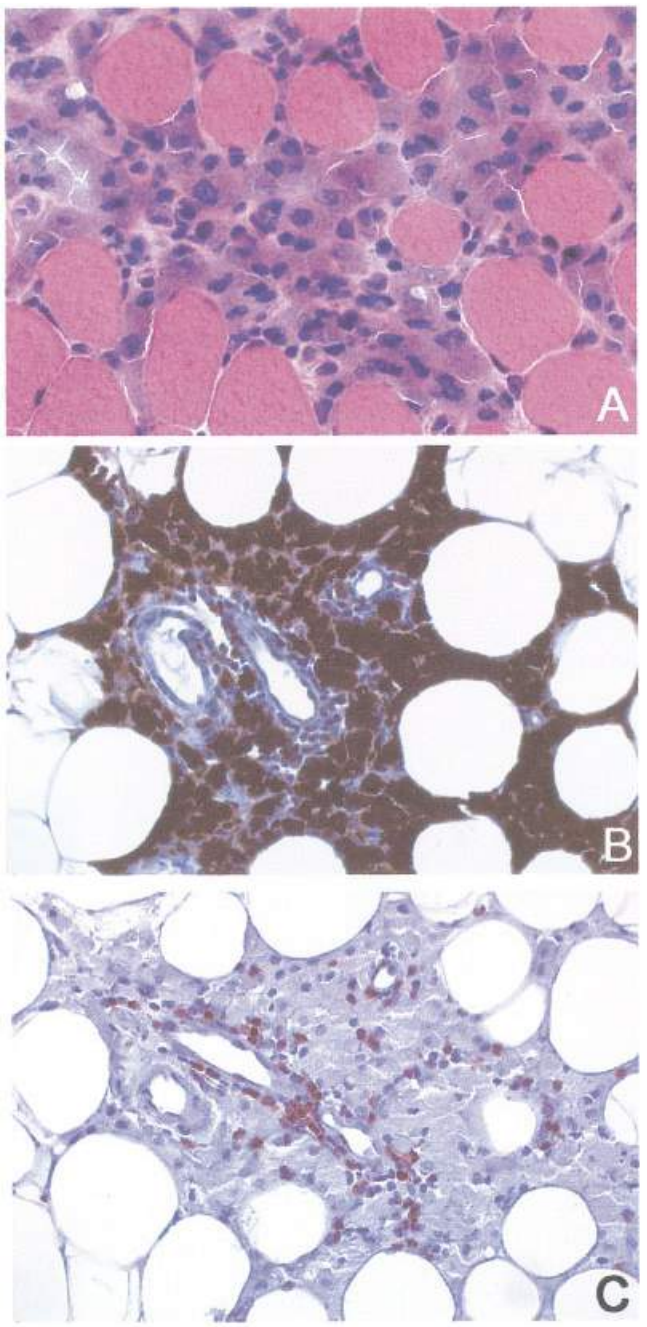

Figure 1. Deltoid muscle biopsy samples from patients with macrophagic myofasciitis (MMF). **A,** Tightly packed, large, basophilic macrophages intermingled with lymphocytes in perifascicular endomysium (frozen section, hematoxylin and eosin stained; original magnification ×400). **B,** MMF lesion in perimuscular adipose tissue showing immunolocalization of the macrophage marker CD68 (paraffin section, immunoperoxidase procedure; original magnification ×400). **C,** Adjacent section of the same biopsy sample showing immunolocalization of the T cell marker CD3 (paraffin section, immunoperoxidase procedure; original magnification ×400).

Table 1. Clinical and laboratory findings in patients with macrophagic myofasciitis*

Chronic fatigue†	28/30 (93)
Severe and disabling	26/30 (87)
Of new onset	25/30 (83)
Leading to substantial reduction in previous levels of activity	24/30 (80)
Present for >50% of the time	19/30 (63)
Not a result of ongoing exertion	18/30 (60)
Affecting both physical and mental functioning	16/30 (53)
Not substantially alleviated by rest	13/30 (43)
Other symptoms†	
Muscle pain	26/30 (87)
Joint pain	17/30 (57)
Sleep disturbance	16/30 (53)
Mood disturbance	16/30 (53)
Subjective memory impairment	15/30 (50)
Headache	14/30 (47)
Unrefreshing sleep	14/30 (47)
CFS criteria fulfilled	16/30 (53)
CDC (1994) (see ref. 4)	14/30 (47)
Oxford (1991) (see ref. 5)	12/30 (40)
Neurologic features suggestive of CNS demyelinating disease	2/20 (10)
Fever	2/20 (10)
Abnormal laboratory findings	
ESR >40 mm/hour	2/14 (14)
CK level >200 IU/liter	4/14 (29)
^{67}Ga scintigraphy	5/5 (100)
Responsive to steroids‡	10/10 (100)

* Values are the number (%) of patients. CFS = chronic fatigue syndrome; CDC = Centers for Disease Control and Prevention; CNS = central nervous system; ESR = erythrocyte sedimentation rate; CK = creatine kinase.
† Part of diagnostic criteria for CFS.
‡ Improvement of both fatigue and myalgias. One patient received intravenous methylprednisolone without significant effect.

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ASIA OR SHOENFELD'S SYNDROME: HIGHLIGHTING DIFFERENT PERSPECTIVES FOR DIFFUSE CHRONIC PAIN

Jozélio Freire Carvalho*, Solange Murta Barros**, Jaime C. Branco***, João Eurico Fonseca****

Is the Gulf War Syndrome (GWS) and the silicone related scleroderma –like syndrome spectres of the same disease? What do they have in common with a rare aluminium induced myopathic syndrome described for the first time in France in 1998? The logic answer was suggested by an elegant integration of the existing evidence into the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) proposed recently by Shoenfeld in a paper published in *Journal of Autoimmunity*[1]. A mosaic of environmental factors can be classified as adjuvants. In fact, we know for decades a variety of compounds that are able to induce autoimmunity in animal models and used in clinical practice to increase the immunogenicity of vaccines, but also known to be able, in genetic susceptible individuals, to induce autoimmune diseases[2,3]. In this vast group of substances bacterial antigens, hormones, aluminium, silicone and several other molecules have been included[4].

The GWS was described in veterans that were suffering from atypical rheumatic symptoms, such as arthralgia, myalgia, lymphadenopaty, chronic fatigue syndrome, malar rash and autoimmune thyroiditis[5].

A cohort study performed 10 years ago compared the titter of anti squalene antibodies of 144 Golf War immunized veterans or medical employees, 48 blood donors, 40 systemic lupus erythematosus patients, 34 silicone breast implant recipients and 30 chronic fatigue syndrome patients. The majority (95%) of overtly ill deployed GWS patients had antibodies to squalene. All (100%) GWS patients immunized for service in Desert Shield/ /Desert Storm who were not in the fighting front had antibodies to squalen. In contrast, none (0%) of the veterans that were in the fighting zone and were not showing signs and symptoms of GWS have antibodies to squalen. Neither patients with idiopathic autoimmune disease nor healthy controls had detectable serum antibodies to squalene. The majority of symptomatic GWS patients had serum antibodies to squalene[6]. The authors proposed that GWS was not a result of the exposition to weapons but rather induced by the intense vaccination program that they were submitted to. It is ironic that more solders were ill due to an oil adjuvant injected in their organisms than fighting against the hostile environment and the armed enemies.

Silicon was considered an inert material and thus unable to induce immune reactions. Recent metanalysis have supported this view, as the risk of silicon exposed individuals for developing a diffuse connective tissue disease is only 0.8%, not significantly higher than the risk of the general population. However, that is not the case for more unspecific symptoms such as arthralgia and myalgia and even some diffuse neurologic manifestations that appear to be more common in individuals exposed to silicon implants[7]. The possible association between chronic fatigue syndrome, fibromyalgia, and previous silicone mammoplasty was proposed almost two decades ago[8].

The post vaccination muscle disease described by Gehardi *et al.* in 1987 is of particular interest as it is based in well defined histologic features[9]. It is a miofasceitis that has the presence of macrophages with aluminum inclusions, which occurs associated with vaccination. Clinically the disease is expressed by systemic symptoms such as fatigue, myalgia, arthalgia, fever and, in some cases, by a demyelinating condition similar to Guillain-Barré, with electromyographic changes. Elevated acute

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Table I. Criteria suggested by Shoenfeld for ASIA diagnosis

Major Criteria

- Exposure to an external stimuli (infection, vaccine, silicone, adjuvant) prior to clinical manifestations
- The appearance of 'typical' clinical manifestations:
  - Myalgia, myositis or muscle weakness
  - Arthralgia and/or arthritis
  - Chronic fatigue, un-refreshing sleep or sleep disturbances
  - Neurological manifestations (especially associated with demyelination)
  - Cognitive impairment, memory loss
  - Pyrexia, dry mouth
- Removal of inciting agent induces improvement
- Typical biopsy of involved organs

Minor Criteria

- The appearance of autoantibodies or antibodies directed at the suspected adjuvant
- Other clinical manifestations (i.e. irritable bowel syndrome)
- Specific HLA (i.e. HLA DRB1, HLA DQB1)
- Evolvement of an autoimmune disease

For ASIA's diagnosis: at least 2 major criteria or 2 minor and 1 major.

phase proteins and creatine quinase also occur. The same group determined that the disease occurs only in HLA DRB1*01 positive individuals[9]. On top of that, it was shown that aluminum can persist in the local of injection, up to 10 years after vaccine administration, which can explain the persistence of this condition in some individuals[10].

These conditions and other observations, regarding for instance de H1N1 vaccination, have motivated the definition of the ASIA syndrome, with the criteria proposed by Shoenfeld listed in Table I[1]. These criteria, if properly validated, are of great clinical relevance, as they raise a major clinical doubt on the classification of some patients with chronic pain syndromes, as chronic fatigue syndrome, or even fibromyalgia. In fact, if we compare the cardinal symptoms of the Shoenfeld's ASIA syndrome with the typical clinical manifestations of patients with diffuse chronic pain we came quickly to the conclusion that reviewing the recent exposition to adjuvants and other potential exogenous stimulus seems to be a wise attitude. This is also in line with the characteristic symptoms of fibromyalgia and chronic fatigue syndrome that frequently occur in patients with well-defined Lyme disease, even after adequate treatment. Lyme disease is caused by an infection due to *Borrelia burgdorferi* spirochete and most of the clinical symptoms are in fact a consequence of an immune response to this infectious agent. Although a biological relationship between Lyme disease and diffuse pain syndromes has not been established, in fact this can be encompassed by the ASIA syndrome[11]. In addition, recent studies have detected the presence of retroviral sequences like xenotropic murine leukemia virus-related virus (XMRV) and polytropic murine leukemia virus related--virus (PMLV) in chronic fatigue syndrome patients, expanding, in fact, the need for thinking on alternative diagnosis in patients classified into these conditions[12]. Consequently, countries such as Australia, Canada, New Zealand and the UK elaborated restrictive guidelines for "blood donors with a history of current diagnosis of CFS". If upcoming research will confirm these observations and validate the ASIA/Shoenfeld criteria, a major paradigm shift will have to occur in the way rheumatologists perceive some cases of diffuse chronic pain. Interestingly, in this issue of Acta Reumatologica Portuguesa 2 case reports related to the ASIA/Shoenfeld are reported[13,14].

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Macrophagic myofaciitis a vaccine (alum) autoimmune-related disease

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Abstract

Macrophagic myofasciitis (MMF) is an immune-mediated condition first reported in 1998. MMF is characterized by post-vaccination systemic manifestations as well as local-stereotyped and immunologically active lesion in the site of inoculation (deltoid muscle). MMF systemic symptoms included myalgias, arthralgias, marked asthenia, muscle weakness, chronic fatigue, and fever. Recently, studies demonstrated that the local lesion is due to persistence for years at site of injection of an aluminum (Al(OH)3) adjuvant commonly used in human vaccines. Time elapsed from last immunization with an Al(OH)3-containing vaccine to muscle biopsy range from 3 months to 8 years; in rare cases, MMF may be diagnosed even 10 years post-vaccination. The discrepancy between the wide applications of aluminum hydroxide-containing vaccines and the very limited number of MMF cases reported may be resolved by observations suggesting that aluminum-containing vaccinations may trigger MMF in genetically susceptible subjects carrying the HLA-DRB1*01. Thus, MMF may be defined as an emerging novel condition that may be triggered by exposure to alum-containing vaccines, in patients with a specific genetic background, and this temporal association may be exhibited from a few months up to 10 years.

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The common immunogenic etiology of chronic fatigue syndrome: from infections to vaccines via adjuvants to the ASIA syndrome

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Abstract

Chronic fatigue syndrome (CFS) is characterized by unexplained fatigue that lasts for at least 6 months with a constellation of other symptoms. Most cases start suddenly, and are usually accompanied by a flu-like illness. It is a symptom-based diagnosis of exclusion, the pathogenesis of which is unknown. Studies have examined and hypothesized about the possible biomedical and epidemiologic characteristics of the disease, including genetic predisposition, infections, endocrine abnormalities, and immune dysfunction and psychological and psychosocial factors. Recently, the AISA (autoimmune/inflammatory syndrome induced by adjuvants) syndrome was recognized, indicating the possible contribution of adjuvants and vaccines to the development of autoimmunity.

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Macrophagic myofasciitis: characterization and pathophysiology

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Summary

Aluminium oxyhydroxide (alum), a nano-crystaline compound forming agglomerates, has been introduced in vaccine for its immunologic adjuvant effect in 1927. Alum is the most commonly used adjuvant in human and veterinary vaccines but mechanisms by which it stimulates immune responses remains incompletely understood. Although generally well tolerated, alum may occasionally cause disabling health problems in presumably susceptible individuals. A small proportion of vaccinated people present with delayed onset of diffuse myalgia, chronic fatigue and cognitive dysfunction, and exhibit very long-term persistence of alum-loaded macrophages at site of previous intra-muscular (i.m.) immunization, forming a granulomatous lesion called macrophagic myofasciitis (MMF). Clinical symptoms associated with MMF are paradigmatic of the recently delineated "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA). The stereotyped cognitive dysfunction is reminiscent of cognitive deficits described in foundry workers exposed to inhaled Al particles. Alum safety concerns will largely depend on whether the compound remains localized at site of injection or may diffuse and accumulate in distant organs. Animal experiments indicate that biopersistent nanomaterials taken-up by monocytes-lineage cells in tissues, e.g. fluorescent alum surrogates, can first translocate to draining lymph nodes, and thereafter circulate in blood within phagocytes and reach the spleen, and, eventually, slowly accumulate in brain.

Keywords: Adjuvants, Immunologic; adverse effects; Alum Compounds; adverse effects; Animals; Fasciitis; chemically induced; immunology; pathology; physiopathology; Humans; Myositis; chemically induced; immunology; pathology; physiopathology; Nanostructures; Phagocytes; metabolism; Syndrome

Introduction

In 1998, a consortium of French myopathologists described an emerging condition of unknown cause characterized by a pathognomonic lesion at muscle biopsy we called macrophagic myofasciitis (MMF).[1] MMF was detected in middle-aged adult patients with diffuse myalgias and fatigue.[1] Macrophages was the major cell type in the lesion, and enclosed agglomerates of nanocrystals in their cytoplasm.[1] Subsequently, these inclusions proved to be a key finding as they were constantly present at electron microscopy, and conspicuously contained aluminium as shown by ionic or X-ray microanalysis.[2] MMF was typically detected in the deltoid muscle, and could be differentiated both clinically and pathologically from Whipple's disease and other infectious histiocytoses, and from diffuse dysimmune fasciitis and panniculitis. [3] The crystalline rather than amorphous ultrastructural appearance of the inclusions was suggestive of aluminium hydroxide. Patients had normal renal function and had no peculiar exposure to aluminium other than previous immunization against hepatitis B (HBV), hepatitis A (HAV) or tetanus toxoid (TT) vaccines (100%), thus strongly suggesting that MMF inclusions correspond to aluminium oxyhydroxide (alum), an adjuvant incorporated in these vaccines to boost immunologic responses.[2] It is now clear that rapid emergence of MMF in France resulted from the specific combination of 3 factors : (1) replacement of the subcutaneous route by the i.m. route of vaccination in the early 1990s; (2) widespread extension of HBV

primovaccination to the French adult population in the same time; and (3) the choice of the deltoid muscle (also used for i.m. vaccination) for routine muscle biopsy in France whereas biceps brachialis and quadriceps femoris muscles are preferred in most other countries. MMF lesion is now universally recognized to assess long-term persistence of alum at site of previous intramuscular (i.m.) immunization.[4] However, alum has been generally considered as safe on the basis of short-term surveys, and exact significance of longstanding MMF detection in a given patient remains uncertain because of (i) apparently "poorly specific" clinical manifestations, which of course does not mean non-disabling ones, and (ii) lack of self-evident link between persistence of alum agglomerates into macrophages at site of immunization and delayed onset of systemic and neurologic manifestations. Formal delineation of "autoimmune/inflammatory syndrome induced by adjuvants" (ASIA),[5] and novel insights into the biodistribution of slowly biodegradable particles taken-up by monocyte-lineage cells in peripheral tissues provide settlement for a better understanding of this rare adverse effect of alum.

MMF histopathology

Deltoid muscle biopsy findings are stereotyped,[1–4] consisting of focal infiltration of the epimysium, perimysium and perifascicular endomysium by well-circumscribed and cohesive sheets of large mononuleated cells of the monocyte and macrophage lineage, usually intermingled with a minor lymphocytic population. The maximum observed section size of the lesion is 1cm. Aluminium salts are positively stained by hematoxylin and, consistently, the cytoplasm of macrophages is basophilic (dark blue) on hematoxylin-eosin stained cryostat sections. Probably due to specific chemical reactions, this is not observed on formalin fixed material in which macrophages exhibit a finely granular grey/beige content. In both cryostat and paraffin sections, macrophages are strongly periodic Acid Schiff $(PAS)^+$. They express CD68 and major histocompatibility complex (MHC) class 1 and MHC class 2 antigens. $CD3^+$ T-cells, mainly $CD8^+$, forming perivascular cuffs are constantly found. Occasional $CD19^+$ B cells, rarely forming lymphoid follicles, and $CD138^+$ plasma cells may be detected. Giant multinucleated cells are not detected except when another foreign material, e.g. coton wool, is present. In rare instances (about 1%) the granuloma may be encircled by thick fibrotic tissue and centered by a large necrotic area, forming a lesion reminiscent of a rheumatoid nodule. Myofibers remote from the infiltrate are typically intact, but MMF may be occasionnaly associated with typical dermatomyositis or autoimmine necrotizing myopathy. At electron microscopy, macrophages appear heavily loaded with submicron/micron-sized agglomerates of spiculated osmiophilic structures surrounded by discontinuous lysosomal membranes. In routine, inclusions can be visualized by the Morin stain for aluminium. Micro-organisms are not detected by appropriate stainings or electron microscopy.

Similar MMF lesions can be detected in the quadriceps muscle in babies and children because this muscle is used for i.m. vaccine administration in young individuals. MMF can be experimentally reproduced by i.m. vaccination in mice, rats and monkeys,[2,6,7] progressively shrinking with time.[6] It is, therefore, important to determine if the MMF lesion is unusually persistent in biopsied patients by precisely recapitulating history of previous vaccinations. In practice we consider MMF to be so when the time elapsed from last vaccine shot to MMF detection is >18 months. This point is particularly important in small children who receive numerous alum-containing vaccine shots in the first year of life, increasing risk of chance associations between MMF lesions and unrelated conditions, e.g. congenital myopathies and muscular dystrophies.[8] The risk also exists in adults but accounts for no more than 5–10% of MMF^+ biopsies, including fully asymptomatic patients and patients investigated for hereditary disorders.

In contrast to i.m. injections, alum-containing vaccines administered by the s.c. route may elicit chronic lesions that are somewhat different from MMF, so-called cutaneous pseudo-lymphoma, associated with a rim of alum-containing macrophages.[9]

From MMF-associated syndrome to ASIA

According to the patient association, about 1000 patients with documented MMF have been identified in France. Occasional cases have been reported in many other countries.[8,10–15] The structure of symptoms was strikingly similar in independent cohorts of French adult patients.[4] We recently reviewed the files of 457 adult MMF patients collected from 1994 to 2011 in our centre. Patients were either investigated and biopsied (n=270) at the Neuromuscular Centre of Créteil (Neuromuscular Reference Centre Garches-Necker-Mondor-Hendaye), or were referred for follow-up or complementary investigation after MMF detection in other French hospitals by one of the myopathologists that had described the lesion (n=187). Most patients were females (70%) and at the middle age at time of biopsy (median 45 years, range 12–83). They had received 1 to 17 i.m. alum-containing vaccine administrations (mean 5.3) in the 10 years before MMF detection, and these included HBV vaccination in 85%. Patients mainly complained of chronic diffuse myalgias >6mois (89%) with or without arthralgias, disabling chronique fatigue >6 months (77%), overt cognitive alterations affecting memory and attention (51%), and dyspnea 50%. As previously reported, onset of these clinical symptoms was always posterior to, and delayed from, immunization, median time elapsed from last vaccine administration being 7 months (range 0.5–84) for initial systemic symptoms, and 11 months (range 0–72) for first myalgia.[4] Time elapsed from last vaccine administration to biopsy was 65 months (range 3–219). Compared to our previous reports, this delay has progressively increased (36 months in the initial series of 2001, 53 months in series of 2003), [4] indicating that MMF patients are chronically diseased and, though mainly vaccinated in the late nineties or early 2000', frequently looked for diagnosis long after onset of symptoms.

Myalgias and fatigue may not be synchronous. Myalgia may follow an exercise of unusual intensity and often begin in lower limbs,[4] and almost never at site of previous vaccine injection. Myalgia progressively extend upward to affect paravertebral muscles and become diffuse at time of biopsy.[4] Muscle weakness is rare. Myopathic electromyogram and CK elevation are found in less than one half of patients. Some fibromyalgic tender points are detected in a minority of patients, but the 1990 ACR criteria for fibromyalgia are rarely fulfilled.[4] Interestingly, ^{67}Gallium scintigrams has shown the presence of subtle radionuclide uptake predominating in the painful areas along the lower limb muscle fascias and in para-articular tissues in all tested patients.[17] This was not found in fibromyalgic controls.

Fatigue, sleep disturbances with unrereshing sleep, and sometimes headaches may be very disabling and often deeply impacts professional and personal life. A case-control study conducted by AFSSAPS pointed out chronic fatigue as more frequent and more pronounced in patients with than without MMF in deltoid muscle (http://afssaps.sante.fr/htm/10/myofasci/etude.pdf). In fact, a majority of patients fulfil international criteria of chronic fatigue syndrome.[18] Consequently, history of exposure to alum-containing vaccines should be checked carefully in patients with CFS, and muscle biopsy searching persistent MMF at site of injection should be considered when chronology is consistent, even many years after onset of symptoms.

CNS involvement is assessed by cognitive dysfunction. Patients complain of subjective memory impairment, difficulties in sustaining attention, and mood disturbancies. Although often disabling, cognitive dysfunction is often underestimated or remains undetected by routine examination. A comprehensive battery of neuropsychological tests in unselected MMF patients without MS showed alterations in all individuals, consistent with mild cognitive impairment (MCI) but including at least one test reaching the dementia threshold in 96%.[19] Compared to arthritis controls matched for pain severity and duration, depression and educational level, MMF patients displayed distinctive impairment of visual memory, working memory and dichotic listening, a pattern suggestive of cortico-subcortical organic damage involving fronto-parieto-thalamo-striatal areas, with deep white matter alterations.[19] Very similar cognitive alterations have been documented workers exposed to inhaled Al fumes or powder.[20–22] These alterations are also reminiscent of those described in HIV- or HCV-infected individuals.[19]

In addition to CFS, 15–20% of patients with MMF concurrently develop an autoimmune disease, the most frequent of which being multiple sclerosis (MS)-like demyelinating disorders [12–23, 23,24] Hashimoto's thyroiditis, and diffuse dysimmune neuromuscular diseases, such as dermatomyositis, necrotizing autoimmune myopathy, myasthenia gravis, and inclusion body myositis. Even in the absence of overt autoimmune disease, low titers of various autoantibodies, increased inflammatory biomarkers, and abnormal iron status are commonly detected.[4]

Taken individually, none of the clinical manifestations commonly associated with persistent MMF is specific of a given cause. Combination of chronic myalgias, fatigue, and cognitive dysfunction is consistent with CFS,[18] a poorly understood condition also known as myalgic encephalomyelitis,[25] which may be triggered by various infectious and non-infectious agents. We previously noted the closely similar structure of symptoms in individuals with MMF and with the so-called Gulf war syndrome[4] which is increasingly recognized as linked to multiple vaccinations,[26,27] with special emphasis put on anthrax vaccine, an alum-adjuvanted vaccine administered in 6 shots, that was recently shown to also induce MMF.[13] On these grounds, we proposed to consider MMF-associated symptoms as an adjuvant-induced syndrome.[28] Therefore, we fully support the term ASIA (autoimmune/inflammatory syndrome induced by adjuvants) coined by Pr Shoenfeld to designate these symptoms, regardless of the nature of the involved immunologic adjuvant (alum, silicone gel, viral components, etc).[5]

Handling and transport of poorly soluble nanomaterials by phagocytes : a possible clue for understanding MMF and ASIA

For decades, aluminium oxyhydroxide, is the most commonly used adjuvant in human and veterinary vaccines. The mechanism by which it stimulates the immune response remains incompletely understood. [29]

Imbalance between the huge number of alum-vaccine receivers and the small number of biopsy-proven MMF cases strongly suggest that individual susceptibility factors play a crucial role in intolerance to alum. In rats, the genetic background strongly influences the size of lesions induced by i.m. injection of alum.[6] Adverse response to alum injection may also depend on susceptibility genes, such as HLA-DRB1*01, that may favour the development of autoimmune diseases.[30] Thus, aluminium likely represents one environmental factor able to trigger adverse effects in individuals with as yet largely unknown susceptibility genes. In keeping with this view, several closely related conditions have been shown to be associated with Al overload, including MMF,[14] idiopathic CFS,[31] and MS.[32] Moreover, strong suspicion of a possible link between Gulf war syndrome and alum administration has been experimentally supported.[33] Quite logically, questions are currently burgeoning about the exact safety level of aluminium adjuvants.[34]

However, if biopersistence of the adjuvant in the body is a priori undesirable, the exact significance of MMF remains uncertain since a conceptual link is still a missing between the observed persistence of particle-loaded MPs at site of previous immunization and the systemic, especially neurologic, clinical manifestations. Alum is potentially highly neurotoxic,[33] but it is used at concentrations viewed as an acceptable compromise between adjuvanticity and toxicity by industry and regulatory agencies. In fact, the potential toxicity of alum will be influenced by whether the bioactive nanomaterial remains localized at injection points or rather scatters and accumulates in distant organs and tissues. Characterization of the fate of i.m. injected particles is therefore crucial for understanding pathophysiology of MMF and related disorders.

A reference study based on isotopic ^{26}Al showed poor ^{26}Al clearance in the urine after i.m. injection of isotopic alum to rabbits (6% at d28 endpoint), and detected ^{26}Al, in an unknown form, in lymph nodes, spleen, liver, and brain.[35] However, as for other slowly biodegradable nanomaterials, the biodistribution of alum particles following injection into muscle is currently unknown.

Aluminium oxyhydroxide is composed of micron/submicron-sized aggregates of nano-sized (ca 13 nm) particles and these aggregates were initially believed to remain extracellular until their complete solubilisation in interstitial fluids.[35] We now know that quite the reverse is the case and that APCs avidly take up alum particles,[36] and, in so-doing, become long-lived cells,[37] and impede alum solubilization.[2] Inflammatory monocytes (MOs) are attracted into muscle by danger signals, becoming macrophages and MO-derived dendritic cells (DCs), before migrating to the draining lymph nodes (DLNs).[38] Since one function of migratory DCs is to transfer antigenic material to a large network of distant resident APCs, we examined if fluorescent nanomaterials injected into muscle could translocate to distant organs as part of a general mechanism linked to phagocytosis.

Preliminary results have substantiated this view.[39,40] We observed that fluorescent surrogates of alum particles injected into mouse muscle were rapidly taken up by macrophages to form a MMF-like granuloma. An important proportion of particles escaped the injected muscle, mainly within immune cells, gaining access to the regional lymph nodes. Then particle-loaded cells exited the lymphatic system to reach the blood stream (presumably through the thoracic duct, a terminal lymphatic vessel plugged to the subclavian vein), allowing them to gain access to distant organs such as spleen, liver and, eventually, the brain. Using lymph node ablation and genetically manipulated animals, we documented that systemic biodistribution of particles injected into muscle necessitates early cell loading in muscle or lymph nodes, and crucially depend on the presence of attracting signals for monocytes (namely the MCP-1/CCL2 chemokine) in tissues. Thus, immune cells loaded with alum-like particles circulate after the i.m. injection and can reach distant tissues such as brain, especially if they produce attracting signals for inflammatory cells or exhibit weak blood brain barrier (BBB).[39,40] This may also apply to other poorly degradable nanomaterials such as silicone, another compound suspected to cause ASIA.[5] Of course, lot remains to be done to determine if, in what conditions, and to what extent alum and other mineral particles gaining access to the brain by a Trojan horse mechanism, as HIV and HCV particles do, can cause significant inflammatory and neurotoxic damage.

In conclusion, MMF revealed an almost complete lack of knowledge on the fate, systemic diffusion, and long-term safety of alum particles. On the grounds of our clinical and experimental data, we believe that increased attention should be paid to possible long-term neurologic effects of continuously escalating doses of alum-containing vaccines administered to the general population. Special emphasis should be put on individuals with immature/altered BBB or inflammatory states.

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Autoimmune (auto-inflammatory) syndrome induced by adjuvants (ASIA)--animal models as a proof of concept

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Abstract

ASIA syndrome, "Autoimmune (Auto-inflammatory) Syndromes Induced by Adjuvants" includes at least four conditions which share a similar complex of signs and symptoms and have been defined by hyperactive immune responses: siliconosis, macrophagic myofasciitis syndrome, Gulf war syndrome and post-vaccination phenomena. Exposure to adjuvants has been documented in these four medical conditions, suggesting that the common denominator to these syndromes is a trigger entailing adjuvant activity. An important role of animal models in proving the ASIA concept has been established. Experimentally animal models of autoimmune diseases induced by adjuvants are currently widely used to understand the mechanisms and etiology and pathogenesis of these diseases and might thus promote the development of new diagnostic, predictive and therapeutic methods. In the current review we wish to unveil the variety of ASIA animal models associated with systemic and organ specific autoimmune diseases induced by adjuvants. We included in this review animal models for rheumatoid arthritis-like disease, for systemic lupus erythematosus-like disease, autoimmune thyroid disease-like disease, antiphospholipid syndrome, myocarditis and others. All these models support the concept of ASIA, as the Autoimmune (Auto-inflammatory) Syndrome Induced by Adjuvants.

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Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) 2013: Unveiling the pathogenic, clinical and diagnostic aspects

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Abstract

In 2011 a new syndrome termed 'ASIA Autoimmune/Inflammatory Syndrome Induced by Adjuvants' was defined pointing to summarize for the first time the spectrum of immune-mediated diseases triggered by an adjuvant stimulus such as chronic exposure to silicone, tetramethylpentadecane, pristane, aluminum and other adjuvants, as well as infectious components, that also may have an adjuvant effect. All these environmental factors have been found to induce autoimmunity by themselves both in animal models and in humans: for instance, silicone was associated with siliconosis, aluminum hydroxide with post-vaccination phenomena and macrophagic myofasciitis syndrome. Several mechanisms have been hypothesized to be involved in the onset of adjuvant-induced autoimmunity; a genetic favorable background plays a key role in the appearance on such vaccine-related diseases and also justifies the rarity of these phenomena. This paper will focus on protean facets which are part of ASIA, focusing on the roles and mechanisms of action of different adjuvants which lead to the autoimmune/inflammatory response. The data herein illustrate the critical role of environmental factors in the induction of autoimmunity. Indeed, it is the interplay of genetic susceptibility and environment that is the major player for the initiation of breach of tolerance.

Keywords: Adjuvant; Autoantibodies; Autoimmune/Inflammatory syndrome induced by adjuvants; Autoimmunity; Saccharomyces cerevisiae; Vaccine.

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Postural Orthostatic Tachycardia With Chronic Fatigue After HPV Vaccination as Part of the "Autoimmune/Auto-inflammatory Syndrome Induced by Adjuvants": Case Report and Literature Review

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Abstract

We report the case of a 14-year-old girl who developed postural orthostatic tachycardia syndrome (POTS) with chronic fatigue 2 months following Gardasil vaccination. The patient suffered from persistent headaches, dizziness, recurrent syncope, poor motor coordination, weakness, fatigue, myalgias, numbness, tachycardia, dyspnea, visual disturbances, phonophobia, cognitive impairment, insomnia, gastrointestinal disturbances, and a weight loss of 20 pounds. The psychiatric evaluation ruled out the possibility that her symptoms were psychogenic or related to anxiety disorders. Furthermore, the patient tested positive for ANA (1:1280), lupus anticoagulant, and antiphospholipid. On clinical examination she presented livedo reticularis and was diagnosed with Raynaud's syndrome. This case fulfills the criteria for the autoimmune/auto-inflammatory syndrome induced by adjuvants (ASIA). Because human papillomavirus vaccination is universally recommended to teenagers and because POTS frequently results in long-term disabilities (as was the case in our patient), a thorough follow-up of patients who present with relevant complaints after vaccination is strongly recommended.

Keywords

Postural orthostatic tachycardia, chronic fatigue, HPV vaccine, Gardasil, ASIA syndrome, vaccine adjuvants, autoimmunity, autoantibodies

Introduction

Postural orthostatic tachycardia syndrome (POTS) is a heterogeneous disorder of the autonomic nervous system in which a change from the supine position to an upright position causes an abnormally large increase in heart rate or tachycardia (30 bpm within 10 minutes of standing or head-up tilt).[1] The tachycardic response in POTS is frequently accompanied by a decrease in blood flow to the brain and hence a spectrum of symptoms associated with cerebral hypoperfusion (Table 1).[1-3] Due to the wide heterogeneity of symptoms and its frequent co-occurrence with other systemic autoimmune diseases, POTS is difficult to diagnose. Moreover, because many of POTS-related symptoms are also observed in chronic anxiety and panic disorders, POTS is frequently underdiagnosed and misdiagnosed.[2]

POTS predominantly affects women of the childbearing age with a 5:1 female–male ratio.[2] The estimated prevalence of POTS is at least 170/100 000. This estimate was based on the finding that 40% of patients with chronic fatigue syndrome (CFS) also suffer from POTS.[4] Indeed, CFS is a frequent and major comorbidity in POTS.[5,6] The 2 conditions frequently appear together, and research shows that there is a clinically identifiable subgroup of patients with CFS and orthostatic intolerance that differs from control subjects and from those with CFS without orthostatic intolerance.[4] In agreement with these observations, Okamoto et al[7] recently found that the majority of patients with POTS also fulfilled the criteria for CFS and that severe fatigue and CFS-defining

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Table 1. Symptoms Associated With Postural Orthostatic Tachycardia Syndrome (POTS).[2,3]

Symptom Category	Present in Current Case
Orthostatic symptoms associated with general hypoperfusion	
Light headedness/dizziness	+
Presyncope and syncope	+
Palpitations	+
Exacerbation by exercise/exercise intolerance	+
Sense of weakness	+
Tremulousness	–
Dyspnea	+
Ventricular fibrillation	–
Myocardial infarction	–
Cold extremities	+
Chest pain	–
Exacerbation associated with menses	Not reported
Hyperhidrosis	Not reported
Loss of sweating	Not reported
Tinnitus	–
Visual disturbances	+
Nonorthostatic symptoms	
Nausea	+
Bloating	–
Diarrhea	–
Constipation	–
Abdominal pain	+
Bladder symptoms	–
Other associated symptoms	
Fatigue	+
Sleep disturbances	+
Migraines	+
Neuropathic type pain	+
Cognitive disturbances	+
Flu-like symptoms	+

symptoms were also common in POTS patients who did not meet all the criteria for CFS. Such typical CFS symptoms that are overrepresented in POTS patients include migraine, incapacitating fatigue, fibromyalgia, unrefreshing sleep, and impaired memory or concentration. Flu-like symptoms associated with CFS such as joint pains, tender lymph nodes, and sore throat are also present in POTS albeit with lesser prevalence.[7] These and other similar observations indicate that POTS with CFS is not a separate clinical entity entirely distinct from POTS but rather a more severe form of this condition.[7,8] Much like POTS, CFS affects predominantly women and can be severely disabling, profoundly impairing patients' ability to function on a daily basis.[6,9]

Genetic as well as nongenetic factors such as trauma, bacterial or viral infection, and pregnancy may predispose to POTS.[1] In addition, it is becoming increasingly recognized that POTS and CFS can also be triggered by various medications (ie, antihypertensive drugs, antipsychotics)[1] and vaccines.[10-15] Herein we describe a case of a 14-year-old girl who presented with POTS/CFS of an autoimmune origin approximately 2 months after receiving her second injection of the quadrivalent human papillomavirus (qHPV) vaccine Gardasil.

Case Report

A 14-year-old previously healthy girl presented with flu-like symptoms, sore throat, low-grade fever, fatigue, swollen glands, and intense headaches in February 2009, approximately 2 months after her second qHPV vaccine injection. Over the course of 1 week, the headache intensified and the patient further presented with photophobia, phonophobia, altered sense of taste, diminished appetite, gait disturbances, leg weakness, and inability to walk without assistance. By March 2009, her condition worsened and she quit regular school attendance due to progressively disabling symptoms. At that time she developed syncope and incapacitating chronic fatigue. Although the patient subsequently resumed attending school (by the end of 2009), her attendance was limited to 2 hours per day due to fatigue, diminished ability to focus, weakness, and severely impaired balance and coordination. She attended school in a wheel-chair and was exhausted after the 2-hour period. Her illness continue to progress, and by the end of 2010, she had the following symptoms: persistent incapacitating headaches, dizziness, recurrent syncope, lower extremity weakness, poor motor coordination, fatigue, neck pain, joint pains, numbness in the legs, blurred vision, photophobia, phonophobia, cognitive impairment, insomnia, tachycardia, dyspnea, impaired thermoregulation, cold extremities, blush discoloration of toes, excessive hair loss, gastrointestinal (GI) disturbances, altered sense of taste, diminished appetite, and weight loss (20 pounds within 3 months of symptoms onset). The psychiatric evaluation in September 2009 ruled out the possibility that the patient's symptom were of psychosomatic origin, and the subsequent evaluation in 2010 found no evident signs of panic and anxiety disorders.

Serological evaluations revealed a number of abnormalities, including an elevated ANA at 1:1280, a positive lupus anticoagulant, and a weakly positive antiphospholipid of 7.3 in October 2009. On clinical examination, the patient presented livedo reticularis. She was then diagnosed with an undifferentiated connective tissue disease and Raynaud's syndrome. Serology results for Epstein–Barr virus, Lyme, Babesia, and Ehrlichia were negative. Titers to *Streptococcus pneumoniae* indicated previous exposure but were however within a normal range, thus ruling out recent exposure.

Over the course of her illness, the patient experienced a complete loss of consciousness with syncope approximately 12 times. These problems were never present prior to the onset of the illness in February 2009. On further testing, the patient was diagnosed with orthostatic intolerance. In

Table 2. The Suggested Criteria of ASIA[29,30] in the Current Case of Post-HPV Vaccine POTS/CFS.

Major Criteria	Present in Current Case
1. Exposure to an external stimuli (infection, vaccine, and/or immune adjuvants) prior to clinical manifestations	+
2. The appearance of "typical" clinical manifestations	
Myalgia, muscle weakness	+
Arthralgia and/joint pain	+
Chronic fatigue, unrefreshing sleep or sleep disturbances	+
Neurological manifestations	+
Cognitive impairment, memory loss	+
Pyrexia	−
3. Removal of inciting agent induces improvement	NA
4. Typical biopsy of involved organs	Not assessed
Minor Criteria	**Present in Current Case**
1. The appearance of autoantibodies	+
2. Other clinical manifestations (gastrointestinal disturbances, livedo reticularis)	+
3. Specific HLA (eg, HLA DRB1, HLA DQB1)	Not assessed
4. Evolvement of an autoimmune disease (undifferentiated connective tissue disease/ Raynaud's, probable secondary antiphospholipid syndrome)	+

particular, on the standing test the patient's lowest heart rate supine was 47 bpm with a blood pressure 103/56 mm Hg. On standing, the patient's heart rate increased immediately to 82 bpm and continued to increase to a maximum of 98 bpm after 9 minutes. According to the electrophysiologist, the patient's recurrent syncope was thus consistent with neurally mediated hypotension, and in December 2009, she was finally diagnosed with vasovagal syncope and associated postural orthostatic tachycardia syndrome. In addition, her illness met the criteria for CSF given her persisting fatigue of over 6 months, new-onset disabling headaches, postexertional worsening of the fatigue, myalgias, cognitive dysfunction, and unrefreshing sleep (Table 1). The patient's relevant medical history includes a family history of Raynaud's (patient's mother) and a personal history of headaches, dizziness, photophobia, and phonophobia in 2007, all of which however resolved completely in the same year.

Discussion

Autoimmune Origin of POTS and CFS

Herein we described a case that clearly fulfilled the criteria for POTS/CFS (Table 1) secondary to qHPV vaccine booster injection. An autoimmune mechanism has been suggested as a causal mechanism in both POTS and CFS due to frequent findings of autoantibodies (including ANA) in POTS/CFS patients.[16,17] Other reported abnormalities in CFS also point to an underlying autoimmune mechanism (ie, increased levels of pro-inflammatory cytokines interleukin-1, tumor necrosis factor-α, and increased levels of nuclear factor-κB).[18] It is estimated that up to 60% of CFS patients suffer from autoimmune responses[18] and that both POTS and CFS frequently co-occur with systemic autoimmune disorders including multiple sclerosis,[19] Sjorgen's syndrome,[20] lupus,[1,21] and Raynaud's.[22] Similarly, our case was diagnosed with Raynaud's, CFS, and neurally mediated hypotension or more specifically, POTS.

Our patient's symptoms began manifesting approximately 2 months following vaccination. An interval of 6 weeks between exposure and outcome is often used as evidence of a plausible causal association; however, immune and autoimmune diseases are chronic diseases that more often than not have a long incubation time.[23] For example, it was reported by Arbuckle et al that systemic lupus erythematosus (SLE) evolves slowly and progressively over many years and only when enough autoantibodies are present.[24] In particular, autoantibodies were found in 88% of SLE patients up to 9.4 years before the clinical diagnosis of the syndrome (mean = 3.3 years).[24] Thus, long-term persistence of elevated titers of autoantibodies was necessary for the emergence of clinically overt signs and symptoms for the diagnosis of SLE. Notably, the accumulation of autoantibodies occurred while patients were still asymptomatic.

Similarly, postvaccination adverse immune phenomena can have long latency periods (ie, month to years following immunization).[25-27] As early as 1982, compelling evidence from epidemiological, clinical, and animal research has emerged to show that autoimmune neuropathies can occur 4 to 10 months following vaccination.[28] In such cases the disease would first manifest with vague symptoms (ie, arthralgia, myalgia, paraesthesia, weakness—note also that these are typical ASIA symptoms; Table 2), which were frequently deemed as insignificant and thus ignored. These symptoms, otherwise known as "bridging symptoms" and consistent with a mild subclinical disease, would progress slowly and insidiously until exposure to a secondary immune stimulus. The latter would then trigger the rapid and acute clinical

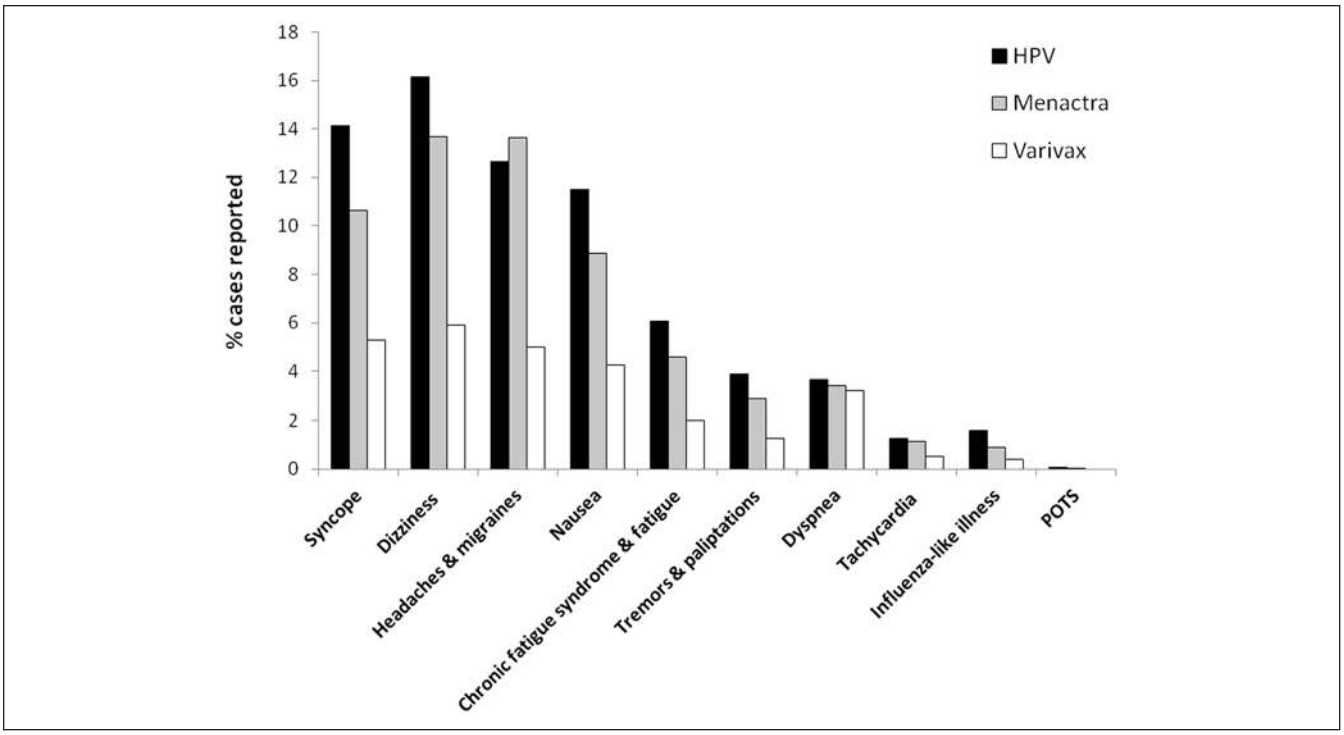

Figure 1. Number of adverse event reports related to POTS/CFS following HPV, Menactra meningococcal polysaccharide diphtheria toxoid conjugate, and Varivax Varicella vaccines in the US Vaccine Adverse Event Reporting System (VAERS) as of February 13, 2013. The VAERS database[33] was searched using the following criteria: (1) Symptoms: syncope (general, exertional, postural); headaches (including migraines); nausea; chronic fatigue syndrome (including general fatigue); tremors and palpitations; dyspnea (general, exertional, at rest); tachycardia (including tachyarrhythmia, tachycardia paroxysomal, heart rate abnormal, heart rate increased, heart rate irregular); influenza-like illness (including viremia, viral infection); POTS; (2) Vaccine products: HPV, HPV2 (human papilloma virus bivalent), HPV4 (human papilloma virus types 6, 11, 16,1 8); MNQ (Meningococcal vaccine Menactra); Varcel (Varivax-Varicella virus live); (3) Gender (all genders); (4) Age (6 to 29 years; target age group for HPV, Menactra and Varivax vaccines); (5) Territory (the United States); (6) Date vaccinated (2007-2013; HPV vaccine postlicensure period).[34] Adverse events related to a particular symptom are reported as percentages of the total number of events reported for the particular vaccine (ie, 14% syncope refers to the 2354 reports of syncope out of a total of 16 644 adverse events associated with the HPV vaccine; the total number of adverse events reported for Varivax and Menactra was 9136 and 8790, respectively).

manifestation of the disease.[28] In other words, it was the secondary anamnestic response that would bring about the acute overt manifestation of an already present subclinical long-term persisting disease.

Consistent with these observations, we recently described several cases of autoimmunity (systemic lupus) following Gardasil where the nonspecific ASIA-related manifestations eventually progressed over time to a full-blown immune disease following subsequent vaccine reexposure.[31] Moreover, in all of our cases, several common features were observed, namely, a personal or familial susceptibility to autoimmunity and an adverse response to a prior dose of the vaccine, both of which were associated with a higher risk of postvaccination full-blown autoimmunity.[31]

POTS and Vaccinations

Ours is the seventh case of POTS associated with the qHPV vaccine Gardasil reported in the literature. In addition, POTS following administration of the novel H1N1 influenza vaccine was reported recently.[13] Recently, Blitshteyn[12] reported six cases of POTS following HPV vaccination. In this case series, all six previously healthy young women (aged 12 to 22 years) developed symptoms of POTS within 6 days to 2 months after vaccination with the Gardasil HPV vaccine. Of further relevance to our case, two out of six cases reported by Blitshteyn also showed a positive ANA and, in all six cases the symptoms were disabling. In particular, three of the patients were not capable of attending school full time and one of them became wheel-chair bound like the patient described in our report. The course of POTS following HPV vaccination was similar in all six patients, with all of them improving in 2 to 3 years' time frame with the use of standard pharmacotherapy for POTS. It is possible as emphasized by Blitshteyn[12] that some patients with POTS are simply undiagnosed or misdiagnosed with anxiety and panic-related disorders, which leads to underreporting and a paucity of data on the incidence of POTS and other autonomic system disorders following vaccination. The analysis of the US VAERS database substantiates this concern. In particular, although the majority of POTS-related symptoms were reported in 4% to 16% of HPV vaccine recipients, POTS was only reported in 0.07% of cases (Figure 1). The highest number of both POTS- and CFS-related symptom reports was associated

with HPV vaccines when compared with 2 other vaccines (Menactra and Varivax), routinely given to adolescents in the United States. On average, the number of VAERS reports related to POTS/CFS symptoms was 3 to 5 times greater for the HPV compared with the Varivax vaccine. A relatively high percentage of POTS/CFS symptom reports was also associated with the Menactra vaccine. If these symptoms were psychogenic and not related to a specific vaccine but rather a reaction to the injection procedure itself, one would expect a more even distribution of reports with different vaccines. In particular, the percentage of POTS/CFS reports for Varivax should be more or less the same as for Menactra especially considering the fact that the total number of VAERS reports associated with these 2 vaccines was roughly the same (9136 and 8790, respectively). As shown in Figure 1, this is not the case. Consistent with our findings, Slade et al[32] found a disproportional reporting of syncope following HPV compared with other vaccines in their 2009 postlicensure analysis of adverse events reported to VAERS and published in JAMA. We are in further agreement with Slade et al[32] who also noted that although VAERS shares inherent limitations of all passive surveillance systems, it is national in scope and can thus provide important signals that may require further attention. Indeed, because both POTS and CFS are frequently severely disabling,[1,6,9,10,13,15] a more thorough follow-up of patients who present with relevant complaints postvaccination seems warranted in order to determine the true incidence of these syndromes with particular vaccines.

Another possible reason for the frequent misdiagnosis of POTS is that patients with this syndrome typically present with complaints that partially overlap with those seen in panic disorders or chronic anxiety.[2] Notably, such symptoms (syncope, hyperventilation, limb jerking, numbness or tingling, palpitations, and tremors) appear to be among the most frequently reported adverse reactions following vaccination with HPV vaccines and may be mistakenly labeled as "psychogenic events."[35] From our patient's medical history, it is evident however that the post-qHPV vaccination phenomena were neither temporary nor psychogenic. Indeed, the psychiatrist's evaluation specifically excluded the latter in addition of finding no relevant signs of anxiety or panic disorders. The highly positive ANA further excludes a psychosomatic origin of the patient's illness; rather, it suggest an immune-/auto-immune-mediated underlying pathology.

Although in our case the patient had a previous history of relevant symptoms (headaches, dizziness, photophobia, and phonophobia) 2 years prior to qHPV vaccination, all of these symptoms resolved in the same year and did not cause long-term disability of the patient. Indeed, at the time of first vaccination the patient was in good general health. Moreover, during the course of her illness, the patient experienced a wide spectrum of new-onset adverse conditions, including recurrent episodes of syncope with complete loss of consciousness, disabling fatigue, neck pain, joint pains, numbness in the legs, cognitive disturbances, blurred vision, unrefreshing sleep, tachycardia, dyspnea, impaired thermoregulation, cold extremities, blush discoloration of toes, excessive hair loss, GI disturbances, diminished appetite, altered sense of taste, and significant weight loss. She also tested positive for ANA, lupus anticoagulant, and antiphospholipid and was subsequently diagnosed with undifferentiated connective tissue disease/Raynaud's. Notably, none of these manifestations were present prior to the onset of her illness in February 2009 following Gardasil vaccination, indicating that the vaccine may have been the triggering, or at the very least, the exacerbating factor.

Although a viral illness cannot be completely excluded as the primary trigger of POTS/CFS in our case, it should be noted that symptoms mimicking viral illness (commonly referred to as flu-like symptoms) are in fact one of the well-recognized symptom categories in CFS.[10,36] Moreover, both flu-like symptoms and CFS are associated with the use of certain vaccines, and more specifically, aluminum and other vaccine adjuvants.[14,15,37] Indeed, because vaccines induce an immune response similarly to infections, they may also just like infections trigger autoimmune diseases.[38] However, unlike infectious agents, vaccines frequently contain adjuvants that further enhance their immune stimulation, above the levels of natural infections.[39] These observations suggest that vaccines may provoke more exaggerated, anarchic immune responses than infections. The latter point is specially relevant in view of the fact that vaccines (including HPV) are typically repeatedly administered over relatively short periods of times (ie, weeks or months). Moreover, vaccines have been reported to precede CFS mainly following exposure to multiple vaccinations and/or as an adverse response to the vaccine adjuvant.[14,15,39,40]

POTS, CFS, and the ASIA Syndrome

It is of further relevance to note that the safety trials for Gardasil (which is an aluminum-adjuvanted vaccine) did not include a true inactive placebo but rather an aluminum-adjuvant-containing placebo,[41] despite much data showing that aluminum in vaccine-relevant exposures can be toxic to humans.[42,43] In the last decade, studies on animal models have repeatedly demonstrated the ability of aluminum adjuvants to inflict immune-mediated diseases by themselves.[44,45] This research culminated in delineation of ASIA (autoimmune/inflammatory syndrome induced by adjuvants), which encompasses several medical conditions with similar set of signs and symptoms and a common exposure to an immune adjuvant.[10,29] Shoenfeld and colleagues proposed 4 major and 4 minor criteria for ASIA (Table 2), and in order to diagnose ASIA, fulfillment of either 2 major or 1 major and 2 minor criteria is required.[29] The criteria for ASIA enable the inclusion of patients with well-defined autoimmune diseases (ie, multiple sclerosis, lupus) as well as those with ill-defined and nonspecific yet clinically relevant conditions (ie, myalgia, chronic fatigue, and cognitive disturbances) under the spectrum of vaccine adjuvant-associated conditions.[30] The

inclusion of the latter category of manifestations under ASIA is of special importance as these nonspecific manifestations are all too easily ignored or disregarded as irrelevant and nonvaccine related not only by patients and physicians but also by scientists involved in design of vaccine trials.[46,47] Nonetheless, many ill-defined medical conditions that fall under the ASIA spectrum are frequently disabling and thus of significant clinical relevance. For example, CFS and cognitive dysfunction associated with the aluminum vaccine adjuvant-induced macrophagic myofasciitis (MMF) syndrome are disabling in 87% and 53% of cases, respectively,[9] and impair both professional activities as well as numerous aspects of daily life.[9,42] Similarly in our case, the patient was unable to attend regular school due to progressive and disabling POTS/CFS symptoms. In addition, some of the nonspecific ASIA manifestations have the potential to progress over time to a full-blown autoimmune disease, especially following subsequent vaccine re-exposure.[31] Of note, our patient fulfilled the first 2 major criteria for ASIA (due to a prior exposure to the HPV vaccine and the obvious appearance of "typical" manifestations) as well as 3 minor criteria, owing to the positive ANA, lupus anticoagulant, and antiphospholipid and the concurrent diagnosis of Raynaud's (Table 2).

In years following licensure, numerous case reports of serious adverse reactions of the autoimmune origin associated with the qHPV vaccine Gardasil have raised concerns about the safety of the vaccine.[12,31,48-52] Postlicensure data from vaccine safety surveillance databases worldwide appear to substantiate these concerns. For example, in the United States, compared with all other vaccines Gardasil alone is associated with >60% of all serious adverse reactions (including 63.8% of all deaths and 81.2% cases of permanent disability) in females younger than 30 years of age.[34] These observations suggest that HPV vaccine risks may not have been fully identified during prelicensure trials.[34,41,53] The unusual frequency of adverse reactions following HPV vaccination cannot solely attributed to the aluminum adjuvant, as many other vaccines also contain aluminum (ie, tetanus, diphtheria, etc) but are not associated with as many adverse reactions. However, it is the aluminum that evokes the enhanced immune reaction necessary for inducing the production of the elevated titers of antibodies. The antigen on its own is not capable of evoking this strong immune response. Because of this, any adverse effect arising from the antigen (or other constituents in the vaccine) is ultimately linked to the action of the adjuvant. For example, Zivkovic et al[54] showed that induction of the antiphospholipid syndrome (APS) syndrome and associated decreased fecundity by tetanus toxoid (TTd) hyperimmunization in C57BL/6 mice critically depends on the aluminum adjuvant. In particular, Zivkovic et al[54] investigated reproductive pathology induced in C57BL/6 mice by TTd hyperimmunization using a combination of different pretreatments (complete Freund's adjuvant or glycerol) and adjuvants (aluminum-hydrogel or glycerol). A decrease in fecundity was recorded in only C57BL/6 mice immunized with aluminum-hydrogel adjuvant, irrespective of the kind of applied pretreatment.

In conclusion, herein we described a case of disabling CFS/POTS secondary to qHPV Gardasil vaccination with symptom onset at 2 months following the second vaccine booster. With the concurrent detection of elevated ANA, lupus anticoagulant, antiphospholipid, and subsequent diagnosis of Raynaud's, this case fully meets the criteria for the recently identified ASIA syndrome (Table 2). Moreover, the case presented here is consistent with other literature supporting an immune-mediated etiology of POTS and CFS.[1,12,13,15-17] To the best of our knowledge, this is the second case of post-HPV vaccine associated POTS described in the literature to date. Due to the wide heterogeneity of symptoms and its frequent co-occurrence with other systemic autoimmune diseases, POTS is difficult to diagnose and hence many cases remain unreported. The relatively high prevalence of POTS/CFS-related symptoms in young women vaccinated with HPV vaccines (Figure 1) should alert physicians to a closer monitoring of post-HPV-related manifestations fitting the POTS/CFS criteria. We also recommend further studies to ascertain whether or not the association between HPV vaccination and POTS is causal.

Authors' Note

An informed consent has been received from the patient to present her case.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: Y. Shoenfeld is currently providing expert testimony in cases involving adverse reactions to the HPV and other vaccines in the US National Vaccine Injury Compensation Program (including this case). LT, SC, and CP declared no potential conflicts of interest.

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Evolution of multiple sclerosis in France since the beginning of hepatitis B vaccination

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Abstract

Since the implementation of the mass vaccination campaign against hepatitis B in France, the appearance of multiple sclerosis, sometimes occurring in the aftermath of vaccinations, led to the publication of epidemiological international studies. This was also justified by the sharp increase in the annual incidence of multiple sclerosis reported to the French health insurance in the mid-1990s. Almost 20 years later, a retrospective reflection can be sketched from these official data and also from the national pharmacovigilance agency. Statistical data from these latter sources seem to show a significant correlation between the number of hepatitis B vaccinations performed and the declaration to the pharmacovigilance of multiple sclerosis occurring between 1 and 2 years later. The application of the Hill's criteria to these data indicates that the correlation between hepatitis B vaccine and multiple sclerosis may be causal.

Figures

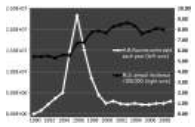

Fig. 1 Evolution of annual incidence rate...

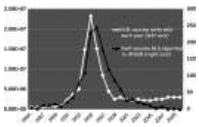

Fig. 2 Sales of Hepatitis B (HB)...

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Chronic fatigue syndrome and fibromyalgia following immunization with the hepatitis B vaccine: another angle of the 'autoimmune (auto-inflammatory) syndrome induced by adjuvants' (ASIA)

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Abstract

The objectives of this study were to gather information regarding demographic and clinical characteristics of patients diagnosed with either fibromyalgia (FM) or chronic fatigue (CFS) following hepatitis B vaccination (HBVv) and furthermore to apply the recently suggested criteria of autoimmune (auto-inflammatory) syndromes induced by adjuvants (ASIA), in the aim of identifying common characteristics that may suggest an association between fibromyalgia, chronic fatigue and HBV vaccination. Medical records of 19 patients with CFS and/or fibromyalgia following HBVv immunization were analyzed. All of which were immunized during 1990-2008 in different centers in the USA. All medical records were evaluated for demographics, medical history, the number of vaccine doses, as well as immediate and long term post-immunization adverse events and clinical manifestations. In addition, available blood tests, imaging results, treatments and outcomes were analyzed. ASIA criteria were applied to all patients. The mean age of patients was 28.6 ± 11 years, of which 68.4 % were females. 21.05 % had either personal or familial background of autoimmune disease. The mean latency period from the last dose of HBVv to onset of symptoms was 38.6 ± 79.4 days, ranging from days to a year. Eight (42.1 %) patients continued with the immunization program despite experiencing adverse events. Manifestations that were commonly reported included neurological manifestations (84.2 %), musculoskeletal (78.9 %), psychiatric (63.1 %), fatigue (63.1 %), gastrointestinal complains (58 %) and mucocutaneous manifestations (36.8 %). Autoantibodies were detected in 71 % of patients tested. All patients fulfilled the ASIA criteria. This study suggests that in some cases CFS and FM can be temporally related to immunization, as part of ASIA syndrome. The appearance of adverse event during immunization, the presence of autoimmune susceptibility and higher titers of autoantibodies all can be suggested as risk factors. ASIA criteria were fulfilled in all patients eluding the plausible link between ASIA and CFS/FM.

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Invited review

Vaccines, adjuvants and autoimmunity

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ABSTRACT

Vaccines and autoimmunity are linked fields. Vaccine efficacy is based on whether host immune response against an antigen can elicit a memory T-cell response over time. Although the described side effects thus far have been mostly transient and acute, vaccines are able to elicit the immune system towards an autoimmune reaction. The diagnosis of a definite autoimmune disease and the occurrence of fatal outcome post-vaccination have been less frequently reported. Since vaccines are given to previously healthy hosts, who may have never developed the disease had they not been immunized, adverse events should be carefully accessed and evaluated even if they represent a limited number of occurrences.

In this review of the literature, there is evidence of vaccine-induced autoimmunity and adjuvant-induced autoimmunity in both experimental models as well as human patients. Adjuvants and infectious agents may exert their immune-enhancing effects through various functional activities, encompassed by the adjuvant effect. These mechanisms are shared by different conditions triggered by adjuvants leading to the autoimmune/inflammatory syndrome induced by adjuvants (ASIA syndrome).

In conclusion, there are several case reports of autoimmune diseases following vaccines, however, due to the limited number of cases, the different classifications of symptoms and the long latency period of the diseases, every attempt for an epidemiological study has so far failed to deliver a connection. Despite this, efforts to unveil the connection between the triggering of the immune system by adjuvants and the development of autoimmune conditions should be undertaken. Vaccinomics is a field that may bring to light novel customized, personalized treatment approaches in the future.

Contents

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1. Introduction

Vaccines have been a preventive treatment option available for over 200 years. They have been proven to be effective in preventing infections that previously had high morbidity and mortality. An example of this is the eradication of small pox, which was mainly attributed to successful vaccination programs. Preventing a high burden disease has since proven to be a cost effective measure and, as such, vaccines have become a part of multiple national health programs. These promising results led to the development of more and more vaccines and to the study of its applicability in other fields such as cancer prevention and treatment.

Vaccines are drugs administered to healthy individuals, and much like other drugs, vaccines are associated with adverse events. Usually the described adverse events are transient and acute, but may rarely present with hypersensitivity and induction of autoimmunity that may be severe and fatal. These adverse events play an important role in the life of the vaccinated patients.

Immune mediated diseases arise from various different sources; these include environmental, genetic, hormonal and immune defects. The combination of these defects can be described as the mosaic of autoimmunity [1]. Patient background can be used as a clue to determine the response that may be elicited following drug administration.

It has been proven that infectious agents may elicit an autoimmune disease in a prone subject through various mechanisms, including, but not limited to, molecular mimicry, epitope spreading and polyclonal activation [2].

Scientific findings suggest that autoimmunity may be triggered by vaccine adjuvants, of which aluminum compounds (aluminum hydroxide and phosphate) have been the most studied and the most widely used. Adjuvants are molecules, which, in combination with antigens, enhance immunological response. This enables an easier and more effective recognition of "non self", which in turn permits the triggering of adaptive and innate immune responses [3].

Recently a new syndrome was described: "Autoimmune/inflammatory syndrome induced by adjuvants" (ASIA). This embodies a spectrum of reactions, which are usually mild but may also be severe. These reactions are attributed to adjuvant stimulation, which can include chronic exposure to silicone, tetramethylpentadecane, pristane, aluminum, infectious components and other adjuvants. All of these environmental factors have been found to induce autoimmunity and inflammatory manifestations by themselves both in animal models and in humans. The mechanisms of this disease will be described in further detail [4].

This review will focus on general mechanism of vaccines, adjuvant-induced autoimmunity, and on vaccines and the specific autoimmune diseases that they may trigger.

2. General mechanisms of vaccines and adjuvant induced autoimmunity

2.1. Adjuvants role in infections and autoimmunity

Adjuvants approved to date for human vaccines are: aluminum, MF59 in some viral vaccines, MPL, AS04, AS01B and AS02A against viral and parasitic infections, virosomes for hepatitis B virus (HBV), human papilloma virus (HPV), hepatitis A virus (HAV), and cholera toxin for cholera.

Adjuvants may be composed of several different compounds. Currently, oil based adjuvants, virosomes, toll-like receptors (TLRs) related adjuvants, MPL, adjuvants made of unmethylated CpG dinucleotides and tuftsin have all been described.

Table 1
Types of adjuvants in development or use.

Type of Adjuvants	Name of compound	Vaccines in test or use
Related to Toll like receptors (TLRs)	Aluminum hydroxide and phosphate	PCV7, PCV13, MenC, HPV, HAV, Hib; tetanus vaccine
	IC31	Influenza [14]
	ASO4 (MPL + QS-21), ASO2A (MPL + Alum), CPG 7907, and GM-CSF RD-529, ISS, Flagellin TLR agonists	Papilloma virus, hepatitis B, malaria [15]
Oil based emulsions	CFA, IFA, MF59™ montanide, adjuvant 65, lipovant, QS-21 [16]	Influenza
	ISCOMs, ADVAX™, algammulin	
Xenobiotic adjuvants	Unmethylated CpG dinucleotides [17]	Hepatitis B, allergens, tumor cells
Tuftsin auto adjuvant	Tuftsin	Influenza, malaria, autoimmune encephalomyelitis, restoration of innate immune system (HIV patients), SLE [18–21]

CFA: complete freund adjuvant; IFA: incomplete freund adjuvant; PCV: pneumococcal conjugated vaccine; MenC: meningitis C; HPV: human papiloma Virus; HAV: hepatitis A virus; Hib: haemophilus influenza type b.

It is of great interest the understanding of the mechanisms related to the adjuvant effect, as well as to aluminum salts. Aluminum acts through multiple pathways, which do not depend solely on TLRs signaling. Each of these pathways leads to an enhanced host immune response [5].

There are many oil based adjuvants. One is incomplete Freund adjuvant (IFA), which contains water in oil emulsion. Another is complete Freund adjuvant (CFA), which is the same as IFA, except that it also contains killed Mycobacteria in addition to water in oil emulsion. Usually, CFA is used for primary vaccination and IFA for boosting. Recent oil based adjuvants that have been developed are MF59 (Novartis®), AS03 (GlaxoSmithKline®), Advax™ which are based on inulin compounds (Vaxine™ Pty) and Qs-21/ISCOMs, which are immune stimulating complexes composed of cholesterol and phospholipid with or without antigen (Table 1).

Virosomes are adjuvants that contain a membrane-bound hemagglutinin and neuraminidase obtained from the influenza virus. Both components facilitate the uptake into antigen presenting cells (APC) and mimic the natural immune response [6].

Leucocyte membranes have membrane bound pattern recognition receptors (PRRs) called TLRs, which are responsible for detecting most (although certainly not all) antigen-mediated infections. Their activation leads to adaptive immune responses. For this reason, many adjuvants that are used today are directed to PRRs. These adjuvants are called TLRs related adjuvants [7].

MPL is a series of 4'monophosphoryl lipid A obtained from the purification of a modified lipopolysaccharide (LPS) of Salmonella Minnesota.

Bacterial deoxyribonucleic acid (DNA) is immunostimulatory due to Unmethylated CpG dinucleotides. Vertebrate DNA has relatively low amounts of unmethylated CpG compared to Bacterial DNA. The adjuvant effect of CpG is enhanced when conjugated to protein antigens. This adjuvant is being tested in vaccines directed at infectious agents, allergens and tumor cells [8–10].

Another type of adjuvant is tuftsin. Tuftsin is an auto adjuvant, which is a natural self-immunostimulating tetrapeptide (Thr–Lys–Pro–Arg). This tetrapeptide is a fraction of the IgG heavy chain molecule produced by enzymatic cleavage in the spleen [11]. Its functions include: binding to receptors on neutrophils and macrophages to stimulate their phagocytic activity, increasing tumor necrosis factor alpha (TNFα) release from human Kupffer cells enhancing secretion of IL1 by activating macrophages, activation of macrophages expressing nitric oxide (NO) synthase to produce NO and enhancement of murine natural cell mediated cytotoxicity in vitro [11–13].

In summary, it is an adjuvant with minor side effects with a promising effect in restoring innate immune mediated response.

2.1.1. Mechanisms of adjuvanticity

Adjuvants may exert their immune enhancing effects according to five immune functional activities:

1. Translocation of antigens to the lymph nodes where they can be recognized by T cells.
2. Antigen protection enabling longer exposure.
3. Enhanced local reaction at the injection site.
4. Induction of the release of inflammatory cytokines.
5. Interaction with PRRs, specifically TLRs [22].

a Adjuvant effect

The term "adjuvant effect" refers to the co-administration of an antigen with a microbial specific factor to enhance an antigen-specific immune response in vivo. The microbial components of adjuvants activate APCs to produce pro-inflammatory cytokines ("non-specific" signal 2) and to up-regulate molecules essential for antigen presentation. These molecules include major histocompatibility complex (MHC) class II (antigen-specific signal 1) and B7-1/2. These innate immune events allow a more effective presentation to the adaptive immune system, resulting in an augmented activation and clonal expansion of T cells [23].

In accordance to this effect, if self-antigens are used, an autoimmune response can be elicited [24]. It has been shown that auto-reactive T-cells that surpass tolerance mechanisms can be triggered by exogenous adjuvants to become auto-aggressive [25].

Infectious agents are able to naturally generate their adjuvant effect and can induce autoimmunity [26]. An example of this is the causality between viral infection and myocarditis. Half the cases of myocarditis are preceded by an acute viral infection. Infectious myocarditis in humans can be reproduced in experimental murine models of myocarditis [27]. It has also been shown that the autoimmune reaction elicited by an infectious agent can be effective in treating cancer. An example of this is that bladder administration of BCG (*bacille Calmette–Guérin*) has been shown to be effective against superficial bladder cancer development [28]. It can be inferred that the adjuvant effect can be used against specific tumor derived molecules, so that these molecules can be recognized as "non self".

2.1.2. Innate immune pattern recognition of pathogens and adjuvants

PRR-PAMP (Pattern Recognition Receptor—Pathogen-Associated Molecular Patterns) interactions activate the APCs to promote antigen-specific lymphocytic responses [29].

The definition of PAMPs has now broadened, in that the recognized structures do not need to be pathogens. Thus the concept of "microbe-associated molecular patterns" (MAMPs) and of "danger/damage-associated molecular patterns" (DAMPs) were defined based on the notion that the endogenous host molecules signal danger or damage to the immune system [30].

2.1.3. Innate immune response mediates the adjuvant effect

TLRs are single-transmembrane PRRs localized on cell surface and endosomal membranes. From all the PRRs, these are the most studied. TLRs play a crucial role in innate immune response to "non self" and are biosensors of tissue damage. The interaction between

the four known TLRs adapters: MyD88, TIRAP/Mal, TRAM and TRIF, in TLR signaling, shape the innate immune response.

Besides PRRs the innate immune system also detects proteolytic enzymes generated during infection [31].

Merging the response to different PRRs signaling may be the pathway for developing customized responses to different aggressions [32].

b Experimental models of adjuvants

Many animals have been used in experimental models of adjuvant-related autoimmune conditions [33]. These include primates, salmons, rabbits and swine; however, the most common are murine models.

Murine models include autoimmune prone strains, models of autoimmune disease and autoimmune resistant strains (Table 2).

An interesting model is that described by Lujan et al. The authors described that a commercial sheep, inoculated repetitively with aluminum-containing adjuvants vaccinations, developed an acute neurological episode with low response to external stimuli and acute meningoencephalitis few days after immunization. An excitatory phase, followed by weakness, extreme cachexia, tetraplegia and death appeared. This was suggested to be part of the spectrum of ASIA syndrome. Moreover, the biopsy of the nervous tissue of experimental animals indicated the presence of alum [48].

c Toxicity of aluminum adjuvants

Aluminum nanoparticles have both a unique capacity of surpassing the blood brain barrier (BBB) and of eliciting immune inflammatory responses. These are probably the reasons why Aluminums' most sensitive target is the brain, and also why documented side effects are mostly neurologic or neuropsychiatric [49,50].

Aluminum is present in nature, not only as a vaccine adjuvant, but also in food, water and cosmetics. It has been described as a neurotoxin because even when a relatively small amount of Aluminium reaches the brain [49], is can act as a genotoxin [51], a prooxidant [52], it can be proinflammatory [51], act as an immunotoxin [5] and also as an endocrine disruptor [53]. Aluminum interferes with many essential cellular processes. Memory, concentration, speech deficits, impaired psychomotor control, reduced seizure tolerance and altered behaviour are manifestations of aluminium neurotoxicity. Moreover, Alzheimer's [54], amyotrophic lateral sclerosis, Parkinsonism dementia [55], multiple sclerosis [56], and neurological impairments in children have been linked to aluminum neurotoxicity [57].

Brain susceptibility to aluminum compounds is possibly due to the brain's high metabolic requirement, to the fact that it possesses a large area of biological membranes and to the relatively low concentration of antioxidants [54].

Aluminum adjuvants exert their immunostimulatory effect through many different pathways that activate both the innate and adaptive immune systems. One of the most significant is the activation of the NLRP3 inflammasome pathway [58]. NLPR3 activation has been shown to trigger type 2 diabetes. By using NLPR3 knockout mice it has been demonstrated that the absence of inflammasome components leads to a better maintenance of glucose homeostasis and higher insulin sensitivity [59]. On the other hand, activation of the inflammasome and its downstream components: pro-inflammatory cytokines IL-1β and IL-18 are strongly implicated in the development of several central nervous system (CNS) disorders [60].

The vast majority of people are consuming higher amounts of aluminum through dietary and parenteral intake than what expert authorities consider safe. Upper limits set by US food and drug administrations (FDA) for aluminum in vaccines are set at no more than 850 μg/dose. These values were not based on toxicity studies, but on the minimum amount needed for aluminum to exert its effect as an adjuvant [51]. The quantities of aluminum to which infants, in their first year of age are exposed, have been considered safe by the FDA. However the scientific basis for this recommendation does not take into account aluminum persistence in the body. The concern about aluminum in dietary intake has been reinforced by the Food and Agriculture (FAO) WHO Expert Committee, which lowered the provisional tolerable weekly intake of aluminum from 7 mg/kg/bw (490 mg/week, for an average 70 kg human) to 1 mg/kg/bw (70 mg/week) [61].

The amount of dietary intake of aluminum has risen in urban societies to up to 100 mg/day considering the widespread use of processed convenience foods. However, only about 0.25% of dietary aluminum is absorbed into systemic circulation and most of it is thereafter eliminated through the kidneys [54]. Absorption of aluminum by the skin from ointments and cosmetics containing aluminum has been shown. Moreover, the presence of aluminum in breast tissue was associated with breast cancer [62].

Aluminum compounds persist for up to 8–11 years post vaccination in human body. This fact, combined with repeated exposure, may account for a hyper activation of the immune system and subsequent chronic inflammation [63].

The clinical and experimental evidence collected so far identify at least three main risks associated with aluminum in vaccines:

1. It can persist in the body.
2. It can trigger pathological immunological responses.
3. It can pass through the BBB into the CNS where it can trigger immuno-inflammatory processes, resulting in brain inflammation and long-term neural dysfunction.

2.2. Allergy and autoimmunity caused by metals

There is a link between allergies and autoimmunity since both are the result of an abnormal immune response [3,4].

Metals such as mercury, aluminum, nickel and gold are known to induce immunotoxic effects in humans. The immunologic effects of these metals include immunomodulation, allergies and autoimmunity. They may act either as immunosuppressants or as immune adjuvants.

Metals bind firmly to cells and proteins and thus have the ability to modify autologous epitopes (hapetenization). T-cells then recognize the proteins as foreign and trigger an autoimmune response [64].

Hypersensitivity caused by metals may be referred to as Type IV delayed hypersensitivity. The reaction is considered delayed because the first symptoms appear 24–48 h after exposure, because it is mostly T-cell mediated and the gold standard for diagnosis of delayed type hypersensitivity is patch testing [65].

In mercury-sensitized patients, even mercury concentrations within the normal range might provoke neuroallergic reactions in the brain [66].

Identifying metal sensitivity and removal of the sensitizing metals, such as dental amalgam, have been proved successful by showing symptom improvement in patients with previous autoimmune diseases. These diseases included fibromyalgia, autoimmune thyroid diseases and orofacial granulomatosis [67–70] (Table 3).

2.3. Genetics and vaccinology

The timeline regarding the field of vaccinology has been divided in two generations, the first regarding the administration of inactivated pathogens in whole or live attenuated forms (e.g., Bacillus Calmette Guerin (BCG), plague, pertussis, polio, rabies, and small-

Table 2
Experimental models of adjuvant autoimmunity

Experimental models		Strain	Disease model or related signs and symptoms	Adjuvant
Murine	Rats	DA (dark agouti) rats	Rheumatoid arthritis	Mineral oil (CFA, pristane, squalene,avridine) [34,35]
			Arthritis	Collagen [36]
		Sprague Dawley rats	Arthritis	CFA [37]
			MMF	Aluminum [38]
	Mice	BALB/c	Plasmacytomas	Mineral oil, pristane [39]
			Sclerosing lipogranulomas	SC injection of mineral oil [40]
			SLE-related autoantibodies	Pristane, CFA, squalene [41]
		C57BL/6	Antiphospholipid-like syndrome	CFA, IFA [42]
		NZB/NZWF1	SLE, lupus like GLN	CFA, alum [43]
Salmons			Impaired growth rate, decreased carcass quality, spinal deformities, uveitis, inflammatory reactions in the abdominal cavity, RF, ANA, ANCA, immune-complex GLN and chronic granulomatous inflammation	Vaccines with adjuvants such as oils [44]
Rabbits			Inflammation at injection site	Vaccine: CFA, IFA, montanide [45]
Swine			Granulomatous inflammation Adverse local reactions	Mineral oils [46]
Primates	Rhesus macaque		Potential delayed acquisition of neonatal reflexes	aluminum contained in pre clinical vaccine testing [47]

C57BL/6 (transgenic factor V Leiden-mutated C57/BL6-back-crossed mice); RF: rheumatoid factor; ANA: antinuclear autoantibodies; ANCA: anti-cytoplasmic autoantibodies; GLN: glumerulonephritis; SLE: systemic lupus erythematous; MMF: macrophagic myofasciitis.

pox) and the second regarding vaccines assembled from purified microbial cell components, also referred as subunit vaccines (e.g., polysaccharides, or protein antigens) [78]. This latter approach relies on recombinant DNA technology and polysaccharide chemistry.

Table 3
Metals reported side effects.

Metal	Derivatives	Main cause of exposure	Side effects
Mercury	Methyl mercury	Skin ointments Dental amalgam fillings	Kidney disease [71]; peripheral neuropathy; multiple sclerosis [72]; ANA positivity [73]
		Polluted fish	
	Thimerosal and phenyl mercury	Antiseptics/ preservatives in eye drops vaccines	Flu like symptoms Eyelid eczema and edema
Gold	Colloidal gold [74]	Treatment for RA	Nephropathy
Nickel [75,76]		Food Jewelry Tobacco	allergic and autoimmune symptoms; scleroderma-related autoantibodies and cutaneous sclerosis
Aluminum [4,77]		Food Vaccines	Neurotoxic; delayed type hypersensitivity; ASIA syndrome; chronic fatigue syndrome; macrophagic myofasciitis

RA: rheumatoid arthritis.

There are obstacles to conventional vaccine development methods such as non-cultivable in vitro pathogens (e.g., hepatitis C, papilloma virus types 16 and 18, and Mycobacterium leprae), antigen hypervariability (e.g., serogroup B meningococcus, gonococcus, malaria), opportunistic pathogens (e.g., Staphylococcus aureus) and rapid evolving pathogens such as Human immunodeficiency virus (HIV) [79].

Vaccine research gained a new perspective as the genomics field emerged over the last decades. Bacterial genomes have been sequenced and analyzed making it possible to choose the best candidate vaccine antigens by using the concept of reverse vaccinology [80].

The main known factors influencing the observed heterogeneity for immune responses induced by vaccines are gender, age, ethnicity, co-morbidity, immune system, and genetic background. The interaction between genetic and environmental components will dictate the response to vaccines.

Studying the vaccine and the host will enable the development of customized treatment options.

The combination of genetics, epidemiology and genomics in vaccine design has been denominated “vaccinomics” [81].

The importance of genetic influence is supported by twins and siblings studies, which show familial aggregation. This suggests that genomics is crucial in inter-individual variations in vaccine immune responses [82].

Both Human leukocyte antigen (HLA) and non-HLA gene markers have been identified as markers for immune response to vaccines. Multiple studies have shown connections between HLA gene polymorphisms and non-responsiveness to the HBV vaccine [83].

HLA region is divided in three sub regions: Class I is associated with the induction and maintenance of cell-mediated immune response, class II is associated with presentation of exogenous antigens to helper T CD4+ cells and class III, where immune non HLA related genes are located. Normal human tissue has at least 12HLA

antigens, and although new recombinant haplotypes may occur, it is inherited mostly intact from progenitors [84].

HLA allelic differences are associated with different responses to vaccines, either by hyper or hypo responsiveness. We can infer that a similar response may be associated with different safety in relation to the development of autoimmune reactions to vaccines, particularly in the patients with genetic predisposition to an enhanced response to vaccine inoculation [85]. Furthermore, patients that share the same HLA, for instance siblings, have been diagnosed with ASIA following similar environmental stimuli [86,87].

2.4. Autoantibodies induced by vaccines

Autoantibodies help to diagnose certain autoimmune diseases, however, they can also be found in healthy individuals. Thus, autoimmune diseases cannot be diagnosed based solely on antibody detection [88].

Inoculation of vaccines triggers autoimmune responses that result in the development of autoantibodies. Many studies have been carried out in animals, healthy subjects and patients with autoimmune diseases to understand if this development is of clinical significance [89–92]. A difference in eliciting the production of autoantibodies in healthy humans has been observed between adjuvanted and non-adjuvanted influenza vaccines [93]. The annual influenza vaccine has been the most heavily researched vaccine, along with HPV and Pneumococcal vaccines as far as their relationship with patients who have previously been diagnosed with an autoimmune disease [94–96]. Autoantibody induction after HPV vaccination was also shown in adolescent girls with systemic lupus erythematosus (SLE) [97].

Although induction of autoantibodies was proven following vaccine administration, there have been no proven relation with disease diagnosis in either of the specific groups studied so far [92,98].

It has been widely demonstrated that autoantibodies can develop years before the manifestation of a full-blown autoimmune disease [99].

Moreover, the development of a specific autoantibody is also genetically determined, and the link between genetic, autoantibodies and vaccines may become an even more intriguing area of research [100].

2.5. Siliconosis and autoimmune (auto-inflammatory) syndrome induced by adjuvants (ASIA)

Silicones are synthetic polymers that can be used as fluids, emulsions, resins and elastomers making them useful in diverse fields. They were thought to be biologically inert substances and were incorporated in a multitude of medical devices such as joint implants, artificial heart valves, catheters, drains and shunts. Of all the silicone-containing products, the most famous are most likely breast implants. Silicon is one of the substances suspected to induce ASIA [5].

It is currently believed that exposure alone is not enough to trigger the disease but that it requires the presence of additional risk factors (e.g., genetic susceptibility, other environmental factors) [4].

Silicone exerts local tissue reactions. Some of these reactions are considered para-physiological, such as capsular tissue formation around an implant. Other reactions are viewed as abnormal, like when capsular contractures and allergic reactions to silicone or platinum (catalyst used in silicone polymerization found in minute concentrations in implants) occur [101]. Cutaneous exposure to silicone with cosmetics or baby bottles could potentially sensitize patients [102].

There is also a systemic component of silicone exposure related to diffusion of silicone through the elastomer envelope, commonly termed "bleeding". It may arouse systemic effects as it degrades and fragments in tissue, it can also spread throughout the body and lead to the development of cancer or autoimmune phenomena [103].

Patients with ruptured implants complain more frequently of pain and chronic fatigue when compared to patients with intact implants [104].

Anti-silicone antibodies were found to be present in human sera more frequently in patients who have undergone silicone breast implants, however, their pathological significance remains uncertain [105]. The same was seen for other antibodies such as autoantibodies directed against dsDNA, ssDNA, SSB/La, silicone and collagen II, which were found to be present in increased levels in patients after exposure to silicone [106].

It has also been shown that the formation of autoantibodies is directly related to implant duration.

Several autoimmune diseases have been linked to silicone exposure including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), polymyositis, systemic sclerosis (SSc) and fibromyalgia. Although ASIA symptoms may arise 24 years after the onset of exposure to silicone implants [107], most of the follow-up periods are short and concluding evidence is yet to come regarding this causality.

2.6. Vaccines and autoimmune diseases

There have been published case reports, epidemiologic and research studies that suggest a connection between several vaccines and certain autoimmune conditions, notwithstanding that, overall the benefits of vaccination outweigh the risks.

3. The vaccines

3.1. Measles, mumps, rubella (MMR) vaccine

Thrombocytopenia has been reported as the main adverse event following MMR vaccine. After MMR vaccine the onset of immune thrombocytopenic purpura (ITP) usually occurred within 6 weeks at a risk rate of 1:22,000–25,000 MMR vaccine doses, while the incidence of ITP following infections is 1:6000 for measles and 1:3000 for rubella [108]. As the risk of thrombocytopenia is higher in patients who experience natural infection with measles, mumps or rubella than in those receiving the vaccine, vaccination is encouraged. Arthralgia complaints have also been reported and they may present as transient arthralgia, acute arthritis and rarely chronic arthritis [109].

Some risk factors have been found to be associated with the development of arthritis in vaccinated patients such as: female gender, older age, prior seronegativity and specific HLA alleles [110].

3.2. Yellow fever (YF) vaccine

YF vaccine is only advisable to people in, or going to endemic areas.

The risk of developing YF vaccine-associated neurologic disease (YEL-AND) is inversely proportional to age [111]. This is why children aged <6 months cannot be vaccinated and <8 months, except during epidemics [112]. Vaccination is not advisable to people >60 years because of possible higher risk of severe adverse effects (SAEs) even though the incidence remains low [113].

3.3. Bacillus Calmette-Guérin (BCG) vaccine

Besides being a vaccine for *Mycobacterium tuberculosis* (TB), the BCG has proved effective as immunotherapy for bladder cancer.

Although the mechanism is yet to be fully understood, it is thought that BCG binds to fibronectin forming complexes that enable the recognition as "non-self" by the innate immune response of Th1 cells. Ultimately the pathways result in the apoptosis of tumor cells [114].

Because of its effect in treating non-muscle-invasive urothelial carcinoma, as well as superficial bladder tumors, it was expected that BCG could play a role in treating other types of cancer, despite data having not corroborated this hypothesis so far. Adverse events vary according to the site and method of administration. Intradermal administration of BCG has been reported to elicit arthritis [115], dermatomyositis [116] and Takayasu's arteritis (TA) [117] among others. Intravesical treatment for bladder cancer can cause reactive arthritis (ReA) [118]. The risk relies on a systemic reaction composed of an early infective phase (PCR positive and response to anti-TB treatment) and a late hypersensitivity reaction [119].

3.4. Hepatitis B virus vaccine (HBVacc)

HBV is a DNA virus of the *Hepadnaviridae* family, responsible for acute and chronic liver disease.

HBV vaccines are considered the first efficient vaccines against a major human cancer. HBV vaccines have reduced the risk of developing chronic infection and they also have proved to reduce the incidence of liver cancer in children [120].

The vaccine has been associated mainly with autoimmune neuromuscular disorders. They include, but are not limited to: optic neuritis, Guillain-Barre syndrome (GBS), myelitis and multiple sclerosis (MS), systemic lupus erythematosus (SLE), arthritis, vasculitis, antiphospholipid syndrome (APS) and myopathy [121].

HBV vaccine is the most common immunization associated with acute myelitis.

There are studies that indicate that the pathogenicity behind such vaccine and autoimmunity might be based on cross-reactivity between HBV antigen (HBsAg) epitopes, yeast antigens, as well as other adjuvants contained in the vaccine itself [122].

3.5. Human papilloma virus (HPV) vaccine

Up to 90% of cervical cancer deaths, occur in developing countries that lack the ability to fully implement the Papanicolau (Pap) screening programs.

HPV poses a special challenge in vaccine safety. HPV is necessary for the development of cervical cancer. However, most women infected with HPV will not develop the disease since 70% of infections will resolve within a year and up to 90% within 2 years without specific treatment. Over the course of decades, cancer may result in a small proportion of the remaining infected women. Death rate from cervical cancer in 9–20 year old girls is zero and long-term benefits are yet to be proven. In this specific case, short term risks to healthy subjects can prove to pose a heavier burden than cervical cancer [123].

There are at least 100 types of HPV strains, 15 of which have been pathologically associated with cancer. Two vaccines, Gardasil™ and Cervarix™, are commercially available against HPV. Both contain the L1 capsid proteins of several HPV strains as antigens. Gardasil™ contains serotypes 16, 18, 6, 11. These antigens are combined with aluminum (Al) hydroxyphosphate sulphate as an adjuvant. Cervarix™ contains a combination of the oil-based adjuvant monophosphoryl lipid A (MPL) and Al hydroxide (ASO4) as adjuvant and is directed at strains 16 and 18 [124].

There have been several reports of post-licensure adverse events, some of which have even been fatal [125]. Compared to other vaccines, an unusually high proportion of adverse drug reactions has been reported associated with HPV vaccines [126].

In 2008, Australia reported an annual ADR rate of 7.3/100,000, the highest since 2003. This increase was almost entirely due to ADRs reported following the commencement of the national HPV vaccination program for females aged 12–26 years in April 2007 (705 out of a total of 1538 ADRs records). The numbers only decreased after the cessation of the catch-up schedule. Although the percentage of convulsions attributable to the HPV vaccine decreased, the overall report remained comparable between 2007 and 2009 (51% and 40% respectively). These reports do not prove the association, but show that there is a higher frequency of ADRs related to HPV vaccines reported worldwide, and that they fit a consistent pattern (i.e., nervous system-related disorders rank the highest in frequency) that deserves further investigation [126–128].

Indeed, several autoimmune diseases have been linked to HPV immunization. Examples include GBS, MS, Acute disseminated encephalomyelitis (ADEM), Transverse Myelitis (TM), postural orthostatic tachycardia syndrome (POTS), SLE, primary ovarian failure (POF), pancreatitis, vasculitis, immune thrombocytopenic purpura (ITP) and Autoimmune hepatitis (AH) [123].

3.6. Influenza

Influenza is an acute viral infection that affects the respiratory tract and is caused by influenza type A–C viruses of the Orthomyxoviridae family [129].

H1N1 mortality rates in the 2009 outbreak showed high risk in those aged 70 years and older, presence of chronic diseases and delayed admission. Risk of infection was lower in those who had been vaccinated for seasonal influenza with 2008/9 trivalent inactivated vaccine [130].

Studies have demonstrated that influenza vaccine is safe and immunogenic in patients with SLE or rheumatoid arthritis (RA), diminishing the risk of respiratory infections [129].

It has been shown that adjuvanted vaccine had more local reactions but did not increase systemic adverse reactions [131].

Molecular mimicry has been suggested as a mechanism to explain an autoimmune response following influenza vaccination. However, a causal relationship between influenza vaccines and induction of autoimmune diseases remains unproved [129].

Diseases or symptoms reported after influenza vaccination include mostly neurological syndromes such as GBS [REF]. Nonetheless, influenza vaccines should be recommended for patients with MS, because influenza infection is associated with increased risk of exacerbations.

That being said, influenza vaccinations showed increased risk of autoimmune responses suggestive of ASIA [132], vasculitis [133] and APS [134] among others.

3.7. Meningococcal vaccines

Meningococcal disease is caused by *Neisseria meningitidis*. One of the following five serogroups causes almost every invasive disease: A–C, Y, and W-135. Vaccines available so far for its prevention encompass either pure polysaccharide vaccines that use purified bacterial capsular polysaccharides as antigens, or protein/polysaccharide conjugate vaccines, which use the polysaccharide molecule plus diphtheria or tetanus toxoid as T-cell-stimulating antigens.

N. meningitidis serogroup B (MenB) MenB glycoconjugate vaccines are not immunogenic and hence, vaccine design has focused on sub-capsular antigens [135].

MenB capsular polysaccharide is composed of a linear homopolymer of $\alpha(2 \rightarrow 8)$ *N*-acetyl-neuroaminic acid (polysialic acid; PSA).

MenB PSA and PSA found on neural cell adhesion molecules are structurally identical. As a result of this, it has been proposed that infection with MenB or vaccination with PSA may be associated with subsequent autoimmune or neurological disease [136].

No evidence of increased autoimmunity was found to be associated with meningococcal serogroup B infection [136]. Regarding vaccination, the inoculation does not cause autoimmune diseases but may unmask autoimmune phenomena in genetically predisposed individuals. Local reactions are more frequent in individuals vaccinated with quadrivalent meningococcal conjugate vaccines compared to plain polysaccharide vaccines. The intramuscular administration of the conjugate vaccine (versus subcutaneous for that of polysaccharide) may, in part, explain the higher reactivity [137].

Diseases previously associated with meningococcal vaccines are GBS [138], Henoch-Schönlein Purpura (HSP) [139] and Bullous pemphigoid (BP) [140].

3.8. Pneumococcal vaccine

Streptococcus pneumoniae (Pneumococcus) is the main cause of bacterial community-acquired pneumonia and meningitis in western countries, as well as the cause of more than 800,000 children deaths in developing countries [141,142].

There are three anti-pneumococcal vaccines commercially available. Two of these are conjugated to a protein carrier (PCV7 and PCV13) and one is not conjugated (PPV23). PPV23 was licensed in 1983 and consists of the capsular polysaccharides of twenty-three different Streptococcus pneumoniae serotypes (1–5, 6B, 7F, 8, 9N, 9 V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F). It does not elicit immunological memory because the immune response it triggers is T-cell independent. It is usually administered to the elderly (above 65 years), as it is believed to be less effective in children.

PCV7 is composed of the most frequent serotypes 4, 6B, 9 V, 14,18C, 19F, and 23F. PCV13 is directed at serotypes 1, 3–5, 6A, 6B, 7F, 9 V, 14, 18C, 19A, 19F, and 23F. Contrary to PPV23 both PCV7 and PCV13 have an aluminum adjuvant in their composition that elicits a T-cell mediated response [143].

Ever since vaccines were introduced in the healthcare system, prevalence, fatality and admissions for invasive pneumococcal disease have decreased significantly [144].

Vaccine adverse events vary depending on whether the vaccine is adjuvanted or not. In a non adjuvanted vaccine, local reactions are present in 9% of people vaccinated intra muscularly and in 24% of those immunized sub-cutaneously [145]. In conjugated vaccines, this percentage rises to 50% [146]. Systemic reactions such as fever, irritability, decreased appetite and sleep disturbances occur in 80–85% of recipients of PCV or PPV. Symptoms like arthralgia, arthritis, myalgia, paresthesia and fatigue are more frequent in patients post PPV. This may be related to the fact that the vaccines are administered to different age groups.

Autoimmune risk following PPV vaccine is very low. Only 14 case reports were found after PPV vaccine. Six of these referred to reactivation of a previous autoimmune disorder. Studies directed to access vaccine safety in subjects with autoimmune diseases showed immunization was safe [147,148].

3.9. Tetanus vaccine

Tetanus toxoid (TT) is a potent exotoxin produced by the bacteria *Clostridium tetani*. The toxin has a predominant effect on inhibitory neurons, inhibiting release of γ-aminobutiric acid (GABA). When spinal inhibitory interneurons are affected the symptoms appear [149]. The vaccine against *C. tetani* contains deactivated tetanus toxoid plus an adjuvant (usually aluminium hydroxide).

The most studied and prevalent disease associated with TT is antiphospholipid syndrome (APS), but CNS complications have also been reported such as optic neuritis, acute myelitis and encephalomyelitis [150].

In mice, the immune response to TT depends on genetic background and to the specific adjuvant used for immunization. Naive BALB/c mice, immunized with TT, developed antibodies directed to TT, dsDNA and β2GPI and were extremely sick [90].

4. The diseases

4.1. Anti-phospholipid syndrome (APS)

APS is an autoimmune disease characterized by the occurrence of thrombotic events. Patients suffering from this condition have recurrent fetal loss, thromboembolic phenomena, thrombocytopenia as well as neurological, cardiac and dermatological involvement [151].

The serological marker of APS is the presence of anti-phospholipid antibodies (aPL), which bind negatively charged phospholipids, platelets and endothelial cells mainly through the plasma protein beta-2-glycoprotein-I (b2GPI). The presence of IgG and IgM anti-cardiolipin antibodies (aCL) and lupus anticoagulant is associated with thrombosis in patients with APS [151].

β2GPI was identified as the most important antigen in APS. β2GPI has several properties in vitro which define it as an anticoagulant (e.g., inhibition of prothrombinase activity, adenosine diphosphate-induced platelet aggregation, platelet factor IX production) [152]. Passive transfer of anti-β2GPI antibodies induce experimental APS in naïve mice and thrombus formation in ex vivo model [153].

Evidence suggests that the molecular mimicry mechanism between β2GPI and TT is one of the possible causes for APS.

Besides TT, APS has also been reported following HBV and influenza virus vaccination, although data are scarce [154,155].

4.2. Systemic lupus erythematosus (SLE)

SLE is a multisystem autoimmune disease characterized by the production of a variety of autoantibodies. IgG isotype antibodies to double-stranded DNA (dsDNA) are thought to be diagnostic markers and their presence correlates with disease pathogenesis. Several factors including genetic, hormonal, environmental and immune defects are involved in the induction of autoantibodies in this disease [156].

Post vaccination manifestations of SLE or lupus like syndrome have been reported and range from autoantibody induction to full blown clinical disease. Reports have been published associating SLE to HBV, MMR, dTP, HPV, influenza, BCG, pneumococcal and small pox vaccinations [157].

Vaccination in SLE diagnosed patients is associated with disease exacerbation and decreased antibody response, which may be due to the underlying disease and the frequent use of immunosuppressive drugs [158].

A temporal link between SLE and HBV vaccination is the only relation that has been demonstrated [159].

Several studies have demonstrated an increased prevalence of HPV in individuals with lupus compared to the general population, which has increased awareness for the need to vaccinate this high-risk population [160]. To do so, the association between immunization with HPV vaccines and SLE like symptoms, as well as the higher incidence of flares in known Lupus patients must be taken into account.

4.3. Vasculitis

Vasculitis is the name given to a group of autoimmune mediated diseases, which involve blood vessels of different types and sizes. They can be categorized according to several disease features indluding: the type of vessel affected, organ distribution, genetic predisposition and clinical manifestation [161].

4.3.1. Large vessels vasculitis

So far, 18 cases of large vessel vasculitis have been detected. This includes 15 cases of giant cell arteritis (GCA) following influenza vaccination, 2 cases of Takayasu disease (TD), and one case of large cell arteritis involving subclavian and renal arteries following HBV vaccines.

Two of these patients had previous received the diagnosis of ankylosing spondylitis and polymyalgia rheumatica (PMR)-like illness [162].

4.3.2. Medium vessels vasculitis

One case of polyarteritis nodosa (PAN) following the administration of Tetanus and BCG vaccine is described. All other cases of PAN in adults follow the administration of HBV vaccine [163–165].

Case reports of medium vessels vasculitis – both polyarteritis nodosa and Kawasaki disease (KD) – have also been published in pediatric patients. KD has been described one day after the second dose of HBV vaccine and following yellow fever vaccine [166,167]. Two cases of pediatric patients with PAN have been reported two months after receiving the HBV vaccine [164,165].

4.3.3. Small vessels vasculitis: ANCA-associated vasculitis

Eosinophilic granulomatosis with polyangiitis (EGPA) after tetanus vaccination [163] and following HBV vaccine [168] have been reported. There are also 3 cases of microscopic polyangiitis (MPA) and 6 cases of granulomatosis with polyangiitis (GPA) following influenza vaccines in the literature [169,170].

4.3.4. Immune complex small vessels vasculitis

Henoch Schönlein purpura (HSP) is the most common vasculitis of childhood. It is generally benign and self-limited. It is mediated by IgA immune complex deposition in various tissues as well as in small-sized blood vessels. Genetic risk factors play an important role in the pathogenesis of the disease: it is associated with HLA-DRB*01, 07 and 11. HSP was associated with seasonal influenza, influenza A (H1N1), pneumococcal and meningococcal disease, hepatitis A virus (HAV), HBV, anti-human papilloma virus (HPV) vaccines, and following multiple combinations of vaccines, such as typhoid, cholera and yellow fever [139,171–173].

Leukocytoclastic vasculitis has been associated with several vaccines, including influenza vaccine [174], HAV vaccine [175], HBV vaccine [176], pneumococcal vaccine [177], varicella [178], rubella, smallpox [179] and the anthrax vaccine [180].

Dermal vasculitis with pan uveitis has also been described following MMR vaccine [181].

4.4. Rheumatoid arthritis (RA)

RA is the most prevalent chronic inflammatory arthritis affecting the synovial membrane of multiple diarthrodial joints. Although its etiology has not been completely clarified, deregulation of the immune system is evident with a preponderance of inflammatory cytokines and immune cells within the joints.

RA has an estimated heritability of 60%, leaving a substantial proportion of risk to environmental factors. Immunizations have previously been proposed as potential environmental triggers for RA. In the Norfolk Arthritis Register database, 19 of the first 588 patients reported receiving a tetanus vaccination within 6 weeks prior to the onset of arthritis. Similarly, a transient rise in RF titer was recorded in 10 out of 245 military recruits 2–3 weeks after receiving concomitant immunization against tetanus, typhoid, paratyphoid, mumps, diphtheria, polio and smallpox. However, only 2 showed a persistent elevation in titer and none developed arthritis [182].

Several mechanisms have been proposed to explain the putative association between vaccination and the initiation of RA, the most prominent of which are molecular mimicry and non-specific immune system activation [182].

Vaccines who have been associated with RA include rubella vaccine in which reactive arthritis occurs in 5% of recipients. Controlled studies failed to show persistent arthritis or arthralgia in these patients [110].

Patients following HBV vaccine showed an increase of arthritis in a VAERS study, but this was not seen in a large retrospective epidemiological study [183].

Data so far suggest that vaccines carry an insignificant role in the pathogenesis of RA.

4.4.1. Vaccines in the therapy of RA

Several mechanisms are being studied to produce vaccines mainly targeting inflammatory cytokines as “antigens” such as TNF, aiming to induce high titers of endogenous neutralizing anti-cytokine antibodies with the goal of breaking natural Th tolerance to auto antigens. Other cytokines, namely IL-1 IL-6, MIF, RANTES, IL-18, MCP-1 are also being tested [184].

Another vaccine related therapy uses autologous T cell lines to induce a specific immune response by the host’s T cells directed against the autoimmune (vaccine) T cells [185]. This strategy has been successful in mouse models and has shown encouraging results in a small pilot study of 15 RA patients, where 10 patients showed a clinical response, defined by ACR 50 improvement criteria [186].

4.5. Undifferentiated connective tissue disease (UCTD)

UCTD is a clinical condition characterized by signs, symptoms and laboratory tests suggestive of a systemic autoimmune disease but that does not fulfill the criteria for any defined connective tissue disease (CTD).

Such patients with clinical manifestations suggestive of systemic connective tissue disease but not fulfilling any existing criteria are quite frequent: 12-20% of the patients initially asking for a rheumatologic evaluation may at least temporarily be diagnosed as affected by ‘undefined’ or ‘undifferentiated’ connective tissue disease.

Comparing studies on these diseases is unfeasible because of the inexistence of defined criteria for diagnosis [187].

Within 5 years of follow-up, patients usually evolve to defined CTDs, which include SLE, systemic sclerosis (SSc), primary Sjögren’s syndrome (pSS), mixed connective tissue disease (MCTD), systemic vasculitis, poly-dermatomyositis (PM/DM) and RA. Maintaining an undefined profile for 5 years makes evolving into CTDs less probable and the diagnosis of “stable UCTD” reliable [188].

Disease etiology is a concern and it has been associated with Vitamin D deficiency and silicone implants, both of which lead to an imbalance in proinflammatory and anti-inflammatory cytokines [189].

Vaccines have also been associated with this disease, namely the HBV vaccine [190].

Etiopathogenesis of UCTD is unknown and it has been suggested it might fall on ASIA spectrum since symptomatic similarities are striking and UCTD etiopathogenesis has been associated with adjuvants [122].

4.6. Alopecia areata (AA)

AA is an autoimmune disease, characterized by one or more well demarcated oval and round non-cicatricial patches of hair loss. The disease may affect any hair bearing part of the body and has a great impact on a patient's self-esteem and quality of life.

Depending on ethnicity and location, AA is the most prevalent skin disease. AA prevalence varies and is estimated to be between 0.1–0.2% in the United States and 3.8% in Singapore [191,192].

As with any other autoimmune disease, the development of AA encompasses genetic and environmental factors. Environmental factors associated with AA development are emotional and/or physical stress, infections and vaccines [193].

Secondary syphilis is one of the most well studied examples, however Epstein Barr Virus [194] and Herpes Zoster [195] infections have also been related to the development of the disease.

As far as vaccines go, HBV vaccine has been associated with AA development. In one study of 60 patients, 48 developed AA after vaccination with HBV vaccine. Of those 48 patients, 16 were re-challenged, and the reappearance of disease was witnessed [196]. In mice this association failed to be established [197]. One case of AA was witnessed following Tetanus Toxoid, as well as two case reports following HPV and MMR vaccine [198–200].

4.7. Immune thrombocytopenic purpura (ITP)

ITP is an autoimmune disease defined by a platelet count of less than 105 platelets/μL without overlapping diseases. It can present with or without anti-platelet–antibodies. Thrombocytopenia is relatively common and the overall probability of developing ITP was 6,9% in a cohort of 260 patients. It was also found that 12% of patients developed an overlapping AID other than ITP [201].

The etiology of the disease is yet to be fully understood but it has been detected following infectious diseases, such as Helicobacter pylori, hepatitis C virus (HCV), novel influenza A infection, rotavirus infection and human immunodeficiency virus (HIV) [202].

ITP onset has also been reported, although rarely, as a severe adverse event following vaccine administration. This was more often observed after measles–mumps–rubella (MMR), hepatitis A and B, diphtheria–tetanus–acellular pertussis (DTaP), and varicella vaccinations [203].

Molecular mimicry has been suggested as a possible mechanism for the development of ITP, namely following Helicobacter Pylori infection. Its eradication has been shown to increase platelet count and diminish the levels of anti-CagA antibody in a subset of H. Pylori infected subjects with ITP [204].

These data point towards a beneficial role of H. pylori eradication in chronic ITP.

Two cases of ITP following anti-rabies vaccine have been reported and one after HPV vaccine. Reactivation of ITP was reported two weeks after a tick-borne encephalitis vaccination [202]. The most consistent association with ITP is with the MMR vaccine [205]. However, it should be emphasized that the number of cases are fewer than expected without vaccination.

4.8. Type 1 diabetes (T1D)

T1D is due to antigen specific reactions against insulin producing beta cells of the pancreas. Much like other autoimmune diseases, T1D results from a combination of genetic, environmental, hormonal and immunological factors. Environmental factors such as pathogens, diet, toxins, stress and vaccines are believed to be involved in the beginning of the autoimmune process [206].

Although the mechanisms by which viral infections cause autoimmune diabetes have not been fully clarified, there is some evidence to suggest a role for natural infections in the pathogenesis of T1D mellitus in susceptible individuals [207].

It has been hypothesized that vaccination could trigger T1D in susceptible individuals. Although post-vaccination T1D may be biologically plausible, cumulative evidence has not supported an increased risk of T1D following any vaccine [208].

Several experimental data have suggested that, depending on the timing, vaccination might exert a protecting or aggravating effect on the occurrence of diabetes [209].

A study suggests that *Haemophillus influenza* type b vaccine might be a risk factor in the induction of islet cell and anti-GAD antibodies measured at one year of age [210] but there are previous studies that show no association between Hib and T1D [211].

In a cohort of American military officers diagnosed with T1D, there was no association found between vaccination and T1D diagnosis [212].

Available data about a relation between the mumps vaccine and T1D are still incomplete and their interpretation is difficult because of miscellaneous confounding factors associated with the development of T1D [213].

Association between Hemagglutinin 1 Neuraminidase 1 (H1N1) vaccines and T1D is so far unproven [214].

In humans, it has been hypothesized that early-age BCG vaccination is associated with the risk of T1D. The few studies conducted to date provided no consistent evidence of an association. There are, however, studies showing a possible temporary boost of the immune function after vaccination [215]. Studies also show that among BCG-vaccinated children who test positive for islet autoantibodies, there is a higher cumulative risk of T1D [216].

In animal experiments it has been observed that BCG seems to have a protective effect against diabetes, however researchers have yet to translate this benefit to humans [217].

In all, studies results do not support any strong association between vaccination and T1D.

4.9. Narcolepsy

Narcolepsy is a sleep disorder described as excessive sleepiness with abnormal sleep pattern characterized by uncontrollable rapid eye movement (REM) events which occur at any time during the day. These event and may or may not be accompanied by a loss of muscle tone (cataplexy) [218].

A plethora of data indicates that narcolepsy is caused by the lack of orexin (also known as hypocretin), an important neurotransmitter, which is involved in the regulation of the sleep cycle. In Narcolepsy patients, a loss of orexin producing neurons in the hypothalamus and low levels of orexin in the cerebrospinal fluid (CSF) has been reported [218].

Narcolepsy has been shown to have an autoimmune background. Antibodies against Tribbles 2 (Trib2) have been found in these patients, which may be related to the pathogenesis of disease. An experimental model of narcolepsy in mice has been made by passive transfer of total IgG from narcolepsy patients into the animal's brains through intra ventricular injection [219].

Environmental factors like Influenza A virus and streptococcal infections have been associated with disease onset. Interestingly, fever by itself without the diagnosis of an infectious etiology was found to be a risk factor for narcolepsy [220].

Several groups have studied and found an increase in the incidence of narcolepsy diagnosis following the introduction of influenza vaccination, specifically, ASO3-adjuvanted Pandemrix™ vaccine. This association was shown in Finland especially in 4–19 year-olds, but also in case reports from other countries [221]. Other studies failed to find an association.

The actual infection with H1N1 has been associated with disease development in China, however no such relationship has been noted in Europe [220].

The above-mentioned associations are specifically related to the ASO3-adjuvanted Pandemrix™ vaccine. The same association has not been reported for other H1N1 adjuvanted or non-adjuvanted vaccines.

The major difference between the ASO3 and the MF59 adjuvants is the presence of the α-tocopherol.

α-tocopherol is unique in that it can achieve the highest and longest antibody response by producing an enhanced antigen-specific adaptive immune response. In vitro it was shown that α-tocopherol could increase the production of orexin as well as increase the proteosome activity. This increased production of orexin fragments may facilitate antigen presentation to MHC class II, thus triggering an autoimmune process [220].

All these data together support the relationship between the H1N1 Pandemrix™ vaccine and the development of narcolepsy.

4.10. Celiac disease

Gluten induced enteropathy, gluten sensitive enteropathy, or more commonly called celiac disease (CD) is a life-long autoimmune condition mainly of the gastrointestinal tract, specifically affecting the small intestine.

The abnormal immune response crates autoantigens which are directed towards Tissue transglutaminase (tTG). The two main autoantibodies and the most widespread serological markers to screen for the disease are anti tTG and anti endomysium. Two additional auto-antibodies, namely: anti deaminated gliadin peptide and anti-neoepitope tTG were found recently to be reliable for CD screening as well [222].

CD is an autoimmune disease induced by well-known nutritional environmental factors. The non-dietary ones are less studied and established. Several infectious disease have been linked to its development, the so-called infectome [193].

A clear cause-effect relation is yet to be established for most of the pathogens associated with CD. What has been shown, however, is that in countries with low economic status, inferior hygiene conditions and higher infectious load, CD prevalence is lower [223].

An epidemiologic relationship was established in 2006 between rotavirus infection and CD. Data showed that in genetically predisposed individuals, rotavirus infection was related to childhood CD development [224].

In subsequent research studies, a celiac peptide was recognized and proved to share homology with rotavirus major neutralizing protein VP7 and with the CD autoantigen tTG. The antibodies directed against the viral protein VP7 were shown to predict the onset of CD and induce typical features of CD in the intestinal epithelial cell-line T84 [225].

It has also been suggested that rotavirus vaccine alters B and T behavior, as the percentage of B-cells was higher in the vaccinated infants [226].

Rotavirus vaccine as an inducer of CD is still in discussion and warrants further study.

4.11. Polymyalgia rheumatica (PMR)

PMR is an autoimmune inflammatory rheumatic disease characterized by raised inflammatory markers with pain and morning stiffness of shoulders and pelvic girdles and synovitis of proximal joints and extra-articular synovial structures. Its diagnosis is clinical and it is typically a disease of the elderly occurring mainly in subjects above 70. Etiopathogenesis of PMR remains unknown, but genetic and environmental factors play a role [227].

A close temporal relationship has been ascertained concerning epidemics of *Mycoplasma pneumoniae*, *Chlamydia pneumonia*, Parvovirus B19 and peaks of cases of PMR and giant cell arteritis, however this is not clearly proven [228].

Cases of PMR following vaccination have rarely been reported. However, it is believed that post vaccination PMR may be underreported due to its symptomatic similarities with the transient effects of vaccines, namely: arthralgia, myalgia and low-grade fever. This leads to failure in establishing a chronological relationship when the disease is diagnosed.

Most of the reported cases are associated with seasonal influenza vaccine (Inf-V). Often, the time interval between vaccine administration and symptoms onset varies from one day, to three months. Three cases were reported with associated Giant Cell arthritis. A case report of relapsing PMR after four years of remission following tetanus vaccination has also been reported [229,230].

4.12. Acute disseminated encephalomyelitis (ADEM)

Acute disseminated encephalomyelitis (ADEM) is an inflammatory demyelinating disease of the central nervous system (CNS).

ADEM is usually poly-symptomatic with encephalopathy (i.e., behavioral change or altered level of consciousness). It affects mostly children and young adults and has higher prevalence in males. Its incidence is 0.6–0.8 per 100 000 per year [231].

Although there is no concrete evidence of a clear pathogenic association, ADEM has been associated with immunization or previous viral infection. Post-vaccination ADEM accounts for only 5-10 percent of all cases, while post-infectious ADEM accounts for 66 percent of all cases of ADEM [232].

The hypothesis that better describes these associations is molecular mimicry. T-cells targeting human herpesvirus-6 (HHV-6), coronavirus, influenza virus and Epstein-Barr virus (EBV) have been shown to cross-react with myelin basic protein (MBP) antigens. Anti-MBP T-cells were detected in patients following vaccination with simple rabies vaccine [233–235].

In a post experimental therapy for Alzheimer's disease with a vaccine that contained aggregates of synthetic Aβ42 fragments of amyloid precursor protein, ADEM was shown to develop in mice [236]. The experimental model of MS, EAE mice, may be induced with injection of Aβ42, but only when the latter is administered together with the complete Freund's adjuvant [237]. This observation points to the importance and central role of the adjuvants in induction of ADEM and autoimmunity in general [238].

The overall incidence of post vaccination ADEM is estimated to be 0.1–0.2 per 100 000 and a higher risk has been reported following immunization against measles. Other vaccines accountable for post-vaccination ADEM include vaccines against the varicella zoster, the rubella, the smallpox and the influenza viruses [239]. Surprisingly, certain vaccines such as anti-tetanus vaccine were shown to have a negative correlation with ADEM (statistically significant decreased risk) [240].

HBV immunization has been studied as a possible cause for ADEM but was later associated with clinically isolated syndrome (CIS) (a first time occurring demyelinating episode that may, or not develop to MS) and complete conversion to MS [241].

As far as case reports are concerned, ADEM was associated with vaccination with influenza, hepatitis A and B, MMR, HPV and tetanus [121,242,243].

4.13. Bullous dermatoses

Bullous dermatoses are characterized by the presence of blisters and autoantibodies against structural components of the skin: desmosomal proteins (in pemphigus), adhesion molecules of the

dermal-epidermal junction (in pemphigoid diseases), and epidermal/ tissue transglutaminase (in dermatitis herpetiformis).

The most frequent autoimmune bullous diseases are bullous pemphigoid (BP) and pemphigus vulgaris (PV). BP is more frequently observed in the elderly, while the age of onset of PV is between 40 and 60 years. Neither of the diseases have any gender preference [244].

BP and PV etiology is, so far, poorly understood. Both diseases have been associated with various environmental factors, which include emotional and/or physical stress, infections and vaccinations [244].

Genetic predisposition has also been studied with overexpression of certain HLA class II alleles. These include HLA-DQB1*0301, DRB1*04, DRB1*1101, and DQB1*0302. These alleles have been found to be more prevalent in BP patients than in the general population [245]. PV is associated with certain HLA class II loci such as HLA-DR4 and HLADR14 alleles (DRB1*0401 and DRB1*0402, which is prevalent in Ashkenazi Jews, Iranian and Sardinian patients). Other loci include DRB1*1401 (common among Japanese and Italian patients) and two DQB1 alleles (DQB1*0302 and DQB1*0503), which are strongly associated with PV.

BP and PV patients' sera were found to have significantly higher prevalence of antibodies to hepatitis B virus, hepatitis C virus, helicobacter pylori, toxoplasma gondii and cytomegalovirus [244].

As far as vaccination is concerned, BP developed in patients following influenza, diphtheria, tetanus, pertussis, hepatitis B, BCG, polio and herpes zoster vaccines [140,246,247] Furthermore, reactivation of BP following influenza vaccination was reported in one case report [248].

New onset PV was associated with: influenza vaccine, hepatitis B vaccine, anthrax vaccine, typhoid booster and rabies vaccination. In addition, exacerbation of PV after vaccination was also reported following influenza vaccine and tetanus vaccine [121].

4.14. Idiopathic inflammatory myopathies (IIM)

IIM compose a group of skeletal muscles diseases in which myositis without a recognized cause occurs. IIM is usually subdivided in 4 entities: dermatomyositis (DM), polymyositis (PM), inclusion body myositis (sIBM) non-specific myositis (NSM) and immune mediated necrotizing myopathy (IAM) [249].

IIM prevalence is around 1.1×10^{-6} cases, with a bimodal age of distribution that peaks in childhood and again between 45 and 55 years. DM is the most common inflammatory myopathy while PM is the least frequent.

Despite exhibiting similar clinical symptoms, the subsets of IIM exhibit significant immunopathological variation. DM begins with the activation of the complement and formation of membrane attack complexes (MAC). In PM and sIBM the fundamental process is related to CD8+ T cells mediated cytotoxicity [249].

It is unclear what breaks the tolerance and drives the immune response to induce IIM. So far, DM, PM and sIBM have been linked to vaccination. Several cases have been reported in the literature associating different vaccines with the development of idiopathic inflammatory myopathies. 119 cases of IIM had been reported to VAERS database up to June 2013. Out of these 119 cases, 33 were classified as PM, 85 as DM and an only one as a sIBM. DM has been reported after almost any vaccine, however only a few studies have attempted to clarify the possible relationship between DM and vaccination. PM is a frequent misdiagnosed disorder. Some reports have associated previous immunization, especially hepatitis B vaccine with PM [250]. Despite being recently differentiated from other IIM, sIBM has already been related to HBV vaccine [250]. Some vaccines associated with myositis are MMR vaccine, smallpox vaccine, Poliomyelitis (IPV), diphtheria and tetanus toxoid, influenza, HPV and BCG [250].

4.15. Fibromyalgia syndrome (FMS)

FMS is an entity that is related to the inability of the CNS to modulate pain.

The conditioned pain modulation process in the CNS appears to be compromised among many FMS patients, which might explain the enhanced pain sensation experienced by these patients [251].

The etiology of FMS is yet to be unveiled. Genetic predisposition, physical trauma (particularly to the cervical spine), emotional stress (to various stressors) as well as a variety of infections have been linked with FMS.

Vaccines have been associated with the triggering of FMS namely rubella and Lyme disease vaccines [252]. There are several reports of fibromyalgia-like disease after vaccination, specifically HPV (Martinez-Lavin Journal of Clinical rheumatology 2014). The medical community and regulatory agencies should be aware of these possible adverse effects aiming at defining their magnitude.

4.16. Chronic fatigue syndrome (CFS)/myalgic encephalomyelitis (ME)/systemic exertion intolerance disease (SEID)

Chronic fatigue syndrome (CFS) is a disease characterized by disabling fatigue, headaches, concentration difficulties and memory deficits (90%). Other symptoms such as sore throat (85%), tender lymph nodes (80%), skeletal muscle pain and feverishness (75%), sleep disruption (70%), psychiatric problems (65%) and rapid pulse (10%) are often observed. It more frequently affects women and has a prevalence of 0.2-2.6% [253].

Although disease etiology is still unknown, there are several pathogens, such as Epstein–Barr virus (EBV), which have been associated with CFS. Patients often have higher titers of IgM to the EBV viral capsid antigen. Cytomegalovirus and human herpes virus 6 antibodies were also detected more often in CFS patients, although other reports failed to replicate these results. Parvovirus B19 infection has also been suggested as a trigger to CFS [253–255].

Vaccine inoculation has also been appointed as a probable cause. Vaccinations against rubella, Q fever and hepatitis B were found to be associated with higher risk of developing CFS while meningococcal vaccine, poliovirus and influenza vaccine were not. Surprisingly, staphylococcus toxoid vaccine appeared to have a protective effect [121,256,257].

4.17. ASIA syndrome

Defined in 2011 by Shoenfeld and Agmon-Levin ASIA syndrome is characterized by hyperactive immune response to adjuvants [4].

As previously stated, ASIA incorporates four known medical conditions: Siliconosis, GWS, MMF, and post-vaccination phenomena [4]. Recently, the sick building syndrome (SBS) was proposed as a candidate for the ASIA spectrum [258]. All of these diseases satisfy several criteria for FMS and SEID [252].

a Macrophagic myofasciitis (MMF)

MMF has been described as an emerging condition of unknown cause characterized by a pathognomonic lesion in muscle biopsy mixing large macrophages with submicron to micron-sized agglomerates of nanocrystals in their cytoplasm and lymphocytic infiltrates. These lesions were related to aluminum deposits in muscle following immunization with aluminum containing vaccines [63].

MMF lesion is now universally recognized as indicative of a long-lasting persistence of aluminum adjuvant at the site of prior

intramuscular immunization. The long-lasting MMF lesion should be considered as a biomarker of aluminum bio persistence in a given individual.

Patients with MMF have higher reported myalgia with incidence being up to 90%. Its etiology is not clear but genuine muscle weakness is rare and the diagnosis of fibromyalgia is also rare. Higher prevalence of chronic fatigue syndrome (CFS) in patients with MMF has been reported as well.

Cognitive impairment has been associated with MMF: in one series of 105 MMF patients, up to 97% had attention and memory complaints and neuropsychological tests were abnormal in 89% [259].

b Gulf War syndrome (GWS)

GWS is a clinical entity specifically related to a certain time and place in history. It was described among veterans of the military conflict occurring in 1990–1991 in the Persian Gulf.

The syndrome is characterized by chronic fatigue, musculoskeletal symptoms, malaise and cognitive impairment. It clinically overlaps with Post Traumatic Stress Disorder (PTSD), FMS, CFS and other functional disorders [260].

The unique conditions that have been associated so far with disease development are the exposure to extreme climate in the Persian Gulf, exposure to various chemicals (pesticides, depleted uranium), stress provoked by prolonged waiting without actual combat and the intense exposure to vaccinations of the soldiers for fear of biological weaponry [260].

Comparing Gulf War veterans and veterans of the Bosnian conflict, multiple vaccinations administered to servicemen in the Gulf War was identified as a unique exposure [261].

The mechanism through which vaccination exposure may lead to the development of functional symptoms is not completely understood. The possibility that a shift from Th1 to Th2 type reactions could be of pathogenic significance was raised and is supported by an increased frequency of allergic reactions, low natural killer cell activity and low levels of interferon γ and IL-2 in these patients [262].

One study with GWS patients showed a connection between anti-squalene antibodies and symptoms development. This was refuted by a larger study that found no association between anti-squalene antibodies and chronic multi-symptom illness [263].

c ASIA registry

A registry is a collection of data related to patients with the same specific characteristic. It is often the first approach in the study of an area of inquiry. In rare diseases, registries are often the way to get a sufficiently sized sample of patients which can be used either for epidemiological or research purposes.

ASIA syndrome may be underreported because of unawareness and failure to connect the syndrome with the exposure. This registry was created to fully understand the clinical aspects of disease and compare patients from all over the world in order to have fully validated criteria for disease diagnosis and also to define demographic and environmental history of disease.

The ASIA Syndrome registry website can be found on the following link: https://ontocrf.costisa.com/en/web/asia. Only cases reported by physicians are accepted.

Table 4
Most common autoimmune inflammatory rheumatic diseases (AIIRDs) and non-inflammatory autoimmune rheumatic diseases (ARDs).

AIIRDs	ARDs
Rheumatoid arthritis	Degenerative spine diseases
Ankylosing spondylitis	Osteoarthritis
Reactive arthritis	Osteoporosis
Connective tissue diseases	Fibromyalgia
Polymyalgia rheumatica	

5. Vaccination in autoimmune diseases.

5.1. Autoimmune rheumatic diseases (Table 4)

To make an informed decision in medicine, there is always a need to weigh the pros and cons. ARDs may play an important role in deciding whether vaccination is or is not appropriate to a patient. In these cases, patients are immunosuppressed on account of their diagnosis and even more so if they are under specific immunomodelatory medication [4].

If the efficacy of vaccination is reduced, there is a potential for development of disease flares following vaccination. In the case of live vaccines, its inoculation may even be enough to trigger disease in the host.

For these specific reasons, live vaccines are generally contraindicated in patients receiving immunosuppressant medication. There is a need for screening and treatment of Latent Tuberculosis Infection (LTBI) before starting anti-TNF-alpha therapy. The same is true for vaccination. Preferably, even recommended vaccination (see Table 5) should be administered before the initiation of Disease-Modifying Anti-rheumatic Drugs (DMARDs) because these may reduce vaccine efficacy [264].

5.2. Autoimmune inflammatory rheumatic diseases (AIIRD)

Immunosuppression equals high risk of infection and lower vaccine efficacy.

Taking into account safety concerns and efficacy, the EULAR recommendations for immunizations in AIIRD patients are:

- Assess vaccination status in initial investigation.
- Administer vaccines in a stable disease phase.
- Live attenuated vaccines are to be avoided especially if immunosuppressive agents are being administered. BCG is not recommended.
- Administer vaccines ideally before starting DMARDs and anti-TNFα agents.
- Influenza and 23-valent polysaccharide pneumococcal vaccination is recommended.
- Tetanus toxoid vaccination is recommended following recommendations of general population, in case of major and/or contaminated wounds in patients receiving rituximab in the previous 24 weeks Tetanus Ig is indicated.
- HPV and Herpes Zoster should be considered.
- In hyposplenic/asplenic patients, influenza, pneumococcal, Haemophilus Influenza b and Meningococcal C are advisable.
- Hepatitis A and B is recommended in patients at risk.
- Travel patients should be immunized according to general population guidelines except for live attenuated vaccines, which are to be avoided [148].

Table 5
Vaccination recommendation in ARDs [265].

Vaccines		Recommended	Not recommended	Special 5emarks
Live	BCG		X	
	Herpes zoster	Previous contact with varicella (vaccine/infection)	Highly immunosuppressed patients[a]	Single dose >50 y
	Yellow fever	Endemic areas [266]		Routine immunization not recommended
	MMR	X		
Non-live	Influenza	X	Allergy to egg or the vaccine itself; GBS up to 6 weeks after vaccination	Annual Rituximab: before starting/6 mts after 1st infusion/4 wks before next dose [148,267]
	Pneumococcal	X		1 Initial dose + 1 booster (5 y later)
	DTaP and DT	X		DTaP every 10 y Tetanus Igb if exp
	Meningococcal	X		Low data support [268]
	Hep A	X		
	Hep B	Neg HBsAg in serum		
	HPV	Adolescents and young women		Preferably before initiating sexual activity
	Hib	X		

X – for all ARDs patients; MMR: measles, mumps and rubella; Hib: haemophilus Influenza type B; DTaP: diphteria, tetanus and pertussis; DT: diphteria and tetanus; Hep: hepatitis; Igb: immunoglobulin; y: years; mts: months; wks: weeks; HPV: human papilloma virus; GBS: guillain barré syndrome; exp: exposure; Neg HBsAG: negative hepatitis B antigen.

[a] Highly immunosuppressed patients: high doses of corticosteroids (>20 mg of prednisone per day or equivalent) for 2 weeks or longer, pulse therapy, cytotoxic or alkylating agents, synthetic DMARDs at doses above those recommended, or immunobiological therapy [264].

6. Conclusions and future perspectives

Vaccines have many beneficial effects in combating infectious diseases and preventing mortality and morbidity. They have also proved to be effective cancer treatments by immunomodulation, as demonstrated by the intravesical administration of BCG to treat superficial bladder cancer [28].

Vaccines are however, linked to autoimmunity. Beneficial outcomes, like the adjuvant effect are based on immunity triggering and enhanced immunity mechanisms. These same responses account for autoimmunity exertion. Vaccines induce the production of autoantibodies, but their pathologic effect is yet to be unveiled.

Although vaccines are widely considered safe, there are subjects with predispositions to whom vaccines pose a bigger threat. An example is the fact that animal models with autoimmune predispositions develop autoimmune disease following adjuvant exposure. As many as 1% of recipients of aluminum containing adjuvants may be sensitized to future exposure [269].

Silicon-induced inflammatory fibro proliferative response is irrefutable and well documented. The presence of anti-silicone antibodies and silicone-associated autoimmune phenomena seems very plausible.

ASIA syndrome and aluminum safety studies show that the use of aluminum containing "placebo" in control groups in vaccine safety studies should be carefully evaluated. New studies must be performed using a proper placebo to adequately test vaccine safety. Another evident failure in vaccine safety studies are the short-term periods which are evaluated. Continued immune system activation has been observed to be a potential mechanism of disease. A disease which is poorly understood so far.

Vaccine recommendations should be reassessed frequently in different subsets of the population. This does not invalidate the need for vaccines, however, the lower the possibility of exerting adverse events, the easier it will be for the potential benefits to outweigh the risks.

Vaccinomics represents a major breakthrough in vaccine development and can lead to the development of targeted vaccines to peptides most likely to be immunogenic [81]. A predictable response to vaccine can be achieved by differentiating the host variability. This can be achieved namely in genetics and pathogen variability. Developing a vaccine accordingly will lead to increased specificity in treatment and leave less room for adverse events. By using immunomodulation, vaccinomics can also give rise to novel therapies for autoimmune diseases.

There are several reports of cases of autoimmunity diseases following vaccines but despite in vitro positive results and due to both the limited number of cases and the long latency period of the diseases, every attempt for an epidemiological study has failed to deliver a connection.

Classification as ASIA syndrome, in detriment of classic specific autoimmune diseases, could be the key to finding effective preventative therapeutic strategies. It will enable the study of bigger patient clusters with earlier diagnoses.

Future studies that could help clarify the association between vaccinations, adjuvants and autoimmunity should ideally have a different design, more long-term data and should include autoimmune phenomena as well as large-scale epidemiological studies of autoimmune diseases.

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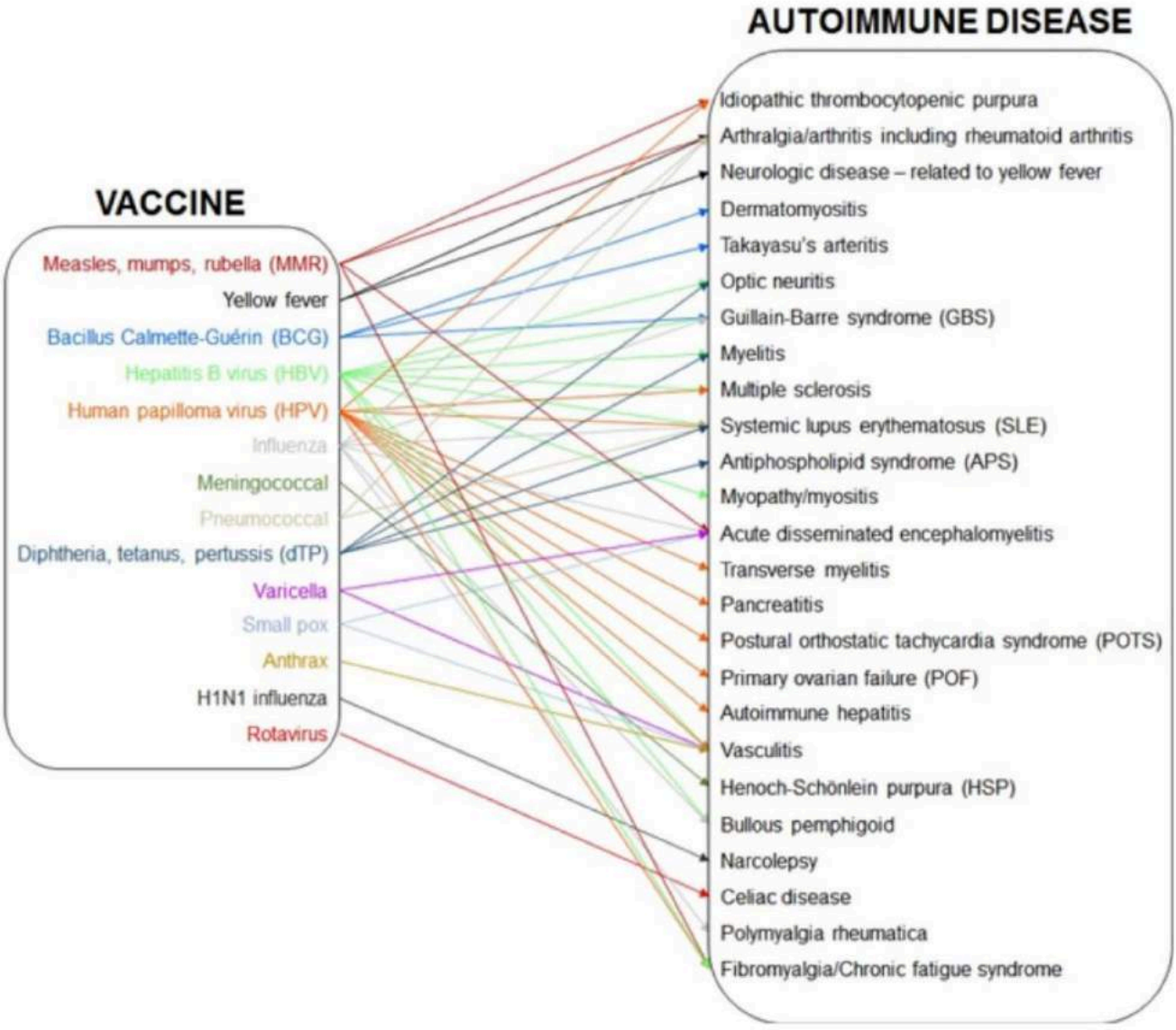
EXAMPLES OF THE MOST RELEVANT ASSOCIATIONS BETWEEN VACCINES AND AUTOIMMUNE DISEASE, AND SIMPLIFIED PATHOMECHANISM



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HPV vaccination syndrome. A questionnaire-based study

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Abstract

Isolated cases and small series have described the development of complex regional pain syndrome, postural orthostatic tachycardia, and fibromyalgia after human papillomavirus (HPV) vaccination. These illnesses are difficult to diagnose and have overlapping clinical features. Small fiber neuropathy and dysautonomia may play a major role in the pathogenesis of these entities. We used the following validated questionnaires to appraise the chronic illness that might appear after HPV vaccination: The 2010 American College of Rheumatology Fibromyalgia Diagnostic Criteria, COMPASS 31 dysautonomia questionnaire, and S-LANSS neuropathic pain form. These questionnaires and a "present illness" survey were e-mailed to persons who had the onset of a chronic ailment soon after HPV vaccination. Forty-five filled questionnaires from individuals living in 13 different countries were collected in a month's period. Mean (±SD) age at vaccination time was 14 ± 5 years. Twenty-nine percent of the cases had immediate (within 24 h) post-vaccination illness onset. The most common presenting complaints were musculoskeletal pain (66%), fatigue (57%), headache (57%), dizziness/vertigo (43%), and paresthesias/allodynia (36%). Fifty-three percent of affected individuals fulfill the fibromyalgia criteria. COMPASS-31 score was 43 ± 21, implying advanced autonomic dysfunction. Eighty-three percent of the patients who had ongoing pain displayed S-LANSS values >12, suggesting a neuropathic component in their pain experience. After a mean period of 4.2 ± 2.5 years post-vaccination, 93% of patients continue to have incapacitating symptoms and remain unable to attend school or work. In conclusion, a disabling syndrome of chronic neuropathic pain, fatigue, and autonomic dysfunction may appear after HPV vaccination.

Keywords: Complex regional pain syndrome; Dysautonomia; Fibromyalgia; Gulf War Illness; HPV vaccine; Myalgic encephalomyelitis; Small fiber neuropathy.

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Adjuvants- and vaccines-induced autoimmunity: animal models

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Abstract

The emergence of autoimmunity after vaccination has been described in many case reports and series. Everyday there is more evidence that this relationship is more than casual. In humans, adjuvants can induce non-specific constitutional, musculoskeletal or neurological clinical manifestations and in certain cases can lead to the appearance or acceleration of an autoimmune disease in a subject with genetic susceptibility. The fact that vaccines and adjuvants can trigger a pathogenic autoimmune response is corroborated by animal models. The use of animal models has enabled the study of the effects of application of adjuvants in a homogeneous population with certain genetic backgrounds. In some cases, adjuvants may trigger generalized autoimmune response, resulting in multiple auto-antibodies, but sometimes they can reproduce human autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, Sjögren syndrome, autoimmune thyroiditis and antiphospholipid syndrome and may provide insights about the potential adverse effects of adjuvants. Likewise, they give information about the clinical, immunological and histologic characteristics of autoimmune diseases in many organs, especially secondary lymphoid tissue. Through the description of the physiopathological characteristics of autoimmune diseases reproduced in animal models, new treatment targets can be described and maybe in the future, we will be able to recognize some high-risk population in whom the avoidance of certain adjuvants can reduce the incidence of autoimmune diseases, which typically results in high morbidity and mortality in young people. Herein, we describe the main animal models that can reproduce human autoimmune diseases with emphasis in how they are similar to human conditions.

Keywords: Adjuvants; Alum; Autoimmunity; Pristane; Squalene; Vaccines.

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The autoimmune/inflammatory syndrome induced by adjuvants (ASIA)/Shoenfeld's syndrome: descriptive analysis of 300 patients from the international ASIA syndrome registry

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Abstract

The autoimmune/inflammatory syndrome induced by adjuvants (ASIA) is a recently identified condition in which the exposure to an adjuvant leads to an aberrant autoimmune response. We aimed to summarize the results obtained from the ASIA syndrome registry up to December 2016, in a descriptive analysis of 300 cases of ASIA syndrome, with a focus on the adjuvants, the clinical manifestations, and the relationship with other autoimmune diseases. A Web-based registry, based on a multicenter international study, collected clinical and laboratory data in a form of a questionnaire applied to patients with ASIA syndrome. Experts in the disease validated all cases independently. A comparison study regarding type of adjuvants and differences in clinical and laboratory findings was performed. Three hundred patients were analyzed. The mean age at disease onset was 37 years, and the mean duration of time latency between adjuvant stimuli and development of autoimmune conditions was 16.8 months, ranging between 3 days to 5 years. Arthralgia, myalgia, and chronic fatigue were the most frequently reported symptoms. Eighty-nine percent of patients were also diagnosed with another defined rheumatic/autoimmune condition. The most frequent autoimmune disease related to ASIA syndrome was undifferentiated connective tissue disease (UCTD). ASIA syndrome is associated with a high incidence of UCTD and positive anti-nuclear antibodies (ANA) test. Clinical and laboratory features differ from the type of adjuvant used. These findings may contribute to an increased awareness of ASIA syndrome and help physicians to identify patients at a greater risk of autoimmune diseases following the exposure to vaccines and other adjuvants. The ASIA syndrome registry provides a useful tool to systematize this rare condition.

Keywords: ANA; Adjuvants; Autoantibodies; Autoimmune diseases; Chronic fatigue syndrome (CFS); Fibromyalgia; Silicone; Systemic lupus erythematosus; Vaccines.

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Cognitive dysfunction associated with aluminum hydroxide-induced macrophagic myofasciitis: A reappraisal of neuropsychological profile

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Abstract

Patients with macrophagic myofasciitis (MMF) present with diffuse arthromyalgias, chronic fatigue, and cognitive disorder. Representative features of MMF-associated cognitive dysfunction include attentional dysfunction, dysexecutive syndrome, visual memory deficit and left ear extinction. Our study aims to reevaluate the neuropsychological profile of MMF. 105 unselected consecutive MMF patients were subjected to a neuropsychological battery of screen short term and long-term memory, executive functions, attentional abilities, instrumental functions and dichotic listening. From these results, patients were classified in four different groups: Subsymptomatic patients (n=41) with performance above pathological threshold (-1.65 SD) in all tests; Fronto-subcortical patients (n=31) who showed pathological results at executive functions and selective attention tests; Papezian patients (n=24) who showed pathological results in storage, recognition and consolidation functions for episodic verbal memory, in addition to fronto-subcortical dysfunction; and Extinction patients (n=9) who had a left ear extinction at dichotic listening test in association to fronto-subcortical and papezian dysfunction. In addition, inter-test analysis showed that patients with apparently normal cognitive functions (Subsymptomatic group) performed significantly worse to attention tests compared to others. In conclusion, our study shows that (i) most patients have specific cognitive deficits; (ii) all patients with cognitive deficit have impairment of executive functions and selective attention; (iii) patients without measurable cognitive deficits display significant weakness in attention; (iv) episodic memory impairment affects verbal, but not visual, memory; (v) none of the patients show an instrumental dysfunction.

Keywords: Aluminum; Attention; Dichotic listening; Dysexecutive syndrome; Episodic memory; Macrophagic myofasciitis.

Demyelination

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Transverse myelitis and vaccines: a multi-analysis

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Abstract

Transverse myelitis is a rare clinical syndrome in which an immune-mediated process causes neural injury to the spinal cord. The pathogenesis of transverse myelitis is mostly of an autoimmune nature, triggered by various environmental factors, including vaccination. Our aim here was to search for and analyze reported cases of transverse myelitis following vaccination. A systematic review of PubMed, EMBASE and DynaMed for all English-language journals published between 1970 and 2009 was preformed, utilizing the key words transverse myelitis, myelitis, vaccines, post-vaccination, vaccination and autoimmunity. We have disclosed 37 reported cases of transverse myelitis associated with different vaccines including those against hepatitis B virus, measles-mumps-rubella, diphtheria-tetanus-pertussis and others, given to infants, children and adults. In most of these reported cases the temporal association was between several days and 3 months, although a longer time frame of up to several years was also suggested. Although vaccines harbor a major contribution to public health in the modern era, in rare cases they may be associated with autoimmune phenomena such as transverse myelitis. The associations of different vaccines with a single autoimmune phenomenon allude to the idea that a common denominator of these vaccines, such as an adjuvant, might trigger this syndrome.

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The spectrum of post-vaccination inflammatory CNS demyelinating syndromes

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Abstract

A wide variety of inflammatory diseases temporally associated with the administration of various vaccines, has been reported in the literature. A PubMed search from 1979 to 2013 revealed seventy one (71) documented cases. The most commonly reported vaccinations that were associated with CNS demyelinating diseases included influenza (21 cases), human papilloma virus (HPV) (9 cases), hepatitis A or B (8 cases), rabies (5 cases), measles (5 cases), rubella (5 cases), yellow fever (3 cases), anthrax (2 cases),meningococcus (2 cases) and tetanus (2 cases). The vast majority of post-vaccination CNS demyelinating syndromes, are related to influenza vaccination and this could be attributed to the high percentage of the population that received the vaccine during the HI1N1 epidemia from 2009 to 2012. Usually the symptoms of the CNS demyelinating syndrome appear few days following the immunization (mean: 14.2 days) but there are cases where the clinical presentation was delayed (more than 3 weeks or even up to 5 months post-vaccination) (approximately a third of all the reported cases). In terms of the clinical presentation and the affected CNS areas, there is a great diversity among the reported cases of post-vaccination acute demyelinating syndromes. Optic neuritis was the prominent clinical presentation in 38 cases, multifocal disseminated demyelination in 30, myelitis in 24 and encephalitis in 17. Interestingly in a rather high proportion of the patients (and especially following influenza and human papiloma virus vaccination-HPV) the dominant localizations of demyelination were the optic nerves and the myelon, presenting as optic neuritis and myelitis (with or without additional manifestations of ADEM), reminiscent to neuromyelitic optica (or, more generally, the NMO-spectrum of diseases). Seven patients suffered an NMO-like disease following HPV and we had two similar cases in our Center. One patient with post-vaccination ADEM, subsequently developed NMO. Overall, the risk of a demyelinating CNS disease following vaccination, although non-negligible, is relatively low. The risk of onset or relapse of CNS demyelination following infections against which the vaccines are aimed to protect, is substantially higher and the benefits of vaccinations surpass the potential risks of CNS inflammation. This does not in any way exempt us from"learning" the lessons taught by the reported cases and searching new and safer ways to improve vaccination techniques and increase their safety profile.

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Hepatitis B vaccine and the risk of CNS inflammatory demyelination in childhood

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PMID: 18843097 DOI: 10.1212/01.wnl.0000335762.42177.07

Abstract

Background: The risk of CNS inflammatory demyelination associated with hepatitis B (HB) vaccine is debated, with studies reporting conflicting findings.

Methods: We conducted a population-based case-control study where the cases were children with a first episode of acute CNS inflammatory demyelination in France (1994-2003). Each case was matched on age, sex, and geographic location to up to 12 controls, randomly selected from the general population. Information on vaccinations was confirmed by a copy of the vaccination certificate. The odds ratios (ORs) of CNS inflammatory demyelination associated with HB vaccination were estimated using conditional logistic regression.

Results: The rates of HB vaccination in the 3 years before the index date were 24.4% for the 349 cases and 27.3% for their 2,941 matched controls. HB vaccination within this period was not associated with an increase in the rate of CNS inflammatory demyelination (adjusted OR, 0.74; 0.54-1.02), neither >3 years nor as a function of the number of injections or brand type. When the analysis was restricted to subjects compliant with vaccination, HB vaccine exposure >3 years before index date was associated with an increased trend (1.50; 0.93-2.43), essentially from the Engerix B vaccine (1.74; 1.03-2.95). The OR was particularly elevated for this brand in patients with confirmed multiple sclerosis (2.77; 1.23-6.24).

Conclusions: Hepatitis B vaccination does not generally increase the risk of CNS inflammatory demyelination in childhood. However, the Engerix B vaccine appears to increase this risk, particularly for confirmed multiple sclerosis, in the longer term. Our results require confirmation in future studies.

Febrile Seizures

Pediatrics. 2010 Jul;126(1):e1-8. doi: 10.1542/peds.2010-0665. Epub 2010 Jun 29.

Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures

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PMID: 20587679 DOI: 10.1542/peds.2010-0665

Abstract

Objective: In February 2008, we alerted the Advisory Committee on Immunization Practices to preliminary evidence of a twofold increased risk of febrile seizures after the combination measles-mumps-rubella-varicella (MMRV) vaccine when compared with separate measles-mumps-rubella (MMR) and varicella vaccines. Now with data on twice as many vaccine recipients, our goal was to reexamine seizure risk after MMRV vaccine.

Methods: Using 2000-2008 Vaccine Safety Datalink data, we assessed seizures and fever visits among children aged 12 to 23 months after MMRV and separate MMR + varicella vaccines. We compared seizure risk after MMRV vaccine to that after MMR + varicella vaccines by using Poisson regression as well as with supplementary regressions that incorporated chart-review results and self-controlled analyses.

Results: MMRV vaccine recipients (83,107) were compared with recipients of MMR + varicella vaccines (376,354). Seizure and fever significantly clustered 7 to 10 days after vaccination with all measles-containing vaccines but not after varicella vaccination alone. Seizure risk during days 7 to 10 was higher after MMRV than after MMR + varicella vaccination (relative risk: 1.98 [95% confidence interval: 1.43-2.73]). Supplementary analyses yielded similar results. The excess risk for febrile seizures 7 to 10 days after MMRV compared with separate MMR + varicella vaccination was 4.3 per 10,000 doses (95% confidence interval: 2.6-5.6).

Conclusions: Among 12- to 23-month-olds who received their first dose of measles-containing vaccine, fever and seizure were elevated 7 to 10 days after vaccination. Vaccination with MMRV results in 1 additional febrile seizure for every 2300 doses given instead of separate MMR + varicella vaccines. Providers who recommend MMRV should communicate to parents that it increases the risk of fever and seizure over that already associated with measles-containing vaccines.

Fertility

Am J Reprod Immunol. 2013 Oct;70(4):309-16. doi: 10.1111/aji.12151. Epub 2013 Jul 31.

Human papilloma virus vaccine and primary ovarian failure: another facet of the autoimmune/inflammatory syndrome induced by adjuvants

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Abstract

Problem: Post-vaccination autoimmune phenomena are a major facet of the autoimmune/inflammatory syndrome induced by adjuvants (ASIA) and different vaccines, including HPV, have been identified as possible causes.

Method of study: The medical history of three young women who presented with secondary amenorrhea following HPV vaccination was collected. Data regarding type of vaccine, number of vaccination, personal, clinical and serological features, as well as response to treatments were analyzed.

Results: All three patients developed secondary amenorrhea following HPV vaccinations, which did not resolve upon treatment with hormone replacement therapies. In all three cases sexual development was normal and genetic screen revealed no pertinent abnormalities (i.e., Turner's syndrome, Fragile X test were all negative). Serological evaluations showed low levels of estradiol and increased FSH and LH and in two cases, specific auto-antibodies were detected (antiovarian and anti thyroid), suggesting that the HPV vaccine triggered an autoimmune response. Pelvic ultrasound did not reveal any abnormalities in any of the three cases. All three patients experienced a range of common non-specific post-vaccine symptoms including nausea, headache, sleep disturbances, arthralgia and a range of cognitive and psychiatric disturbances. According to these clinical features, a diagnosis of primary ovarian failure (POF) was determined which also fulfilled the required criteria for the ASIA syndrome.

Conclusion: We documented here the evidence of the potential of the HPV vaccine to trigger a life-disabling autoimmune condition. The increasing number of similar reports of post HPV vaccine-linked autoimmunity and the uncertainty of long-term clinical benefits of HPV vaccination are a matter of public health that warrants further rigorous inquiry.

Keywords: Autoantibodies; autoimmune/inflammatory syndrome induced by adjuvants; autoimmunity; human papilloma virus; primary ovarian failure.

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Association of spontaneous abortion with receipt of inactivated influenza vaccine containing H1N1pdm09 in 2010-11 and 2011-12

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Abstract

Introduction: Inactivated influenza vaccine is recommended in any stage of pregnancy, but evidence of safety in early pregnancy is limited, including for vaccines containing A/H1N1pdm2009 (pH1N1) antigen. We sought to determine if receipt of vaccine containing pH1N1 was associated with spontaneous abortion (SAB).

Methods: We conducted a case-control study over two influenza seasons (2010-11, 2011-12) in the Vaccine Safety Datalink. Cases had SAB and controls had live births or stillbirths and were matched on site, date of last menstrual period, and age. Of 919 potential cases identified using diagnosis codes, 485 were eligible and confirmed by medical record review. Exposure was defined as vaccination with inactivated influenza vaccine before the SAB date; the primary exposure window was the 1-28days before the SAB.

Results: The overall adjusted odds ratio (aOR) was 2.0 (95% CI, 1.1-3.6) for vaccine receipt in the 28-day exposure window; there was no association in other exposure windows. In season-specific analyses, the aOR in the 1-28days was 3.7 (95% CI 1.4-9.4) in 2010-11 and 1.4 (95% CI 0.6-3.3) in 2011-12. The association was modified by influenza vaccination in the prior season (post hoc analysis). Among women who received pH1N1-containing vaccine in the previous influenza season, the aOR in the 1-28days was 7.7 (95% CI 2.2-27.3); the aOR was 1.3 (95% CI 0.7-2.7) among women not vaccinated in the previous season. This effect modification was observed in each season.

Conclusion: SAB was associated with influenza vaccination in the preceding 28days. The association was significant only among women vaccinated in the previous influenza season with pH1N1-containing vaccine. This study does not and cannot establish a causal relationship between repeated influenza vaccination and SAB, but further research is warranted.

Keywords: Influenza; Influenza vaccine; Pregnancy; Spontaneous abortion.

Genetic Susceptibilities

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Genetic Basis for Adverse Events Following Smallpox Vaccination

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Abstract

Background—Although vaccinia immunization is highly effective in preventing smallpox, post-vaccination reactions are common. Identifying genetic factors associated with AEs might allow screening before vaccinia administration and provide a rational basis for the development of improved vaccine candidates.

Methods—Two independent clinical trials in healthy, vaccinia-naïve adult volunteers were conducted with the Aventis Pasteur smallpox vaccine (APSV). Volunteers were assessed repeatedly for local and systemic AEs to vaccine and were genotyped using the same panel of 1442 single-nucleotide polymorphisms (SNPs).

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Results—In the first study, thirty-six SNPs in 26 genes were associated with systemic AEs (p-value ≤ 0.05). In the second study, only those SNPs associated with AEs in the first sample were tested. In the final analysis, three SNPs were associated consistently with AEs in both studies. A nonsynonymous SNP in methylenetetrahydrofolate reductase (*MTHFR*) was associated with AE risk in both trials (odds ratio [OR]; 95% confidence interval [CI]); p-value [p]): (OR=2.3; CI=1.1–5.2; p=0.04) and (OR=4.1; CI=1.4–11.4; p<0.01). Two SNPs in the interferon regulatory factor 1 (*IRF1*) gene were associated with AE risk in both sample sets: (OR=3.2; CI=1.1–9.8; p=0.03) and (OR=3.0; CI=1.1–8.3; p=0.03).

Conclusions—Genetic polymorphisms in an enzyme previously associated with adverse reactions to a variety of pharmacologic agents (*MTHFR*) and an immunological transcription factor (*IRF1*) were associated with AEs after smallpox vaccination in two independent study samples. These findings highlight common genetic variants with promising clinical significance that merit further investigation.

Keywords

adverse events; vaccination; smallpox; genetics; epidemiology

INTRODUCTION

Although reactions following inoculation with vaccinia virus were common in the recent population-wide vaccination programs [1], the biological basis for these adverse events (AEs) is not well understood. The performance of two independent clinical studies of a single vaccinia vaccine at our study site afforded us the unique opportunity to assess genetic factors that might predict systemic AEs. All of the vaccinia-naïve subjects enrolled developed pock formation at the vaccination site, and a subset experienced systemic reactions including fever, rash or regional lymphadenopathy. Since poxviruses have evolved multiple mechanisms to evade host immune responses, such as targeting of primary innate immunity and manipulating intracellular signal transduction pathways [2], we questioned whether subjects encountering AEs exhibited unique genetic polymorphisms in these pathways that made them more susceptible to these reactions.

In earlier studies, we characterized humoral and cellular immune responses and outlined patterns of systemic cytokine expression following smallpox vaccination [3–8]. In the current report, we utilized data collected during two independent studies to identify stable genetic factors associated with AEs. Since many genetic association studies fail to replicate during subsequent studies, we sought to repeat the assessment on an additional study group [9,10]. Independent replication of the results of our first study with the second strengthens the plausibility of these genetic associations. An identical panel of candidate single-nucleotide polymorphisms (SNPs) was evaluated in each of the studies. Subjects with systemic AEs including fever, lymphadenopathy, or generalized acneiform rash, were compared with those who did not experience these reactions. For both studies, the data were genotypes at 1442 SNPs across at least 386 candidate genes. This investigation provides important preliminary findings in two independent data sets addressing the contribution of common genetic variants to a complex clinical phenotype, which also bears substantial importance with respect to public health.

METHODS

Study Subjects

Vaccines, study subjects, and study design for both of the clinical trials have been described previously in detail. Both trials were conducted at Vanderbilt University in the NIH-funded

Vaccine and Treatment Evaluation Unit (VTEU) [4,8,11]. The first study [7] enrolled 85 healthy vaccinia-naïve adults in genotyping studies and the second study [11] also enrolled 46 healthy vaccinia-naïve adults. In both studies, individuals were asked to self-identify ethnic background. Both studies complied with the Internal Review Board policies of Vanderbilt and the NIH, and written consent was obtained for all individuals.

Clinical Assessments

For both studies, the same team of trained physicians and nurses used the same forms to obtain medical history and to record local and systemic AEs after vaccination. Subjects were examined at regular intervals (days 3–5, 6–8, 9–11, 12–15, and 26–30 after vaccination). Local and systemic AEs were recorded. Subjects with an oral temperature of greater than 38.3 °C anytime during the study, generalized skin eruptions on non-contiguous areas to the site of vaccination [11], or enlarged or tender regional lymph nodes associated with vaccination were defined as those experiencing systemic AEs.

Identification of Genetic Polymorphisms

We used a previously described custom SNP panel based on the NCI SNP500 Cancer project [12]; specifically, this panel targets investigation of soluble factor mediators and signaling pathways, many of which have known immunological significance [13]. There is a heavy weighting towards non-synonymous SNPs in this panel (*i.e.*, those that result in an amino acid substitution). Genotyping for single nucleotide polymorphisms (SNPs) was performed using DNA amplified directly from EBV-transformed B cells generated from peripheral blood samples collected from each subject. Genotyping was performed at the Core Genotyping Facility of the National Cancer Institute (NCI) in Gaithersburg, MD. Genotypes were generated using the Illumina™ GoldenGate assay technology. Of the 1536 SNPs assayed, a total of 1442 genotypes passed quality control filters for both the first and second sample sets. A complete list of the SNPs examined in this study is found in Supplemental Table 1.

Statistical Analysis

Demographic characteristics including age, gender, and race were compared between the first and second study using Student's t-test (for age) and two-sample tests of proportions (for AE status, gender, and race). Allele frequencies were estimated from the total number of copies of individual alleles divided by the number of all alleles in the sample, and compared between the two studies using a two-sample test of proportions. Deviations in the fitness for Hardy-Weinberg proportion were evaluated using the exact test described in Wigginton *et al* [14].

We chose a two-stage design for identifying and replicating genetic associations in the independent clinical trials. This study design was selected with the goal of minimizing Type I errors (false positives). For comparison, we also performed the genetic association analysis in a single pooled sample. In the first study, potential associations were tested between each of the 1442 SNPs passing quality control filters and the occurrence of AEs using logistic regression. For each SNP in the first sample set, we recorded the odds ratio estimate and p-value of the likelihood ratio test for a univariate logistic model. No correction for multiple comparisons was made in our first set, because we reserved the second study sample set for determination of probable true positives. In the second sample set, we tested only those SNPs having an AE-associated p-value ≤ 0.05 in the first study. We considered a significant SNP association in the first study to have replicated if it met the following criteria in the second study: an odds ratio that consistently associated AE risk with the same genotypes and a p-value ≤ 0.05. To obtain an empirical probability of meeting our replication criteria purely by chance, we generated 1,000 simulated data sets from both study sample sets by permuting case-control labels. An additional association with p-value 0.06 is discussed below because of its high biologic plausibility.

Patterns of linkage disequilibrium (LD) between replicated SNPs on the same chromosome were assessed using Haploview [15]. Haplotypes were inferred for SNPs in high LD using the iterative approach described in Lake *et al* [16]. The resulting haplotypes were tested for association with AEs using univariate logistic models. Statistical analyses and simulations were performed using R version 2.5.1, Stata version 9 (Stata Corp, College Station, TX), and Haploview version 3.32 [15,17,18].

RESULTS

Demographic Characteristics of Subjects Included in Genetic Analysis

In both studies, all participants were invited to donate genetic samples. In the first study, of the 148 vaccinia-naïve participants enrolled in the clinical trial, a total of 96 individuals gave consent for the genetic substudy. Of those 96 subjects with genetic data, 16 experienced *systemic* AEs following immunization. An additional 11 genotyped subjects who reported only a localized rash near the inoculation site were removed from the analysis to focus only on systemic AEs. The other 69 reporting no AEs were used as controls. Thus the first study included analysis of 85 subjects. In the second study, which included 48 vaccinia-naïve healthy adults, 46 gave consent for genotyping and were enrolled. Of the 46 individuals, 24 experienced systemic AEs.

Table 1 summarizes age, race, gender, and AE status decompositions of both studies. Table 1 also describes the results of the demographic comparisons between the first and second studies. As the table indicates, there was no statistical difference in age, gender, or race between the two study populations. In the first study, 40 (47%) individuals were male, 84 (99%) were white and 1 (1%) was Asian. In the second study, 27 (59%) individuals were male, 44 (96%) were white, 1 (2%) was black, and 1 (2%) was Asian.

Genetic Associations with Adverse Events

A total of 36 SNPs (within 26 genes) that showed significant associations in the first study were tested for potential associations in the second study. Three variant genotypes were confirmed to be associated with AEs in the second study. These included one SNP in *MTHFR* ($p < 0.01$) and two SNPs in *IRF1* ($p = 0.03$). The strong significance of the association in the replication study suggested a high level of plausibility that the gene products were involved in the pathogenesis of the AEs. The results of our simulation study indicated that the probability of meeting our replication criteria (an odds ratio that consistently associated AE risk with the same genotypes and a p-value ≤ 0.05) entirely by chance was $p < 0.001$. It is important to note that we also reanalyzed the data as a single pooled sample and found the same pattern of statistically significant associations. The statistical results that replicated in the second study are shown alongside those from the first study in Table 2.

Three SNPs in a third gene, *IL4*, had p-values equal to 0.06 in the second study. While not significant using a strict requirement for $p \leq 0.05$, we thought this association of great interest because of the prior biologic studies showing a central role for this cytokine in poxvirus biology [19–21]. Considering the reduced size of the second sample and the fact that the AE risk associated with variant genotypes was consistent across studies, these *IL4* SNPs warrant further study, because additional variants in linkage disequilibrium could also be associated with AE outcomes (Table 3).

The SNPs located in *IRF1* and *IL4* are located in the same chromosomal region (5q31.1), suggesting an indirect association with one or more functional variants in that region. Because of the close physical proximity of the associated variants in the two genes, Haploview [15] software was used to examine the patterns of LD among those variants in each sample. Figure

1 shows that the LD plots for SNPs in the two genes follow the same pattern in each study sample. While there is strong LD between SNPs within the two genes, there is little evidence for LD between the two genes, indicating that the associations for each gene are statistically separate signals.

This region of chromosome 5q31 contains discrete haplotype blocks [22]. Accordingly, haplotypes were inferred for AE-associated SNPs in *IRF1* (rs839 and rs9282763) and *IL4* (rs2070874, rs2243268, rs2243290). In both studies, two *IRF1* haplotypes accounted for all subjects. The common *IRF1* haplotype listed in Table 4 represented 71% of the first sample set and 63% of the second sample set. The rare *IRF1* haplotype was significantly associated with AEs in both studies ($p = 0.03$). Across both studies, two different three-SNP haplotypes in *IL4* accounted for 99% of subjects. The common *IL4* haplotype listed in Table 4 represented 78% of the first set and 87% of the second set. The rare *IL4* haplotype was significantly associated with risk of AEs in the first study ($p = 0.05$); the association was similar in the second study ($p = 0.06$).

DISCUSSION

The candidate genes identified with the strongest association with AEs in both studies include a metabolism gene previously associated with adverse reactions to a variety of pharmacologic agents (*MTHFR*) and an immunological transcription factor (*IRF1*). The statistical results from these studies have strong biological plausibility and are in agreement with previous work on the immune response to poxviruses.

MTHFR

A SNP in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene (rs1801133) was associated strongly with AE risk in both studies. This non-synonymous SNP in exon 5 causes an amino acid change from alanine to valine, and functional characterization of this SNP demonstrated that it is thermolabile and affects both the quantity and activity of the MTHFR enzyme [23]. The enzyme catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is a co-substrate for homocysteine remethylation to methionine. *MTHFR* function provides pools of methyl groups that are crucial for the control of DNA synthesis and repair mechanisms [24]. *MTHFR* is a key enzyme in homocysteine metabolism, which plays a major role in regulating endothelial function. It may be of interest in the future to examine the association of genetic variation in this gene with the rare cardiac events that occur after vaccination.

Genetic variation of *MTHFR* has been associated with a range of clinical outcomes, including altered cardiovascular function, organ transplantation, toxicity of immunosuppressive drugs, and systemic inflammation [25–28]. Elevated plasma homocysteine levels stimulate endothelial inflammatory responses, which could contribute to systemic AEs. Alternatively, since vaccination elicits immune responses involving the rapid proliferation of cells, demand for DNA synthesis metabolites would be elevated, and alterations in the level or activity of *MTHFR* enzyme may exert significant influence over this process.

Interferon regulatory factor-1

The interferon regulatory factor-1 (*IRF1*) gene is part of the immunological gene cluster on chromosome 5q31. We found two SNPs in *IRF1* that are significantly associated with AEs in both study samples. The *IRF1* gene encodes an important member of the interferon regulatory transcription factor (IRF) family. The IRF family regulates interferons and interferon-inducible genes. *IRF1* activates transcription of the Type I interferons α and β as well as genes induced

by the Type II interferon γ [29]. Many viruses target IRFs to evade host immune responses by binding to cellular IRFs and blocking transcriptional activation of IRF targets [30].

Polymorphisms in the gene coding for a transcription factor with such far-reaching effects as *IRF1* could have profound effects on the proper immune response and clearance of vaccinia virus. Mice deficient in interferon receptors are especially susceptible to vaccinia virus infection, suggesting an important role for these molecules in controlling vaccinia infection [31]. Vaccinia dedicates several host modifying genes to counteracting interferons. For example, the viral gene B18R encodes a protein that serves as a viral IFN-α/β binding protein that binds interferons from several species [32]. This protein also can bind to the cell surface after secretion, thus preventing host interferon from binding to cellular interferon receptors [33]. Although the SNPs identified in *IRF1* and *IL4* do not change amino acids in the encoded proteins, recent evidence suggests that synonymous SNPs, such as rs839, can alter regulation of mRNA or splice junctions [34,35]. It is also plausible that one or both SNPs are in LD with the causal variant not tested in this study.

Interleukin-4

Genetic polymorphisms in this major cytokine gene involved in adaptive immunity to viruses also may be associated with AEs, however with a p-value of 0.06 in our relatively small replication study. We found three SNPs in *IL4* that may be associated with AEs in both studies. There was high intragenic LD ($r^2 > 0.9$) between the tested SNPs within each gene, *IRF1* and *IL4*, and haplotypes inferred separately for each of these genes mirrored the significant risk patterns of the SNPs observed individually. Thus, the fact that multiple SNPs in high LD were identified in regions of *IRF1* and *IL4* strongly suggest that there are additional markers in LD, several of which could functionally contribute to the risk for AEs.

The *IL4* gene encodes a pleiotropic cytokine produced by a variety of immune cells, especially activated T cells. *IL4* controls humoral immune responses, isotype switching, and suppression of cytotoxic T cell function and expansion. Thus, genetic polymorphisms related to inappropriate regulation of *IL4* expression and/or activity of IL-4 cytokine could be associated with over-stimulated inflammatory responses leading to the development of clinical AEs. Previous studies on the role of *IL4* in poxvirus pathogenesis have shown it to have a central role in altering the adaptive immune response. *IL4* over-expression during infection with recombinant poxviruses encoding *IL4* suppresses the induction of cytotoxic T cell activity by inhibiting CD8+ T cell proliferation, which increased the pathogenicity of such recombinant viruses even in previously immunized animals [36]. *IL4* also plays a role in preventing optimum innate immune responses to poxviruses. IL-4 secretion during vaccinia virus infection of individuals with atopic dermatitis alters the cytokine milieu, resulting in a block of production of the antimicrobial peptide LL-37, accounting in part for the increased risk of vaccinia virus infection in subjects with atopic dermatitis [37].

Model of pathogenesis

Since the outcome of interest here was the aggregation of specific AEs, it is logical that more than one gene may be involved. The genes with variants for which we discovered an association with AEs are all potentially involved in pathways that are in line with our previously hypothesized mechanism of AEs involving excess stimulation of inflammatory pathways and the imbalance of tissue damage repair pathways. This model was developed from studies of circulating cytokines and relevant immunological effector cells [3–5]. For subjects experiencing AEs, vaccination appears to trigger an acute inflammatory response that is excessive. Antigen presentation to T cells in the dermis leads to the release of T-cell cytokines that trigger a cascade of cytokines and chemokines whose release enhances the inflammatory response by promoting the migration of monocytes into the lesion and their maturation into

macrophages and by further attracting T cells [38,39]. Taken together, these previous findings suggest that systemic AEs following smallpox vaccination may be consistent with low-grade macrophage activation syndrome caused by virus replication and vigorous tissue injury and repair.

There are limitations to this study. The subject numbers are small for a genetic association study of low-penetrance high-frequency alleles. The association of the *IL4* variations with AEs was weaker than that of the other genes. Nevertheless, findings of the same variants in two independent clinical trials, the high biologic plausibility of these associations in light of what is known about poxvirus biology, and the potential public health significance suggest the findings are of interest.

Conclusions and Future Directions

These data present the rare opportunity to study two independent cohorts of smallpox vaccinees relating common genetic variation to the occurrence of post-vaccination AEs. Statistical analysis of the first study revealed potentially significant associations between SNPs in biologically interesting candidate genes. Of the AE-associated genes identified in the first study, two replicated in an independent study, with one additional candidate gene just beyond our statistical significance cut-off but with a high level of biologic plausibility. It is possible that our findings could be due to chance, but we avoided multiple testing issues by testing only the most promising results in the validation sample. While all SNPs were tested in the first study, only those SNPs significantly associated with AEs were tested in the second study, and our empirically derived probability of replication by chance alone was less than 0.1%. The association of SNPs in three genes across both studies and their biologically plausible connection with AEs lends credence to the reproducibility of these associations.

As with any statistical association, follow-up studies are needed to identify the particular genetic susceptibility variants and examine the functional consequences of polymorphisms in the AE-associated genes. Since we found multiple AE-associated SNPs in regions of *IRF1* and *IL4*, focused studies should be undertaken to characterize the genetic variability in these candidate regions. Indeed, haplotypes in *IRF* and *IL4* displayed altered susceptibility to a specific systemic AE (fever) after smallpox vaccination [40]. While the association of AEs with a non-synonymous polymorphism in the gene for *MTHFR* points toward functional significance of this SNP, fine mapping of this locus should determine whether this is indeed the case. For all three candidate genes, both follow-up replication and functional studies are needed to establish the plausibility of the association of common genetic polymorphisms with the hypothesized etiological pathways.

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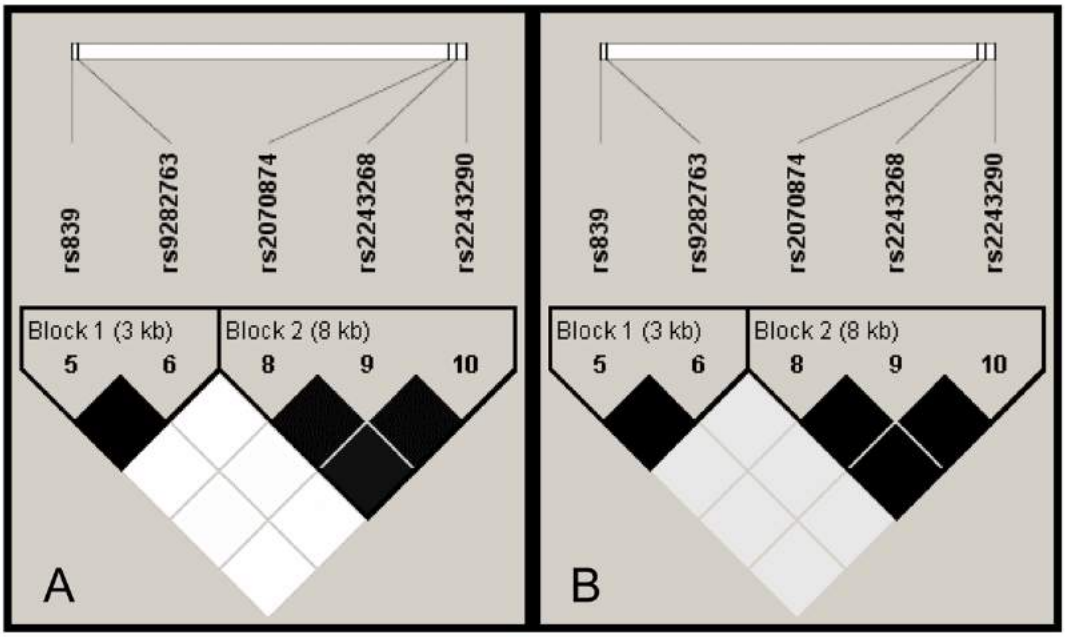

Figure 1. Haploview plot of SNPs at chromosome 5q31.1

Panel A =first study; panel B =second study. Squares are shaded to indicate strength of evidence for LD between the pairwise markers. Dark = strong evidence ($r^2 > 0.90$), light gray = weak evidence ($r^2 < 0.10$), white = no evidence ($r^2 < 0.0$). The same two LD blocks are apparent in both studies, encompassing SNPs in *IRF1* (rs839 and rs9282763) or *IL4* (rs2070874, rs2243268, and rs2243290).

Table 1

Summary of AE status, age, gender, and race for both studies.

Dataset	AE/nonAE	Age[a]	Gender (M/F)	Race (W/B/A)[b]
First study (*N* = 85)	16/69	23.2 (3.9)	40/45	84/0/1
Second study (*N* = 46)	24/22	24.2 (3.8)	27/19	44/1/1
[c] P-value of difference	< 0.01	0.15	0.20	0.25

[a] Mean (standard deviation)

[b] W = white, B = black, A = Asian

[c] Two-sided p-value for t-test (age) or two-sample test of proportions (AE/nonAE, gender, race)

Table 2

Genetic polymorphisms associated with AEs in both studies.

Gene	SNP (rs#)	SNP Location (Base pair) [a]	Chromosomal Location	First Study		Second Study	
				Odds Ratio[b]	p-value (X^2)[b]	Odds Ratio[b]	p-value (X^2)[c]
MTHFR	1801133	6393745	1p36.3	2.3 (1.1–5.2)	0.04	4.1 (1.4–11.4)	< 0.01
IRF1	9282763	34237146	5q31.1	3.2 (1.1–9.8)	0.03	3.0 (1.1–8.3)	0.03
	839	34234139	5q31.1	3.2 (1.1–9.8)	0.03	3.0 (1.1–8.3)	0.03

[a] Base pair according to dbSNP (NCBI Human Genome Build 36.1).

[b] Estimated odds ratio (95% confidence interval)

[c] Likelihood ratio chi-square (X^2) test with one degree of freedom

Table 3

Distribution of genotypes at SNPs in *MTHFR*, *IRF1*, and *IL4*.

Gene	SNP (rs #)	SNP Location (Base Pair)	Genotype	First Study Count (Percent)	Second Study Count (Percent)
MTHFR	1801133	6393745	CC	36 (42)	18 (39)
			CT	39 (46)	21 (46)
			TT	10 (12)	7 (15)
IRF1	9282763	34237146	AA	39 (46)	17 (37)
			AG	43 (51)	24 (52)
			GG	3 (4)	5 (11)
	839	34234139	GG	39 (46)	17 (37)
			AA	43 (51)	24 (52)
			AG	3 (4)	5 (11)
IL4	2070874	34424723	CC	52 (62)	34 (74)
			CT	28 (33)	12 (26)
			TT	4 (5)	0 (0)
	2243268	34428976	AA	52 (62)	34 (74)
			AC	27 (32)	12 (26)
			CC	5 (6)	0 (0)
	2243290	34433182	CC	53 (62)	34 (74)
			AA	26 (31)	12 (26)
			AC	6 (7)	0 (0)

Table 4

Haplotypes inferred for AE-associated SNPs in *IRF1* (rs839 and rs9282763) and *IL4* (rs2070874, rs2243268, rs2243290).

Gene	SNP (rs#)	Baseline Haplotype[a]	Risk Haplotype[b]	First Study		Second Study	
				Odds Ratio[c]	p-value (X^2) [d]	Odds Ratio[c]	p-value (X^2) [d]
IRF1	9282763	A	G	3.2 (1.0–10.2)	0.03	3.0 (1.0–9.0)	0.03
	839	G	A				
IL4	2070874	C	T	2.4 (1.0–5.7)	0.05	3.8 (1.0–14.4)	0.06
	2243268	A	C				
	2243290	C	A				

[a] Most common haplotype considering 2 SNPs in *IRF1* or 3 SNPs in *IL4*

[b] Rare (variant) haplotype considering 2 SNPs in *IRF1* or 3 SNPs in *IL4*

[c] Estimated odds ratio comparing risk haplotype to baseline haplotype (95% confidence interval)

[d] Likelihood ratio chi-square (X^2) test with one degree of freedom

HYPERSENSITIVITY TO VACCINATION

The purpose of a vaccine is to induce immunity by means of the reaction of the immune system and for that reason its administration can give rise to certain undesired effects.

It should be remembered that all drugs, including vaccines, are not exempt to cause mild, moderate or serious adverse reactions during their administration. There are certain factors intrinsic to the product, genetic, immune and environmental factors that can interact with each other and, therefore, interfere in the individual response of each person with its administration.

Vaccines, unlike other medicines, are administered to healthy people with a preventive purpose and therefore it is necessary an optimum safety profile of the drug. In addition, it is important to know the precautions and contraindications of each vaccine in order to avoid risks in the vaccinated population.

Most of the adverse effects produced by vaccination are mild and transient, linked to local reactions that are limited to transient pain, swelling and/or redness in the area of administration.

The adverse reactions that can appear after the vaccination, are classified according to the WHO, in the following groups.

-Reactions induced by vaccination:

Local and systemic (fever, irritability, malaise, systemic symptoms, headache, arthralgia). These adverse reactions can be subdivided into common reactions that are usually mild, and rare that can be more serious (seizures, type I hypersensitivity reactions and II, neurological reactions, thrombocytopenia).

-Reactions due to defects in the quality of the vaccine:

Due to the intrinsic characteristics of the vaccine, the maintenance in optimal conditions of the preservatives, antibiotics and other substances that allow its stabilization.

-Reactions due to program errors (storage, transport, handling or administration)

-Reactions due to anxiety for the same act of vaccination:

Vasovagal syncope is described as a secondary reaction at the time or after the application, due to a feeling of fear to the application of an injectable.

In order to cope with this situation, there is an important educational, preventive and surveillance function. In addition, the knowledge of the intrinsic characteristics of the person, together with the genetic susceptibility of the same, can help in the resolution of these reactions, with their identification and anticipation, contributing the opportune measures in each moment.

Identifying the genetic factors associated with the adverse effects, would allow a screening and knowledge prior to the administration of vaccines, which could stratify and foresee the individual susceptible effects in order to optimize and resolve them.

GENE OR REGION STUDIED

- MTHFR
- IL1A
- IL1R1

Association of MTHFR Gene Variants with Autism

Marvin Boris, M.D.; Allan Goldblatt, P.A.; Joseph Galanko, Ph.D.; S. Jill James, Ph.D.

ABSTRACT

Autism is a complex neurodevelopment disorder with numerous possible genetic and environmental influences.

We retrospectively examined the laboratory data of 168 children sequentially referred to our facility with a confirmed diagnosis of autism or pervasive developmental disabilities (PDD). Since folate and methylation (single carbon metabolism) are vital in neurological development, we routinely screened children for the common mutations of the methylenetetrahydrofolate reductase gene (MTHFR), which regulates this pathway. All children had polymerase chain reaction (PCR) DNA evaluation to determine the frequency of the 677 and 1298 common polymorphisms in the MTHFR gene.

We observed a significantly increased frequency of the homozygous mutation 677CT allele (TT): 23% in the autistic children compared to 11% in the control population ($P<0.0001$). Additionally, the heterozygous 677CT allele (CT) was present in 56% of the autistic children compared to 41% in the control population ($P<0.0001$). Somewhat paradoxically, the normal 1298AA allele was significantly higher in the autistic group, 55%, compared to the controls, 44% ($P<0.05$). Despite the increased frequency of normal 1298AA alleles, the compound 677CT/1298AC heterozygous mutations were more prevalent in the autistic population, 25%, than in controls, 15% ($P=0.01$).

Overall, the data show an increased risk of autism spectrum disorder (ASD) associated with common mutations affecting the folate/methylation cycle. These associations by themselves may provide a partial explanation for a subgroup of children genomically at risk for ASD disorders. Increased folinic acid during pregnancy and early development may offset the genomic risk factors, and this deserves further study. Further, since folate-dependent methylation provides, in part, the methyl group for inactivation of monoamine neurotransmitters via the catecholamine-O-methyltransferase (COMT) system, this observation may help to further differentiate subtypes within the broad phenotype of ASD. A search for additional genomic and environmental risk factors should be undertaken. In particular, the methylation/transsulfation and COMT pathways should be investigated.

Background

It is generally accepted that the prevalence of autism and pervasive developmental disorders (PDD) has risen significantly in the last two decades. These disorders interfere with normal development of language and socialization. Atypical patterns of stereotypic and restricted activities are common features of these syndromes. Multiple theories regarding causality have been generated, and typically these focus on genetic vulnerability and environmental risk factors. As yet, no theory has gained wide acceptance.

Clinically available testing for methylenetetrahydrofolate reductase (MTHFR) gene mutations (polymorphisms) has recently become available and had been incorporated into our evaluation process for developmentally delayed children. The MTHFR gene codes for an essential enzyme in folate metabolism. To further understand this condition, we retrospectively evaluated our findings regarding the genomic variations in the gene. MTHFR enzyme catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. Methyltetrahydrofolate is essential in one-carbon-donor metabolism for the remethylation of homocysteine to methionine and the generation of metabolically active tetrahydrofolate in the methionine synthase reaction.[1] Common polymorphisms in the MTHFR gene have been associated with reduced enzyme activity. A detailed review of folate metabolism and MTHFR is available from Schiver et al.[2]

MTHFR is located on chromosome 1 at 1p36.3. Common single nucleotide polymorphisms of the 677C→T and the 1298A→C alleles in the MTHFR gene decrease the activity of the enzyme.[3] The 677C→T allele has been associated with neural tube defects,[4-7] cerebrovascular and cardiovascular disease,[8-15] inflammatory bowel disease,[16] colorectal cancer,[17-18] and psychiatric disorders.[19-20]

The 677C→T and the 1298AC gene variants are prevalent in many populations. The heterozygous 677CT genotype ranges from 13% in Africans to 51% in Italians and 44% in North American Caucasians.[2 1] The homozygous 677TT rate in the last group is 12%. The prevalence of heterozygous genotype 1298AC among Caucasians in the United States was 47%, and that of the homozygous 1298AA mutant allele was 7.9%.[2 2]

The 677C→T allele is characterized by a mutation of a cytosine to a thymine giving rise to an amino acid replacement of valine for alanine in the catalytic domain of the enzyme. Homozygosity for the mutant T allele is associated with a 60% reduction in enzyme activity.[2 3] The 1298A→C mutant allele has a cytosine substitution for adenine, resulting in a glutamate to alanine change within the C-terminal regulatory domain.[2 4] Compound heterozygosity for both the 677CT and 1298AC is associated with a decrease of approximately 50%-60% in MTHFR activity.[2 5]

In a study by Ramaekers et al., low 5-methyltetrahydrofolate levels in the spinal fluid of children who had normal neurodevelopment until age 4 to 6 months was associated with subsequent neurological regression.[2 6] Addition of folinic acid as a dietary supplement corrected the symptoms. The observed favorable response to folinic acid further supports a central role for methylation in at least some developmental disorders.

Materials and Methods: Population

All the 168 Caucasian children, whose charts were retrospectively analyzed, were in the private practice of the principal investigator. A diagnosis of autism (73.8%) or PDD (26.2%) was previously made either by a neurologist, psychiatrist, neuropsychologist, or developmental pediatrician, and was confirmed by the investigator after referral. All the children meet

Table 1. MTHFR C677T and A1298C Genotypes in 168 Children with Autism

MHTFR 1298AC Alleles	MHTFR 677→T Alleles		
	CC	CT	TT
AA	2	52	39
AC	23	42	–
CC	10	–	–

Table 2. Frequency of 677CT Genotypes in Autistic and Control Populations

677C→T	CC	CT	TT	T ALLELE FREQUENCY
Autistic	35 (21%)*	94 (56%)*	39 (23%)*	0.51*
Control	2570 (48%)	2213 (41%)	606 (11%)	0.32

*P<0.0001

Table 3. Distribution of 1298AC Genotype in Autistic and Control Populations

1298AC	AA	AC	CC	C ALLELE FREQUENCY
Autistic	93 (55%)*	65(39%)	10 (6%)	0.25
Controls	70 (44%)	75 (47%)**	14 (9%)	0.32

*P=0.0005
**P=0.04

Table 4. Frequency of Compound Heterozygous Genotypes in Autistic and Control Populations

	677CT and 1298AC	NON 677CT and 1298AC
Autistic	42(25%)*	126 (75%)
Non-Autistic	43(15%)*	236(85%)

*P=0.01

the DSM-IV criteria for their psychiatric diagnosis. In the study, these groups will be referred to as autistic spectrum disorders (ASD). All parents gave informed consent prior to testing, and ethical approval for the retrospective chart review was granted by a private Investigational Review Board (Arizona State University, Tempe, Arizona).

In the study group, there were 142 males (84.5%) and 26 females (15.5%). The distribution by gender was statistically similar for both autism and PDD. Among the 168 children, 149 were diagnosed with regressive autism, and 19 showed no evidence of regression.

The genetic frequencies in the control population for the 677C→T genotypes were derived from Ogino and Wilson's data of MTHFR genotypes in a Caucasian population of 5,389 persons.[27] The 1298A→C polymorphism frequencies in U.S. Caucasians were utilized as reported by Rady et al.[29] The control compound polymorphism (677CT+1298AC) rates were obtained from Weisberg et al.[24]

Laboratory Methods

Blood specimens were previously processed by PCR DNA analysis for MTHFR alleles at the Mayo Clinic (20.2%), North Shore University Hospital-Long Island Jewish Hospital Core Laboratory (58.3%), or Quest Laboratories (21.4%). All laboratories utilize standardized, commercially available PCR primer kits, with accepted internal controls. Laboratory selection was determined by the participants' insurance relationships with the various laboratories utilized. There were no significant differences in the frequencies of reported polymorphism between any of the laboratories.

Statistical Analysis

The Fisher's Exact Test was applied to a two-way frequency table. A null hypothesis of interest was stated, and a *P*-value was calculated. For each of 677C→T and 1298A→C variant alleles the following were compared:

a) Overall distribution of ASD and controls,
b) Proportion of homozygous in ASD and controls,
c) Proportion of variant (i.e., homozygous or heterozygous) in ASD and controls, and
d) Allele frequency in ASD and controls.

Results

The frequency of the MTHFR 677C→T and 1298A→C genotypes in the 168 ASD children are shown in Table 1. The homozygous 677TT allele was present in 39 (23%) of the ASD children and in 606 (11%) of the controls (P<0.0001) (Table 2). The heterozygous 677CT allele occurred in 56% of children in the ASD group. This was significantly greater than the 41% prevalence in the control group (P=0.0001). The 677C→T allele frequency in the 168 ASD children was 0.51 compared to an allele frequency of 0.32 among the 5389 controls (P<0.0001) (Table 2).

The homozygous and heterozygous 1298A→C mutant alleles (1298CC and 1298AC) were similar in the ASD and control groups (Table 3). However, the normal 1298AA allele was significantly higher in the ASD group, being present in 93 (55%) affected children compared to 70 (44%) controls (P=0.0005). The 1298A→C allele frequency was significantly lower in ASD (0.25) than the controls (0.32), with P=0.04.

Heterzygosity for both the 677CT and 1298AC was identified in 25% of the ASD children, but only 15% of the controls (Table 4). This was significant, with P=0.01.

Discussion

The data demonstrate that 677C→T polymorphisms, whether homozygous or heterozygous, are significantly associated with ASD. The homozygous (TT) individuals are reported to have an approximately 50% decrease in MTHFR enzyme activity, and the heterozygous (CT) a 30% decrease in enzyme activity as measured in their lymphocytes.[2 12,2]

The 1298AA normal alleles are more prevalent in the control population than in children with ASD. The compound heterozygous state, 677CT/1298AC, which lowers enzyme activity by 50-60%,[2 2] was found to be significantly more prevalent in the autistic group. Notably, only 2% of children with ASD in our study presented without at least one polymorphism in the MTHFR gene.

It is unlikely that any single polymorphism accounts for the majority of autistic risk factors. The high natural prevalence of MTHFR variants in the absence of autistic symptoms could be interpreted in various ways. Given the rising prevalence of ASD, it may indicate emergence of a new environmental risk factor that exposes this genomic vulnerability commonly present in the folate

pathway. Multiple studies on Down syndrome have shown that polymorphisms in the folate pathway are associated with this syndrome.[2 83-0] Low plasma levels of transcobalamin combined with polymorphisms in methionine synthase reductase interact with MTHFR to increase the risk of neural tube defects.[3 1] This study does not take into account the numerous potential influencing cofactors, which may be additive to the MTHFR observations, e.g. dietary folate, serum folate, dietary B vitamin intakes, amino acid deficiencies, environmental exposures, or heavy-metal exposure. It is likely some combination of these influences the phenotypic expression (ASD symptoms) of the genomic risk factors (MTHFR polymorphisms).

The data support the hypothesis that ASD syndromes are associated with single nucleotide mutations of the MTHFR gene in some cases. Although 677C→T variant alleles (677CT or 677TT) and the heterozygous compound allele (677CT/1298AC) are significantly increased in the ASD group, it is unlikely that this association alone is sufficient to produce the complex array of symptoms associated with ASD. Therefore, a search for additional genomic, metabolic, epigenetic, transposon, and environmental risk factors should be undertaken.

Based on the observed MTHFR-related genetic variations in children with ASD, it is reasonable to evaluate dietary supplementation with folinic acid and its cofactors in the methylation cycle, e.g. B vitamins and trimethylglycine (Betaine), for these children. This would be particularly important in the subgroup shown to carry MTHFR polymorphisms.

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Association of methylenetetrahydrofolate reductase (MTHFR) gene C677T polymorphism with autism: evidence of genetic susceptibility

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Abstract

Autism (MIM 209850) is a heterogeneous neurodevelopmental disease that manifests within the first 3 years of life. Numerous articles reported that dysfunctional folate-methionine pathway enzymes may play an important role in the pathophysiology of autism. Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme of this pathway and MTHFR C677T polymorphism reported as risk factor for autism in several case control studies. However, controversial reports were also published. Hence the present meta-analysis was designed to investigate the relationship of the MTHFR C677T polymorphism with the risk of autism. Electronic databases were searched for case control studies with following search terms - 'MTHFR', 'C677T', in combination with 'Autism'. Pooled OR with its corresponding 95 % CI was calculated and used as association measure to investigate the association between MTHFR C677T polymorphism and risk of autism. Total of thirteen studies were found suitable for the inclusion in the present meta-analysis, which comprises 1978 cases and 7257 controls. Meta-analysis using all four genetic models showed significant association between C677T polymorphism and autism (ORTvs.C = 1.48; 95 % CI: 1.18-1.86; P = 0.0007; ORTT + CT vs. CC = 1.70, 95 % CI = 0.96-2.9, p = 0.05; ORTT vs. CC = 1.84, 95 % CI = 1.12-3.02, p = 0.02; ORCT vs.CC = 1.60, 95 % CI = 1.2-2.1, p = 0.003; ORTT vs.CT+CC = 1.5, 95 % CI = 1.02-2.2, p = 0.03). In total 13 studies, 9 studies were from Caucasian population and 4 studies were from Asian population. The association between C677T polymorphism and autism was significant in Caucasian (ORTvs.C = 1.43; 95 % CI = 1.1-1.87; p = 0.009) and Asian population (ORTvs.C = 1.68; 95 % CI = 1.02-2.77; p = 0.04) using allele contrast model. In conclusion, present meta-analysis strongly suggested a significant association of the MTHFR C677T polymorphism with autism.

Keywords: Autism; C677T polymorphism; Homocysteine; MTHFR; Meta-analysis; Methylation.

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PLoS one

Adverse Events following 12 and 18 Month Vaccinations: a Population-Based, Self-Controlled Case Series Analysis

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Abstract

Background: Live vaccines have distinct safety profiles, potentially causing systemic reactions one to 2 weeks after administration. In the province of Ontario, Canada, live MMR vaccine is currently recommended at age 12 months and 18 months.

Methods: Using the self-controlled case series design we examined 271,495 12 month vaccinations and 184,312 18 month vaccinations to examine the relative incidence of the composite endpoint of emergency room visits or hospital admissions in consecutive one day intervals following vaccination. These were compared to a control period 20 to 28 days later. In a post-hoc analysis we examined the reasons for emergency room visits and the average acuity score at presentation for children during the at-risk period following the 12 month vaccine.

Results: Four to 12 days post 12 month vaccination, children had a 1.33 (1.29–1.38) increased relative incidence of the combined endpoint compared to the control period, or at least one event during the risk interval for every 168 children vaccinated. Ten to 12 days post 18 month vaccination, the relative incidence was 1.25 (95%, 1.17–1.33) which represented at least one excess event for every 730 children vaccinated. The primary reason for increased events was statistically significant elevations in emergency room visits following all vaccinations. There were non-significant increases in hospital admissions. There were an additional 20 febrile seizures for every 100,000 vaccinated at 12 months.

Conclusions: There are significantly elevated risks of primarily emergency room visits approximately one to two weeks following 12 and 18 month vaccination. Future studies should examine whether these events could be predicted or prevented.

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Introduction

The measles, mumps and rubella (MMR) have been used extensively in children and have been demonstrated to be safe and effective in preventing disease [1]. However, because it is a live vaccine the MMR vaccine has the potential to cause adverse events one to 2 weeks following vaccination [2]. Most reactions to this vaccine will be mild with fevers occurring in 5 to 15% and rashes in 5% [3]. More serious reactions are extremely rare and may not be identified during pre-licensure trials [4]. Post market surveillance has identified an incidence of febrile seizures following the MMR vaccine of 25 to 34 per 100 000 vaccinated and a two to three-fold increased relative risk [5,6]. However, at a population level, mass exposures to a vaccine with a rare side effect profile could have detectable important population level effects. No study has examined the impact on aggregate health service utilization following the MMR vaccination.

In the province of Ontario, Canada, the MMR and meningococcal C vaccines are currently recommended at 12 months of age and a second dose of MMR vaccine along with a booster dose of pentavalent (diphtheria, acellular pertussis, tetanus, polio and *Haemophilus influenzae* type b) vaccine is recommended at 18 months of age. We sought to examine the population wide effects of these

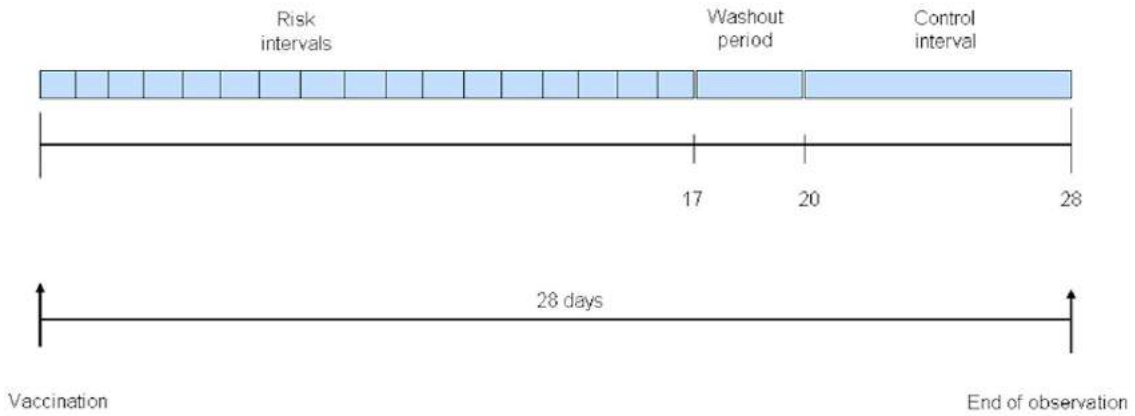

Figure 1. Illustration of the self-controlled case series design. The observation period for each patient begins with pediatric vaccination date (leftmost upward arrow) and continues for a total of 28 days. In the primary analyses, each day post vaccination is considered a *risk interval*, and consecutive days with a statistically significant t elevation in relative incidence were pooled to create a combined risk interval. Days 20–28 comprise the *control interval*. The intervening days represent the wash-out period.
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vaccinations on the combined endpoint of emergency room visits and hospital admissions in selected periods post-vaccination.

Methods

Design

The overall goal of this study was to determine the risk of serious adverse events in all children vaccinated in Ontario at 12 and 18 months of age with recommended pediatric vaccines. This was measured by comparing the risk of either presentation to emergency room (ER), or hospital admission in consecutive one day periods after the date of vaccination compared to a later control period. This analysis was conducted on all children born between April 1st 2006 and March 31st 2009. Our primary analysis of the composite risk of ER visits and hospitalizations was conducted using the *self-controlled case-series design*, described by

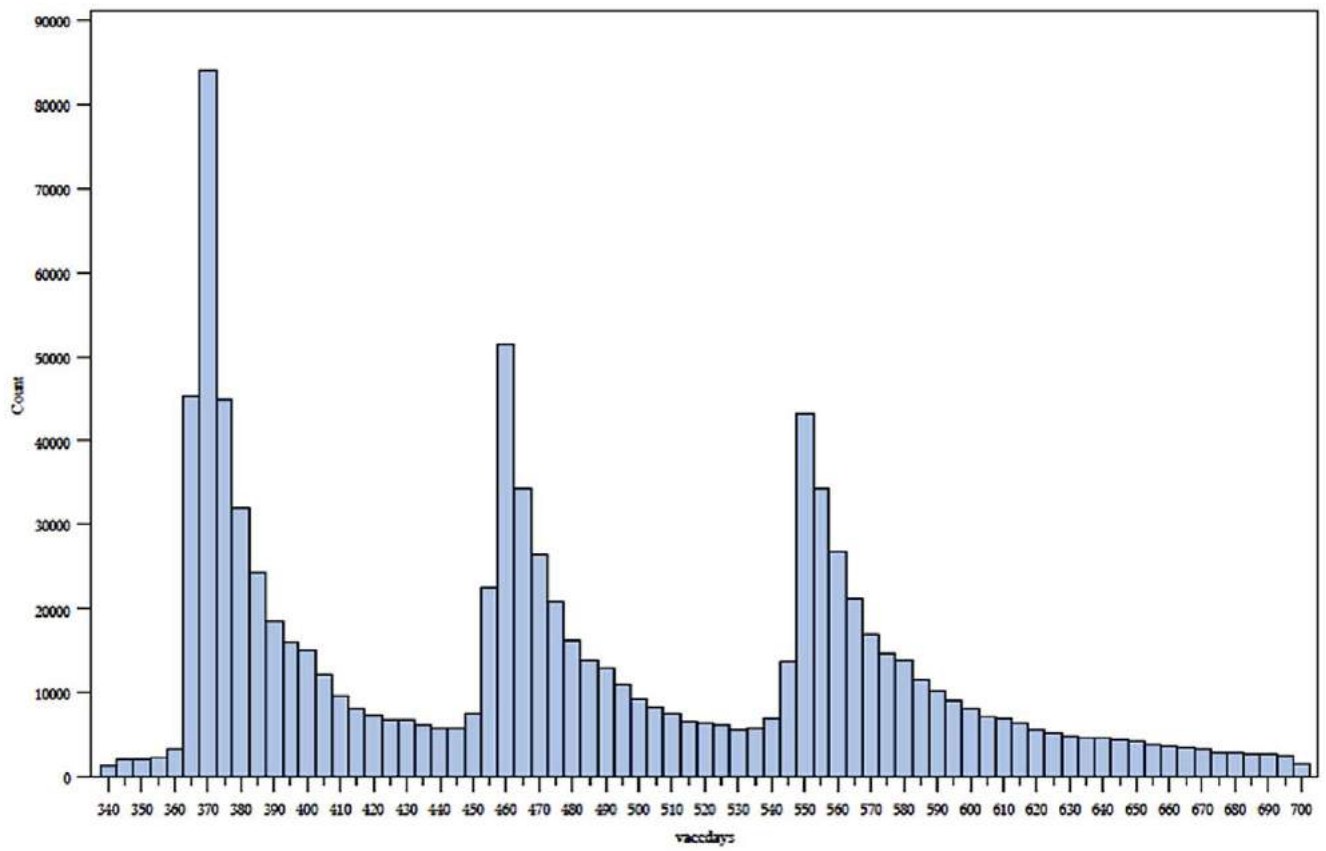

Figure 2. Vaccination events by days since birth from days 340 to 700. Count = number of individuals vaccinated on a given day. **Days** = number of days after date of birth.
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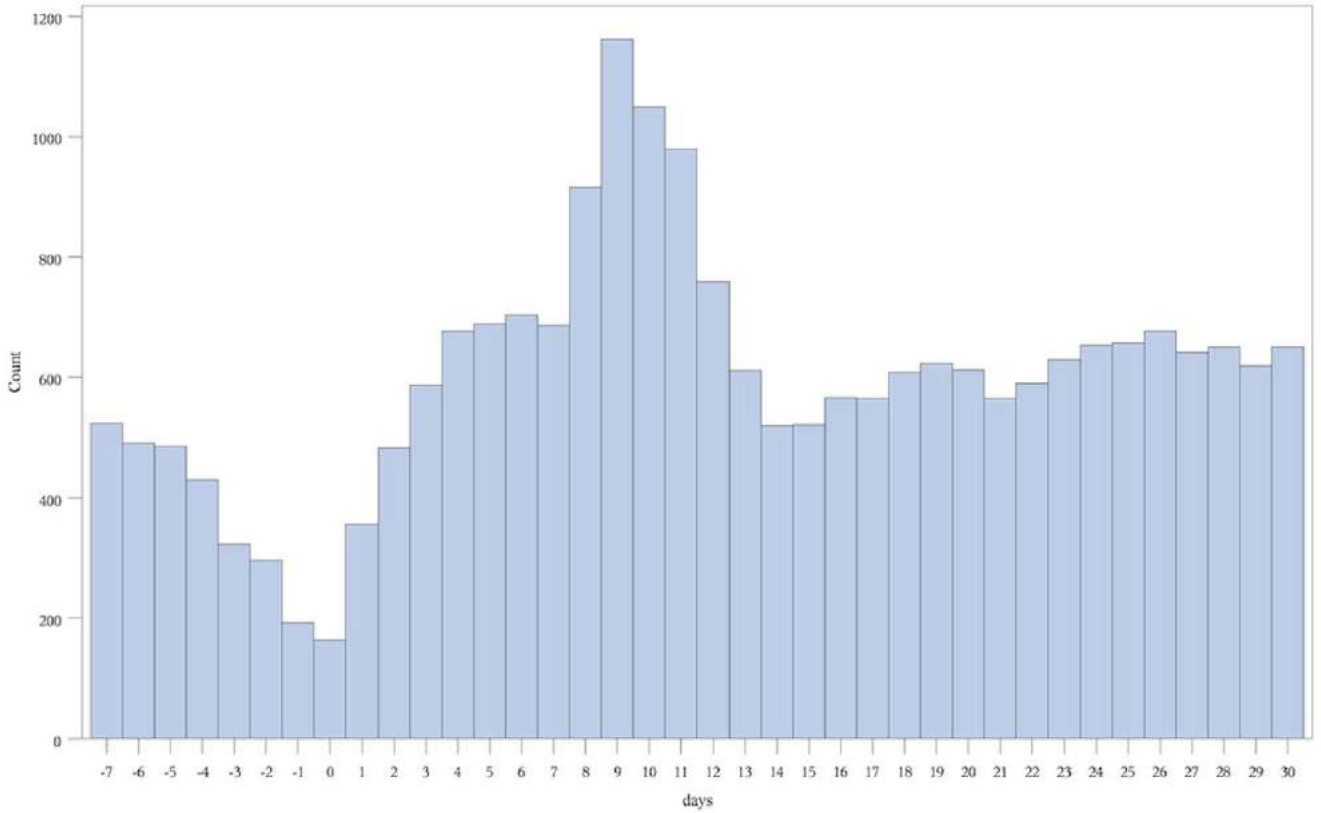

Figure 3. Number of combined endpoints versus days before/after 12 month vaccination. Count = number of combined endpoints of emergency room visit or hospitalization. **Days** = number of days before or after vaccination, day 0 being the day of vaccination.
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Farrington and associates [7,8]. We analyzed events following the 12 and 18 month vaccinations separately.

Data

Our study cohort included all children in the Newborn Screening Ontario data set between April 1st 2006 and March 31st 2009. This database captures over 99% of Ontario births. Our exposure of interest, pediatric vaccination, was identified using the Ontario Health Insurance Plan (OHIP) database. We used codes for general vaccination, as, except for influenza, vaccine-specific codes are not available. To identify the 12 and 18 month vaccinations separately we identified vaccination occurring on exactly the respective due dates as well as vaccinations occurring up to 60 days after the respective date. To allow adequate follow-up after the 12 month vaccination, only vaccinated children born on or before December 31st 2008 could be included in the analysis (N = 271,495 children). Likewise, only vaccinated children born on or before June 30th 2008 could be included in the analysis of adverse events after the 18 month vaccination (N = 184,312 children). Only subjects with both vaccinations and events in the observation period contribute to the conditional self-controlled case series analysis, therefore infants with no ER visits or hospitalizations in close proximity to the vaccination were not included. If infants had more than one vaccination in the database during the two month target period the first vaccination was used as the index vaccination. If another vaccination occurred within the observation period (0 to 28 days after the index vaccination), or the infant died, then this individual was excluded from analysis (see Appendix S1).

The Canadian Institute for Health Information's (CIHI) Discharge Abstract Database (DAD) captures all hospital admissions, including children in both tertiary and community hospitals, and was used to ascertain hospital admission. CIHI's National Ambulatory Care Registration System (NACRS) was used to ascertain ER visits, the Canadian Triage and Acuity Score (CTAS) rating and the diagnosis made by the most responsible physician for the visit. The Registered Persons Database was used to ascertain cases of death. These datasets are housed at the Institute for Clinical Evaluative Sciences (ICES), and linkage between datasets was achieved using encrypted health card numbers as unique identifiers. The study was performed within ICES' status as a Prescribed Entity in Ontario's privacy legislation and Research Ethics Board approval was received at OHRI and ICES (Sunnybrook).

Analysis

We graphed the number of combined endpoint events in the days before and after vaccination. In the self-controlled case series model, the date of vaccination serves as the index date for exposure for each patient. Previous studies have identified that children are at increased risk for systemic reactions at different times from 5–14 days after vaccination [5,6,9,10]. Because *a priori* we did not know with certainty the time period following vaccination for which there would be an increased risk of our combined endpoint, we modified the standard self-controlled case series approach by looking for an elevation in risk during each post-vaccination day up to day 17 (Figure 1). We then classified days 20–28 as unexposed, establishing a washout period in

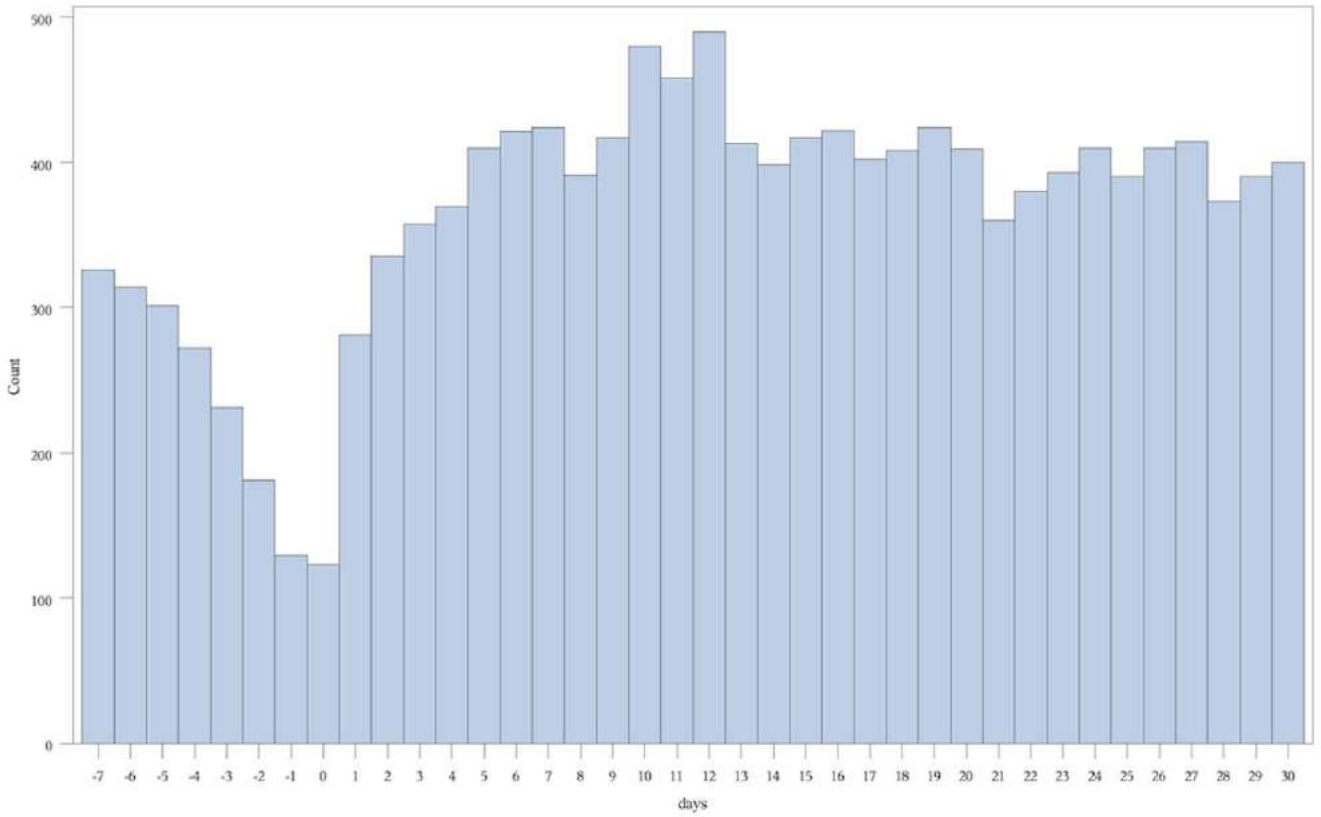

Figure 4. Number of combined endpoints versus days before/after 18 month vaccination. Count = number of combined endpoints of emergency room visit or hospitalization. **Days** = number of days before or after vaccination, day 0 being the day of vaccination.
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between the exposed and unexposed periods (Figure 1). When multiple events occurred to a given individual, the first occurrence of the composite outcome in the post-vaccination period was used (eg., someone attending the ER who was then admitted would have one event counted in that period). The relative incidence rate of the composite endpoint during the exposed period compared with the unexposed period was analyzed using a fixed effects Poisson regression model. This model included a term for exposure period and a term for patient, thereby allowing each individual to serve as his or her own control and accounting for intra-individual correlation. An offset term was also included to account for the differing durations of the exposed and unexposed periods. Deaths after the 12 and 18 month vaccinations were explored in a separate analysis due to the fact that a subject dying effectively truncates their follow-up potentially biasing the results of the SCCS analysis. As noted above, children who died during the follow-up period were excluded from the SCCS analysis of ER visits and hospitalizations.

To define the at-risk period we combined consecutive days with statistically significant elevations in relative incidence. We considered statistical significance to be a p-value less than or equal to 0.001 based on a Bonferroni correction to account for multiple testing (38 separate tests) [11]. We conducted separate analyses for the 12 and 18 month vaccinations. We also conducted secondary analyses to determine the association between vaccination and ER visits, hospital admissions, and deaths separately. All p values were 2 sided, and analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC).

In order to assess the types of cases captured by our endpoints we conducted a post-hoc analysis where we compiled the reasons for presentation to the ER as determined by the most responsible physician for the risk period for the 12 month vaccination. This was compared to the prevalence of the same diagnoses in the control period. We examined a tracer condition, ear/face nose injury, for which we do not expect a difference in rates. We also identified the CTAS ratings for presentations during the affected period and compared them to those during the control period using the Wilcoxon Rank-Sum test. CTAS ratings range from 1 to 5 with 1 representing a severe condition requiring resuscitation and 5 representing a less severe condition requiring non-urgent care [12]. In another post-hoc analysis we graphically examined the pattern of events following 12 and 18 month vaccination in the years 2002–2005 when the MMR vaccine was still given at 12 months, however, the booster was given at five years and not eighteen months.

Results

In total, we examined 455,807 separate vaccination events in these 413,957 children that occurred at 12 and 18 months plus 60 days (Figure 2). We present the number of endpoint events versus days pre and post vaccination graphically for each of the vaccine periods (Figures 3 and 4).

12 month analysis

271,495 children received vaccinations between 365 and 425 days of age. Consecutive statistically significant elevations in combined endpoints began on day 4 and continued to day 12. A

Table 1. Relative incidence of combined endpoint (hospital admission or emergency room visit) following 12 month vaccination.

Risk interval*	Endpoints during risk interval (n)	Relative Incidence (95% CI)	P value
Day 4	621	1.15 (1.06–1.25)	0.0008
Day 5	641	1.19 (1.10–1.29)	<0.0001
Day 6	647	1.20 (1.11–1.31)	<0.0001
Day 7	644	1.20 (1.10–1.30)	<0.0001
Day 8	870	1.62 (1.50–1.74)	<0.0001
Day 9	1096	2.04 (1.91–2.17)	<0.0001
Day 10	991	1.84 (1.72–1.97)	<0.0001
Day 11	923	1.72 (1.60–1.84))	<0.0001
Day 12	713	1.32 (1.22–1.43)	<0.0001
Days 4 to 12** (Combined risk interval)	6462	1.33(1.29–1.38)	<0.0001
Days 20–28 (Control Interval)	4845	NA	NA

*Risk and control intervals expressed as days following vaccination.
**Total number of endpoints in the combined risk interval are less than the cumulative individual day event total because some children may have experienced events in multiple days and only the first event is counted.
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total of 6462 children experienced at least one of the combined endpoints during the combined 9 day at risk period compared to 4845 during the 9 day control period. The relative incidence of the combined endpoint was 1.33 (1.29–1.38) (Table 1). The highest relative incidence during the at-risk period occurred between days 8 and 11 peaking at 2.04 (1.91–2.17) on day 9. Overall, an excess of 595 children experienced at least one of the combined endpoints during the risk interval per 100,000 vaccinated, or one additional child experiencing at least one endpoint during the risk interval for every 168 children who received their 12 month vaccinations (Table 2). Examining the historical graph of the events post 12 month vaccination in the years 2002–2005 demonstrated a similar peak in events (Figure 5).

The primary reason for the elevation in the combined endpoint was an increase in ER visits (relative incidence 1.34(1.29–1.39)). There were an excess of 598 children experiencing 1 or more ER visits during the risk interval per 100,000 vaccinations or 1 additional child for every 168 children vaccinated. There was no increase in hospital admissions (relative incidence 1.08 (0.93–1.25)). There were five or fewer deaths (Table 3). The average CTAS score for ER visits during the risk period was 3.27 compared to 3.26 for the control period. ($p = 0.74$), suggesting no differences in severity of presentation between ER visits in the risk and control periods. There was an increase in presentation for multiple conditions during the risk period compared to the control period. The largest relative risk was associated with febrile seizures (relative incidence = 2.34, fever (RI = 2.31) and viral exanthem (RI = 2.23). We calculated that there were approximately 20 additional febrile seizures during the risk interval for every 100 000 children vaccinated. There was no increase in our tracer condition (ear/face/nose injury).

18 month analysis

184,312 children received vaccinations between 545 and 605 days of age. Consecutive statistically significant elevations in combined endpoints began on day 10 and continued to day 12. A total of 1275 children experienced at least one event included in the combined endpoint during the combined three day at risk period compared to 3065 during the nine day control period. The relative incidence of the combined endpoint was 1.25 (1.17–1.33) (Table 4). The highest relative incidence during the at-risk period was 1.34 (1.21–1.47) which occurred on day 12. Overall, an additional 137 children experienced at least one combined endpoint during the three day risk period per 100,000 vaccinated, or one additional child experiencing at least one excess event for every 730 children vaccinated (Table 3). Examining the historical graph of the events post 18 month vaccination in the years 2002–2005, when the booster dose of the MMR vaccine was not given, demonstrated no similar peak in events (Figure 5).

The primary reason for the elevation in the combined endpoint was an increase in ER visits (relative incidence 1.25(1.18–1.34)). There were an excess of 139 children experiencing one or more ER visits during the risk interval or one excess visit for every 719 children vaccinated. There was not a significant increase in hospital admissions (relative incidence 1.23(0.94–1.59)) (Table 4). No deaths occurred in the risk or control periods.

Discussion

Our analysis demonstrated that the 12 and 18 month vaccinations are not associated with an increase in adverse events immediately following vaccination. Instead it showed a reduced risk in this period, which is likely a result of the previously

Table 2. Increased risk of combined endpoints from vaccination.

Vaccination	Additional children experiencing at least one event (per 100,000 vaccinations)	Number vaccinated	Number vaccinated per excess event
12 months	595	271,495	168
18 months	137	184,312	730

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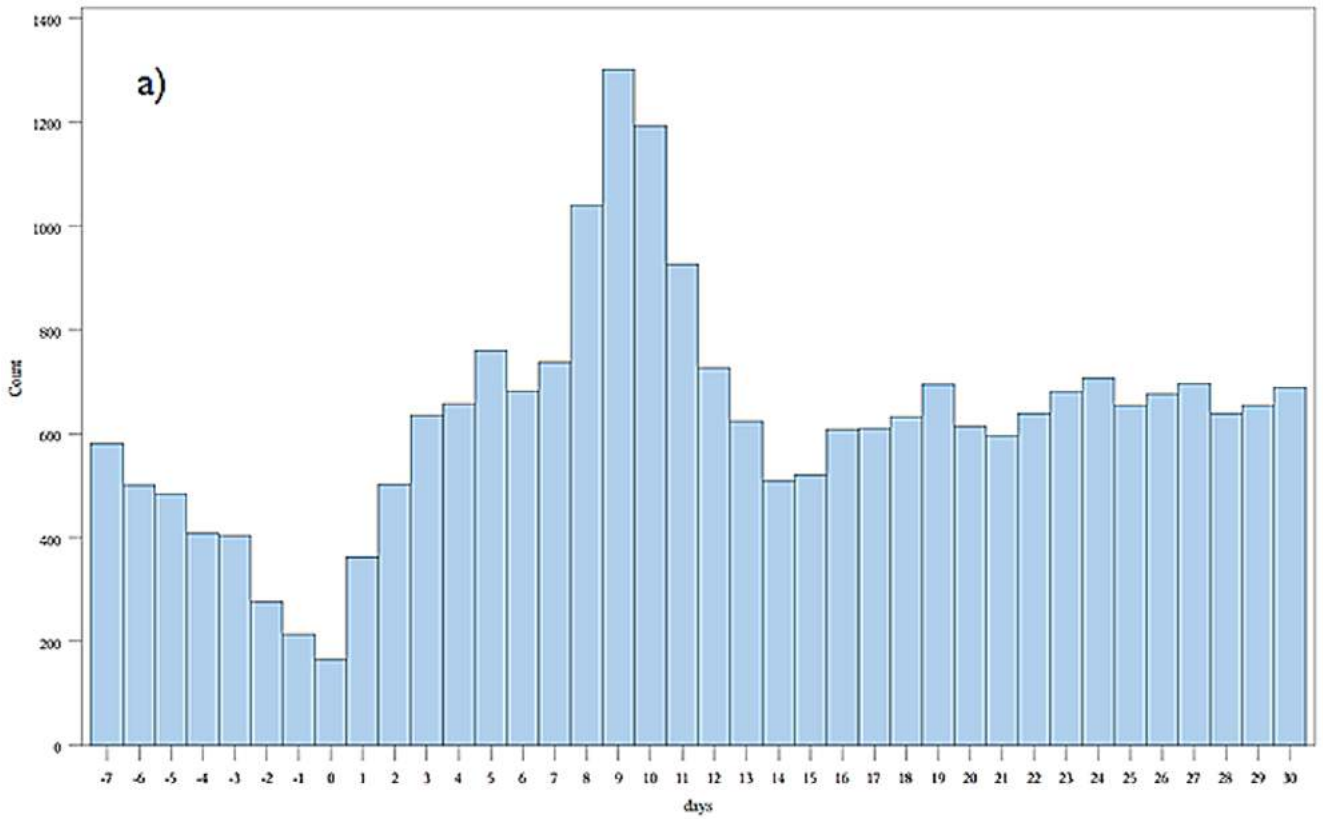
a)
Count
days

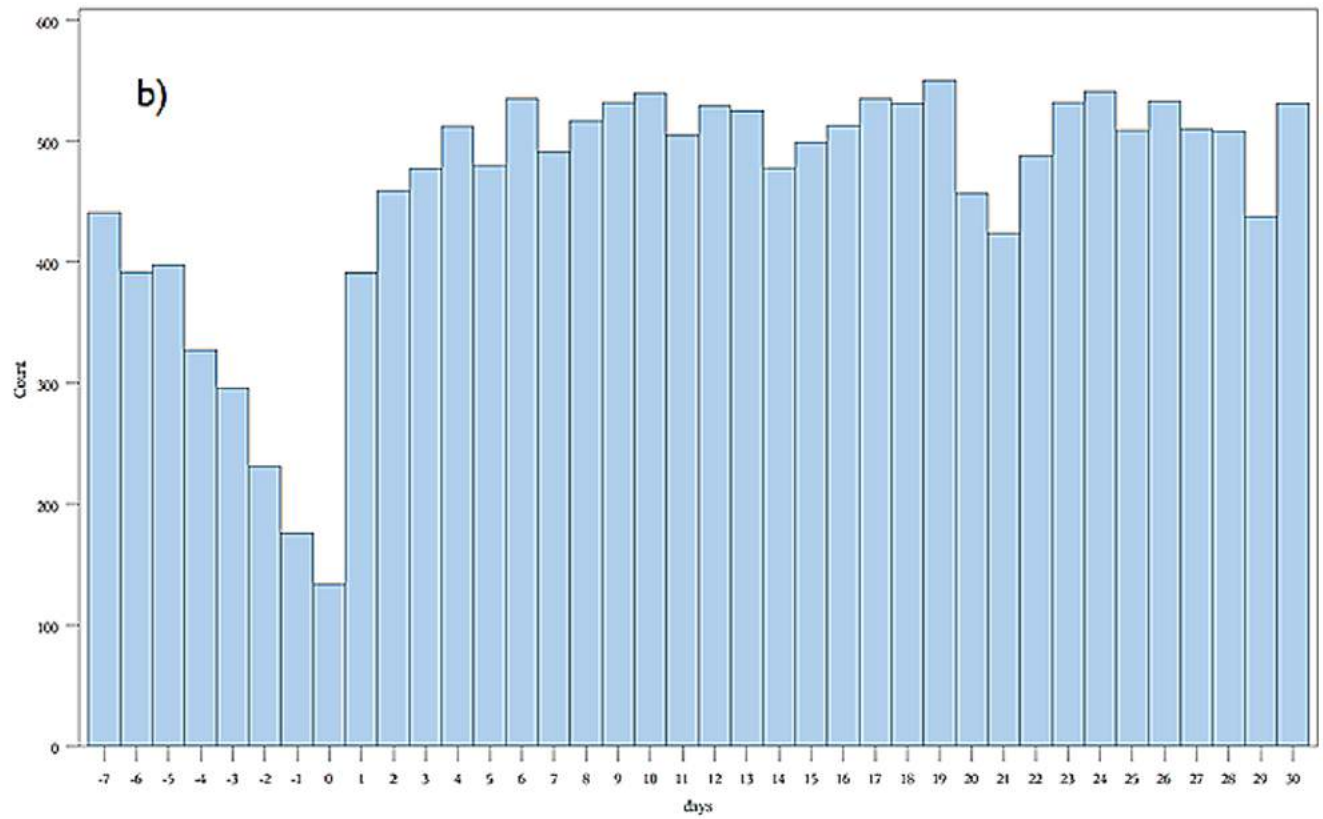
b)
Count
days

Figure 5. Historical analysis of combined endpoints versus days following 12 and 18 month vaccination: April 2002–March 2005. a) Before/after 12 month vaccination. b) Before/after 18 month vaccination. **Count** = number of combined endpoints of emergency room visit or hospitalization. **Days** = number of days before or after vaccination, day 0 being the day of vaccination.
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documented healthy vaccinee effect [9,13,14]. We identified an increase in events occurring between 4 and 12 days post-vaccination for the 12 month and, to a lesser extent and for a shorter time period for the 18 month vaccines. The majority of these events represented ER visits and at their peak, on day 9 following the 12 month vaccine, were approximately twice the baseline rate. Although there was an increase in hospital admission in each period, none of these increases were statistically significant. Overall the increase in event rate following the 12 month vaccines accounted for approximately 598 extra children experiencing one or more ER visits during the risk interval per 100,000 vaccinations. The average acuity of patients presenting to the emergency room was similar to that in the control period. The conditions for which there were the largest increase in risk for presentation to the emergency room during the risk interval compared to the control interval following the 12 month vaccine were febrile convulsions, fever and viral exanthema, consistent with the known adverse event profile of MMR and varicella vaccines. There were 20 additional febrile seizures for every 100,000 children vaccinated at 12 months.

The development of an inflammatory response approximately one week after vaccination is recognized in the literature. For example, the Centres for Disease Control and Prevention list days 7 to 12 post vaccination as the highest risk period for developing fever and possibly a rash [15]. This closely coincides with our observation of the time period during which emergency room visits peaked. A previous twin study also identified the development of systemic symptoms between days 6 and 14 and peaking on day 10 [9]. A study of febrile seizures following MMR vaccination identified the highest at risk period to be 8 to 14 days following vaccination and a relative risk of 2.83 and other studies have made similar observations [5,6,16]. These are consistent with our findings. While it is known that vaccines can produce these adverse events, our study demonstrated the population wide impact of this effect and that these events are resulting in an increase in health services utilization. The estimated 595 additional children experiencing at least one event for every 100 000 vaccinated translates into approximately one child experiencing at least one event per 168 children vaccinated. The explanation for this effect is likely the controlled replication of the virus creating a mild form of the illness the vaccine is designed to prevent. The top diagnoses for the presentations to the emergency room during the 12 month risk interval would all be consistent with a mild viral illness.

The reduced effect at 18 months is likely due to this vaccination in most instances being a second exposure to the antigen to which the vast majority of children would have developed adequate immunity. Residual events during this period may represent the small percentage of children who did not immunologically respond to the first dose of the vaccine.

Our study has several strengths. The use of the self-controlled case series design allows for individuals to serve as their own controls implicitly controlling for all fixed covariates [8,17]. Seasonal confounding is unlikely to have influenced our findings since the 12 and 18th month vaccines are provided throughout the year. The potential for confounding due to co-existent exposures at 12 and 18 months exists, however, if such an exposure were to be significant we would have expected to observe an effect at 18 months in our historical analysis. Our study included nearly all children born in Ontario during the study period which strengthens the generalizability of these findings. The combination of the self-controlled case series design and our sample size increased the power of our study to identify small effects. While our study cannot establish causality it has many features that support a causal relationship between vaccination and delayed adverse events. These include the consistency with other studies and a compelling biological model which explains the diagnoses in the affected children and the reduction in effect with the 18 month vaccinations. Furthermore, our historical analysis demonstrates that the effect seen at 18 months after MMR vaccination in 2006–2009 is not present in 2002–2005, when the MMR vaccine was given only at 12 months and not at 18 months. The effect is still clearly visible after the 12 month vaccination in the 2002–2005 data.

There are important limitations of this study. The first is that, as mentioned, the healthy vaccinee effect may have masked an association in the immediate post-vaccination period. Second, we cannot know whether a specific vaccine was associated with the adverse events as multiple vaccines are typically administered at each visit. However, we have previously demonstrated the safety of the pentavalent vaccine which is given with the 18 month MMR vaccine [18]. It is possible that the effects seen at 12 month are in part due to the potential co-administration of the meningococcal C vaccine, however, this is not a live vaccine and should create inflammation in the immediate post-vaccination period as opposed to one week later. Third, the codes we used for identifying the reasons for presentation to the emergency room have not been validated. However, we would expect that the diagnoses of febrile convulsion to have a low misclassification error and has previously been validated as a useful ER code in a separate dataset [19]. We also did not look for increases in visits to physician offices that did not result in presentation to the emergency room or admission and cannot comment on the impact of immunization on that outcome.

Table 3. Relative incidences of individual endpoints (emergency room visit, hospital admission, death) during highest risk interval compared to control period.

Outcome	12 months	Events (risk/control)	18 months	Events (risk/control)
Emergency visits	1.34 (1.29–1.39)	6395/4772	1.25 (1.18–1.34)	1264/3024
Admissions	1.08 (0.93–1.25)	356/330	1.23 (0.94–1.59)	78/191
Deaths	-	< = 5/< = 5	-	0/0

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Table 4. Relative incidence of combined endpoint (hospital admission or emergency room visit) following 18 month vaccination.

Risk interval*	Endpoints during risk interval (n)	Relative Incidence (95% CI)	P value
Days 10	447	1.31 (1.19–1.45)	<0.0001
Days 11	428	1.26 (1.14–1.39)	<0.0001
Days 12	455	1.34 (1.21–1.47)	<0.0001
Days 10 to 12 (Combined risk interval)	1275	1.25 (1.17,1.33)	<0.0001
Days 20 to 28 (Control Interval)	3065	NA	NA

*Risk and control intervals expressed as days following vaccination.
doi:10.1371/journal.pone.0027897.t004

Our findings have important implications for those providing care to children. The immediate risk of a serious adverse event following immunization is low with both the vaccination visits that contain the MMR and varicella vaccines. However, the 12 month vaccines which typically contain the first dose of the MMR vaccine is associated with an increased risk of an emergency room visit approximately 4 to 12 days after immunization, peaking between days 8 and 11. This increase in rate of a child experiencing at least one event for every 158 vaccinated individuals is associated with a similar acuity as the control period. If the presentation to the emergency room was due to parental anxiety we would have expected to see a reduction in acuity during the risk period. The findings also suggest that the reactions are not severe since acuity was not higher than the control period and furthermore, there were few hospital admissions. Additional reassurance can be derived from previous studies that identified no long-term consequences related to vaccine associated febrile seizures [5,6]. The increase in ER visits we observed could be a result of insufficient information being provided to parents who may not expect their child to develop a reaction a week after vaccination. In particular, the likelihood of this risk may be underestimated by physicians. Our study also reinforces the reduced risk of events following the second dose of MMR vaccine.

Given the effectiveness of the MMR vaccine in eliminating both measles and rubella, and the highly infectious nature of these diseases, high vaccination coverage is essential. The diseases that the vaccines are preventing are not benign and vaccination can eliminate many of the serious sequelae of these infections [20]. Complications from measles include otitis media (7–9% of cases), pneumonia (1–6% of cases), encephalitis (1 per 1,000–2,000 cases), subacute sclerosing panecephalitis (1 per 100,000 cases), and death (1 per 3000 cases) [3,21]. Further studies attempting to predict which children develop post-vaccination reactions, as well as determining the effectiveness of prophylactic treatment with antipyrectics prior to the high risk period for symptom development are warranted.

Supporting Information

Appendix S1 Figure A1: Flowchart Describing SCCS Study Cohort.
(TIF)

Author Contributions

Conceived and designed the experiments: KW SH DM JK SD NC. Performed the experiments: SH. Analyzed the data: KW SH CV DM JK. Wrote the paper: KW SH JK MP SD NC BP PC.

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Children Who Get Flu Vaccine Have Three Times Risk Of Hospitalization For Flu, Study Suggests

Date: May 20, 2009

Source: American Thoracic Society

Summary: The inactivated flu vaccine does not appear to be effective in preventing influenza-related hospitalizations in children, especially the ones with asthma. In fact, children who get the flu vaccine are more at risk for hospitalization than their peers who do not get the vaccine, according to new research. While these findings do raise questions about the efficacy of the vaccine, they do not in fact implicate it as a cause of hospitalizations, according to researchers.

FULL STORY

The inactivated flu vaccine does not appear to be effective in preventing influenza-related hospitalizations in children, especially the ones with asthma. In fact, children who get the flu vaccine are more at risk for hospitalization than their peers who do not get the vaccine, according to new research that will be presented on May 19, at the 105th International Conference of the American Thoracic Society in San Diego.

Flu vaccine (trivalent inactivated flu vaccine—TIV) has unknown effects on asthmatics.

"The concerns that vaccination maybe associated with asthma exacerbations have been disproved with multiple studies in the past, but the vaccine's effectiveness has not been well-established," said Avni Joshi, M.D., of the Mayo Clinic in Rochester, MN. "This study was aimed at evaluating the effectiveness of the TIV in children overall, as well as the children with asthma, to prevent influenza-related hospitalization."

The CDC's Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics (AAP) recommend annual influenza vaccination for all children aged six months to 18 years. The National Asthma Education and Prevention Program (3rd revision) also recommends annual flu vaccination of asthmatic children older than six months.

In order to determine whether the vaccine was effective in reducing the number of hospitalizations that all children, and especially the ones with asthma, faced over eight consecutive flu seasons, the researchers conducted a cohort study of 263 children who were evaluated at the Mayo Clinic in Minnesota from six months to 18 years of age, each of whom had had laboratory-confirmed influenza between 1996 to 2006. The investigators determined who had and had not received the flu vaccine, their asthma status and who did and did not require hospitalization. Records were reviewed for each subject with influenza-related illness for flu vaccination preceding the illness and hospitalization during that illness.

They found that children who had received the flu vaccine had three times the risk of hospitalization, as compared to children who had not received the vaccine. In asthmatic children, there was a significantly higher risk of hospitalization in subjects who received the TIV, as compared to those who did not ($p = 0.006$). But no other measured factors—such as insurance plans or severity of asthma—appeared to affect risk of hospitalization.

"While these findings do raise questions about the efficacy of the vaccine, they do not in fact implicate it as a cause of hospitalizations," said Dr. Joshi. "More studies are needed to assess not only the immunogenicity, but also the efficacy of different influenza vaccines in asthmatic subjects."

Story Source:

Materials provided by **American Thoracic Society**. *Note: Content may be edited for style and length.*

Infections

Increased Risk of Noninfluenza Respiratory Virus Infections Associated With Receipt of Inactivated Influenza Vaccine

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We randomized 115 children to trivalent inactivated influenza vaccine (TIV) or placebo. Over the following 9 months, TIV recipients had an increased risk of virologically-confirmed non-influenza infections (relative risk: 4.40; 95% confidence interval: 1.31-14.8). Being protected against influenza, TIV recipients may lack temporary non-specific immunity that protected against other respiratory viruses.

Influenza vaccination is effective in preventing influenza virus infection and associated morbidity among school-aged children [1, 2]. The potential for temporary nonspecific immunity between respiratory viruses after an infection and consequent interference at the population level between epidemics of these viruses has been hypothesized, with limited empirical evidence to date, mainly from ecological studies [3–15]. We investigated the incidence of acute upper respiratory tract infections (URTIs) associated with virologically confirmed respiratory virus infections in a randomized controlled trial of influenza vaccination.

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METHODS

Recruitment and Follow-up of Participants

In a double-blind randomized controlled trial, we randomly allocated children aged 6–15 years to receive 2008–2009 seasonal trivalent influenza inactivated vaccine (TIV; 0.5 mL Vaxigrip; Sanofi Pasteur) or placebo [16]. Serum specimens were obtained from participants before vaccination from November through December 2008, a month after vaccination, in midstudy around April 2009, and at the end of the study from August through October 2009. Participants were followed up for illnesses through symptom diaries and telephone calls, and illness reports in any household member triggered home visits during which nasal and throat swab specimens (NTSs) were collected from all household members. We defined the follow-up period for each participant from 14 days after receipt of TIV or placebo to collection of midstudy serum samples as the winter season and from collection of midstudy samples through final serum sample obtainment as the summer season.

Proxy written informed consent was obtained for all participants from their parents or legal guardians, with additional written assent from those ≥8 years of age. The study protocol was approved by the Institutional Review Board of Hong Kong University.

Laboratory Methods

NTSs were tested for 19 respiratory viruses by the ResPlex II Plus multiplex array [17–19] and for influenza A and B by reverse-transcription polymerase chain reaction (RT-PCR) [16, 20] (Supplementary Appendix). We refer to infections determined by these assays as "confirmed" infections. Information on influenza serology is provided in the Supplementary Appendix .

Statistical Analysis

We defined an acute respiratory illness (ARI) determined by self-reported signs and symptoms as ≥2 of the following signs or symptoms: body temperature ≥37.8°C, headache, sore throat, cough, presence of phlegm, coryza, and myalgia [16]. We defined febrile acute respiratory illness (FARI) as body temperature ≥37.8°C plus cough or sore throat. Because duration of follow-up varied by participant, we estimated the incidence rates of ARI and FARI episodes and confirmed viral infections overall and during the winter and summer seasons and estimated the relative risk of these episodes for participants who received TIV versus placebo with use of the incidence rate ratio using Poisson regression (Supplementary

Table 1. Characteristics of Participants and Duration of Follow-up

Characteristic	TIV (n = 69)	Placebo (n = 46)
Age group, No. (%)		
6–8 years	19 (28)	16 (35)
9–11 years	41 (59)	27 (59)
12–15 years	9 (13)	3 (7)
Female sex, No. (%)	30 (43)	23 (50)
Median duration of follow-up, days	272	272
Mean no. of individuals per household	3.7	3.6

Abbreviation: TIV, trivalent inactivated influenza vaccine.

Appendix). All statistical analyses were conducted using R, version 2.11.0 (R Development Core Team, Vienna, Austria). Data and syntax to reproduce these statistical analyses are available on the corresponding author's Web site.

RESULTS

Among the 115 participants who were followed up, the median duration of follow-up was 272 days (interquartile range, 264–285 days), with no statistically significant differences in age, sex, household size, or duration of follow-up between TIV and placebo recipients (Table 1). We identified 134 ARI episodes, of which 49 met the more stringent FARI case definition. Illnesses occurred throughout the study period (Supplementary Appendix Figure 1). There was no statistically significant difference in the risk of ARI or FARI between participants who received TIV and those who received placebo, either during winter or summer 2009 (Table 2).

We were able to collect 73 NTSs for testing from participants for 65 of 134 (49%) ARI episodes, which included 22 of 49 (45%) FARI episodes. The mean delay between ARI onset and collection of first NTS was 1.22 days, and 5% of NTSs were collected >3 days after illness onset, with no statistically significant differences between TIV and placebo recipients. We detected respiratory viruses in 32 of 65 NTSs (49%) collected during ARI episodes, which included 12 of 22 (55%) FARI episodes. We collected 85 NTSs from participants at times when one of their household contacts reported an acute URTI but the participants were not ill, and identified viruses in 3 of the specimens (4%), including influenza A (H3N2), coxsackie/echovirus, and coronavirus 229E.

There was no statistically significant difference in the risk of confirmed seasonal influenza infection between recipients of TIV or placebo, although the point estimate was consistent with protection in TIV recipients (relative risk [RR], 0.66; 95% confidence interval [CI], .13–3.27). TIV recipients had significantly lower risk of seasonal influenza infection based on serologic evidence (Supplementary Appendix). However, participants who received TIV had higher risk of ARI associated with confirmed noninfluenza respiratory virus infection (RR, 4.40; 95% CI, 1.31–14.8). Including 2 additional confirmed infections when participants did not report ARI, TIV recipients had higher risk of confirmed noninfluenza respiratory virus infection (RR, 3.46; 95% CI, 1.19–10.1). The majority of the noninfluenza respiratory virus detections were rhinoviruses and coxsackie/echoviruses, and the increased risk among TIV recipients was also statistically significant for these viruses (Table 3). Most respiratory virus detections occurred in March 2009, shortly after a period of peak seasonal influenza activity in February 2009 (Figure 1).

Table 2. Incidence Rates of Acute Upper Respiratory Tract Infection Among 115 Participants Aged 6–15 Years Who Received Trivalent Inactivated Influenza Vaccine or Placebo

	TIV (n = 69)		Placebo (n = 46)			
Variable	Rate[a]	(95% CI)	Rate[a]	(95% CI)	Relative Risk (95% CI)	*P* Value
Winter 2009						
ARI[b] episodes	2080	(1530–2830)	2260	(1550–3300)	0.92 (.57–1.50)	.74
FARI[b] episodes	609	(346–1070)	753	(392–1450)	0.81 (.34–1.92)	.63
Summer 2009						
ARI[b] episodes	1510	(1130–2020)	1160	(757–1780)	1.30 (.78–2.18)	.31
FARI[b] episodes	658	(424–1020)	442	(221–884)	1.49 (.65–3.38)	.33

Abbreviations: ARI, acute respiratory illness; CI, confidence interval; FARI , febrile acute respiratory illness; TIV, trivalent inactivated influenza vaccine.

[a] Incidence rates were estimated as the number of ARI or FARI episodes per 1000 person-years of follow-up.

[b] ARI was defined as at least 2 of the following symptoms: body temperature ≥37.8°C, cough, sore throat, headache, runny nose, phlegm, and myalgia; FARI was defined as body temperature ≥37.8°C plus cough or sore throat.

Table 3. Incidence Rates of Respiratory Virus Detection by Reverse-Transcription Polymerase Chain Reaction and Multiplex Assay

Variable	TIV (n = 69)			Placebo (n = 46)			*P* Value
	No.	Rate[a]	(95% CI)	No.	Rate[a]	(95% CI)	
Any seasonal influenza	3	58	(19–180)	3	88	(28–270)	.61
Seasonal influenza A (H1N1)	2	39	(10–160)	2	59	(15–240)	.68
Seasonal influenza A (H3N2)	1	19	(3–140)	0	0	(0–88)	.31
Seasonal influenza B	0	0	(0–58)	1	29	(4–210)	.17
Pandemic influenza A (H1N1)	3	58	(19–180)	0	0	(0–88)	.08
Any noninfluenza virus[b]	20	390	(250–600)	3	88	(28–270)	<.01
Rhinovirus	12	230	(130–410)	2	59	(15–240)	.04
Coxsackie/echovirus	8	160	(78–310)	0	0	(0–88)	<.01
Other respiratory virus[c]	5	97	(40–230)	1	29	(4–210)	.22
ARI episode with specimen collected but no virus detected	19	369	(235–578)	14	412	(244–696)	.75
ARI episode with no specimen collected	41	796	(586–1080)	28	824	(569–1190)	.89

Incidence rates are from respiratory specimens collected from 115 participants aged 6–15 years who received trivalent influenza vaccine or placebo during 134 acute respiratory illness episodes.

Abbreviations: ARI, acute respiratory illness; CI, confidence interval; TIV, trivalent inactivated influenza vaccine.

[a] Incidence rates were estimated as the no. of virus detections or illness episodes per 1000 person-years of follow-up. ARI was defined as at least 2 of the following symptoms: body temperature ≥37.8°C, cough, sore throat, headache, runny nose, phlegm, and myalgia.

[b] In TIV recipients there were 4 detections with both rhinovirus and coxsackie/echovirus, and 1 detection with both coxsackie/echovirus and coronavirus NL63.

[c] Including positive detections of coronavirus, human metapneumovirus, parainfluenza, respiratory syncytial virus (RSV). The ResPlex II multiplex array tested for 19 virus targets including influenza types A and B (including 2009-H1N1), RSV types A and B, parainfluenza types 1–4, metapneumovirus, rhinovirus, coxsackievirus/echovirus, adenovirus types B and E, bocavirus, and coronavirus types NL63, HKU1, 229E, and OC43.

DISCUSSION

In the prepandemic period of our study, we did not observe a statistically significant reduction in confirmed seasonal influenza virus infections in the TIV recipients (Table 3), although serological evidence (Supplementary Appendix) and point estimates of vaccine efficacy based on confirmed infections were consistent with protection of TIV recipients against the seasonal influenza viruses that circulated from January through March 2009 [16]. We identified a statistically significant increased risk of noninfluenza respiratory virus infection among TIV recipients (Table 3), including significant increases in the risk of rhinovirus and coxsackie/echovirus infection, which were most frequently detected in March 2009, immediately after the peak in seasonal influenza activity in February 2009 (Figure 1).

The increased risk of noninfluenza respiratory virus infection among TIV recipients could be an artefactual finding; for example, measurement bias could have resulted if participants were more likely to report their first ARI episode but less likely to report subsequent episodes, whereas there was no real difference in rhinovirus or other noninfluenza respiratory virus infections after the winter influenza season. The increased risk could also indicate a real effect. Receipt of TIV could increase influenza immunity at the expense of reduced immunity to noninfluenza respiratory viruses, by some unknown biological mechanism. Alternatively, our results could be explained by temporary nonspecific immunity after influenza virus infection, through the cell-mediated response or, more likely, the innate immune response to infection [21–23]. Participants who received TIV would have been protected against influenza in February 2009 but then would not have had heightened nonspecific immunity in the following weeks. They would then face a higher risk of certain other virus infections in March 2009, compared with placebo recipients (Figure 1). The duration of any temporary nonspecific immunity remains uncertain [13] but could be of the order of 2–4 weeks based on these observations. It is less likely that the interference observed here could be explained by reduced community exposures during convalescence (ie, behavioral rather than immunologic factors) [14].

The phenomenon of virus interference has been well known in virology for >60 years [24–27]. Ecological studies have reported phenomena potentially explained by viral interference [3–11]. Nonspecific immunity against noninfluenza respiratory viruses was reported in children for 1–2 weeks after receipt of live attenuated influenza vaccine [28]. Interference in respiratory and gastrointestinal infections has been reported after receipt of live oral poliovirus vaccine [29–32].

Our results are limited by the small sample size and the small number of confirmed infections. Despite this limitation, we were able to observe a statistically significant increased risk

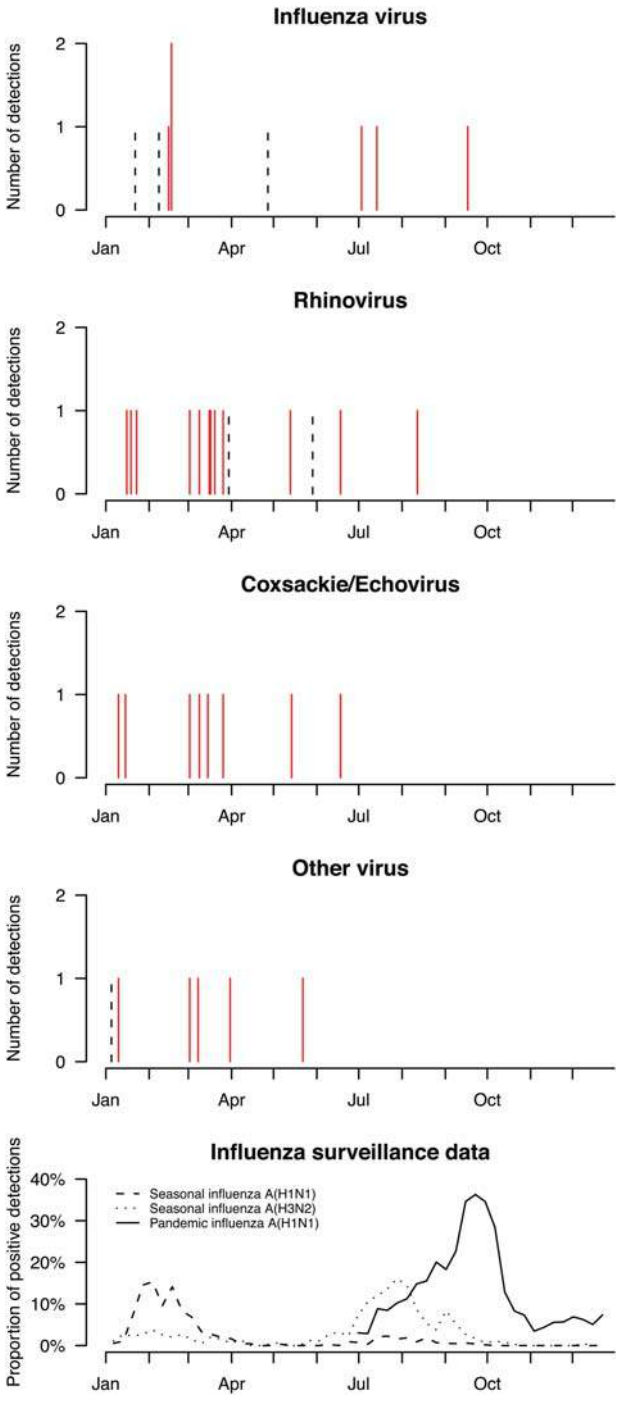

Figure 1. Timing of influenza and other respiratory virus detections in 115 participants aged 6–15 years (*A–D*), compared with local influenza surveillance data (*E*). Solid red bars indicate detections in 69 participants who received 2008–2009 trivalent inactivated influenza vaccine, and black dashed bars indicate detections in 46 participants who received placebo. The bottom panel shows local laboratory surveillance data on the proportion of influenza virus detections among specimens submitted to the Public Health Laboratory Service (PHLS). Less than 2% of PHLS specimens were positive for influenza B throughout the year. "Other viruses" included coronavirus, human metapneumovirus, parainfluenza, and respiratory syncytial virus.

of confirmed noninfluenza respiratory virus infection among TIV recipients (Table 3). A negative association between serologic evidence of influenza infection and confirmed noninfluenza virus infection in winter 2009 was not statistically significant (odds ratio, 0.27; 95% CI, .01–2.05) (Supplementary Appendix). One must be cautious in interpreting serology in children who have received TIV [2, 33]. Finally, acute URTI incidence was based on self-report with regular telephone reminders, and we may have failed to identify some illnesses despite rigorous prospective follow-up.

Temporary nonspecific immunity leading to interference between epidemics of respiratory viruses could have important implications. First, as observed in our trial, TIV appeared to have poor efficacy against acute URTIs (Table 2), apparently because the protection against influenza virus infection conferred by TIV was offset by an increased risk of other respiratory virus infection (Table 3). Second, interference between respiratory viruses could suggest new approaches to mitigating epidemics [32]. Mass administration of live polio vaccine in children has been used to control enterovirus 71 epidemics [10, 31]. Finally, viral interference could bias estimates of influenza vaccine effectiveness in test-negative case-control studies (Supplementary Appendix) [2, 34–43]. One test-negative study reported an association between receipt of TIV and the risk of influenza-like illness associated with a noninfluenza virus [38].

Additional work is required to more fully characterize temporary nonspecific immunity overall and in specific groups, such as children. Animal studies [44–50] and volunteer adult human challenge studies [51] could provide useful evidence. Additional community-based observational cohort studies and community-based experimental studies, such as our vaccine trial, may be particularly suitable for investigating temporary nonspecific immunity, because most acute URTIs do not require medical attention.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online (http://www.oxfordjournals.org/our_journals/cid/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Live Attenuated Influenza Vaccine Enhances Colonization of *Streptococcus pneumoniae* and *Staphylococcus aureus* in Mice

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ABSTRACT **Community interactions at mucosal surfaces between viruses, like influenza virus, and respiratory bacterial pathogens are important contributors toward pathogenesis of bacterial disease. What has not been considered is the natural extension of these interactions to live attenuated immunizations, and in particular, live attenuated influenza vaccines (LAIVs). Using a mouse-adapted LAIV against influenza A (H3N2) virus carrying the same mutations as the human FluMist vaccine, we find that LAIV vaccination reverses normal bacterial clearance from the nasopharynx and significantly increases bacterial carriage densities of the clinically important bacterial pathogens *Streptococcus pneumoniae* (serotypes 19F and 7F) and *Staphylococcus aureus* (strains Newman and Wright) within the upper respiratory tract of mice. Vaccination with LAIV also resulted in 2- to 5-fold increases in mean durations of bacterial carriage. Furthermore, we show that the increases in carriage density and duration were nearly identical in all aspects to changes in bacterial colonizing dynamics following infection with wild-type (WT) influenza virus. Importantly, LAIV, unlike WT influenza viruses, had no effect on severe bacterial disease or mortality within the lower respiratory tract. Our findings are, to the best of our knowledge, the first to demonstrate that vaccination with a live attenuated viral vaccine can directly modulate colonizing dynamics of important and unrelated human bacterial pathogens, and does so in a manner highly analogous to that seen following wild-type virus infection.**

IMPORTANCE **Following infection with an influenza virus, infected or recently recovered individuals become transiently susceptible to excess bacterial infections, particularly *Streptococcus pneumoniae* and *Staphylococcus aureus*. Indeed, in the absence of preexisting comorbidities, bacterial infections are a leading cause of severe disease during influenza epidemics. While this synergy has been known and is well studied, what has not been explored is the natural extension of these interactions to live attenuated influenza vaccines (LAIVs). Here we show, in mice, that vaccination with LAIV primes the upper respiratory tract for increased bacterial growth and persistence of bacterial carriage, in a manner nearly identical to that seen following wild-type influenza virus infections. Importantly, LAIV, unlike wild-type virus, did not increase severe bacterial disease of the lower respiratory tract. These findings may have consequences for individual bacterial disease processes within the upper respiratory tract, as well as bacterial transmission dynamics within LAIV-vaccinated populations**

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The conventional view of pathogen dynamics posits that pathogen species act independently of one another. More recently, however, community interactions between pathogens have been recognized as necessary to modulate both health and disease (1–7). These interactions might be expected to be most prevalent within gut, respiratory, and other mucosal surfaces that harbor complex populations of commensal and, occasionally, pathogenic microbes. In the respiratory tract, for example, viral infections are known to predispose to secondary bacterial invasive disease and pneumonia from pathogens that are most commonly benign but occasionally become virulent, particularly following a viral infection (8–10). A well-known example is the often lethal synergy between influenza virus and pneumococcal or staphylococcal bacterial secondary infections.

Infection with influenza viruses increases susceptibility to severe lower and upper respiratory tract (LRT and URT, respectively) bacterial infections resulting in complications, such as pneumonia, bacteremia, sinusitis, and acute otitis media (11). Bacterial infections may be a primary cause of mortality associated with influenza virus infection in the absence of preexisting comorbidity (12, 13). Primary influenza virus infection increases acquisition, colonization, and transmission of bacterial pathogens (14), most notably the pneumococcus *Streptococcus pneumoniae* and *Staphylococcus aureus* (11, 15).

Although the underlying mechanisms, while well studied, are not entirely defined, they likely include a combination of influenza virus-mediated cytotoxic breakdown of mucosal and epithelial barriers (16–18) and aberrant innate immune responses to

bacterial invaders in the immediate postinfluenza state, characterized by uncontrolled pro- and anti-inflammatory cytokine production, excessive leukocyte recruitment, and extensive immunopathology (11, 19–22). When coupled with diminished epithelial and mucosal defenses, such an environment becomes increasingly hospitable for bacterial pathogens to flourish and invade in the days and first few weeks following influenza virus infection.

Increasingly, evidence is linking the early innate immune response triggered by infection or vaccination to sustained adaptive immunity (23). Thus, a broad goal of vaccination is to elicit an immune response analogous to that of the pathogen itself, without subsequent disease (24). The intranasally administered live attenuated influenza vaccine (LAIV) contains temperature-sensitive and attenuated virus designed to replicate efficiently in the cooler temperatures of the upper respiratory tract (URT) but which fails to do so in the warmer temperatures of the lower respiratory tract (LRT) (25, 26). Through selective replication in the URT, LAIV proteins are exposed to the host immune system in their native conformation, eliciting highly robust (IgA), serum (IgG), and cellular immune responses mimicking those of the pathogenic virus itself (27).

Although an innate immune response to vaccination is beneficial for long-term protection from influenza virus (28) and influenza virus-bacterial (29) coinfections, the direct consequences of such a response to a viral vaccine, with respect to secondary colonization and disease due to entirely unrelated bacterial pathogen species, are unknown. As increased susceptibility to and transmission of bacterial pathogens following influenza are due in large part to the innate immune response and breakdowns of the epithelial barriers of the URT, it is important to understand whether similar effects, elicited by live attenuated virus replication, may also predispose to bacterial infection. We sought here to determine the effects of a live attenuated influenza vaccine on URT and LRT bacterial infections. In particular, we ask whether LAIV vaccination alters bacterial colonization dynamics of the upper respiratory tract or disease in the lower respiratory tract of mice.

RESULTS

Using a live attenuated influenza A virus vaccine, HK/Syd 6:1:1 (LAIV), which contains many of the same mutations and demonstrates similar growth dynamics to those in the commercially available human FluMist vaccine (MedImmune, Gaithersburg, MD) (see reference 30 and Fig. S1 in the supplemental material for vaccine details), we evaluated the effects of LAIV and its wild-type (WT) HK/Syd parent strain (referred to as WT virus) on *Streptococcus pneumoniae* (the pneumococcus) and *Staphylococcus aureus* replication and disease.

LAIV virus is restricted in growth at 37°C but not at 33°C. To determine whether LAIV virus grows efficiently at temperatures seen within the nasopharynx (NP) while remaining restricted in growth at warmer temperatures of the LRT, WT influenza virus and its LAIV derivative were grown in MDCK cells at 37°C. As expected (30), a >3-log decrease in viral titers was measured for LAIV relative to the WT parent strain ($P < 0.001$) (Fig. 1A). However, when LAIV was propagated at 33°C, a temperature often associated with the nasopharyngeal environment (31), viral replication was no different from that of WT virus titers measured at 37°C.

HK/Syd 1:1:6 LAIV vaccination is safe and effective in mice. Although LAIV is attenuated, inoculation with very high doses may cause morbidity and weight loss. Via a series of dosing experiments (data not shown), a vaccinating dose of 2e6 tissue culture infective doses ($TCID_{50}$) of LAIV in 40 μl phosphate-buffered saline (PBS) vehicle was determined to be safe, with no weight loss or other detectable signs of morbidity in mice (Fig. 1B). This dose is in agreement with previous studies (28, 30). Inoculation with the same dose of the WT parent virus led to significant morbidity and mortality (5/12 mice succumbed by day 7 postinfection) (Fig. 1B), demonstrating the attenuated nature of the LAIV.

The vaccine efficacy and antibody response using this LAIV strain were described previously (30). To phenotypically confirm efficacy here, groups of 8 4-week-old mice were inoculated with LAIV or the PBS control and 4 weeks later with a lethal dose of the WT virus. Early vaccination with LAIV conferred complete protection from any detectable morbidity or weight loss due to infection with the WT strain, versus 100% mortality in unvaccinated control mice (Fig. 1C).

LAIV is restricted in growth in the lower but not the upper respiratory tract. To determine whether the differences in replication seen *in vitro* also occur *in vivo* in the upper (~33°C) versus lower (~37°C) respiratory tract, groups of 5 mice were vaccinated with LAIV, and viral titers were measured in whole lung and whole NP homogenates (Fig. 1D). By 3 days postvaccination, NP titers were 10,000-fold greater than in the lungs (1.3e6 versus 1.2e2 $TCID_{50}$; $P < 0.001$). In contrast, the WT virus grew to high viral titers in both the NP and lungs (>5e5 $TCID_{50}$) (data not shown), in agreement with previous reports (32), which led to significant morbidity and mortality, as demonstrated in the controls in Fig. 1B. Overall, maximal NP titers occurred earlier and were nearly 400-fold greater than maximum lung titers (1.3e6 versus 3.4e3 $TCID_{50}$; $P < 0.001$). Importantly, these NP viral dynamics are in agreement with viral shedding in NP aspirates from human subjects following vaccination with the FluMist vaccine (33).

LAIV cytokine response in the nasopharynx and lungs. While LAIV replication in the NP induces a robust systemic inflammatory response (34, 35), the cytokine response in the NP has, to our knowledge, not been observed. Nasopharyngeal homogenates and bronchoalveolar lavage (BAL) specimen cytokines were measured in groups of 5 mice each at days 0, 3, 5, and 7 postvaccination (Fig. 1E). Of particular interest, the type I interferon (IFN-β) was significantly increased in the NP and BAL specimens following LAIV vaccination, and this cytokine has been demonstrated to play a pivotal role in excess bacterial colonization of the nasopharynx following WT influenza virus infection (36). As well, macrophage inflammatory protein 1β (MIP-1β) was also significantly upregulated following LAIV, similar to what was seen following influenza virus-pneumococcal coinfections of human middle ear epithelial cells (37). In general, the responses measured here in the NP are similar to those measured from nasopharyngeal washes in humans infected naturally with seasonal influenza A viruses (38).

LAIV enhances pneumococcal bacterial dynamics in the URT in a manner highly analogous to WT influenza virus. Numerous previous investigations have demonstrated that replication of WT influenza virus within the URT predisposes to excess bacterial replication and colonization within the NP, particularly by *Streptococcus pneumoniae* (36, 39, 40). Because, as demonstrated above, LAIV replicates to near WT levels when in the cooler temperatures of the URT, we sought to study effects of LAIV on bacterial carriage density within the NP of mice and compared them to the changes in bacterial carriage following WT

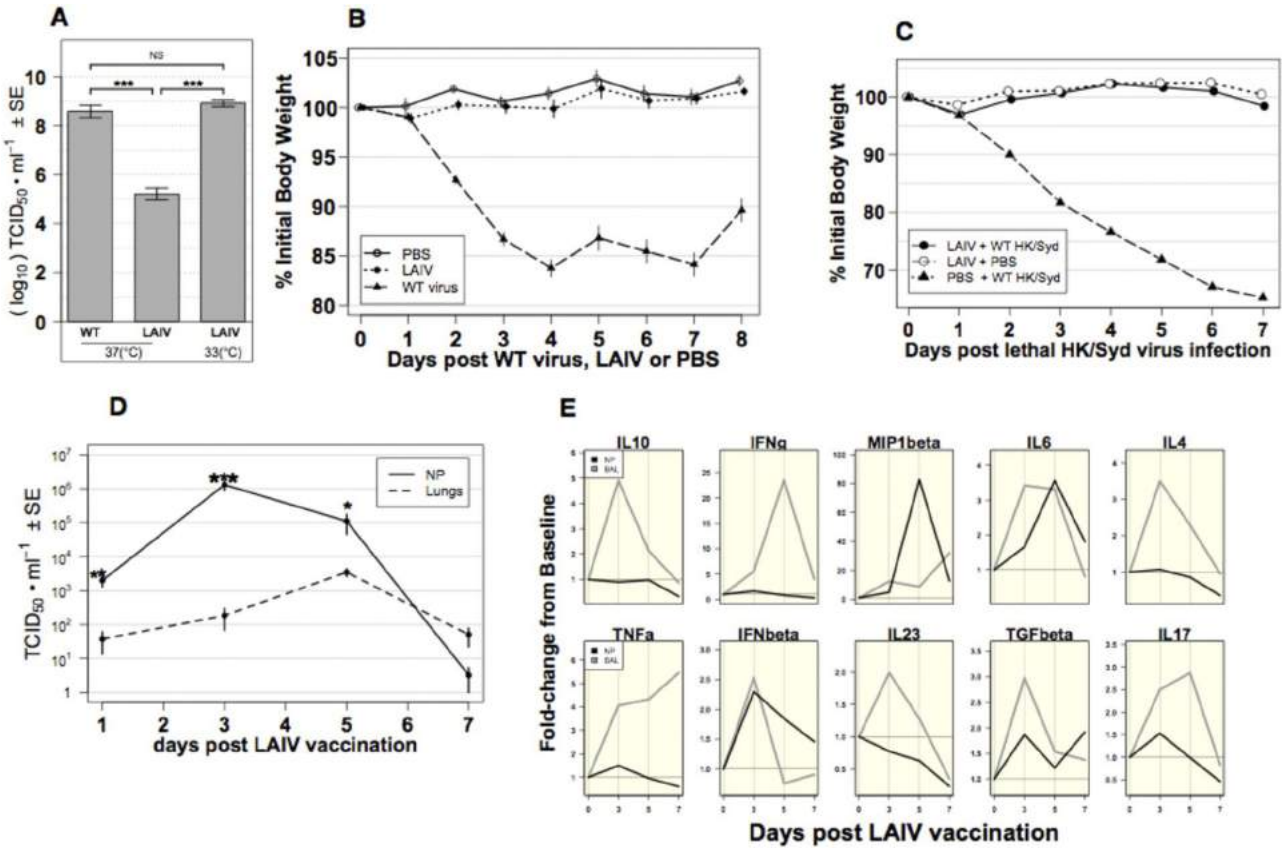

FIG 1 LAIV is safe, effective, replicates well within the URT, and elicits a robust cytokine response. (A) WT and LAIV HK/Syd viruses were grown in MDCK cells at 37°C and LAIV virus was grown at 33°C, and viral titers were measured via the median TCID50 (n = 3 per group). (B) Groups of 12 to 14 8-week-old BALB/c mice were inoculated with 2e6 TCID50 LAIV, WT HK/Syd virus, or PBS and monitored for weight loss. Three of 12 mice and 2/12 mice died at 4 and 7 days postinfection with WT HK/Syd virus, respectively, while no mice died following LAIV or PBS inoculation. (C) Groups of 8 4-week-old BALB/c mice were inoculated with 2e6 TCID50 of LAIV (2 of the 3 groups) or PBS and 4 weeks later infected with a lethal dose (5e7 TCID50) of WT HK/Syd virus or the PBS control. Infection was considered lethal if body weight fell below 70% of the initial body weight. (D) Four groups of 5 mice each were vaccinated with LAIV, and whole lung and NP viral titers were measured at 1, 3, 5, and 7 days postvaccination. (E) Four groups of 5 mice were vaccinated with LAIV, and NP and BAL specimen cytokines were measured at day 0 (unvaccinated mice) and days 3, 5, and 7 following vaccination. Error bars represent standard errors (SE) of the mean. Asterisks indicate statistically significant differences from controls by two-sided Student's t test. *, $P < 0.05$; **, $P < 0.001$; ***, $P < 0.0001$. NS, not significant (no difference between groups).

virus infection. LAIV vaccination or sublethal infection with the WT parent strain was delivered 7 days following inoculation with a common nasopharynx-colonizing strain of pneumococcus type 19F (Fig. 2A to C) included in the current pneumococcal conjugate vaccine (41). Following vaccination, normal bacterial clearance from the NP was halted, and bacteria reverted to exponential growth within 3 days postvaccination (Fig. 2B). Receipt of LAIV significantly increased the density of bacterial carriage and extended the mean duration of colonization from 35 to 57 days (Fig. 2C). Of particular importance, these effects were nearly identical in all aspects to the effects of the WT influenza virus on bacterial carriage density and duration (Fig. 2B and C). Although no detectable morbidity was associated with vaccination alone (Fig. 1B), vaccination in the presence of bacterial colonization resulted in very mild, though sustained weight loss (~3 to 5%; $P < 0.05$) relative to colonized, unvaccinated controls (see Fig. S2 in the supplemental material) that corresponded with time of greatest excess bacterial proliferation.

To test whether order and timing of vaccination relative to bacterial acquisition are important, LAIV or WT virus was administered 7 days before (rather than after) 19F colonization (Fig. 2D to F). Early vaccination or infection with WT virus led to immediate excess bacterial outgrowth following pneumococcal inoculation relative to that in mice pretreated with PBS vehicle (Fig. 2E). This increase was generally more pronounced following LAIV vaccination relative to WT virus infection, but the difference only reached statistical significance at day 1 post-bacterial infection. Increases in mean durations of carriage were also demonstrated and were similar between the two groups, with duration extending from 38 days following treatment with PBS to 63 or 65 days following LAIV or WT virus infection, respectively (Fig. 2F).

To further define the temporal nature of these interactions and simultaneously test whether this response is strain specific, vaccination was given at either 1 or 7 days prior to infection with a slightly more invasive type 7F pneumococcus (Fig. 3A). The maximum bacterial density in both groups of vaccinated mice reached a near 100-fold increase versus that in PBS controls. When inoculation with bacteria followed only 1 day (versus 7 days) postvaccination, similar but delayed dynamics (Fig. 3A) and cumulative bacterial titers (Fig. 3B) were measured. Interestingly, the delay

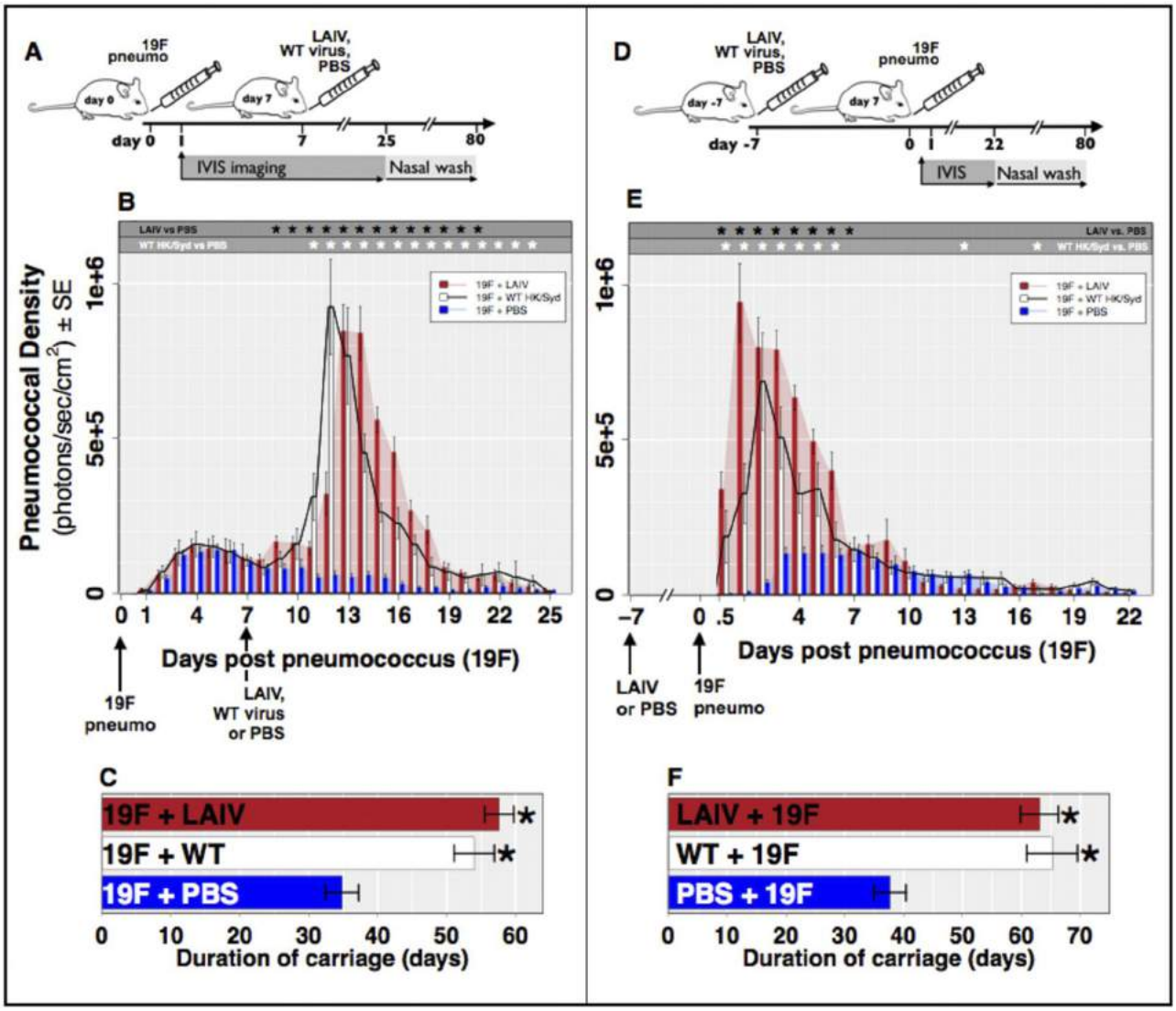

FIG 2 LAIV and WT influenza virus infection similarly enhance 19F pneumococcal carriage density and duration of colonization. Groups of 12 to 14 mice were vaccinated with LAIV and infected with WT influenza virus or PBS vehicle at 7 days following colonization with 19F pneumococcus (A to C) or 7 days prior to colonization with 19F (D to F). Bacterial strains constitutively expressed luciferase, and nasopharyngeal carriage density was measured via in vivo imaging (IVIS) at 12 h postbacterial infection and daily thereafter (B and E). Duration of colonization (C and F) was measured via bacterial plating of nasal washes taken daily after carriage density decreased below the limit of detection for IVIS imaging (~1e4 CFU/ml). Asterisks indicate significant differences between vaccinated (black asterisks in panels B and E) or WT influenza virus-infected (white asterisks in panels B and E) versus control groups ($P < 0.05$ by Students t test), and error bars represent standard errors around the mean.

was consistent with the difference in times from vaccination to bacterial inoculation between the two groups.

We sought to understand whether these effects of LAIV vaccination on bacterial proliferation would continue over a longer duration. Mice were infected with pneumococcus 28 days following LAIV vaccination—well after viral clearance from the NP was complete (~7 days postvaccination). Despite the 28-day lag between LAIV and pneumococcal infection, LAIV continued to yield immediate excess bacterial proliferation relative to PBS controls (Fig. 3C); however, the effect was modest and short-lived, with only 2- to 4-fold increases over PBS controls measured between days 1 and 3 postinfection, respectively. By day 4, bacterial density in the NP returned to control levels, and the duration of colonization was not increased.

LAIV enhances *Staphylococcus aureus* dynamics in the URT. We next sought to test the effects of LAIV on carriage of an entirely distinct but important Gram-positive bacterium, *Staphylococcus aureus*. LAIV was administered 7 days prior to infection with *S. aureus* strain Wright (Fig. 4A and B) or Newman (Fig. 4C and D). Similar to the previous experiments using two strains of pneumococcus, the density of these two strains of *S. aureus* following vaccination was increased at all measured time points for both the Wright and Newman strains (Fig. 4A and C), and duration of colonization was significantly extended 3- to 5-fold over that in the PBS controls (Fig. 4B and D).

LAIV does not increase morbidity or mortality from bacterial LRT infections. Given the severe and often lethal interaction seen between circulating influenza virus strains and bacterial

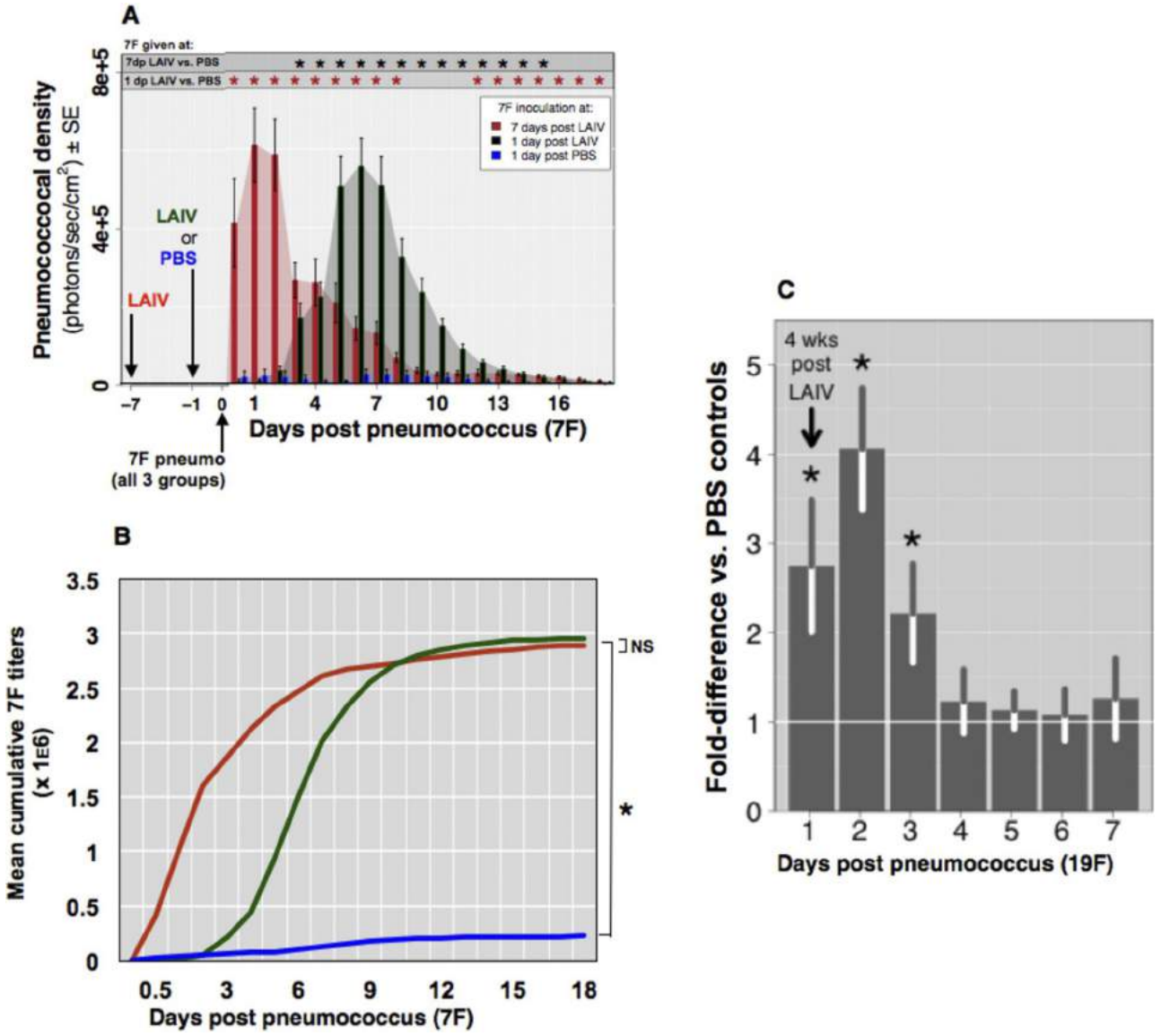

FIG 3 LAIV enhancement of pneumococcal density is time dependent and long lasting. Groups of 12 to 14 mice were vaccinated with LAIV or PBS vehicle at 1 or 7 days prior to colonization with pneumococcal (pneumo) serotype 7F. Bacterial strains constitutively expressed luciferase, and bacterial NP density was measured via IVIS *in vivo* imaging (A and B). Mean cumulative bacterial titers in panel B were calculated by first calculating the cumulative bacterial titers per individual mouse NP at each time point and then calculating the average and SE across the individual cumulative titers per time point, rather than simply averaging the areas under the mean density curves shown in panel A. Asterisks indicate significant differences in bacterial densities between the vaccinated and PBS control groups (dark green indicates LAIV given 7 days prior and red indicates LAIV given 1 day prior to 7F inoculation; $P < 0.05$ by two-tailed Student's *t* test). (C) Groups of mice were vaccinated with LAIV ($n = 20$) or PBS vehicle control ($n = 30$), respectively, at 28 days prior to colonization with 19F pneumococcus. Fold differences per day between mean bacterial densities measured in mice treated 28 days prior with LAIV versus PBS are reported. Error bars indicate standard errors of the mean and asterisks indicate significant differences ($P < 0.05$) from PBS controls (by two-tailed single-sample *t* test).

lower respiratory tract infections (LRIs) (11, 42), we assessed the effects of LAIV on bacterial LRIs and mortality and compared these effects to those seen following WT influenza virus-bacterial coinfection and single infections with bacteria. Mice received LAIV, WT influenza virus, or PBS control and 7 days later (a time known to maximize the lethal effects of influenza virus-bacterial coinfections [43]) were inoculated with a sublethal dose of either of the highly invasive type 2 or 3 pneumococcal serotypes D39 or A66.1, respectively (Fig. 5A to C).

In contrast to the 100% mortality observed when sublethal inoculation with D39 or A66.1 followed pretreatment with wild-type influenza virus, bacterial inoculation following pretreatment with LAIV demonstrated no increases in morbidity (i.e., weight loss; data not shown) or mortality (Fig. 5B and C) relative to bacterial infection alone.

DISCUSSION

The potent and often lethal effects of an antecedent influenza virus infection on secondary bacterial disease have been reported previously (11, 21, 44–46). Viral replication induced epithelial and mucosal degradation, and the ensuing innate immune response yield diminished capacity to avert secondary bacterial infections.

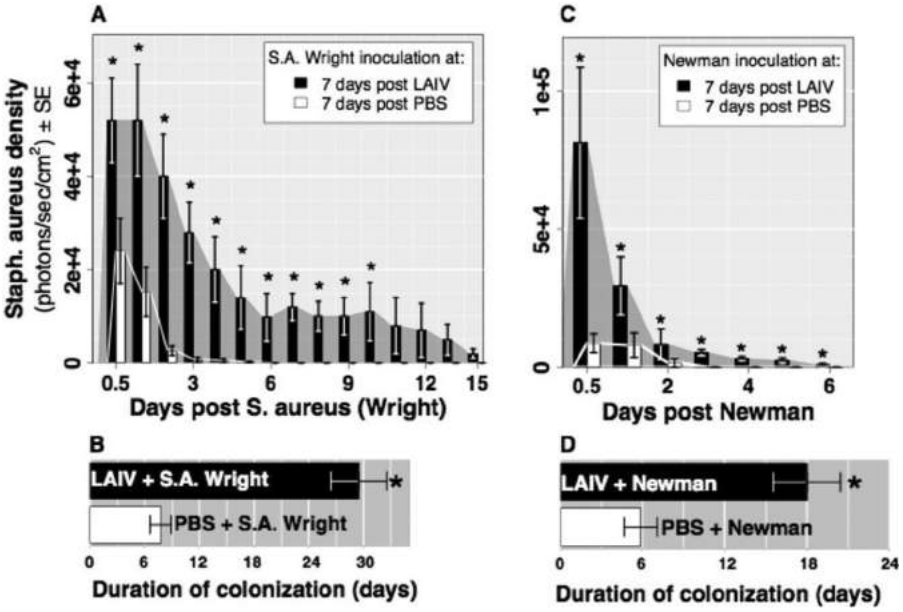

FIG 4 LAIV enhances bacterial load and duration of staphylococcal carriage. Groups of 12 to 14 mice were vaccinated with LAIV or PBS vehicle 7 days prior to colonization with *S. aureus* (S.A.) strain Wright (A and B) or Newman (C and D). *S. aureus* constitutively expressed luciferase, and bacterial density was measured via IVIS *in vivo* imaging. Duration of colonization (B and D) was measured via bacterial plating of nasal washes taken daily after the carriage density decreased below the limit of detection for IVIS imaging. Asterisks indicate significant differences between vaccinated and control groups ($P < 0.05$ by two-sided Student's *t* test), and error bars represent standard errors around the mean.

Recent clinical and experimental data suggest that influenza virus infection may exert its influence beginning in the URT by enhancing susceptibility to bacterial colonization (14, 47, 48) and increasing NP carriage density (36).

Although vaccination with LAIV, in the longer term, thwarts secondary bacterial infections by inhibiting primary infections with influenza virus (29, 49), the immediate effects of LAIV on bacterial replication and disease have never before been described. Indeed, although vaccines are among our greatest achievements in the constant battle against microbial pathogens, the effects of vaccination on distinct pathogen species unrelated to vaccine-targeted pathogens have, until now, remained entirely unexplored. LAIV viruses selectively replicate in the URT, partially denude the epithelium (50), and induce robust innate immune responses that ultimately contribute to long-term protective immunity (28). In so doing, LAIV viruses may, like WT influenza viruses, condition the site of replication for enhanced secondary bacterial colonization.

Here, we demonstrated that vaccination with LAIV, like a WT influenza virus, induces swift increases in bacterial density within the URT, with no discernible differences in effects on bacterial dynamics in the NP between the two virus strains. A lag between viral inoculation and excess bacterial replication of at least 3 to 5 days was consistently measured, no matter the bacterial strain. Of particular interest, the type I interferon, IFN-β, known to play a pivotal role in excess pneumococcal colonization following WT influenza virus infections (36), was maximally upregulated at 3 days post-LAIV vaccination, coincident with commencement of excess bacterial proliferation. After the 3- to 5-day threshold following vaccination was met, the murine NP remained conditioned for excess pneumococcal replication for at least 28 days (our furthest time point out) post-vaccination. However, as the delay between vaccination and bacterial infection was increased, the magnitude of the effects of vaccination on bacterial dynamics became considerably more modest, although statistically significant excess growth was measured even when acquisition followed 28 days post-vaccination.

While the studies described here are limited in scope to murine models, enhanced bacterial load in the URT following LAIV may agree with human data (51), where LAIV has been associated with increases in adverse upper respiratory tract symptoms. Although adverse URT symptoms following administration of FluMist are considered to be of viral etiology, they are most evident in children <5 years of age, where rates of bacterial carriage are greatest (52). Potentially corroborating this are data from a large prospective double-blind trial of FluMist (trial no. MI-CP111 [53]) that assessed reactogenicity and adverse URT events within the first 28 days following vaccination in ~3,000 children between the ages of 6 and 59 months. This trial demonstrated a bimodal increase in URT symptoms following FluMist vaccination, the first between days 2 and 4 post-vaccination and the second between days 5 and 10 post-vaccination (53). While these increased URT events (relative to controls receiving trivalent inactivated influenza vaccine) were considered normal reactions to the live vaccine, the bimodal nature of the increased symptoms suggests that two distinct mechanisms may be in place. In the context of the current findings, the first peak may correspond with viral replication, while the second, more sustained peak may, at least in part, be driven by symptoms due to excess bacterial carriage.

Perhaps the most important finding from our study, with regard to the health of the public and potential concerns regarding vaccination, is that LAIV did not enhance lower respiratory tract infections, morbidity, or mortality following bacterial infections, which are, by most accounts, the most significant issues to be

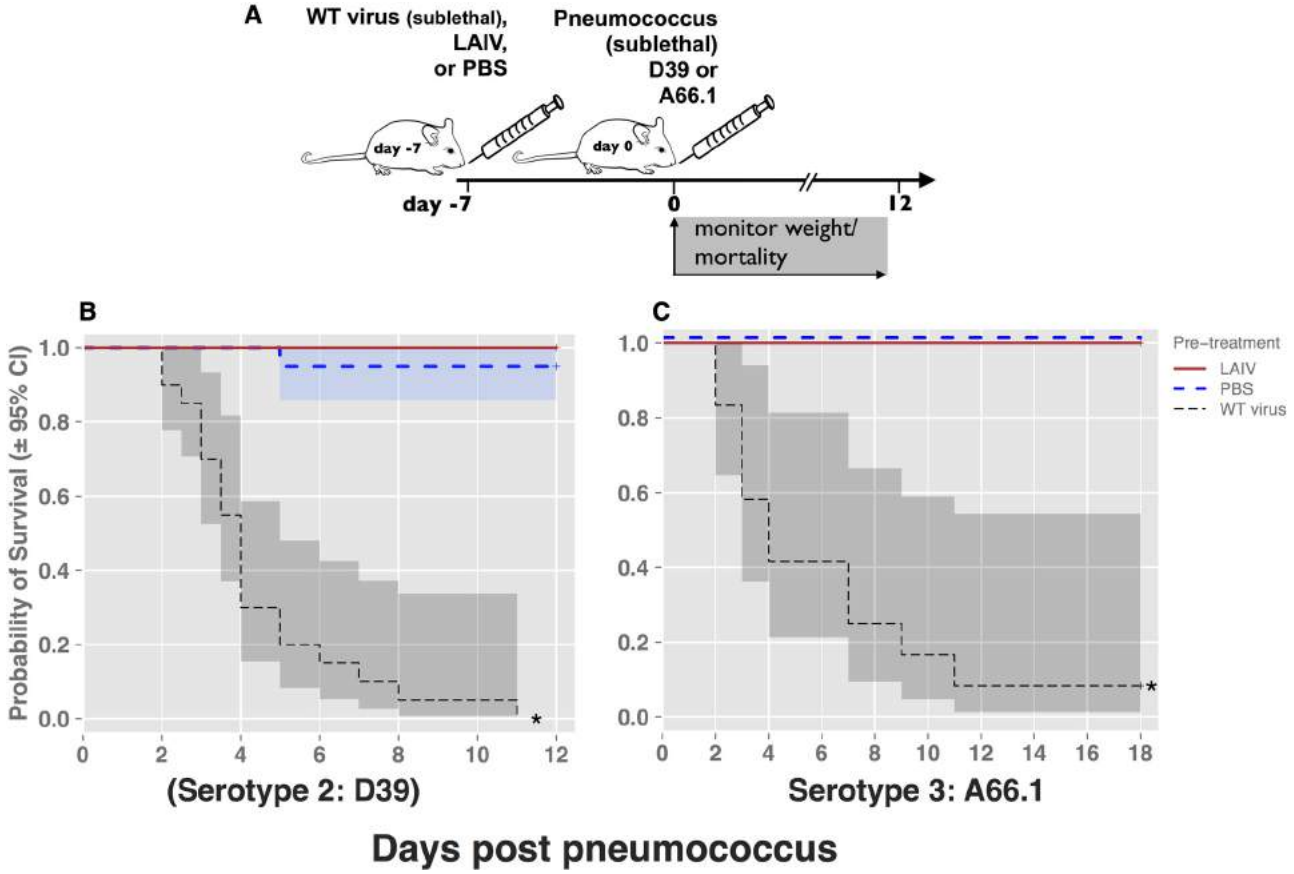

FIG 5 LAIV does not increase severe bacterial disease or mortality. Groups of mice received intranasal LAIV vaccination (solid red curves), sublethal infection with WT influenza virus (broken black curves), or PBS (broken blue curves) 7 days prior to inoculation with a sublethal dose of *Streptococcus pneumoniae* type 2 (1e5 CFU D39; $n = 20$ per group) (B) or type 3 (1e3 CFU A66.1; $n = 12$ to 15 per group) (C), and body weight and mortality were observed at least every 12 h for the first 4 days postpneumococcal inoculation and daily thereafter. Kaplan-Meier survival curves with 95% confidence intervals (CI) were constructed, and asterisks indicate statistically significant differences ($P < 0.05$ by log rank test) between LAIV- or WT virus-infected groups versus PBS controls.

concerned with in terms of respiratory tract bacterial disease. Indeed, this finding is consistent with numerous epidemiological reports all failing to detect any serious adverse sequelae of LAIV vaccination in humans (51, 54). Furthermore, this finding is consistent with significantly diminished LAIV virus replication within the lower respiratory tract, suggesting that viral replication is a requirement for the synergistic response seen between WT influenza viruses and bacterial LRT infections.

While care should be taken to not overgeneralize the data described here to all vaccines, the broad implications suggest that live attenuated viral vaccines may have unintended consequences on important human bacterial pathogens unrelated to the vaccine target species. Furthermore, our findings suggest a role for laboratory models of multispecies interactions with vaccine strains to inform future vaccine monitoring and evaluation programs aimed at identifying thus far entirely unrealized "unconventional" effects, both beneficial and detrimental, of live attenuated viral vaccines and cross-species microbial dynamics.

MATERIALS AND METHODS

Infectious agents and vaccines. Viral infections were carried out with an H3N2 1:1:6 reassortant virus developed as described previously (30), containing the surface glycoproteins hemagglutinin (HA) and neuraminidase (NA) from A/Hong Kong/1/68 (HK68) and A/Sydney/5/97 (Syd97) isolates, respectively, and the six internal protein gene segments from A/Puerto Rico/8/34 (or PR8; referred to here as WT influenza virus). LAIV vaccinations consisted of a temperature-sensitive (*ts*) attenuated variant of HK/Syd, HK/Syd$_{att/ts}$ (LAIV) that contains site-specific mutations in the PB1 and PB2 RNA segments of the genome (see Fig. S1 in the supplemental material) as described previously (30). These are the same mutations found in the attenuated A/Ann Arbor/6/60 master donor strain used to produce the influenza A virus strains found in the commercial product FluMist (30). WT and LAIV viruses were propagated in 10-day-old embryonated chicken eggs at 37 and 33°C, respectively) and characterized in Madin-Darby canine kidney cells to determine the 50% infective tissue culture dose (TCID$_{50}$) in wells. The pneumococcal carrier isolates ST425 (serotype 19F) and ST191 (serotype 7F), chosen based on their colonizing potential as previously described (14), were used for colonization experiments. The highly invasive type 2 and type 3 pneumococcal isolates D39 and A66.1, respectively, were used for pneumonia and survival studies. The 19F and 7F strains were engineered to express luciferase, as described previously (14). *Staphylococcus aureus* strains Wright (ATCC 49525) and Newman (ATCC 25905) were engineered to express luciferase by Caliper Life Sciences (Alameda, CA).

Ethics statement. All experimental procedures were approved by the Institutional Animal Care and Use Committee (protocol no. 353) at St. Jude Children's Research Hospital (SJCRH) under relevant institutional

and American Veterinary Medical Association guidelines and were performed in a biosafety level 2 facility that is accredited by the American Association for Laboratory Animal Science (AALAS).

Animal and infection models. Eight-week-old BALB/c mice (Jackson Laboratories, Bar Harbor, ME) were used in all experiments, with the exception of mice treated with early vaccination to demonstrate vaccine efficacy and effectiveness. In these cases, 4-week-old BALB/c mice were vaccinated or administered PBS and monitored for 4 weeks before further inoculation. All inoculations and vaccinations were via the intranasal route under general anesthesia with inhaled 2.5% isoflurane (Baxter Healthcare, Deerfield, IL). LAIV vaccination consisted of 2e6 $TCID_{50}$ HK/$Syd_{att/ts}$ LAIV in 40 μl PBS. The lethal and sublethal doses of WT HK/Syd were 5e7 and 1e5 $TCID_{50}$ in 50 μl PBS, respectively. Pneumococcal infections with 19F and 7F were performed as described previously (14), except inoculation was in 40 μl PBS. Infection with *S. aureus* strains Wright and Newman contained 1e7 CFU in 40 μl PBS. Mortality studies were performed as described previously (43) with sublethal doses of the invasive type 2 and type 3 pneumococcal serotype D39 and A66.1 isolates, consisting of 1e5 and 1e3 CFU in 100 μl PBS (to ensure bacterial entry into the lower lungs), respectively. Animals were monitored for body weight and mortality at least once per day for all survival studies. Mice were sacrificed if body weight fell below 70% initial weight.

Bacterial CFU titers for duration studies. Bacterial CFU titers were measured in nasal washes using 12 μl of PBS administered and retrieved from each nare and quantitated by serial dilution plating on blood agar plates. Washes were performed daily only after the pneumococcal density fell below the limit of detection for IVIS imaging (~1e4 CFU/ml).

Determination of bacterial and viral titers in lungs and nasopharyngeal homogenates. Viral and bacterial titers were measured in whole lung and nasopharyngeal (NP) homogenates. Whole lungs were harvested and homogenized using a gentleMACS system (Miltenyi Biotech), as per the manufacturer's protocol. NP was isolated via careful dissection dorsally across the frontal bones, laterally via removal of the zygomatic bone, posteriorly by dislocation of the upper jaw from the mandible, and inferiorly just posterior to the soft palate. Isolated NP was homogenized via plunging in 1.5 ml PBS through a 40-μm-mesh strainer. Bacterial titers were measured via plating of serial dilutions, and viral titers were measured by determining the $TCID_{50}$ as previously described (30).

Determination of cytokine levels in the NP and BAL specimens by enzyme-linked immunosorbent assay. Nasopharyngeal isolates and BAL specimens were collected as described above, and cytokines were measured using commercially available kits from R&D systems (macrophage inflammatory protein 1β [MIP-1β], transforming growth factor β [TGF-β], and beta interferon [IFN-β]) or eBiosciences (interleukin-4 [IL-4], IL-6, IL-10, IL-17, IL-23, and gamma interferon [IFN-γ]).

Bioluminescent imaging. Mice were imaged using an IVIS charge-coupled device (CCD) camera (Xenogen) as described previously (14, 29). Nasopharyngeal bacterial density was measured as total photons/s/cm^2 in prespecified regions covering the NP, and background (calculated for each mouse on a region of equal area over the hind limb) was subtracted. Each NP measurement represents an average of two pictures, one for each side of the mouse head. Quantitation was performed using LivingImage software (version 3.0; Caliper Life Sciences) as described previously (14).

Statistical analyses. All statistical analyses were performed within the R statistical computing environment (version 2.14R; R Foundation for Statistical Computing, R Development Core Team, Vienna, Austria). The specific statistical tests used are as indicated in the legend to each figure. The R package Survival was used for all survival analyses, Kaplan-Meier (KM) plots, and KM log rank tests. All other statistical tests were performed using R base functions.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01040-13/-/DCSupplemental.

Figure S1, TIFF file, 0.2 MB.

Figure S2, TIFF file, 0.3 MB.

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MAJOR ARTICLE

Live Attenuated Influenza Virus Increases Pneumococcal Translocation and Persistence Within the Middle Ear

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Background. Infection with influenza A virus (IAV) increases susceptibility to respiratory bacterial infections, resulting in increased bacterial carriage and complications such acute otitis media, pneumonia, bacteremia, and meningitis. Recently, vaccination with live attenuated influenza virus (LAIV) was reported to enhance subclinical bacterial colonization within the nasopharynx, similar to IAV. Although LAIV does not predispose to bacterial pneumonia, whether it may alter bacterial transmigration toward the middle ear, where it could have clinically relevant implications, has not been investigated.

Methods. BALB/c mice received LAIV or phosphate-buffered saline 1 or 7 days before or during pneumococcal colonization with either of 2 clinical isolates, 19F or 7F. Middle ear bacterial titers were monitored daily via in vivo imaging.

Results. LAIV increased bacterial transmigration to and persistence within the middle ear. When colonization followed LAIV inoculation, a minimum LAIV incubation period of 4 days was required before bacterial transmigration commenced.

Conclusions. While LAIV vaccination is safe and effective at reducing IAV and coinfection with influenza virus and bacteria, LAIV may increase bacterial transmigration to the middle ear and could thus increase the risk of clinically relevant acute otitis media. These data warrant further investigations into interactions between live attenuated viruses and naturally colonizing bacterial pathogens.

Keywords. live attenuated influenza virus; middle ear bacterial colonization; bacterial transmigration; acute otitis media; pneumococcus; coinfection.

Infection with influenza A virus (IAV) increases susceptibility to severe lower and upper respiratory tract (URT) bacterial infections, resulting in complications such as pneumonia, bacteremia, sinusitis, and bacterial acute otitis media (AOM) [1]; the latter is a major contributor to the global burden of pediatric disease and remains one of the most common diagnoses leading to the prescription of antimicrobial agents in the United States [2]. While bacterial AOM often occurs in isolation, increasing evidence suggests that primary or concurrent viral respiratory infections of the URT may play uniquely important roles in enhancing bacterial acquisition, colonization, and, ultimately, progression from asymptomatic bacterial carriage to AOM [3], notably from *Streptococcus pneumoniae* and *Staphylococcus aureus* [1, 4].

Although the mechanisms underlying influenza virus–mediated susceptibility to bacterial AOM are not entirely defined, they likely include a combination of IAV-mediated cytotoxic breakdown of mucosal and epithelial barriers of the URT [5–8] and aberrant innate immune responses to bacterial invaders in the immediate postinfluenza state, characterized by uncontrolled proinflammatory and antiinflammatory cytokine production, excessive leukocyte recruitment, and immunopathology [1, 9–13]. When coupled with diminished mucosal defenses, such an environment becomes increasingly hospitable for bacterial pathogens to flourish

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and cause clinical disease in the days and weeks following influenza virus infection.

Increasing evidence links the early innate immune response triggered by vaccination to long-term vaccine efficacy [14]. Thus, a goal of vaccination is to elicit an immune response as close to that elicited by the pathogen itself, without subsequent disease. The intranasally administered live attenuated influenza vaccine (LAIV) is composed of 1:1:6 reassortant viruses containing the hemagglutinin (HA) and neuraminidase (NA) surface proteins from wild-type viruses on a temperature-sensitive and attenuated backbone designed to enable efficient viral replication in the cooler temperatures of the URT but not the warmer temperatures of the lower respiratory tract (LRT) [15, 16]. Through selective replication in the URT, LAIV proteins are exposed to the host immune system in their native conformation, eliciting highly robust immunoglobulin A (IgA), serum immunoglobulin G (IgG), and cellular immune responses mimicking those of the pathogenic virus itself [17], without subsequent virus-mediated disease in the LRT [18, 19].

Recently, we demonstrated that LAIV, while safely providing long-term immunity against influenza and significantly reducing postinfluenza secondary bacterial infections [20], inadvertently enhances the duration and density of bacterial carriage in the nasopharynx of mice [21], a finding that has since been shown in humans [22]. Importantly, in contrast to wild-type IAV infections, LAIV did not alter bacterial outgrowth in the LRT and demonstrated no increases in the incidences of bacterial pneumonia or bacteremia. What is not known is whether LAIV virus replication in the URT may, like that of the wild-type IAV, inadvertently catalyze bacterial migration from the nasopharynx, where it is largely asymptomatic, into the middle ear, where it can increase the risk of symptomatic AOM [13, 23, 24]. Such an effect of the attenuated virus could result from LAIV-mediated inflammation of the epithelial cells of the pharyngotympanic tube [13] or from elevated bacterial density within the nasopharynx [25].

MATERIALS AND METHODS

Vaccinations and Infectious Agents

LAIV viruses were developed from a parent H3N2 1:1:6 reassortant virus developed as described previously [26]. The surface glycoproteins HA and NA were from the A/Hong Kong/1/68 (HK68) and A/Sydney/5/97 (Syd97) isolates, respectively, and the 6 internal protein gene segments were from A/Puerto Rico/8/34 or PR8 (referred to hereafter as WT virus). LAIV consisted of a temperature-sensitive (*ts*) attenuated variant of the WT virus (HK/Syd_{ts}) that contains site-specific mutations in the PB1 and PB2 RNA segments of the genome as described previously [26, 27]. These mutations are found in the attenuated A/Ann Arbor/6/60 master donor strain used to produce the commercial product known as FluMist (MedImmune, Gaithersburg, Maryland) [16]. LAIV viruses were propagated in 10-day-old embryonated chicken eggs at 33°C and quantitated in Madin–Darby canine kidney cells using the median tissue culture infective dose ($TCID_{50}$). In vitro and in vivo growth dynamics have been reported elsewhere [21]. The pneumococcal carrier isolates ST425 (serotype 19F) and ST191 (serotype 7F) have been previously described [3]. These strains were engineered to express luciferase, as described elsewhere [3, 28].

Animal and Infection Models

Eight-week-old BALB/c mice (Jackson Laboratories, Bar Harbor, Maine) were used in all experiments. All inoculations were via the intranasal route. LAIV consisted of 2×10^6 $TCID_{50}$ HK/Syd_{ts} virus in 40 μL of phosphate-buffered saline (PBS). Pneumococcal infections with 19F and 7F *Streptococcus pneumoniae* were as described previously [3]. Briefly, bacterial cultures were grown in Todd–Hewitt broth (Difco Laboratories, Detroit, Michigan) containing 0.5% yeast (THY) until mid- to late-log phase (OD, approximately 0.3,) and aliquots were stored at −80°C in 10% glycerol and quantified via serial dilution on blood agar plates. Inoculations were prepared from frozen aliquots and consisted of 1×10^6 and 1×10^5 colony-forming units of serotype 19F and 7F pneumococci, respectively, in 25 μL of PBS. Infections were initialized via careful administration of 12.5 μL of bacteria to each naris under general anesthesia with 2.5% inhaled isoflurane (Baxter Healthcare, Deerfield, Illinois). All experiments were conducted in biosafety level 2 facilities in a manner in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals.

Bioluminescent Imaging

Mice were imaged using an IVIS CCD camera (Xenogen) as described elsewhere [3]. Middle ear bacterial density was measured as total photons sec^{-1} cm^{-2} in prespecified regions covering the middle ear canal, and background (calculated for each mouse on a region of equal area over the hind limb) was subtracted. Positivity for bacteria within the middle ear was defined as a value of >40 000 photons sec^{-1} cm^{-2}. This threshold has been previously described for this infection model, using the same instruments and laboratory environment [29]. Quantitation was performed using Living Image software (v. 3.0; Caliper Life Sciences) as described previously [3].

Although bioluminescent imaging of *lux*-expressing bacteria has previously been shown to be an efficient and accurate method for measuring bacterial density in the nasopharynx and lungs of mice and ferrets in vivo [3, 21, 29, 30], to ensure imaging was also appropriate for measuring bacterial presence and density within the middle ear, we compared values obtained from imaging to bacterial titers obtained by traditional methods. In short, the middle ear was dissected and completely homogenized in 1 mL of PBS, and serial dilutions were plated on 5% blood agar plates for quantification. Bacterial counts

obtained from serial dilution plating were plotted against values obtained via IVIS just before dissection and showed strong linear correlation ($R^2 = 0.92$; Supplementary Figure 1).

A single episode of bacterial middle ear colonization (MEC) was defined as any continuous detection of bacteria that was not interrupted by an interval of >2 days. This 2-day interval was important to account for normal fluctuations in bacterial density, whereby densities can temporarily fall below the threshold of detection (described above) without actually being cleared and then return to high levels. Additionally, episodes were categorized as early or late onset. Early onset was defined as an initial episode of MEC in a given mouse that occurred within 5 days of bacterial inoculation. Late onset was defined as any episode that commenced at least 2.5 days after clearance of a previous episode or at least 5 days after pneumococcal infection.

Statistical Analyses

All statistical analyses were performed within the R statistical computing environment (R, version 2.14; R Foundation for Statistical Computing, R Development Core Team, Vienna, Austria). Kaplan–Meier curves were constructed for freedom from MEC for each mouse per group, and the log-rank test was used to calculate statistically significant differences between groups. The frequency of MEC was plotted using Loess smoothing (span 0.2), and differences between daily frequencies in the vaccinated groups and those in the PBS controls were calculated using the Fisher exact test for differences in proportions. Differences in mean duration of MEC were calculated using 2-tailed 2-sample Students *t* tests. The false detection rate was used to adjust for multiple comparisons where appropriate, and statistical significance was considered when the calculated probability had an α level of <0.05.

RESULTS

LAIV Increases the Incidence of MEC in Mice Colonized With Pneumococci Before LAIV Receipt

Nasopharyngeal carriage of pneumococcus is believed to be a prerequisite for MEC and subsequent pneumococcal AOM, and elevated bacterial density has been associated with transition from asymptomatic carriage to middle ear infections [25]. To determine whether LAIV vaccination of pneumococci-colonized mice may enhance bacterial transmigration to the middle ear, groups of 12–14 mice were colonized with serotype 19F pneumococcus (a clinical isolate often found colonizing the nasopharynx of children and a well-established model organism for colonization and AOM in mice [3]) 7 days before LAIV or PBS inoculation. A delay of 7 days was used because this was shown to be a sufficient interval over which bacteria reached stable colonization, as assessed via IVIS imaging of the nasopharynx and as previously reported [31]. Within 12 hours after LAIV inoculation, mice demonstrated an increased incidence of MEC (Figure 1), as determined by in vivo imaging of the middle ear (see "Materials and Methods" section). By day 4 after LAIV receipt, 85% of mice had at least 1 episode of MEC, compared with only 25% of PBS controls. In the majority of cases, initial onset of MEC in the LAIV group occurred within the first 4 days following vaccination, and freedom from MEC stabilized in both groups after day 5 (with the exception of a single new case in the PBS group, which was detected on day 9). By day 10 following LAIV or PBS inoculation, the incidence of MEC in LAIV recipients remained significantly greater than that in PBS controls (85% in LAIV recipients vs 50% in PBS controls; $P = .017$).

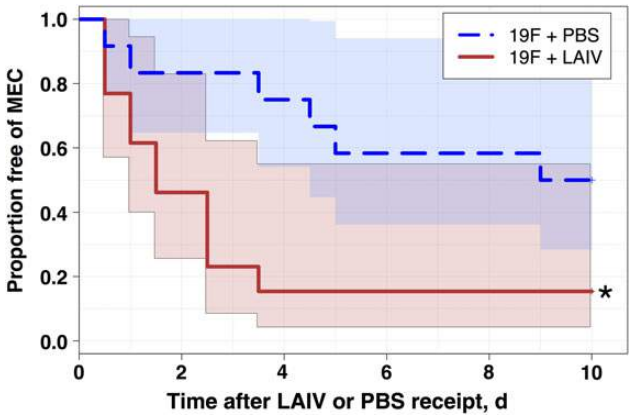

Figure 1. Live attenuated influenza virus (LAIV) enhances the incidence of bacterial middle ear colonization (MEC) in precolonized mice. Groups of 12–14 8-week-old BALB/c mice were colonized intranasally with serotype 19F pneumococcal bacteria engineered to express luciferase. Seven days later, mice were inoculated with LAIV or phosphate-buffered saline (PBS) vehicle as a control. MEC was measured via in vivo imaging of the middle ear at 12-hour intervals for the first 2 days following LAIV or PBS receipt and daily thereafter. Initial onset of bacterial MEC was recorded for each mouse, and Kaplan–Meier survival curves were constructed. Data are reported as freedom from MEC after LAIV or PBS inoculation, and the log-rank test was used to determine statistically significant differences between groups. *$P < .05$, compared with PBS controls.

Antecedent Receipt of LAIV Predisposes to Bacterial Transmigration

To address whether antecedent inoculation with LAIV predisposes to MEC after bacterial infection, and to ensure that the effect of LAIV on bacterial transmigration is not specific to serotype 19F pneumococci, mice received a colonizing dose of pneumococcal serotype 7F (a slightly more invasive clinical strain and a well-described model organism for pneumococcal AOM in mice [3]) at either 7 days or 1 day after LAIV receipt (n = 26 for each group) or 1 day after PBS receipt (n = 20; Figure 2). Inoculation with LAIV 7 days before pneumococcal infection led to immediate increases in the incidence of MEC, with only 30% (8 mice) remaining free from bacterial MEC

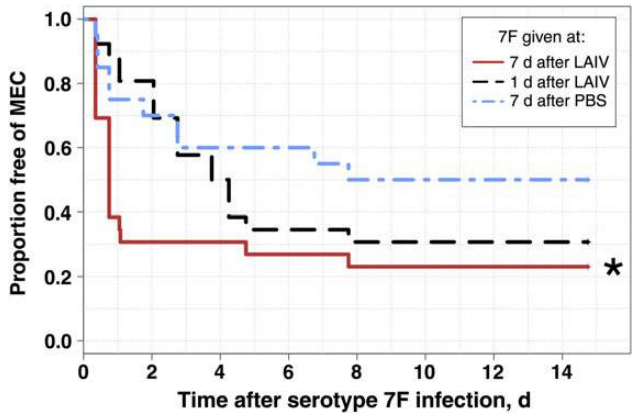

Figure 2. Freedom from middle ear colonization (MEC) following bacterial infection in recently vaccinated mice. Groups of 8-week-old BALB/c mice received live attenuated influenza virus (LAIV) 7 days (n = 26), LAIV 1 day (n = 26), or phosphate-buffered saline (PBS) 1 day (n = 20) before inoculation with serotype 7F pneumococcal bacteria engineered to express luciferase. In vivo imaging was used to detect bacterial MEC every 12–15 hours for the first 2 days following pneumococcal infection and at least daily thereafter. Initial onset of pneumococcal MEC was recorded for each mouse, and Kaplan–Meier survival curves were constructed to describe freedom from pneumococcal MEC. *$P < .05$, by the log-rank test, compared with PBS controls, corrected for multiple comparisons using the false-discovery rate.

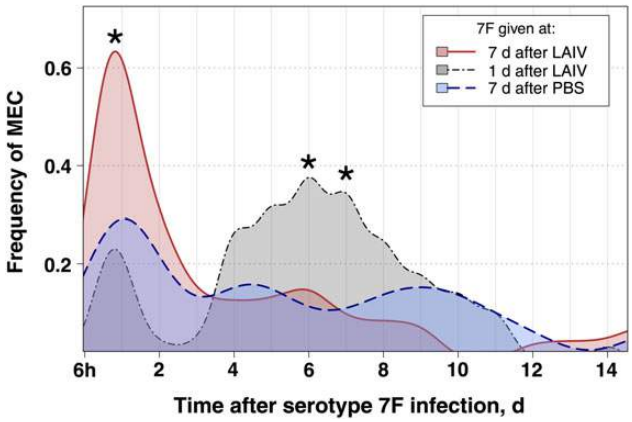

Figure 3. Frequency of middle ear colonization (MEC) following bacterial infection in recently vaccinated mice. Mice were inoculated with live attenuated influenza virus (LAIV) 7 days (n = 26), LAIV 1 day (n = 26), or phosphate-buffered saline (PBS) 1 day (n = 20) before infection with serotype 7F pneumococcus engineered to express luciferase, and in vivo imaging of the middle ear was performed to measure the presence of pneumococcal MEC. The frequency of MEC is plotted for each group, and differences in the daily frequency between groups were tested for statistical significance using the Fisher exact test for differences in proportions. *$P < .05$, compared with the PBS group.

24 hours after infection; compared with 81% (21 mice) infected 1 day after LAIV receipt and 75% (15 mice) infected 1 day after PBS receipt. Following initial enhancement of MEC in mice infected 7 days after LAIV receipt, only 2 new cases (ie, cases in mice previously free from MEC) were seen over the following 2 weeks, at days 5 and 8 after bacterial infection.

An increased incidence of MEC was also detected in the group infected 1 day after LAIV receipt, but onset was distributed in this group, commencing between days 3 and 5 after infection, which corresponded to days 4–6 after LAIV receipt, a time previously demonstrated to maximize bacterial colonization of the nasopharynx [21].

LAIV-Mediated Enhancement of Bacterial Transmigration Is Delayed After Vaccination

To better understand the dynamics of bacterial transmigration and MEC, we investigated the overall frequency per day of MEC for each group (Figure 3), which differs from our Kaplan–Meier analysis above in that the Kaplan–Meier analysis considers only time of first onset in a given mouse, rather than overall proportion with MEC at any particular time in our experimental groups. Consistent with the Kaplan–Meier analysis, mice vaccinated 7 days before pneumococcal infection had significantly increased frequencies of MEC for the first 24–48 hours after infection, compared with PBS controls. The frequency peaked in this group approximately 24 hours after infection, with slightly >60% (16 mice) with MEC. In contrast, only 20–30% of mice receiving LAIV or PBS 1 day before bacterial infection had evidence of MEC, and these episodes were very short lived, with almost no MEC in these groups by day 2. While the maximum frequency of MEC was reached 24 hours after infection in the group infected 7 days after LAIV receipt, mice infected only 1 day following LAIV receipt had a second wave of MEC episodes that began 4 days after LAIV receipt (Figure 3). This second wave of MEC, while lower in maximum frequency (approximately 40%) than in the group infected 7 days after LAIV receipt, had a broader and more sustained peak that lasted from day 4 to day 8 after bacterial infection.

LAIV Increases the Persistence of MEC

The duration of MEC was measured for each episode per mouse, as defined above, and mean durations were calculated for each group. The duration was significantly increased across all vaccinated groups, regardless of pneumococcal strain (ie, serotype 19F or 7F) or whether LAIV was given before or following pneumococcal infection. When LAIV or PBS was administered to mice with preestablished serotype 19F colonization, bacteria persisted in the middle ears nearly 2-fold longer than in PBS controls (2.3 vs 1.2 days; $P < .05$; Figure 4*A*). Similarly, when mice received LAIV 7 days or 1 day before bacterial infection, the mean durations of MEC episodes were 3-fold and 2-fold greater, respectively, than those for PBS controls ($P < .05$ for each comparison; Figure 4*B*). Interestingly, when episodes were classified into early and late onset (see "Materials and Methods" section for classification criteria), durations of early onset cases in the group infected 7 days after

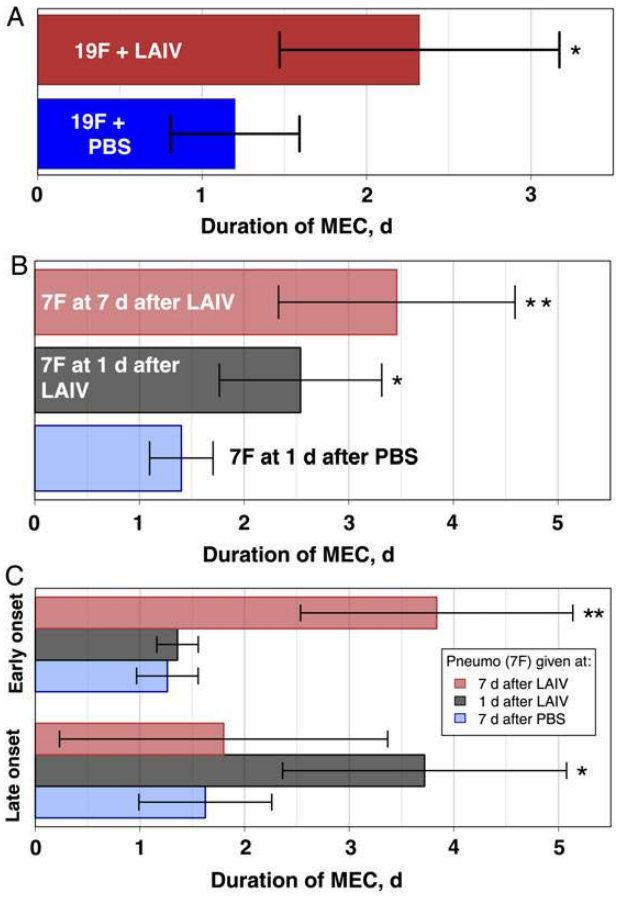

Figure 4. Live attenuated influenza virus (LAIV) enhances persistence of middle ear colonization (MEC). *A* and *B*, Groups of mice were colonized with serotype 19F pneumococcus 7 days before inoculation with LAIV (n = 14) or phosphate-buffered saline (PBS; n = 12; *A*) or received LAIV 7 days (n = 26), LAIV 1 day (n = 26), or PBS 1 day (n = 20) before infection with serotype 7F pneumococcus (*B*). The durations of MEC episodes were measured, and mean durations reported for serotype 19F (*A*) and serotype 7F (*B*) MEC, in which a single episode was defined as any continuous detection in a given mouse that was not interrupted by >2 days. *C*, Episodes of serotype 7F MEC were further classified as early onset (onset within the first 5 days following infection) or late onset (>2 days following termination of an early episode or >5 days after infection), and mean durations reported for each group. Statistically significant differences (vs PBS controls) were tested using 2-tailed 2-sample Student *t* tests with correction for multiple comparisons, using the false-discovery rate. Error bars represent 95% confidence intervals around the mean. **P* < .05, ***P* < .001.

LAIV receipt were almost identical to durations of late-onset cases in the group infected 1 day after LAIV receipt (approximately 3.75 days in each group) and, in each case, the duration was >2-fold greater than that for their respective PBS controls (approximately 1.5 days; $P < .05$; Figure 4*C*). Alternatively, the duration of early onset episodes in the group infected 1 day after LAIV receipt and the duration of late-onset episodes in the group infected 7 days after LAIV receipt were no different than for PBS controls. Taken together with the findings of Kaplan–Meier analyses described above, these data demonstrate a strong influence of time since LAIV inoculation, rather than time since bacterial infection, with a minimum of 4 days after vaccination required before enhanced bacterial transmigration to and colonization of the middle ear is detected.

DISCUSSION

The potent and often lethal effects of a previous influenza virus infection on secondary pneumococcal invasive disease and pneumonia have been reported [1, 11, 32–34]. Viral replication induced epithelial and mucosal degradation, and the ensuing innate immune response yield diminished capacity to avert secondary bacterial infections. Recent clinical and experimental data suggest that influenza viruses may exert their influence, beginning in the URT, by enhancing susceptibility to bacterial colonization [3, 30, 35], increasing nasopharyngeal carriage density [23], and enhancing the incidence of AOM [13].

Although LAIV, in the longer-term, thwarts influenza virus and bacterial coinfections by inhibiting the viral infection [18, 31], LAIV vaccines have recently been found to enhance the density and duration of bacterial colonization within the nasopharynx of mice, and evidence has also been put forth for humans [21, 22, 36]. Importantly, unlike WT IAV, LAIV did not result in increased bacterial proliferation or disease in the LRT, presumably because of the temperature-sensitive nature of LAIV viruses, abrogating viral growth within the warmer temperatures of the lungs. Although LAIV did not effect clinical bacterial LRT infections, the effects of LAIV on transition from colonization to bacterial disease within the URT, a region where LAIV replicates efficiently, had not been studied.

Here, we found that vaccination with a mouse-adapted LAIV significantly increased bacterial transmigration to the middle ear and the duration of MEC, irrespective of bacterial serotype or order of viral versus bacterial inoculation. Interestingly, a minimum period of approximately 4 days was required before enhancement in pneumococcal transmigration and MEC was noted, when LAIV preceded pneumococcal infection.

The dynamics of increased MEC, with regard to time since vaccination, closely match increased pneumococcal colonizing dynamics of the nasopharynx following WT IAV or LAIV virus [21, 31] and support the notion that nasopharyngeal colonizing density may be associated with progression to AOM. Interestingly, the delay in increased onset of migration and MEC in mice vaccinated only 1 day before bacterial inoculation was approximately the same as the time to peak LAIV viral titers in the URT [21]. Thus, a majority of excess MEC occurs during or soon after viral clearance from the URT. This finding supports numerous reports [1, 10–12, 23] that point toward a complex coupling of poorly coordinated antibacterial innate immune defenses and epithelial damage following influenza

virus infection, underlying the excess susceptibility to bacterial disease after influenza virus infection.

On the other hand, the steady increase in onset of MEC measured immediately following LAIV vaccination in serotype 19F–precolonized mice suggests that introduction of LAIV virus in the presence of existing bacterial colonization yields enhanced MEC that is concurrent with viral replication and precedes viral-mediated enhanced nasopharyngeal colonization, which tend to increase beginning on day 4 after LAIV inoculation. This suggests that the mechanisms of virus-induced bacterial AOM may differ according to order of inoculation. Indeed, it may be that even low levels of viral replication in the URT, while not immediately affecting overall bacterial carriage density in the nasopharynx, may rapidly disrupt a delicate balance that naturally exists to prevent asymptomatic carriage from transitioning to bacterial AOM.

It must be clearly emphasized here that any animal study, particularly mouse studies [37], must be viewed in light of the many caveats that exist when extrapolating findings from animal studies to humans. Although animal studies have been integral to our understanding of infectious diseases (and many other biological systems), the individual processes and dynamics often differ between the animal model—mice, in this case—and the human system, as has been shown [37].

While our data suggest that LAIV may enhance pneumococcal transmigration into the middle ear, it is clear that the overall effect of LAIV measured in humans has been that of significant reductions in viral influenza infections and otitis media [38]. While our data suggest a potential effect of LAIV to increase bacterial transmigration to the middle ear, a lack of detection in numerous large clinical trials in humans suggests that any effect is largely subclinical. As well, LAIV-mediated protection from primary influenza virus infections significantly reduces the opportunity for worse secondary bacterial infections [20], further reducing the incidence of LRT and URT bacterial disease, including bacterial AOM.

While we are confident that the overall effects of LAIVs are beneficial to reduce all-cause AOM across populations, as has been reported [39], our data here and previous reports [21] suggest a need for future investigations to more closely evaluate the effects of LAIV on bacterial respiratory pathogen dynamics, including unintended beneficial effects [20]. Indeed, as medicine becomes increasingly personalized [40], it may become possible to tailor classes of vaccines and avenues of vaccine delivery to the individual. In this particular example, considering the benefits of LAIV over inactivated injectable influenza vaccines [41], one could envision that the choice between a killed injectable vaccine and an intranasal LAIV might incorporate the risk of pneumococcal carriage or acquisition (based on factors such as the number of children in the household, the age of the vaccine recipient, and proximity to immunocompromised individuals) as a potential variable in the decision-making process.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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Effect of pneumococcal conjugate vaccine on nasopharyngeal bacterial colonization during acute otitis media

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Abstract

The heptavalent pneumococcal conjugate vaccine (PCV7) has been shown to reduce the incidence of acute otitis media (AOM) caused by Streptococcus pneumoniae by 34% and reduces the overall incidence of AOM by 6% to 8%. More recent studies have shown increases in the proportion of Haemophilus influenzae and Moraxella catarrhalis in the middle-ear fluid of PCV7-immunized children. There has been no report on the effect of PCV7 on all 3 bacterial pathogens combined, either in the middle-ear fluid or nasopharynx of individual children with AOM. We investigated the impact of PCV7 on nasopharyngeal colonization with bacterial pathogens during AOM in the pre-PCV7 and post-PCV7 vaccination eras. Four hundred seventeen children (6 months to 4 years of age) were enrolled onto AOM studies between September 1995 and December 2004. Of these, 200 were enrolled before the vaccine use (historical controls), and 217 were enrolled after the initiation of PCV7 vaccination (101 were underimmunized, and 116 were immunized). Although the nasopharyngeal colonization rate for S pneumoniae was not different between the 3 groups, a significantly higher proportion of PCV7-immunized children with AOM were colonized with M catarrhalis. Overall, the mean number of pathogenic bacteria types isolated from immunized children (1.7) was significantly higher than in controls (1.4). The increase in bacterial colonization of the nasopharynx during AOM could be associated with an increase in AOM pathogens and theoretically can predispose PCV7-immunized children with AOM to a higher rate of antibiotic treatment failure or recurrent AOM.

Meningitis

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Case Report

Haemophilus influenzae Type b Meningitis in the Short Period after Vaccination: A Reminder of the Phenomenon of Apparent Vaccine Failure

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We present two cases of bacterial meningitis caused by *Haemophilus influenzae* type b (Hib) which developed a few days after conjugate Hib vaccination. This phenomenon of postimmunization provocative time period is reviewed and discussed. These cases serve as a reminder to clinicians of the risk, albeit rare, of invasive Hib disease in the short period after successful immunization.

1. Introduction

Haemophilus influenzae type b (Hib) was the leading cause of bacterial meningitis in children worldwide until the introduction of the Hib conjugate vaccine in the early 1990s [1]. Since then, the incidence of Hib disease has declined dramatically in high-income countries and virtually eliminated in parts of the United States and Europe [1].

In 1994, the Hib-conjugated vaccine was introduced into the Israeli National Immunization Program. In 1997, a four-dose vaccine schedule was adopted, given at 2, 4, 6, and 12 months of age. Prospective surveillance estimated that vaccine effectiveness was 95% (95% CI 92–96%) against any invasive disease and 97% (95% CI 93–98%) against bacterial meningitis [2].

Nevertheless, over the past 20 years, there have been some reports of invasive Hib disease within a short period after administration of the vaccine [3–5]. This report describes two children in whom Hib meningitis developed a few days after vaccination. These cases serve as a reminder for clinicians of a phenomenon of elevated risk for infection and apparent vaccine failure in the short period after Hib immunization.

2. Case Reports

2.1. Case 1. A 10-week-old girl presented to another hospital with fever, refusal to eat, grunting respirations, and hypertonicity of 48-hour duration. All symptoms began one day after she had received the first dose of the combination Infanrix-IPV+Hib vaccine (a combined vaccine against diphtheria, tetanus, pertussis, polio, and Hib infections). Her parents reported that she had been perfectly healthy the day before vaccination.

Past medical history revealed that the patient had been born at 31 weeks' gestation after premature rupture of the membranes; maternal fever was documented during delivery. She was hospitalized in the neonatal intensive care unit and treated with empiric antibiotics for 3 days pending blood culture results. The rest of her hospitalization was uneventful, and she was discharged at the age of 5 weeks in good medical condition.

At the present admission to the other hospital, bacterial meningitis was suspected on the basis of abnormal cerebrospinal fluid (CSF) cell count (2358/mm^3, with neutrophil predominance 60%), protein, and glucose (235 mg/dL, 1 mg/dL, resp.) despite negative findings on

direct microscopy of a CSF sample. Empiric treatment with ceftriaxone, vancomycin, and dexamethasone was started. Two days later, both blood and CSF cultures grew *Haemophilus influenzae*, which was identified as type b using latex agglutination-based antigen detection test. The patient's clinical status gradually improved over the next 4 days, when a secondary fever was noted in addition to new-onset seizures. Treatment with phenobarbital was initiated, and the patient was transferred to our tertiary medical center.

At admission to our department, magnetic resonance imaging (MRI) study revealed subdural fluid collections in the posterior fossa and around the hemispheres. Given the patient's clinical and neurological deterioration as well as the high levels of inflammatory markers, a tentative diagnosis of subdural empyema was made. The patient was transferred to the neurosurgery department where she underwent bilateral craniotomy. Findings included a subdural empyema with severe brain edema. The empyema was drained. The antibiotic treatment was continued and combined with anticonvulsant and supportive treatment, leading to gradual improvement.

The patient was discharged from our institute after 20 days, during which she received ceftriaxone. On her discharge, she was clinically stable and had normal findings on neurologic examination except for mild hypertonicity of the left arm and mild left torticollis. On follow-up visits, 2 months later and at age 1 year, brainstem-evoked response audiometry (BERA) was within normal range. There was a mild global developmental delay with normal findings on neurologic examination.

2.2. Case 2. A 5-month-old boy presented to our hospital with fever, apathy, vomiting, and diarrhea of 24-hour duration. All symptoms began 6 days after he received the second dose of the Infanrix-IPV+Hib vaccine. His parents reported that he had been perfectly healthy on the day before vaccination.

Past medical history was unremarkable. The patient was born after a normal term pregnancy and vaginal delivery. He received the first dose of Infanrix-IPV+Hib vaccine at age of 2 months without adverse events.

At admission, the patient was febrile and apathetic, with grunting respiration and a bulging fontanel. Lumbar puncture revealed a white blood cell count of 4,000 cells/mm^3, 95% segmented neutrophils, and glucose level of 8.4 mg/dL (protein level was not calculated because of technical problem). Gram staining of the CSF was negative. Empiric treatment with ceftriaxone, vancomycin, and dexamethasone was started. After 36 hours, blood culture grew *Haemophilus influenzae*, which was later identified as type b using latex agglutination-based antigen detection test.

Over the next days, the patient continued treatment with ceftriaxone, with gradual improvement. BERA study was normal. He was discharged home after 11 days in excellent condition, with no neurologic deficits.

3. Discussion

The Hib vaccine targets the organism's capsular polysaccharide, polyribosylribitol phosphate (PRP). To increase immunogenicity and induce immune memory, several conjugate vaccines were developed through covalent linkage of PRP to a carrier protein. Four conjugated vaccines were found safe and were introduced into routine immunization programs worldwide [1].

While the introduction of conjugate vaccine against Hib has had a substantial impact on Hib infection, over the past 20 years, sparse reports of cases of invasive disease after Hib vaccination have been published [3–5]. Booy et al. [3] investigated all cases of invasive Hib infection that occurred over a 3-year period in children in the United Kingdom after they received at least one dose of the Hib-conjugate vaccine. They identified two kinds of vaccine failures: apparent (early) and true (late). True failures were defined as Hib invasive disease occurring either >1 week after a child up to the age of 12 months received at least two doses of the vaccine, or >2 weeks after a single dose was received by a child >12 months of age. Hib invasive infections that occurred within one week after the administration of one or two doses of vaccine were considered apparent vaccine failures. Thus, in the present report, both cases represent apparent (early) vaccine failures.

The "apparent vaccine failure" was a known phenomenon of the early polysaccharide vaccine [6], but relatively rare when attributed to conjugate vaccine. In Booy's work [3], they reported of 46 apparent vaccine failures out of the 164 cases of invasive disease among the entire population of United Kingdom vaccinated children. Singleton et al. reviewed data from Alaska's Statewide Disease Surveillance conducted during 1980–2004 [4]. Study population included 103,000 children younger than 10 years of age. They reported of 3 early vaccine failures out of 44 cases of invasive disease in immunized children. Cowgill et al. reviewed hospitalization data of a main district hospital in Kenya and reported of 24 cases of invasive disease in immunized children, 12 of them early failures [5].

Already in 1901, Wright [7] coined the term "negative phase" to describe the decrease in bactericidal activity; he observed 1 to 21 days after administration of typhoid vaccine. This phenomenon of postimmunization provocative disease was also confirmed in early studies of conjugated and unconjugated Hib vaccines which reported that subjects with preexisting anticapsular antibodies showed a decrease in antibody concentrations after immunization [8, 9]. The nadir in antibody decline was reached 2-3 days after immunization, and concentrations normalized by day 7. The magnitude of the decline was negatively correlated with the preimmunization concentration [9]. This decrease is presumed to occur with all 4 available Hib conjugate vaccines [9]. Some authors attributed these findings to the formation of a complex between the vaccine antigens and the preexisting serum antibodies, which induces a transient decline in antibody concentration [10]. This could pose a risk of invasive disease if it occurs during a period of asymptomatic colonization with Hib [10].

In order to understand whether the individual having received the Hib vaccine is adequately protected against the organism, the level of anti-PRP antibodies should be assessed. The exact mechanism underlying the invasive infection in our patients could not be determined because

the concentration of Hib antibodies was not measured in either case before or after immunization. However, these cases are reported to serve as a reminder to clinicians of the risk, albeit rare, of invasive Hib disease in the short period after successful immunization. Clinicians should bear this possibility in mind when starting empiric antibiotic treatment in children who present with signs of infection within a week of receiving the vaccine. Large-scale studies that focus on this time frame are still needed.

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Neuropsychiatric Disorder

Vaccines linked to mental disorders by Yale study

KEVIN WANG | 1:18 AM, FEB 21, 2017

STAFF REPORTER

A recent Yale study has called into question the safety of vaccines and could lend fuel to anti-vaccine advocates like Robert F. Kennedy Jr., who has already written a piece covering the study on the news site EcoWatch.

The study, published last month in the journal Frontiers in Psychiatry, reports that patients diagnosed with neuropsychiatric disorders like obsessive-compulsive disorder and anorexia nervosa were more likely to have received vaccinations three months prior to their diagnoses. Though the collaboration between researchers at Pennsylvania State University and the Yale Child Study Center yielded results that seem to dispute the safety of vaccines, the authors asserted that the study needs replication on a larger scale and does not establish a causal relationship between vaccines and neuropsychiatric disorders.

“There’s a fair amount of interest in the vaccine safety question, so let’s try to be critical and do further studies that will help examine this issue in a more thorough way,” said James Leckman, professor of pediatrics and one of the study’s five authors.

Using information from a health insurance claims database, Leckman and his co-authors examined the correlations between specific vaccines and various neurological disorders in six- to 15-year-old children. Children with open wounds and broken bones were used as the two control groups.

While only about 10 percent of children with open wounds had received vaccinations, vaccines had been given to over 20 percent of children later diagnosed with anorexia. Higher numbers of vaccinated children were also found among those who were diagnosed with OCD, anxiety disorder and ADHD as soon as three months after their vaccinations.

Other findings in the study, however, reveal that these correlational results should be taken with a grain of salt.

The broken bone control group also included a higher percentage of vaccinated children, though not as high as that of the anorexia group. Furthermore, vaccinations were more likely to be associated with a lower incidence of major depression and bipolar disorder.

The researchers found correlations for one vaccine in particular: the influenza vaccine, which was associated with higher rates of OCD, anorexia, anxiety disorder and tic disorder.

A biological explanation for these correlations has not been found, but a potential mechanism could lie in the body's immune response to vaccines, the study suggested.

Vaccines work by prodding the immune system to produce antibodies against viruses and bacteria, thus priming the body against these pathogens before they enter it. Some antibodies, however, can react against not only the intended pathogen proteins, but also against human proteins — a phenomenon called cross-reactivity. A 2015 study published in Science Translational Medicine discovered that antibodies elicited by the Pandemrix influenza vaccine cross-reacted with a human brain protein — hypocretin receptor 2.

Autoimmunity, in which antibodies attack human proteins, is also known to play a critical role in normal brain development, Leckman noted. According to Leckman, if children were experiencing inflammation — a process that promotes autoimmunity — at the time of vaccination, the combination of inflammation and vaccination could have deleterious effects on brain development. Such data on vaccination timing was not included in the database on which the study was based.

Another biological explanation could involve genetic factors, Leckman said. Prior studies in Scandinavian countries and China found that the H1N1 influenza vaccine was associated with narcolepsy. The influence of multiple genes found in specific populations could be responsible, he added.

Yale professor of pathology John Rose suggested that the act of vaccine administration, rather than the vaccine itself, could even have an effect on neuropsychiatric development, recalling his childhood experience of being one of the first children to receive the polio vaccine.

"We had to line up in school, and we were getting needles stuck in our arms," Rose said. "That kind of trauma could be leading to these kinds of neuropsychiatric disease. The age range of the children in the study is quite sensitive."

Rose, who developed a vaccine template that was used for the development of the current Ebola vaccine, said he trusts the current process of drug development to establish safety measures for vaccines. On average, a vaccine takes 15–20 years to be fully approved, Rose said.

Leckman said the accuracy of the diagnoses reported by the administrative database could also be questioned.

John Treanor, chief of infectious diseases at the University of Rochester Medical Center, voiced concerns about the database, citing issues of immeasurable confounding variables and the extent to which the control groups actually serve as effective controls. Nevertheless, he emphasized the importance of vaccine safety and further research to understand it.

Rose expressed concern that the study would “activate anti-vaccine people in a very serious way” and agreed with the study’s assertion that the results are very preliminary and do not establish a cause and effect relationship. Animal models, Leckman noted, could help establish such a cause and effect relationship by allowing researchers to manipulate and control for multiple variables.

Even the authors noted that the results of the study are too inconclusive to warrant any reconfiguration of public health strategies.

“Given the modest magnitude of these findings in contrast to the clear public health benefits of the timely administration of vaccines in preventing mortality and morbidity in childhood infectious diseases, we encourage families to maintain vaccination schedules according to [the Centers for Disease Control and Prevention] guidelines,” they wrote in the study.

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Temporal Association of Certain Neuropsychiatric Disorders Following Vaccination of Children and Adolescents: A Pilot Case–Control Study

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Background: Although the association of the measles, mumps, and rubella vaccine with autism spectrum disorder has been convincingly disproven, the onset of certain brain-related autoimmune and inflammatory disorders has been found to be temporally associated with the antecedent administration of various vaccines. This study examines whether antecedent vaccinations are associated with increased incidence of obsessive–compulsive disorder (OCD), anorexia nervosa (AN), anxiety disorder, chronic tic disorder, attention deficit hyperactivity disorder, major depressive disorder, and bipolar disorder in a national sample of privately insured children.

Methods: Using claims data, we compared the prior year's occurrence of vaccinations in children and adolescents aged 6–15 years with the above neuropsychiatric disorders that were newly diagnosed between January 2002 and December 2007, as well as two control conditions, broken bones and open wounds. Subjects were matched with controls according to age, gender, geographical area, and seasonality. Conditional logistic regression models were used to determine the association of prior vaccinations with each condition.

Results: Subjects with newly diagnosed AN were more likely than controls to have had any vaccination in the previous 3 months [hazard ratio (HR) 1.80, 95% confidence interval 1.21–2.68]. Influenza vaccinations during the prior 3, 6, and 12 months were also associated with incident diagnoses of AN, OCD, and an anxiety disorder. Several other associations were also significant with HRs greater than 1.40 (hepatitis A with OCD and AN; hepatitis B with AN; and meningitis with AN and chronic tic disorder).

Conclusion: This pilot epidemiologic analysis implies that the onset of some neuropsychiatric disorders may be temporally related to prior vaccinations in a subset of individuals. These findings warrant further investigation, but do not prove a causal role of antecedent infections or vaccinations in the pathoetiology of these conditions. Given the modest magnitude of these findings in contrast to the clear public health benefits of

the timely administration of vaccines in preventing mortality and morbidity in childhood infectious diseases, we encourage families to maintain vaccination schedules according to CDC guidelines.

Keywords: anorexia nervosa, obsessive–compulsive disorder, anxiety disorder, tic disorder, vaccination, influenza, meningococcus

INTRODUCTION

There is a considerable body of scientific evidence indicating that the immune system plays a key role in normal brain development and in the pathobiology of several neuropsychiatric disorders (1). These include obsessive–compulsive disorder (OCD) (2, 3), anorexia nervosa (AN) (4), tic disorders (5), attention deficit hyperactivity disorder (ADHD) (6), major depressive disorder (7), and bipolar disorder (8). The precise role immune mechanisms play in these disorders remains to be determined.

In light of the role of the immune system in these central nervous system (CNS) conditions, the impact of vaccines on childhood-onset neuropsychiatric diseases had been considered and was mainly addressed with regards to the administration of the measles, mumps, and rubella (MMR) vaccine (and its various components) and the subsequent development of autism spectrum disorder (ASD). Although the controversy over MMR vaccination and ASD still exists for some members of the public, this association has been convincingly disproven (9, 10). On the other hand, the onset of a limited number of autoimmune and inflammatory disorders affecting the CNS has been found to be temporally associated with the antecedent administration of various vaccines (11). These disorders include idiopathic thrombocytopenic purpura, acute disseminated encephalomyelitis, and Guillain–Barré syndrome among others (12–16). More recently, data have emerged indicating an association between the administration of the H1N1 influenza vaccine containing the AS03 adjuvant and the subsequent new onset of narcolepsy in several northern European countries (17, 18). The immune mechanisms and host factors underlying these associations have not been identified or fully characterized, although preliminary data are beginning to emerge (18–23).

Given this growing body of evidence of immunological involvement in CNS conditions, and despite the controversy concerning the link between ASD and MMR and the clear public health importance of vaccinations, we hypothesized that some vaccines could have an impact in a subset of susceptible individuals and aimed to investigate whether there is a temporal association between the antecedent administration of vaccines and the onset of several neuropsychiatric disorders, including OCD, AN, tic disorder, anxiety disorder, ADHD, major depressive disorder, and bipolar disorder using a case–control population-based pediatric sample (children aged 6–15 years). To assess the specificity of any statistical associations, we also determined whether or not there were any temporal associations between antecedent vaccine administration and the occurrence of broken bones or open wounds.

MATERIALS AND METHODS

Data were obtained from the MarketScan® Commercial Claims and Encounters database, which is constructed and maintained by Truven Health Analytics. Data from 2002 to 2007 were used for the study. MarketScan consists of de-identified reimbursed health-care claims for employees, retirees, and their dependents of over 250 medium and large employers and health plans. Hence, individuals included in the database are covered under private insurance plans; no Medicaid or Medicare data are included. The database includes claims information describing the health-care experiences for approximately 56 million covered lives per year. The database is divided into subsections, including inpatient claims, outpatient claims, outpatient prescription drug claims, and enrollment information. Claims data in each of the subsections contain a unique patient identifier and information on patient age, gender, geographic location (including state and three-digit zip code), and type of health plan.

The inpatient and outpatient services subsections of the MarketScan database contain information on all services performed in an inpatient or outpatient setting. These data include information on dates of services, the diagnoses associated with the claim, and the procedures performed. The outpatient services subsection includes information for all services performed in a doctor's office, hospital outpatient clinic, emergency room, or other outpatient facility. Previous studies have used the MarketScan database to examine health-care service use and costs for children (24–29).

Study Population

The study sample consisted of children aged 6–15 with a diagnosis of one of the following conditions (ICD-9 codes in parentheses): OCD (300.3), AN (307.1), anxiety disorder (300.0–300.2), tic disorder (307.20 or 307.22), ADHD (314), major depression (296.2–296.3), and bipolar disorder (296.0–296.2, 296.4–296.8). To test the specificity of the models, we also included children with broken bones (800–829) and open wounds (870–897). To identify new cases, we further limited the sample in each diagnostic group to children who were continuously enrolled for at least 1 year prior to their first diagnosis for the condition (the index date). Next, a matched one-to-one control group was constructed for each diagnostic group consisting of children who did not have the condition of interest and were matched with their corresponding case on age, gender, date of the start of continuous enrollment, and three-digit zip code. Because vaccines tend to occur during certain times of year (such as before summer camps or the beginning of school), controls were also required to have an outpatient visit at which they did not receive a vaccine within 15 days of the date that the corresponding case was first diagnosed with the

condition, in an effort to control for seasonality. The date of this visit was the index date for children in the control group.

For each diagnostic group and their corresponding controls, individuals who were vaccinated in the 3, 6, or 12 months before the index date were identified. Exposure to vaccines was measured using CPT codes (list available from the authors upon request) and ICD-9 codes (V03–V06 or V07.2). Exposure to specific vaccines, including influenza, tetanus and diphtheria (TD), hepatitis A, hepatitis B, meningitis, and varicella, was tracked.

Statistical Analysis

The analyses were performed for each diagnostic group (and their controls) separately. Children with multiple conditions (e.g., ADHD and tic disorder) were included in each of the corresponding analytic groups. First, the proportion of children who were exposed to vaccines in the period before the index date was compared across the case and control groups. Next, bivariate conditional logistic regression models were estimated to determine the hazard ratios (HRs) and 95% confidence intervals (95% CIs) associated with the effect of vaccine exposure on having the condition of interest. Separate models were run for the 3-, 6-, and 12-month periods preceding the index date for each diagnostic group. The study was approved by the Penn State College of Medicine Institutional Review Board.

RESULTS

Characteristics of each of the diagnostic groups are presented in **Table 1**. Sample sizes ranged from 551 children diagnosed with AN to 85,151 children with a broken bone. The average age ranged from 9.5 ± 2.5 for children with tic disorder to 13.3 ± 1.7 for children with AN. Not surprisingly, the distribution of sex varied considerably across diagnostic groups, with higher percentages of females in the AN (86.6%) and major depression (56.3%) categories and higher proportions of males in the tic disorder (76.4%), ADHD (66.8%), open wound (62.2%), broken bone (58.4%), OCD (56.6%), and bipolar disorder (54.1%) categories.

Rates of receipt of vaccines in the 6 months before the first diagnosis of the disorder are also reported in **Table 1** and varied considerably across diagnostic groups. Receipt of any vaccine in the previous 6 months was highest for children with AN (21.4%), followed by OCD (15.9%) and tic disorder (15.8%), and was lowest for children with open wounds (10.3%). Rates of receipt of specific vaccines were fairly low, ranging from 0.5% for the hepatitis vaccine among children with tic disorder to 8.4% for the influenza vaccine among children with tic disorder. In general, vaccination rates were highest among children in the AN, OCD, and tic disorder groups and were lowest for children in the open wound or bipolar disorder groups.

Table 2 presents HRs from the bivariate associations of receipt of vaccine within the 3-, 6-, and 12-month periods preceding the index date for each diagnostic group compared to their matched controls. Children with OCD, AN, anxiety disorder, or ADHD were more likely to have had a vaccination in each of the preceding periods than their matched controls, and children with tic disorder were more likely to have had a vaccination in the

TABLE 1 | Characteristics of the sample.

Characteristic	Broken bone		Open wound		OCD		Anorexia nervosa		Anxiety disorder		Tic disorder		ADHD		Major depression		Bipolar disorder	
	N	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
N	85,151		73,290		3,222		551		23,462		2,547		46,640		13,295		5,892	
Age, mean ± SD	11.1 ± 2.7		10.6 ± 2.9		11.1 ± 2.6		13.3 ± 1.7		11.3 ± 2.8		9.5 ± 2.5		10.3 ± 2.8		12.9 ± 2.2		12.3 ± 2.6	
Gender																		
Male	49,689	58.4	45,562	62.2	1,825	56.6	74	13.4	11,357	48.4	1,945	76.4	31,170	66.8	5,811	43.7	3,185	54.1
Female	35,462	41.6	27,728	37.8	1,397	43.4	477	86.6	12,105	51.6	602	23.6	15,470	33.2	7,484	56.3	2,707	45.9
Receipt of vaccine[a]																		
Any vaccine	10,308	12.1	7,577	10.3	512	15.9	118	21.4	3,389	14.4	402	15.8	5,536	11.9	1,700	12.8	682	11.6
Influenza	3,550	4.2	2,783	3.8	246	7.6	42	7.6	1,418	6.0	214	8.4	2,366	5.1	548	4.1	247	4.2
TD	2,061	2.4	1,358	1.9	68	2.1	27	4.9	520	2.2	53	2.1	913	2.0	405	3.0	159	2.7
HepA	1,950	2.3	1,462	2.0	86	2.7	14	2.5	570	2.4	62	2.4	1,005	2.2	285	2.1	100	1.7
HepB	713	0.8	550	0.8	20	0.6	12	2.2	201	0.9	14	0.5	366	0.8	150	1.1	60	1.0
Meningitis	1,325	1.6	835	1.1	61	1.9	24	4.4	422	1.8	36	1.4	495	1.1	223	1.7	88	1.5
Varicella	1,002	1.2	750	1.0	52	1.6	8	1.5	323	1.4	44	1.7	577	1.2	93	0.7	42	0.7

[a]*Reciept of vaccine in the 6 months before first diagnosis of the disorder.*

OCD, obsessive–compulsive disorder; ADHD, attention deficit hyperactivity disorder; TD, tetanus and diphtheria; Hep, hepatitis.

TABLE 2 | Bivariate associations of receipt of vaccine with new diagnosis.[a]

Vaccine	Broken bone *N* = 85,151			Open wound *N* = 73,290			OCD *N* = 3,222			Anorexia nervosa *N* = 551			Anxiety disorder *N* = 23,462			Tic disorder *N* = 2,547			ADHD *N* = 46,640			Major depression *N* = 13,295			Bipolar disorder *N* = 5,892		
	Hazard ratio (HR)	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI		HR	95% CI	
Any vaccine																											
3 months	**1.04**	**1.00**	**1.08**	0.96	0.92	1.00	**1.23**	**1.02**	**1.49**	**1.80**	**1.21**	**2.68**	**1.12**	**1.04**	**1.20**	1.11	0.90	1.38	**1.06**	**1.00**	**1.12**	**0.88**	**0.80**	**0.97**	0.87	0.75	1.01
6 months	**1.08**	**1.05**	**1.11**	0.97	0.94	1.01	**1.27**	**1.10**	**1.47**	**1.63**	**1.17**	**2.27**	**1.13**	**1.07**	**1.19**	**1.25**	**1.06**	**1.47**	**1.04**	**1.00**	**1.09**	**0.92**	**0.86**	**0.99**	**0.82**	**0.73**	**0.91**
12 months	**1.07**	**1.04**	**1.09**	**0.97**	**0.94**	**1.00**	**1.23**	**1.09**	**1.38**	**1.47**	**1.12**	**1.93**	**1.14**	**1.09**	**1.19**	**1.19**	**1.04**	**1.36**	**1.08**	**1.05**	**1.12**	**0.89**	**0.84**	**0.95**	**0.87**	**0.79**	**0.95**
Influenza																											
3 months	1.03	0.96	1.11	0.93	0.86	1.01	**1.36**	**1.02**	**1.82**	**2.20**	**1.10**	**4.38**	**1.23**	**1.10**	**1.38**	1.24	0.91	1.67	0.98	0.91	1.07	**0.81**	**0.68**	**0.96**	**0.71**	**0.55**	**0.92**
6 months	**1.07**	**1.02**	**1.13**	0.96	0.91	1.02	**1.48**	**1.21**	**1.83**	**1.83**	**1.07**	**3.15**	**1.24**	**1.14**	**1.35**	**1.27**	**1.02**	**1.58**	0.97	0.91	1.02	0.89	0.79	1.00	**0.84**	**0.70**	**1.00**
12 months	**1.06**	**1.02**	**1.09**	0.97	0.93	1.01	**1.35**	**1.16**	**1.59**	1.52	0.99	2.34	**1.27**	**1.19**	**1.35**	**1.28**	**1.08**	**1.50**	1.04	0.99	1.09	0.93	0.84	1.02	0.87	0.76	1.00
TD																											
3 months	1.02	0.94	1.11	0.92	0.83	1.02	1.15	0.72	1.84	1.70	0.78	3.71	0.95	0.80	1.13	0.86	0.47	1.60	1.04	0.91	1.19	0.95	0.78	1.16	0.83	0.62	1.12
6 months	1.07	1.00	1.14	0.94	0.87	1.01	1.07	0.75	1.51	1.77	0.90	3.49	0.91	0.80	1.03	1.24	0.82	1.88	1.03	0.93	1.13	0.96	0.83	1.10	0.82	0.66	1.02
12 months	**1.07**	**1.02**	**1.12**	**0.93**	**0.88**	**0.98**	0.99	0.77	1.26	**1.63**	**1.05**	**2.52**	0.98	0.90	1.07	0.93	0.67	1.30	1.04	0.97	1.11	0.90	0.82	1.00	**0.80**	**0.68**	**0.93**
HepA																											
3 months	1.02	0.94	1.12	0.97	0.88	1.07	1.47	0.92	2.33	1.60	0.52	4.89	1.00	0.85	1.18	1.13	0.70	1.83	1.03	0.92	1.17	0.86	0.68	1.08	1.03	0.73	1.47
6 months	1.05	0.98	1.12	0.99	0.92	1.07	**1.43**	**1.02**	**2.01**	1.09	0.48	2.51	1.08	0.95	1.22	1.35	0.92	1.98	1.05	0.96	1.15	0.95	0.81	1.13	0.79	0.60	1.03
12 months	**1.08**	**1.02**	**1.13**	0.99	0.93	1.05	**1.40**	**1.07**	**1.82**	1.73	0.89	3.37	1.00	0.91	1.10	1.17	0.88	1.56	**1.09**	**1.02**	**1.18**	0.97	0.86	1.11	0.81	0.66	1.00
HepB																											
3 months	1.08	0.93	1.25	1.05	0.89	1.24	0.71	0.32	1.61	3.00	0.61	14.86	1.01	0.76	1.34	1.40	0.44	4.41	1.13	0.91	1.39	1.05	0.77	1.43	0.97	0.61	1.56
6 months	1.02	0.92	1.13	1.02	0.91	1.15	0.80	0.45	1.44	1.71	0.68	4.35	1.01	0.83	1.23	1.17	0.54	2.52	1.07	0.92	1.24	0.89	0.71	1.11	0.91	0.64	1.29
12 months	1.03	0.95	1.11	1.00	0.91	1.09	0.93	0.60	1.44	1.55	0.72	3.30	1.01	0.89	1.16	1.19	0.61	2.31	1.06	0.95	1.18	1.00	0.85	1.18	1.07	0.83	1.38
Meningitis																											
3 months	1.05	0.95	1.17	1.04	0.92	1.19	1.10	0.67	1.80	1.71	0.68	4.35	1.06	0.88	1.27	1.46	0.72	2.95	1.16	0.98	1.38	0.89	0.70	1.13	0.87	0.57	1.33
6 months	1.08	1.00	1.17	1.02	0.92	1.12	1.15	0.78	1.71	1.75	0.86	3.56	1.12	0.97	1.29	**1.94**	**1.08**	**3.46**	1.08	0.95	1.23	0.88	0.73	1.05	0.82	0.61	1.11
12 months	1.06	0.99	1.14	1.02	0.94	1.10	1.34	0.96	1.87	1.42	0.79	2.56	**1.14**	**1.01**	**1.29**	**1.73**	**1.07**	**2.80**	1.06	0.95	1.18	**0.81**	**0.70**	**0.94**	0.85	0.67	1.09
Varicella																											
3 months	**0.88**	**0.79**	**0.99**	0.90	0.79	1.03	1.33	0.79	2.26	1.00	0.20	4.96	1.06	0.85	1.31	0.73	0.42	1.27	1.06	0.90	1.24	0.85	0.58	1.24	1.08	0.63	1.87
6 months	0.97	0.88	1.06	0.96	0.87	1.07	1.38	0.89	2.15	2.66	0.71	10.04	1.17	0.99	1.38	0.91	0.59	1.40	1.09	0.97	1.23	0.85	0.64	1.14	0.79	0.52	1.21
12 months	1.00	0.93	1.08	0.93	0.85	1.01	1.36	0.92	1.99	1.29	0.48	3.45	1.11	0.97	1.28	0.97	0.67	1.40	1.06	0.95	1.17	0.84	0.66	1.06	0.74	0.53	1.05

[a]*Cases and controls matched on date (±15 days, see text) of the start of continuous enrollment, year of birth, gender, and three-digit zip code. N's represent cases only. Results in bold are statistically significant at $p < 0.05$.*
OCD, obsessive–compulsive disorder; ADHD, attention deficit hyperactivity disorder; TD, tetanus and diphtheria; Hep, hepatitis.

preceding 6- and 12-month periods than their matched controls. HRs associated with receipt of any vaccine were highest for children with AN, ranging from 1.47 (95% CI 1.12–1.93) for the 12-month preceding period to 1.80 (95% CI 1.21–2.68) for the 3-month preceding period, followed by OCD, which ranged from 1.23 for both the 12-month (95% CI 1.12–1.93) and 3-month (95% CI 1.02–1.49) preceding periods to 1.27 (95% CI 1.10–1.47) for the 6-month preceding period. However, children with broken bones were also more likely to have had a vaccination in the preceding period, although the HRs were smaller, ranging from 1.04 (95% CI 1.00–1.08) for the 3-month preceding period to 1.08 (95% CI 1.05–1.11) for the 6-month preceding period. The other control condition, open wounds, showed no increased incidence following vaccinations. In addition, children with major depression were *less* likely to have had a vaccination in all 3 preceding periods, and children with bipolar disorder were also *less* likely to have had a vaccination in the 6- or 12-month preceding periods.

There were fewer statistically significant results when looking at the effects of the individual vaccines. Children with OCD were more likely to have received the influenza vaccine in each of the preceding periods, or the hepatitis A vaccine in the previous 6 or 12 months. Children with AN were also more likely to have received the influenza vaccine in the preceding 3 or 6 months, or the TD vaccine in the previous 12 months. Children with anxiety disorder were more likely to have received the influenza vaccine in the previous 12 months. Children with tic disorder were more likely to have received an influenza or a meningococcal vaccine in the previous 6 or 12 months. However, children with broken bones were also slightly more likely to have received the influenza vaccine during the previous 3-, 6-, and 12-month intervals. In contrast, children with major depression were *less* likely to have received the influenza vaccine in the previous 3 months or the meningitis vaccine in the previous 12 months. Similarly, children with bipolar disorder were also *less* likely to have received the influenza vaccine in the previous 3 or 6 months. Antecedent vaccination with any vaccine and with the TD vaccine during the previous 12 months was very modestly associated with a *decreased* incidence of open wounds (**Table 2**).

DISCUSSION

The principal findings of this study are as follows: (i) children with OCD, AN, anxiety disorder, and tic disorder were more likely to have received influenza vaccine during the preceding 1-year period (for OCD in the preceding 3-, 6-, and 12-month periods; for AN in the preceding 3- and 6-month periods; for anxiety disorder in the preceding 6- and 12-month periods; for tic disorder in the preceding 6- and 12-month periods) and (ii) HRs associated with receipt of any vaccine were highest for children with AN, ranging from 1.47 for the 12-month preceding period to 1.80 for the 3-month preceding period, followed by OCD, which ranged from 1.23 for both the 12- and 3-month preceding periods to 1.27 for the 6-month preceding period. However, if we apply a high standard [so that the upper limit of the of the 95% CI of the HR observed for the association between the administration of any vaccine and the subsequent occurrence of a broken bone (1.11) falls below the lower limit of the 95% CI observed for any of the HRs for any of the neuropsychiatric disorders], only the findings for AN pass this stringent threshold (**Table 2**). Applying a similar high standard for the individual vaccines, the only associations that pass this threshold concern the influenza vaccine given in the preceding 6- and 12-month periods for OCD and anxiety disorders.

Our findings showing that children with AN, OCD, or a tic disorder were more likely to have received the influenza vaccine in the preceding periods were noteworthy given the findings of increased incidence of narcolepsy in Finland, Sweden, Ireland, Norway, England, and France after vaccination with AS03-adjuvanted H1N1 vaccine (17, 18). Studies also show a threefold increase in the incidence of narcolepsy after following the 2009 H1N1 pandemic in China (30). Although the strong association between HLA class II and narcolepsy suggests that narcolepsy may be an autoimmune disorder, the exact mechanism leading to immune-related narcolepsy is not completely understood and other host factors are likely to play an important role (31, 32). Investigators have made use of *in silico* techniques to begin to identify potential causal pathways and the relevant host factors (19). More recently, Ahmed et al. (23) have shown that the H1N1 influenza vaccine containing the AS03 adjuvant triggers antibodies that bind to hypocretin receptor 2a. Additional work is needed to replicate and extend these findings.

It is also of note that the observed association between the antecedent administration of the influenza vaccine and the new onset of AN and OCD may suggest that aberrant immune functioning may be a common pathogenetic pathway for OCD and AN. The high comorbidity rates between OCD and AN, common cortico-striatal abnormalities in neuroimaging studies, and anti-putamen antibodies both in OCD and AN cases are some of the shared features of these two disorders worth considering (33–35). In addition, the increased risk for autoimmune disorders (such as type 1 diabetes mellitus, Crohn's disease, and celiac disease) in eating disorders (36) and the documented comorbidity of OCD and autoimmune diseases (such as systemic lupus erythematosus, thyroid dysfunction, and multiple sclerosis) (35) indicate the possible shared host factors and the role of immune-mediated mechanisms in the development of AN and OCD. We also note the findings of Zastrow and colleagues that vaccination to prevent H1N1 influenza is recommendable even in extremely underweight AN patients (37).

Limitations of this study include that we were unable to control for the fact that providers may designate ICD-9 insurance billing codes for vaccines generally without specifying the particular vaccine. Additionally, we were unable to match claims by providers in order to control for the diagnostic predilections of individual physicians and account for the possibility that some physicians might be more (or less) likely to diagnose one or more neuropsychiatric disorders and/or recommend specific vaccinations. The results of this study are further qualified by the limitations of the administrative retrospective data used in this study, rather than from systematically obtained clinical data, especially around diagnostic classification. This is a shortcoming inherent in studies that rely on secondary analyses to secure large sample sizes. Furthermore, early vaccines are grouped together in the first 15 months of infancy, some of them given

simultaneously at one visit and received by most of the infants. This leads to a limitation in analyzing the possibility of the temporal association between individual vaccines and the onset of neuropsychiatric disorders. We deliberately chose our sample from children aged 6–15 years in order to overcome this limitation. Another limitation concerns changes in vaccine guidelines during the time interval used in this analysis. For example, the American Academy of Pediatrics first recommended the use of the conjugate meningococcal vaccine in August 2005 and the varicella booster in April 2007. As a consequence, the size of the cohorts who received these vaccines is smaller in comparison to other vaccines. Another issue concerns the fact that the influenza vaccination is an annual vaccination using a vaccine specific for a given year to protect against the highly variable influenza virus. As a consequence, it is also the most frequently administered vaccine that indeed may well have disproportionately "driven" the "any vaccine" findings (**Table 1**). Given its variability and prevalence, in future studies, it will be important to look year-by-year. Perhaps the largest limitation and potential threat to the study's validity has to do with the fundamental impossibility of detecting a causal relationship within the context of such a case–control study. Indeed, this provides no more than a relative perspective of the potential risk, as opposed to the absolute risk (the real proportion of individuals who had a vaccination and then developed one or more of the investigated conditions) that might be expected to be reasonably small.

Moving forward, our findings require replication in a larger population-based sample, possibly including assessments of various potentially important host factors, e.g., the individual's genomic and epigenomic background, the individual's microbiome, their history of antecedent psychosocial stress, infections, as well as other potentially simultaneously administered vaccinations, the differences in vaccine types, and the route of administration (e.g., intramuscular or intranasal administration of influenza vaccine) as different routes of administration may lead to a difference in immune responses in the host.

It will also be critically important to determine whether or not newly acquired infectious diseases against which the children were vaccinated may themselves lead to an increased incidence to one or more of these neuropsychiatric disorders. In fact, it would not be surprising if the diseases *per se* represent a stronger risk factor than vaccinations. The documented increase in the incidence of narcolepsy following the 2009 H1N1 pandemic in China provides a clear example (30). Our earlier epidemiological study documenting a temporally related modest increase in the incidence of OCD, tic disorders, and ADHD following a prior streptococcal infection provides another example (25). Future epidemiologic investigations are needed to address this important question.

The present study has the potential to extend our knowledge about the role of the immune system in some pediatric-onset neuropsychiatric disorders. However, our findings do not demonstrate a causal role of vaccination in the pathoetiology of any of these conditions. This is especially important given the clear public health benefits of the timely administration of vaccines in preventing mortality and morbidity (38). Vaccines are among the most successful and cost-effective preventive public health interventions (39). Vaccination has led to eradication of smallpox, and we are close to the eradication of poliomyelitis across the world. Since most of the vaccine-preventable diseases are contagious from person to person, the increase in numbers of vaccinated individuals will decrease the chance of a disease to spread. Proper vaccination not only protects our generation but also protects future generations against epidemics of diseases. It should always be kept in mind that vaccines are crucial for eradicating infectious diseases and preventing the higher rates of morbidity and mortality due to infections. However, care should be taken to ensure that children scheduled to receive vaccinations are in good health and that recommended precautions are taken at the time a vaccine is to be administered.

Clinical Significance

These findings provide preliminary epidemiologic evidence that the onset of some pediatric-onset neuropsychiatric disorders, including AN, OCD, anxiety disorders, and tic disorders, may be temporally related to prior vaccinations. Each of these conditions is etiologically heterogeneous, and host factors likely play an important role in a small subset of vulnerable individuals. However, these findings, even if replicated in future studies, do not prove a causal role of vaccination in the pathoetiology of any of these conditions. Indeed, antecedent infections may also increase the risk of developing one or more of these disorders in vulnerable individuals. Finally, given the modest magnitude of these findings and the clear public health benefits of the timely administration of vaccines in preventing mortality and morbidity in childhood, we encourage families to maintain the currently recommended vaccination schedules while taking all necessary precautions as documented by the Centers for Disease Control and Prevention (http://www.cdc.gov/vaccines/recs/vac-admin/contraindications.htm).

AUTHOR NOTES

Data were obtained from the MarketScan® Commercial Claims and Encounters database, which is constructed and maintained by Truven Health Analytics.

AUTHOR CONTRIBUTIONS

DL, RK, BR, and JL designed the study and wrote the protocol. SG commented on the protocol. DL undertook the statistical analysis. BR, DL, and JL wrote the first draft of the manuscript. All the authors commented on the manuscript. All the authors contributed to and have approved the final manuscript.

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Optic Neuritis

Journal of Medical Case Reports

CASE REPORT Open Access

Optic neuritis following diphtheria, tetanus, pertussis, and inactivated poliovirus combined vaccination: a case report

Preston O'Brien[1*] and Robert W. Wong[1,2*]

Abstract

Background: Diphtheria, tetanus, pertussis, and inactivated poliovirus combined vaccine is widely used in young children as part of a series of immunizations before they start attending school. Case studies of demyelinating conditions following administration of diphtheria, tetanus, pertussis, and polio vaccine have been reported, but none so far resulting in optic neuritis. This report further contributes to the database of central nervous system demyelinating conditions affiliated with receipt of vaccines.

Case presentation: A previously healthy 27-year-old Hispanic man presented to an emergency department with headache, periorbital pressure, pain with ocular movements, and intermittent blurred vision that developed 1 day after administration of the diphtheria, tetanus, pertussis, and inactivated poliovirus combined vaccine. A diagnosis of optic neuritis was made via ophthalmic examination with fundus photography and automated Humphrey visual field analysis. His vision recovered following treatment with high-dose intravenously administered methylprednisolone followed by a tapered dose of orally administered prednisolone.

Conclusions: Although the association between immunizations and the onset of central nervous system demyelinating conditions is well documented, this report, to the best of our knowledge, is the first case of optic neuritis following diphtheria, tetanus, pertussis, and inactivated poliovirus combined vaccination. Inclusion of this case report in the medical community will allow for broader understanding of possible conditions that may present shortly after receipt of vaccination.

Keywords: Diphtheria, Tetanus, Pertussis, Virus, Optic neuritis

Background

Diphtheria, tetanus, pertussis, and inactivated poliovirus combined vaccine (DTaP-IPV) is widely used in young children as part of a series of immunizations before they start attending school. Although clinical trials have shown an excellent safety profile [1], there have been reports of encephalitis, angioneurotic edema, seizures, and serious local reactions following its administration [1, 2]. Although cases of central nervous system (CNS) demyelinating conditions following DTaP-IPV vaccine have been reported [3], to the best of our knowledge, we present the first case of optic neuritis.

Case presentation

A 27-year-old Hispanic man with no significant past medical history presented to an emergency department with a 5-day history of headache, pain with ocular movements, and intermittent blurred vision starting 1 day after being immunized with DTaP-IPV. Magnetic resonance imaging and a magnetic resonance venogram of his brain were unremarkable. A lumbar puncture revealed a normal opening pressure and cerebrospinal fluid studies were positive for

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It is our aim with the submission of this case report to the *Journal of Medical Case Reports* to present a new association between receipt of the diphtheria, tetanus, pertussis, and inactivated poliovirus combined vaccine by our patient and his presenting with optic neuritis. It is important that we make efforts to ensure that the medical community is aware of potential central nervous system demyelinating conditions coinciding with receipt of vaccines so that they can follow the observations, treatment, and precautions in dealing with similar circumstances.
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myelin basic protein but negative for oligoclonal bands and neuromyelitis optica autoantibody serology.

On examination, his best corrected vision was 20/100 in his right eye and 20/70 in his left eye. Intraocular pressures, pupil examination, ocular alignment, and extraocular movements were normal. Confrontational visual fields were restricted in both eyes. Posterior segment examination showed optic nerve swelling and hyperemia in both eyes (Fig. 1) and two microaneurysms in the mid periphery of his left eye. No evidence of vitritis, retinal vasculitis, or choroiditis was seen in either eye.

Serum laboratory testing showed elevated glycated hemoglobin (A1C) at 6.9%, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). Other liver tests including bilirubin, alkaline phosphatase, and hepatitis serologies were normal. Tests for infectious and inflammatory etiologies including angiotensin-converting enzyme (ACE), lysozyme, antinuclear antibody (ANA), cytoplasmic antineutrophil cytoplasmic antibodies (c-ANCA), perinuclear antineutrophil cytoplasmic antibodies (p-ANCA), lupus panel, rapid plasma reagin (RPR), fluorescent treponemal antibody absorption (FTA-ABS), chest X-ray, and QuantiFERON Gold assay, which were normal. Over the next 5 days, his vision declined to counting fingers at 30.5 cm (1 foot) in both eyes. A relative afferent pupil defect and dyschromatopsia developed on the left. Automated Humphrey visual field (HVF) testing demonstrated global depression in both eyes (Fig. 2).

He was diagnosed as having DTaP-IPV vaccination-related optic neuritis and started on intravenously administered Solu-Medrol (methylprednisolone). One week later, his headache resolved and vision improved to 20/20 in his right eye and 20/25 in his left eye with less optic nerve hyperemia and swelling. He was discharged on a prednisone taper and an orally administered diabetic medication. One month later, his vision improved to 20/20 with resolution of the optic neuritis without residual visual field deficit in both eyes.

Discussion

In 2008, the DTaP-IPV vaccine was licensed and indicated for use in children of 4–6 years in age. From 2009 to 2012, a large-scale trial monitoring for adverse events found no significant increased risk of meningitis or encephalitis following DTaP-IPV [1]. Although the overall risk of developing a demyelinating CNS syndrome after vaccination is relatively low

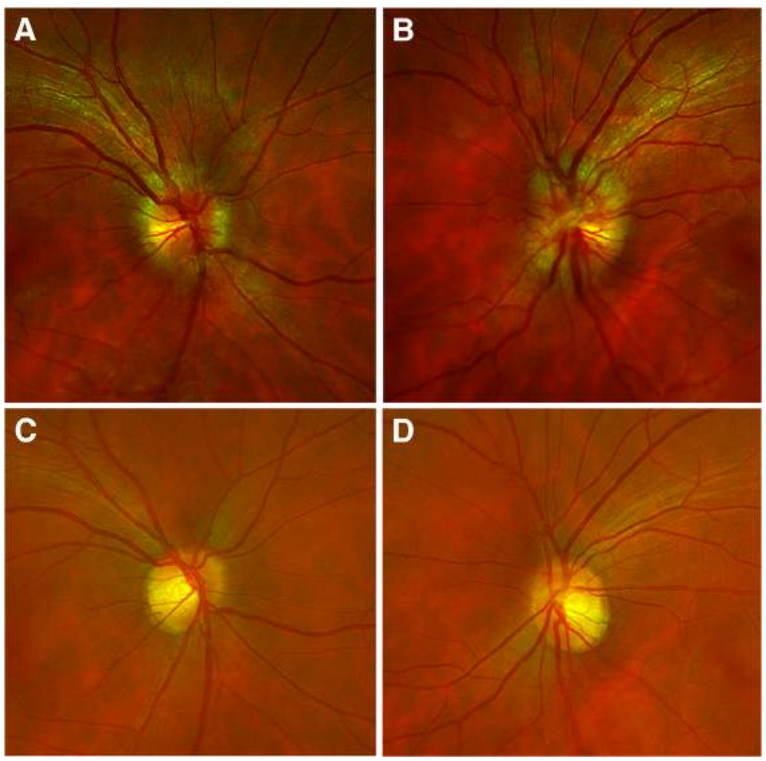

Fig. 1 Color fundus photography of the optic nerve 5 days after initial presentation when vision dropped to counting fingers at 30.5 cm (1 foot) in both eyes of (**a**) right eye and (**b**) left eye. Resolution of optic nerve hyperemia seen on the right eye (**c**) and the left eye (**d**) after treatment with corticosteroids

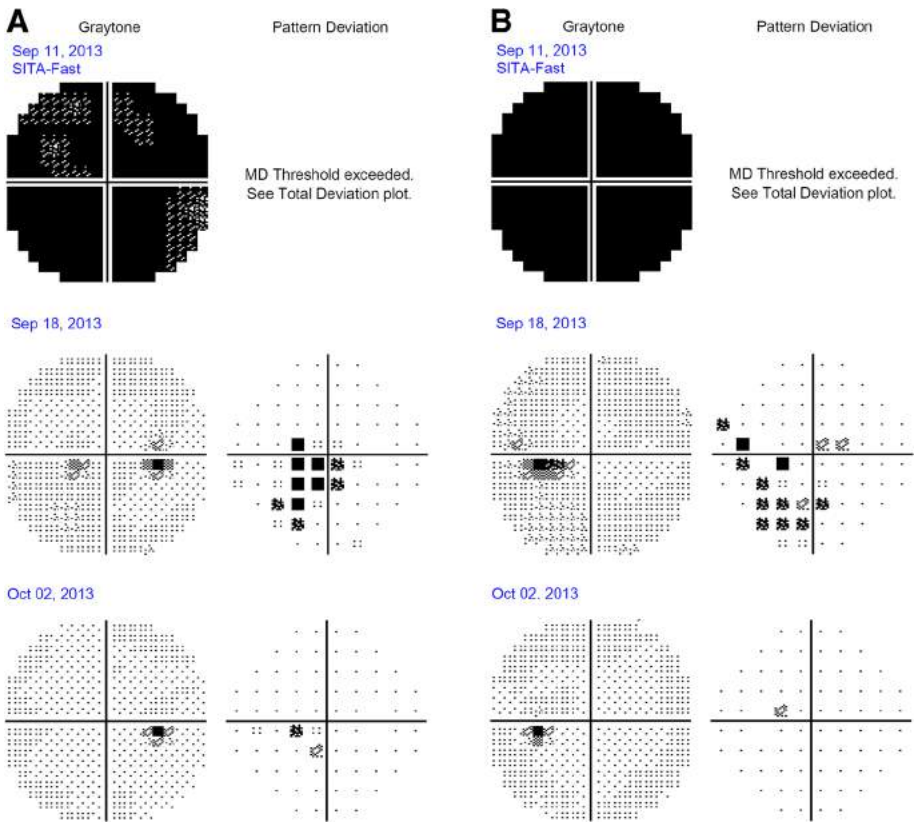

Fig. 2 Automated 30–2 Humphrey visual field testing using Swedish Interactive Testing Algorithm-Fast protocol of (**a**) the right eye and (**b**) the left eye showing gray tone and pattern deviation. The patient was started on intravenously administered Solu-Medrol (methylprednisolone) on September 11, 2013 (*top line*). Follow-up perimetry 1 week (*middle line*) and 3 weeks (*bottom line*) after the initiation of systemic corticosteroids showing improvement and resolution of the visual field deficits. *MD* mean deviation, *SITA* Swedish Interactive Testing Algorithm

(estimated to be 0.1%), it is not negligible [3]. Molecular mimicry from the viral proteins or the adjuvants used in the preparation of the vaccine have been suspected in the development of demyelinating disease following vaccination [3, 4]. Molecular mimicry occurs when similarities exist between proteins of viruses used in vaccinations and the components of CNS myelin which may disrupt self-tolerance and cause production of autoantibodies resulting in CNS inflammation including optic neuritis [3, 5]. Our case is consistent with other cases of post-vaccination optic neuritis, most of which develop 1–3 weeks after vaccination, typical of an immune-triggered mechanism [3].

In most cases, symptoms of optic neuritis were mostly resolved after treatment with steroids such as intravenously administered methylprednisolone followed by tapered oral prednisolone for several weeks [3, 5]. Early recognition of ocular signs and symptoms of optic neuritis following DTaP-IPV vaccination may lead to prompt treatment and preserved vision.

Conclusions

Although the association between immunizations and the onset of CNS demyelinating conditions is well documented, this report, to the best of our knowledge, is the first case of optic neuritis following DTaP-IPV vaccination. Inclusion of this case report in the medical community will allow for broader understanding of possible conditions that may present shortly after receipt of vaccination.

Abbreviations

A1C: Glycated hemoglobin; ACE: Angiotensin-converting enzyme; ALT: Alanine aminotransferase; ANA: Antinuclear antibody; AST: Aspartate aminotransferase; c-ANCA: Cytoplasmic antineutrophil cytoplasmic antibodies; CNS: Central nervous system; DTaP-IPV: Diphtheria, tetanus, pertussis and inactivated poliovirus combined vaccine; FTA-ABS: Fluorescent treponemal antibody absorption; p-ANCA: Perinuclear antineutrophil cytoplasmic antibodies; RPR: Rapid plasma reagin

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Availability of data and materials
The authors agree to making the images and data described in the manuscript freely available for use.

Authors' contributions
Both PO and RW contributed equally to the design, drafting, and editing of this manuscript. Both authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was sent to the University of Texas at Austin Institutional Review Board and need for further approval was waived.

Consent for publication
Written and informed consent was obtained from the patient for publication of the case report and the accompanying images. Copies of the written consent forms are available for review by the Editor-in-Chief of this journal.

Competing interests
The authors declare that they have no competing interests.

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Optic neuritis in pregnancy after Tdap vaccination: Report of two cases

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Affiliations
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Abstract

Two pregnant women developed one-eye blurring vision within three weeks after Tdap vaccination. Neurophtalmologic and MR examination confirmed an unilateral optic neuritis without evidence of underlying disease. Both patients had a full recovery, one after intravenous metilprednisolone. This is the first report of optic neuritis related with Tdap vaccination in pregnancy.

Keywords: Pertussis vaccination; Postvaccination optic neuritis; Tdap vaccination.

Case Reports Pediatr Infect Dis J. 2011 Jan;30(1):84-6. doi: 10.1097/INF.0b013e3181f11126.

Adverse neurologic reactions after both doses of pandemic H1N1 influenza vaccine with optic neuritis and demyelination

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Affiliations
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Abstract

When a neurologic condition develops after vaccination of a patient, the causal relationship is difficult to determine. We report an unusual case in which neurologic signs occurred in a previously healthy child after both doses of H1N1 2009 influenza vaccine, culminating in bilateral optic neuritis and disseminated encephalomyelitis. A causal association is more likely with repeated injury following influenza vaccination.

Acta Neurol Scand. 1989 Nov;80(5):425-31. doi: 10.1111/j.1600-0404.1989.tb03904.x.

The role of infection and vaccination in the genesis of optic neuritis and multiple sclerosis in children

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Abstract

This article describes the association between previous infection and/or vaccination and the development of optic neuritis (ON) in 18 children. Ten of these children subsequently developed clinically definite multiple sclerosis (MS), while in 8 patients a clinically definite etiology could not be confirmed. Vaccination preceded the first ON attack in 6 patients, all but one of whom subsequently developed MS. It also preceded subsequent demyelinating events in 6 patients. Ten of the patients had a bacterial or viral infection within the 2 weeks prior to the first symptoms of ON. Intrathecal antibody synthesis against 2 or more viruses could be shown in 5 out of 8 patients studied; 5 out of 6 patients had oligoclonal antibodies in CSF and 12 out of 16 patients a high IgG index. Neither intrathecal antibody synthesis against 2 or more viruses nor elevated IgG indexes could be found in the control patients. Measles and mumps occurred at a significantly later age in the children who subsequently developed MS than in the control children, and these patients had significantly more events that might have impaired the blood-brain barrier than the controls. These results indicate that immunological events leading to MS may be triggered during childhood. Vaccination and infection often precede ON in childhood. Intrathecal viral antibody production can occur already in childhood at the time of the first symptoms of MS.

Seizures (Nonfebrile)

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CASE REPORT

Nonfebrile Seizures after Mumps, Measles, Rubella, and Varicella-Zoster Virus Combination Vaccination with Detection of Measles Virus RNA in Serum, Throat, and Urine

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We report the case of a child presenting with nonfebrile seizures 6 and 13 days after the first vaccination with a measles, mumps, rubella, and varicella (MMRV) combination vaccine. Measles virus RNA was detected in the patient's serum, throat, and urine. Genotyping revealed the Schwarz vaccine virus strain.

CASE REPORT

An 11-month-old boy was presented to the pediatric unit after experiencing three seizures in the morning of the same day. The seizures were initiated by a sharp outcry with symmetric tonic-clonic movement of the arms and legs. During the seizures, the child was not reacting to his mother and had cyanotic lips. Seizures stopped spontaneously, without the administration of anticonvulsants, after approximately 1 to 2 min. Immediately after the seizures, body temperature, as measured by the mother as well as by the emergency physician, was not elevated (37.3°C). Upon admission, the child was sleepy but conscious and without signs of meningitis. The child had a slight rash on his trunk and pale skin color; otherwise, the clinical examination was unremarkable.

There was no history of seizures before or any other known medical conditions. Six days before the seizure, the first vaccination with the regular measles, mumps, rubella, and varicella (MMRV) vaccine (Priorix-Tetra; GlaxoSmithKline) was performed. In the meantime, there were no signs of infection or fever. All blood parameters on admission were unremarkable except slight leukopenia of $4.3 \times 10^3/\mu l$ (normal range, 6.0×10^3 to $17.0 \times 10^3/\mu l$). All values determined by testing the cerebrospinal fluid (CSF) taken on admission were within the range of normal (CSF protein, 221 mg/liter [normal range, 150 to 450 mg/liter]; glucose, 64 mg/dl [normal range, 50 to 75 mg/dl]; lactate, 1.4 mmol/liter [normal range, 1.2 to 2.1 mmol/liter]; leukocyte count, 2 cells/μl [normal, <4 cells/μl]; erythrocyte count, 0 cells/μl [normal, 0/μl]). CSF tested negative by PCR or reverse transcription (RT)-PCR for herpes simplex viruses 1 and 2, varicella-zoster virus, rubella virus, mumps virus, and measles virus (MeV) (Table 1). A cranial magnetic resonance scan revealed no pathological findings. In a blood sample and a throat swab taken upon admission as well as in a urine sample collected the following day, MeV RNA was detected by real-time RT-PCR by amplifying a 114-nucleotide fragment of the MeV nucleoprotein N gene.

Viral concentration was low in serum and urine but remarkably higher in the throat swab. Genotyping by amplification of a total of 507 nucleotides of the variable genomic region of the MeV nucleoprotein N gene was performed by using one nested and two heminested PCRs and revealed an MeV genotype A virus. The amplified sequence included the 450 nucleotides encoding the C-terminal 150 amino acids of the MeV nucleoprotein N; this is the minimum amount of data required for determining the MeV genotype, as recommended by the WHO (1). The MeV nucleotide sequence was identical to that of the Schwarz MeV vaccine strain (Fig. 1).

TABLE 1 Detection of measles, mumps, rubella, herpes simplex viruses 1 and 2, and varicella-zoster virus by PCR or RT-PCR in serum, throat swab, urine, and CSF

RNA or DNA[a]	Detection of RNA or DNA in clinical specimens[b]			
	Serum[c]	Throat swab[c]	Urine[d]	CSF[d]
Measles virus RNA	+; <1,000 copies/ml	+; 5.81×10^5 copies/ml	+; <1,000 copies/ml	−
Mumps virus RNA	−	−	−	−
Rubella virus RNA	−	−	−	−
Herpes simplex virus 1 and 2 DNA	−	−	−	−
Varicella-zoster virus DNA	−	−	−	−

[a] Primers and protocols are available upon request.
[b] +, detection of viral genome; −, viral genome not detected.
[c] Specimen collected 6 days postvaccination.
[d] Specimen collected 7 days postvaccination.

No IgG antibodies against measles, mumps, and rubella viruses were detectable upon admission (detection of MeV antibodies was performed by IgG and IgM enzyme-linked immunosorbent assay [ELISA; Enzygnost anti-measles virus/IgG and Enzygnost anti-measles virus/IgM; Siemens Healthcare Diagnostics, Eschborn, Germany]).

After an unremarkable hospital course, a fourth seizure episode occurred on the 13th day after the vaccination, while the child was still in the hospital. While the seizures did not fulfill all criteria of a provoked seizure due to the absence of fever, antiepileptic treatment with levetiracetam was started. The remaining

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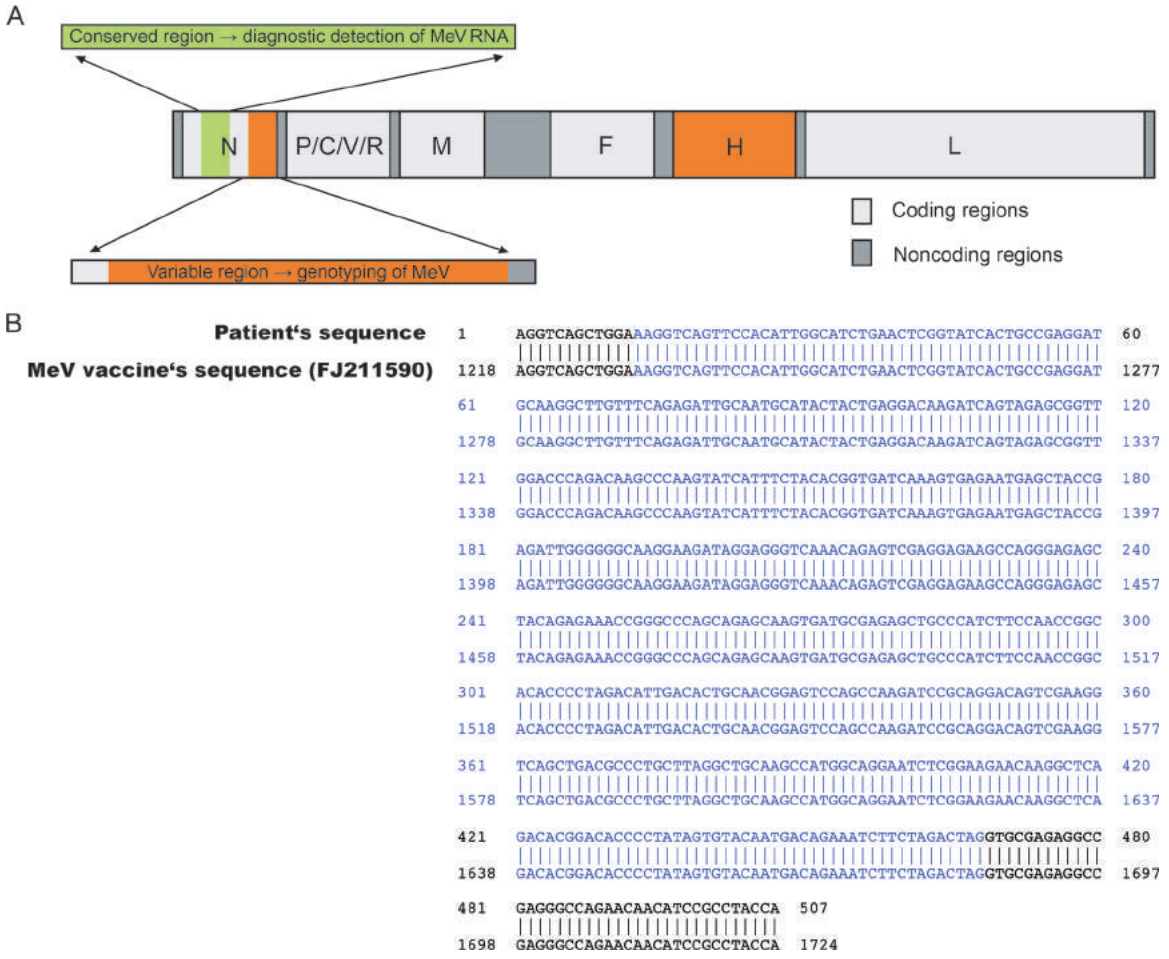

FIG 1 Strategy for identification and genotyping of the measles virus (MeV). (A) Scheme of the MeV genome. The conserved and variable regions used for MeV virus screening and genotyping, respectively, are indicated with arrows. (B) Comparison of the nucleotide sequence for the 507-bp MeV genome fragment obtained from the case patient by the genotyping RT-PCRs (upper rows) and the sequence of the Schwarz MeV vaccine strain (lower rows) (GenBank accession no. FJ211590) using the BLAST algorithm (http://www.ncbi.nlm.nih.gov/blast). Numbers before and after each row refer to the nucleotide position of the respective nucleotide sequence. The minimum requirement for MeV genotyping as defined by WHO within the sequenced genome fragment is indicated in blue.

course of hospitalization was uneventful, and the child was discharged on the 9th day of hospitalization in good health. Temperature was measured regularly during the complete course of disease and was elevated only once, up to 38.3°C, on the 3rd day of hospitalization; however, no seizure was observed in association with this episode.

Regular follow-up visits have not revealed any signs of epilepsy so far, and now, more than 1 year after the vaccination, the child remains well. The therapy with levetiracetam was continued without any side effects. In a blood sample taken 3 months after the vaccination, high antibody values against mumps, measles, and rubella viruses were found, but no antibodies against varicella-zoster virus could be detected.

MeV is one of the most contagious infectious diseases in humans and among the leading causes of death in children (2). Vaccination with live attenuated measles vaccine is the most effective measure for control and eradication (3, 4). Most vaccines used today are based on the Schwarz vaccine strain (genotype A) (5).

Fever is the most common complication of immunization and occurs most often after administration of live attenuated vaccines, toxin-containing vaccines, or whole-cell preparations (6). Adverse events after vaccination against measles, mumps, rubella, and varicella are generally mild. Besides a local reaction at the site of injection, fever, and rash, the most common neurologic adverse events are febrile seizures, commonly 7 to 10 days after vaccination (7, 8). Febrile seizures in general have a favorable outcome and are not associated with neurologic sequelae. While a higher risk for febrile seizures was observed with the MMRV combination vaccine than with the MMR vaccine (8), nonfebrile seizures in association with MMRV or MMR vaccination have not been described so far.

Therefore, a search was performed in the database of the German Federal Institute for Vaccines and Biomedicines (Paul Ehrlich Institute [PEI], Langen, Germany), which collects and evaluates the reports of adverse events, and two further cases were revealed. In the first case, a 9-year-old male experienced convulsions leading to hospitalization 14 days after he had received the second dose of MMRV (MMRVaxPro; Sanofi Pasteur MSD). His symptoms

resolved (the duration of symptoms was unspecified), and the patient was discharged after 2 days of hospitalization. The second case was an 11-month-old female who presented with a tonic-clonic seizure, allergic reaction, and exanthema 1 day after having received an unspecified dose of MMRV (Priorix-Tetra; GlaxoSmithKline) on 10 February 2011. Further seizures without fever in the same child occurred on two more occasions, two and three days after having received vaccinations on 14 March 2011 against diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, hepatitis B, poliomyelitis (Infanrix hexa; GlaxoSmithKline), and *Streptococcus pneumoniae* (Synflorix; GlaxoSmithKline), without any further pathological findings noted in the hospital report. So far, it is not possible based on these cases to assess a causal relationship between nonfebrile seizures and vaccination. Further awareness is necessary to evaluate whether nonfebrile seizures temporally associated with vaccine exposure have to be considered a potential adverse effect. However, it should be stressed that all children with seizures, either febrile or nonfebrile, had a favorable outcome according to available follow-up data.

Even though live attenuated measles vaccines have been used for more than 40 years, data are scarce on the extent to which vaccine virus replicates in or is shed by vaccinees (5). Isolation of infectious vaccine virus from the blood and pharynx of vaccinated children by propagation on canine renal cell culture was successfully performed in early studies with the Edmonston strain (9), from experimentally vaccinated *Cynomolgus* monkeys after vaccination with the Schwarz vaccine strain (10), and in a study evaluating fever and rash appearing 3 to 9 days after measles vaccination (11). In this study, in 6 of 7 children, wild-type virus was isolated from peripheral blood leukocytes or throat swabs, suggesting vaccination during the incubation period of wild-type MeV. In only 1 of 7 patients, vaccine virus (strain Handai) was isolated from blood leukocytes, and this child had the mildest clinical course (mild fever without rash appearing on day 7 after vaccination) (11). It is not stated in the above-mentioned study if the children, in whom Edmonston vaccine virus isolation was achieved, presented with any symptoms or were asymptomatic (9). Further, Edmonston vaccine virus RNA was detected by RT-PCR 13 days after vaccination in the serum of an HIV-positive, 1-year-old boy who presented with measles-like illness 10 days after MMR vaccination (12).

For the Schwarz vaccine strain, there are two case reports about healthy children that describe demonstration of vaccine virus in the throat of a 3-year-old boy (13) and detection of vaccine virus RNA in the throat and urine of a 14-month-old child (14). The first child presented with fever, pharyngitis, and adenopathy 8 days after vaccination. MeV was isolated in cell culture from a throat swab taken 4 days after fever onset. The 14-month-old child in the second case report presented with facial erythema without fever 5 days after vaccination, followed by fever and rash 8 days after vaccination. MeV RNA was detected by RT-PCR from a throat swab taken 5 days and from a urine sample taken 6 days after the onset of fever. In both children, the virus RNA could be characterized as the Schwarz strain, and both children had a favorable follow-up. Taken together, the results from the three reports, including ours, show that Schwarz vaccine strain RNA is present in blood at least at day 6 postvaccination and is detectable in throat and urine at days 7 to 15 postvaccination. However, the clinical relevance of detection of vaccine virus or its RNA from the different body compartments, if any, remains unclear. To the best of our knowledge, so far there are no reports of transmission of vaccine measles virus.

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We report no conflicts of interest.

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SIDS / Death

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Simultaneous sudden infant death syndrome

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Abstract

The simultaneous sudden deaths of twins rarely occur and therefore it has received limited attention in the medical literature. When the deaths of the twins meet the defined criteria for sudden infant death syndrome (SIDS) independently and take place within the same 24 h range it can be called as simultaneous SIDS (SSIDS). The case(s): Twin girls (3.5-month-old) were found dead by their mother in their crib, both in supine position. The infants were identical twins and delivered at a hospital by cesarean section. Both infants were healthy and did not have any serious medical history. Two days prior to the incident, the twins had received the second dose of oral polio, DPT and the first dose of hepatitis B vaccines and they had fever on the first day of the vaccination and been given teaspoonful of acetaminophen. Death scene investigation, judicial investigation, parental assessment, macroscopic and microscopic autopsy findings and the toxicological analysis did not yield any specific cause of death. The case(s) were referred to a supreme board composed of multidisciplinary medical professionals at the Institute of Forensic Medicine, Ministry of Justice, in Istanbul. The Board decided that the available data was consistent with SIDS. These SIDS case(s) are presented because twin SIDS are rare and this is the first time that a simultaneous twin SIDS have been reported in Turkey. Simultaneous SIDS cases have many implications regarding definition, diagnosis and medico-legal approach.

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Infant mortality rates regressed against number of vaccine doses routinely given: Is there a biochemical or synergistic toxicity?

Neil Z Miller and Gary S Goldman

Abstract

The infant mortality rate (IMR) is one of the most important indicators of the socio-economic well-being and public health conditions of a country. The US childhood immunization schedule specifies 26 vaccine doses for infants aged less than 1 year—the most in the world—yet 33 nations have lower IMRs. Using linear regression, the immunization schedules of these 34 nations were examined and a correlation coefficient of $r = 0.70$ ($p < 0.0001$) was found between IMRs and the number of vaccine doses routinely given to infants. Nations were also grouped into five different vaccine dose ranges: 12–14, 15–17, 18–20, 21–23, and 24–26. The mean IMRs of all nations within each group were then calculated. Linear regression analysis of unweighted mean IMRs showed a high statistically significant correlation between increasing number of vaccine doses and increasing infant mortality rates, with $r = 0.992$ ($p = 0.0009$). Using the Tukey-Kramer test, statistically significant differences in mean IMRs were found between nations giving 12–14 vaccine doses and those giving 21–23, and 24–26 doses. A closer inspection of correlations between vaccine doses, biochemical or synergistic toxicity, and IMRs is essential.

Keywords

infant mortality rates, sudden infant death, SIDS, immunization schedules, childhood vaccines, drug toxicology, synergistic effects, linear regression model

Introduction

The infant mortality rate (IMR) is one of the most important measures of child health and overall development in countries. Clean water, increased nutritional measures, better sanitation, and easy access to health care contribute the most to improving infant mortality rates in unclean, undernourished, and impoverished regions of the world.[1–3] In developing nations, IMRs are high because these basic necessities for infant survival are lacking or unevenly distributed. Infectious and communicable diseases are more common in developing countries as well, though sound sanitary practices and proper nutrition would do much to prevent them.[1]

The World Health Organization (WHO) attributes 7 out of 10 childhood deaths in developing countries to five main causes: pneumonia, diarrhea, measles, malaria, and malnutrition—the latter greatly affecting all the others.[1] Malnutrition has been associated with a decrease in immune function. An impaired immune function often leads to an increased susceptibility to infection.[2] It is well established that infections, no matter how mild, have adverse effects on nutritional status. Conversely, almost any nutritional deficiency will diminish resistance to disease.[3]

Despite the United States spending more per capita on health care than any other country,[4] 33 nations have better IMRs. Some countries have IMRs that are less than half the US rate: Singapore, Sweden, and Japan are below 2.80. According to the Centers for Disease Control and Prevention (CDC), "The relative position of the United States in comparison to countries with the lowest infant mortality rates appears to be worsening."[5]

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Table 1. 2009 Infant mortality rates, top 34 nations[8]

Rank	Country	IMR
1	Singapore	2.31
2	Sweden	2.75
3	Japan	2.79
4	Iceland	3.23
5	France	3.33
6	Finland	3.47
7	Norway	3.58
8	Malta	3.75
9	Andorra	3.76
10	Czech Republic	3.79
11	Germany	3.99
12	Switzerland	4.18
13	Spain	4.21
14	Israel	4.22
15	Liechtenstein	4.25
16	Slovenia	4.25
17	South Korea	4.26
18	Denmark	4.34
19	Austria	4.42
20	Belgium	4.44
21	Luxembourg	4.56
22	Netherlands	4.73
23	Australia	4.75
24	Portugal	4.78
25	United Kingdom	4.85
26	New Zealand	4.92
27	Monaco	5.00
28	Canada	5.04
29	Ireland	5.05
30	Greece	5.16
31	Italy	5.51
32	San Marino	5.53
33	Cuba	5.82
34	United States	6.22

CIA. Country comparison: infant mortality rate (2009). *The World Factbook*. www.cia.gov (Data last updated 13 April 2010).[8]

There are many factors that affect the IMR of any given country. For example, premature births in the United States have increased by more than 20% between 1990 and 2006. Preterm babies have a higher risk of complications that could lead to death within the first year of life.[6] However, this does not fully explain why the United States has seen little improvement in its IMR since 2000.[7]

Nations differ in their immunization requirements for infants aged less than 1 year. In 2009, five of the 34 nations with the best IMRs required 12 vaccine doses, the least amount, while the United States required 26 vaccine doses, the most of any nation. To explore the correlation between vaccine doses that nations routinely give to their infants and their infant mortality rates, a linear regression analysis was performed.

Methods and design

Infant mortality

The infant mortality rate is expressed as the number of infant deaths per 1000 live births. According to the US Central Intelligence Agency (CIA), which keeps accurate, up-to-date infant mortality statistics throughout the world, in 2009 there were 33 nations with better infant mortality rates than the United States (Table 1).[8] The US infant mortality rate of 6.22 infant deaths per 1000 live births ranked 34th.

Immunization schedules and vaccine doses

A literature review was conducted to determine the immunization schedules for the United States and all 33 nations with better IMRs than the United States.[9,10] The total number of vaccine doses specified for infants aged less than 1 year was then determined for each country (Table 2). A vaccine dose is an exact amount of medicine or drug to be administered. The number of doses a child receives should not be confused with the number of 'vaccines' or 'injections' given. For example, DTaP is given as a single injection but contains three separate vaccines (for diphtheria, tetanus, and pertussis) totaling three vaccine doses.

Nations organized into data pairs

The 34 nations were organized into data pairs consisting of total number of vaccine doses specified for their infants and IMRs. Consistent with biostatistical conventions, four nations—Andorra, Liechenstein, Monaco, and San Marino—were excluded from the dataset because they each had fewer than five infant deaths, producing extremely wide confidence intervals and IMR instability. The remaining 30 (88%) of the data pairs were then available for analysis.

Nations organized into groups

Nations were placed into the following five groups based on the number of vaccine doses they routinely give their infants: 12–14, 15–17, 18–20, 21–23, and 24–26 vaccine doses. The unweighted IMR means of all nations as a function of the number of vaccine

Table 2. Summary of International Immunization Schedules: vaccines recommended/required prior to one year of age in 34 nations

Nation	Vaccines prior to one year of age	Total[b] doses	Group (range of doses)
Sweden	DTaP (2), Polio (2), Hib (2), Pneumo (2)	12	1 (12–14)
Japan	DTaP (3), Polio (2), BCG	12	
Iceland	DTaP (2), Polio (2), Hib (2), MenC (2)	12	
Norway	DTaP (2), Polio (2), Hib (2), Pneumo (2)	12	
Denmark	DTaP (2), Polio (2), Hib (2), Pneumo (2)	12	
Finland	DTaP (2), Polio (2), Hib (2), Rota (3)	13	
Malta	DTaP (3), Polio (3), Hib (3)	15	2 (15–17)
Slovenia	DTaP (3), Polio (3), Hib (3)	15	
South Korea	DTaP (3), Polio (3), HepB (3)	15	
Singapore	DTaP (3), Polio (3), HepB (3), BCG, Flu	17	
New Zealand	DTaP (3), Polio (3), Hib (2), HepB (3)	17	
Germany	DTaP (3), Polio (3), Hib (3), Pneumo (3)	18	3 (18–20)
Switzerland	DTaP (3), Polio (3), Hib (3), Pneumo (3)	18	
Israel	DTaP (3), Polio (3), Hib (3), HepB (3)	18	
Liechtenstein[a]	DTaP (3), Polio (3), Hib (3), Pneumo (3)	18	
Italy	DTaP (3), Polio (3), Hib (3), HepB (3)	18	
San Marino[a]	DTaP (3), Polio (3), Hib (3), HepB (3)	18	
France	DTaP (3), Polio (3), Hib (3), Pneumo (2), HepB (2)	19	
Czech Republic	DTaP (3), Polio (3), Hib (3), HepB (3), BCG	19	
Belgium	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (2)	19	
United Kingdom	DTaP (3), Polio (3), Hib (3), Pneumo (2), MenC (2)	19	
Spain	DTaP (3), Polio (3), Hib (3), HepB (3), MenC (2)	20	
Portugal	DTaP (3), Polio (3), Hib (3), HepB (3), MenC (2), BCG	21	4 (21–23)
Luxembourg	DTaP (3), Polio (3), Hib (3), HepB (2), Pneumo (3), Rota (3)	22	
Cuba	DTaP (3), Polio (3), Hib (3), HepB (4), MenBC (2), BCG	22	
Andorra[a]	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), MenC (2)	23	
Austria	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), Rota (2)	23	
Ireland	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (2), MenC (2), BCG	23	
Greece	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), MenC (2)	23	
Monaco[a]	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), HepA, BCG	23	
Netherlands	DTaP (4), Polio (4), Hib (4), Pneumo (4)	24	5 (24–26)
Canada	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), MenC (2), Flu	24	
Australia	DTaP (3), Polio (3), Hib (3), HepB (4), Pneumo (3), Rota (2)	24	
United States	DTaP (3), Polio (3), Hib (3), HepB (3), Pneumo (3), Rota (3), Flu (2)	26	

[a] These four nations were excluded from the analysis because they had fewer than five infant deaths.
[b] DTaP is administered as a single shot but contains three separate vaccines (for diphtheria, tetanus, and pertussis). Thus, DTaP given three times in infancy is equivalent to nine vaccine doses.
Immunization schedules are for 2008–2009.[9,10]

doses were analyzed using linear regression. The Pearson correlation coefficient (r) and coefficient of determination (r^2) were calculated using GraphPad Prism, version 5.03 (GraphPad Software, San Diego, CA, USA, www.graphpad.com). Additionally, the F statistic and corresponding p values were computed to test if the best fit slope was statistically significantly non-zero. The Tukey-Kramer test was used to determine whether or not the mean IMR differences between the groups were statistically significant. Following the one-way ANOVA (analysis of variance) results from the Tukey-Kramer test, a post test for the overall linear trend was performed.

Results

Nations organized into data pairs

A scatter plot of each of the 30 nation's IMR versus vaccine doses yielded a linear relationship with a correlation coefficient of 0.70 (95% CI, 0.46–0.85) and $p < 0.0001$ providing evidence of a positive correlation: IMR and vaccine doses tend to increase together.

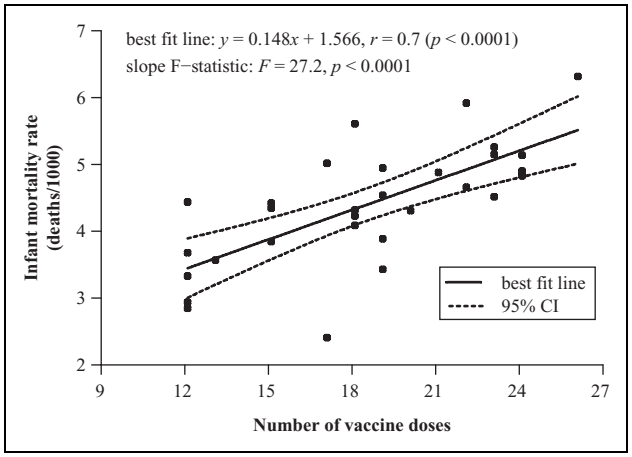

Figure 1. 2009 Infant mortality rates and number of vaccine doses for 30 nations.

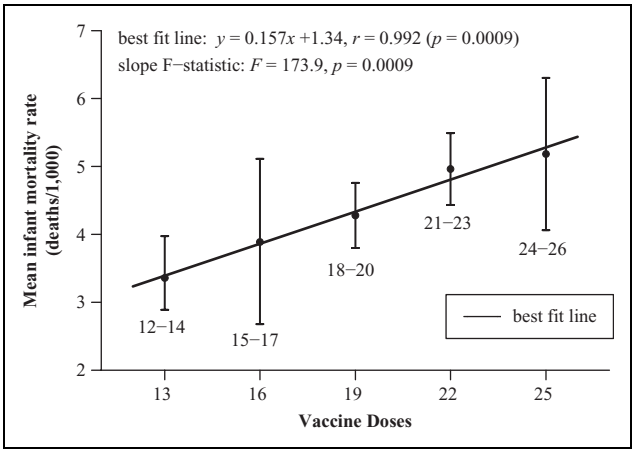

Figure 2. 2009 Mean infant mortality rates and mean number of vaccine doses (five categories).

The F statistic applied to the slope [0.148 (95% CI, 0.090–0.206)] is significantly non-zero, with $F = 27.2$ ($p < 0.0001$; Figure 1).

Nations organized into groups

The unweighted mean IMR of each category was computed by simply summing the IMRs of each nation comprising a group and dividing by the number of nations in that group. The IMRs were as follows: 3.36 (95% CI, 2.74–3.98) for nations specifying 12–14 doses (mean 13 doses); 3.89 (95% CI, 2.68–5.12) for 15–17 doses (mean 16 doses); 4.28 (95% CI, 3.80–4.76) for 18–20 doses (mean 19 doses); 4.97 (95% CI, 4.44–5.49) for 21–23 doses (mean 22 doses); 5.19 (95% CI, 4.06–6.31) for 24-26 doses (mean 25 doses; Figure 2). Linear regression analysis yielded an equation of the best fit line, $y = 0.157x + 1.34$ with $r = 0.992$ ($p = 0.0009$) and $r^2 = 0.983$. Thus, 98.3% of the variation in mean IMRs is explained by the linear model. Again, the F statistic yielded a significantly non-zero slope, with $F = 173.9$ ($p = 0.0009$).

The one-way ANOVA using the Tukey-Kramer test yielded $F = 650$ with $p = 0.001$, indicating the five mean IMRs corresponding to the five defined dose categories are significantly different ($r^2 = 0.510$). Tukey's multiple comparison test found statistical significance in the differences between the mean IMRs of those nations giving 12–14 vaccine doses and (a) those giving 21–23 doses (1.61, 95% CI, 0.457–2.75) and (b) those giving 24–26 doses (1.83, 95% CI, 0.542–3.11).

Discussion

Basic necessities for infant survival

It is instructive to note that many developing nations require their infants to receive multiple vaccine doses and have national vaccine coverage rates (a percentage of the target population that has been vaccinated) of 90% or better, yet their IMRs are poor. For example, Gambia requires its infants to receive 22 vaccine doses during infancy and has a 91%–97% national vaccine coverage rate, yet its IMR is 68.8. Mongolia requires 22 vaccine doses during infancy, has a 95%–98% coverage rate, and an IMR of 39.9.[8,9] These examples appear to confirm that IMRs will remain high in nations that cannot provide clean water, proper nutrition, improved sanitation, and better access to health care. *As developing nations improve in all of these areas a critical threshold will eventually be reached where further reductions of the infant mortality rate will be difficult to achieve because most of the susceptible infants that could have been saved from these causes would have been saved.* Further reductions of the IMR must then be achieved in areas outside of these domains. As developing nations ascend to higher socio-economic living standards, a closer inspection of all factors contributing to infant deaths must be made.

Crossing the socio-economic threshold

It appears that at a certain stage in nations' movement up the socio-economic scale—after the basic necessities for infant survival (proper nutrition, sanitation, clean water, and access to health care) have been met—a counter-intuitive relationship occurs between

the number of vaccines given to infants and infant mortality rates: nations with higher (worse) infant mortality rates give their infants, on average, more vaccine doses. This positive correlation, derived from the data and demonstrated in Figures 1 and 2, elicits an important inquiry: are some infant deaths associated with over-vaccination?

A closer inspection of infant deaths

Many nations adhere to an agreed upon International Classification of Diseases (ICD) for grouping infant deaths into 130 categories.[11–13] Among the 34 nations analyzed, those that require the most vaccines tend to have the worst IMRs. Thus, we must ask important questions: is it possible that some nations are requiring too many vaccines for their infants and the additional vaccines are a toxic burden on their health? Are some deaths that are listed within the 130 infant mortality death categories really deaths that are associated with over-vaccination? Are some vaccine-related deaths hidden within the death tables?

Sudden infant death syndrome (SIDS)

Prior to contemporary vaccination programs, 'Crib death' was so infrequent that it was not mentioned in infant mortality statistics. In the United States, national immunization campaigns were initiated in the 1960s when several new vaccines were introduced and actively recommended. For the first time in history, most US infants were required to receive several doses of DPT, polio, measles, mumps, and rubella vaccines.[14] Shortly thereafter, in 1969, medical certifiers presented a new medical term—sudden infant death syndrome.[15,16] In 1973, the National Center for Health Statistics added a new cause-of-death category—for SIDS—to the ICD. SIDS is defined as the sudden and unexpected death of an infant which remains unexplained after a thorough investigation. Although there are no specific symptoms associated with SIDS, an autopsy often reveals congestion and edema of the lungs and inflammatory changes in the respiratory system.[17] By 1980, SIDS had become the leading cause of postneonatal mortality (deaths of infants from 28 days to one year old) in the United States.[18]

In 1992, to address the unacceptable SIDS rate, the American Academy of Pediatrics initiated a 'Back to Sleep' campaign, convincing parents to place their infants supine, rather than prone, during sleep. From 1992 to 2001, the postneonatal SIDS rate dropped by an average annual rate of 8.6%. However, other causes of sudden unexpected infant death (SUID) increased. For example, the postneonatal mortality rate from 'suffocation in bed' (ICD-9 code E913.0) increased during this same period at an average annual rate of 11.2%. The postneonatal mortality rate from 'suffocation-other' (ICD-9 code E913.1-E913.9), 'unknown and unspecified causes' (ICD-9 code 799.9), and due to 'intent unknown' in the External Causes of Injury section (ICD-9 code E980-E989), all increased during this period as well.[18] (In Australia, Mitchell et al. observed that when the SIDS rate decreased, deaths attributed to asphyxia increased.[19] Overpeck et al. and others, reported similar observations.)[20,21]

A closer inspection of the more recent period from 1999 to 2001 reveals that the US postneonatal SIDS rate continued to decline, but *there was no significant change in the total postneonatal mortality rate.* During this period, the number of deaths attributed to 'suffocation in bed' and 'unknown causes,' increased significantly. According to Malloy and MacDorman, "If death-certifier preference has shifted such that previously classified SIDS deaths are now classified as 'suffocation,' the inclusion of these suffocation deaths and unknown or unspecified deaths with SIDS deaths then accounts for about 90 percent of the decline in the SIDS rate observed between 1999 and 2001 and results in a non-significant decline in SIDS"[18] (Figure 3).

Is there evidence linking SIDS to vaccines?

Although some studies were unable to find correlations between SIDS and vaccines,[22–24] there is some evidence that a subset of infants may be more susceptible to SIDS shortly after being vaccinated. For example, Torch found that two-thirds of babies who had died from SIDS had been vaccinated against DPT (diphtheria–pertussis–tetanus toxoid) prior to death. Of these, 6.5% died within 12 hours of vaccination; 13% within 24 hours; 26% within 3 days; and 37%, 61%, and 70% within 1, 2, and 3 weeks, respectively. Torch also found that unvaccinated babies who died of SIDS did so most often in the fall or winter while vaccinated babies died most often at 2 and 4 months—the same ages when initial doses of DPT were given to infants. He concluded that DPT "may be a generally unrecognized major cause of sudden infant and early childhood death, and that the risks of immunization may outweigh its potential benefits. A need for re-evaluation and possible modification of

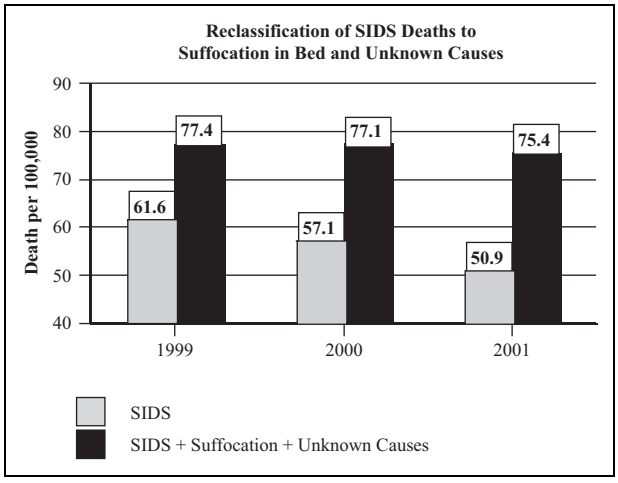

Figure 3. Reclassification of sudden infant death syndrome (SIDS) deaths to suffocation in bed and unknown causes. The postneonatal SIDS rate appears to have declined from 61.6 deaths (per 100,000 live births) in 1999 to 50.9 in 2001. However, during this period there was a significant increase in postneonatal deaths attributed to suffocation in bed and due to unknown causes. When these sudden unexpected infant deaths (SUIDs) are combined with SIDS deaths, the total SIDS rate remains relatively stable, resulting in a non-significant decline.

current vaccination procedures is indicated by this study."[25] Walker et al. found "the SIDS mortality rate in the period zero to three days following DPT to be 7.3 times that in the period beginning 30 days after immunization."[26] Fine and Chen reported that babies died at a rate nearly eight times greater than normal within 3 days after getting a DPT vaccination.[27]

Ottaviani et al. documented the case of a 3-month-old infant who died suddenly and unexpectedly shortly after being given six vaccines in a single shot: "Examination of the brainstem on serial sections revealed bilateral hypoplasia of the arcuate nucleus. The cardiac conduction system presented persistent fetal dispersion and resorptive degeneration. This case offers a unique insight into the possible role of hexavalent vaccine in triggering a lethal outcome in a vulnerable baby." Without a full necropsy study in the case of sudden, unexpected infant death, at least some cases linked to vaccination are likely to go undetected.[28]

Reclassified infant deaths

It appears as though some infant deaths attributed to SIDS may be vaccine related, perhaps associated with biochemical or synergistic toxicity due to over-vaccination. Some infants' deaths categorized as 'suffocation' or due to 'unknown and unspecified causes' may also be cases of SIDS reclassified within the ICD. Some of these infant deaths may be vaccine related as well. This trend toward reclassifying ICD data is a great concern of the CDC "because inaccurate or inconsistent cause-of-death determination and reporting hamper the ability to monitor national trends, ascertain risk factors, and design and evaluate programs to prevent these deaths."[29] If some infant deaths are vaccine related and concealed within the various ICD categories for SUIDs, is it possible that other vaccine-related infant deaths have also been reclassified?

Of the 34 nations that have crossed the socio-economic threshold and are able to provide the basic necessities for infant survival—clean water, nutrition, sanitation, and health care—several require their infants to receive a relatively high number of vaccine doses and have relatively high infant mortality rates. These nations should take a closer look at their infant death tables to determine if some fatalities are possibly related to vaccines though reclassified as other causes. Of course, all SUID categories should be re-inspected. Other ICD categories may be related to vaccines as well. For example, a new live-virus orally administered vaccine against rotavirus-induced diarrhea—Rotarix®—was licensed by the European Medicine Agency in 2006 and approved by the US Food and Drug Administration (FDA) in 2008. However, in a clinical study that evaluated the safety of the Rotarix vaccine, *vaccinated babies died at a higher rate than non-vaccinated babies*—mainly due to a statistically significant increase in pneumonia-related fatalities.[30] (One biologically plausible explanation is that natural rotavirus infection might have a protective effect against respiratory infection.)[31] Although these fatalities appear to be vaccine related and raise a nation's infant mortality rate, medical certifiers are likely to misclassify these deaths as pneumonia.

Several additional ICD categories are possible candidates for incorrect infant death classifications: unspecified viral diseases, diseases of the blood, septicemia, diseases of the nervous system, anoxic brain damage, other diseases of the nervous system, diseases of the respiratory system, influenza, and unspecified diseases of the respiratory system. All of these selected causes may be repositories of vaccine-related infant deaths reclassified as common fatalities. All nations—rich and poor, industrialized and developing—have an obligation to determine whether their immunization schedules are achieving

their desired goals. Progress on reducing infant mortality rates should include monitoring vaccine schedules and medical certification practices to ascertain whether vaccine-related infant deaths are being reclassified as ordinary mortality in the ICD.

How many infants can be saved with an improved IMR?

Slight improvements in IMRs can make a substantial difference. In 2009, there were approximately 4.5 million live births and 28,000 infant deaths in the United States, resulting in an infant mortality rate of 6.22/1000. If health authorities can find a way to reduce the rate by 1/1000 (16%), the United States would rise in international rank from 34th to 31st and about 4500 infants would be saved.

Limitations of study and potential confounding factors

This analysis did not adjust for vaccine composition, national vaccine coverage rates, variations in the infant mortality rates among minority races, preterm births, differences in how some nations report live births, or the potential for ecological bias. A few comments about each of these factors are included below.

Vaccine composition

This analysis calculated the total number of vaccine doses received by children but did not differentiate between the substances, or quantities of those substances, in each dose. Common vaccine substances include antigens (attenuated viruses, bacteria, toxoids), preservatives (thimerosal, benzethonium chloride, 2-phenoxyethanol, phenol), adjuvants (aluminum salts), additives (ammonium sulfate, glycerin, sodium borate, polysorbate 80, hydrochloric acid, sodium hydroxide, potassium chloride), stabilizers (fetal bovine serum, monosodium glutamate, human serum albumin, porcine gelatin), antibiotics (neomycin, streptomycin, polymyxin B), and inactivating chemicals (formalin, glutaraldehyde, polyoxyethylene). For the purposes of this study, all vaccine doses were equally weighted.

Vaccine coverage rates

No adjustment was made for national vaccine coverage rates—a percentage of the target population that received the recommended vaccines. However, most of the nations in this study had coverage rates in the 90%–99% range for the most commonly recommended vaccines—DTaP, polio, hepatitis B, and Hib (when these vaccines were included in the schedule). Therefore, this factor is unlikely to have impacted the analyses.[9]

Minority races

It has been argued that the US IMR is poor in comparison to many other nations because African–American infants are at greater risk of dying relative to White infants, perhaps due to genetic factors or disparities in living standards. However, in 2006 the US IMR for infants of all races was 6.69 and the IMR for White infants was 5.56.[13] In 2009, this improved rate would have moved the United States up by just one rank internationally, from 34th place to 33rd place.[8] In addition, the IMRs for Hispanics of Mexican descent and Asian–Americans in the United States are significantly lower than the IMR for Whites.[6] Thus, diverse IMRs among different races in the Unites States exert only a modest influence over the United States' international infant mortality rank.

Preterm births

Preterm birth rates in the United States have steadily increased since the early 1980s. (This rise has been tied to a greater reliance on caesarian deliveries, induced labor, and more births to older mothers.) Preterm babies are more likely than full-term babies to die within the first year of life. About 12.4% of US births are preterm. In Europe, the prevalence rate of premature birth ranges from 5.5% in Ireland to 11.4% in Austria. Preventing preterm births is essential to lower infant mortality rates. However, it is important to note that some nations such as Ireland and Greece, which have very low preterm birth rates (5.5% and 6%, respectively) compared to the United States, require their infants to receive a relatively high number of vaccine doses (23) and have correspondingly high IMRs. Therefore, reducing preterm birth rates is only part of the solution to reduce IMRs.[6,32]

Differences in reporting live births

Infant mortality rates in most countries are reported using WHO standards, which do not include any reference to the duration of pregnancy or weight of the infant, but do define a 'live birth' as a baby born with any signs of life for any length of time.[12] However,

four nations in the dataset—France, the Czech Republic, the Netherlands, and Ireland—do not report live births entirely consistent with WHO standards. These countries add an additional requirement that live babies must also be at least 22 weeks of gestation or weigh at least 500 grams. If babies do not meet this requirement and die shortly after birth, they are reported as stillbirths. This inconsistency in reporting live births artificially lowers the IMRs of these nations.[32,33] According to the CDC, "There are some differences among countries in the reporting of very small infants who may die soon after birth. However, it appears unlikely that differences in reporting are the primary explanation for the United States' relatively low international ranking."[32] Nevertheless, when the IMRs of France, the Czech Republic, the Netherlands, and Ireland were adjusted for known underreporting of live births and the 30 data pairs retested for significance, the correlation coefficient improved from 0.70 to 0.74 (95% CI, 0.52–0.87).

Ecological bias

Ecological bias occurs when relationships among individuals are inferred from similar relationships observed among groups (or nations). Although most of the nations in this study had 90%–99% of their infants fully vaccinated, without additional data we do not know whether it is the vaccinated or unvaccinated infants who are dying in infancy at higher rates. However, respiratory disturbances have been documented in close proximity to infant vaccinations, and lethal changes in the brainstem of a recently vaccinated baby have been observed. Since some infants may be more susceptible to SIDS shortly after being vaccinated, and babies vaccinated against diarrhea died from pneumonia at a statistically higher rate than non-vaccinated babies, there is plausible biologic and causal evidence that the observed correlation between IMRs and the number of vaccine doses routinely given to infants should not be dismissed as ecological bias.

Conclusion

The US childhood immunization schedule requires 26 vaccine doses for infants aged less than 1 year, the most in the world, yet 33 nations have better IMRs. Using linear regression, the immunization schedules of these 34 nations were examined and a correlation coefficient of 0.70 ($p < 0.0001$) was found between IMRs and the number of vaccine doses routinely given to infants. When nations were grouped into five different vaccine dose ranges (12–14, 15–17, 18–20, 21–23, and 24–26), 98.3% of the total variance in IMR was explained by the unweighted linear regression model. These findings demonstrate a counter-intuitive relationship: *nations that require more vaccine doses tend to have higher infant mortality rates*.

Efforts to reduce the relatively high US IMR have been elusive. Finding ways to lower preterm birth rates should be a high priority. However, preventing premature births is just a partial solution to reduce infant deaths. A closer inspection of correlations between vaccine doses, biochemical or synergistic toxicity, and IMRs, is essential. All nations—rich and poor, advanced and developing—have an obligation to determine whether their immunization schedules are achieving their desired goals.

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In the United States Court of Federal Claims

OFFICE OF SPECIAL MASTERS

Filed: July 10, 2017

CHASE BOATMON & MAURINA CUPID, *parents of* J.B., *deceased*,	*	PUBLISHED DECISION
	*	No. 13-611V
Petitioners,	*	Special Master Gowen
v.	*	Entitlement Decision; Diphtheria-Tetanus-acellular Pertussis (DTaP) Vaccine; Inactivated Polio Vaccine (IPV); Haemophilus Influenzae (HiB) Vaccine; Pneumococcal Conjugate (PCV) Vaccine; Rotavirus Vaccine; Sudden Infant Death Syndrome (SIDS).
SECRETARY OF HEALTH AND HUMAN SERVICES,	*	
Respondent.	*	

Ronald C. Homer & Joseph M. Pepper, Conway, Homer P.C., Boston, MA, for petitioners. Lara A. Englund & Ryan M. Pyles, United States Department of Justice, Washington, DC, for respondent.[1]

RULING ON ENTITLEMENT[2]

On August 27, 2013, Chase Boatmon and Maurina Cupid ("petitioners"), as the representatives of the estate of their deceased minor child, J.B., filed a petition under the National Vaccine Injury Compensation Program ("Vaccine Act" or the "Program"),[3] 42 U.S.C. § 300aa-10 *et. seq.* (2012). Petitioners allege that as a result of receiving vaccinations for

[1] Mr. Homer is petitioners' attorney of record, while his colleague Mr. Pepper appeared at the entitlement hearing. Similarly, for respondent, Ms. Englund has always been the attorney of record, but Mr. Pyles appeared at the entitlement hearing.

[2] Because this decision contains a reasoned explanation for the action in this case, the undersigned intends to post it on the website of the United States Court of Federal Claims, pursuant to the E-Government Act of 2002, *see* 44 U.S.C. § 3501 note (2012). The court's website is at http://www.uscfc.uscourts.gov/aggregator/sources/7. Before the decision is posted on the court's website, each party has 14 days to file a motion requesting redaction "of any information furnished by that party: (1) that is a trade secret or commercial or financial in substance and is privileged or confidential; or (2) that includes medical files or similar files, the disclosure of which would constitute a clearly unwarranted invasion of privacy." Vaccine Rule 18(b). "An objecting party must provide the court with a proposed redacted version of the decision." *Id.* If neither party files a motion for redaction within 14 days, the decision will be posted on the court's website. *Id.*

[3] The National Vaccine Injury Compensation Program is set forth in Part 2 of the National Childhood Vaccine Injury Act of 1986, Pub. L. No. 99-660, 100 Stat. 3705, codified as amended, 42 U.S.C. §§ 300aa-1 to -34 (2012). All citations in this decision to individual sections of the Vaccine Act are to 42 U.S.C. § 300aa.

Diphtheria-Tetanus-acellular Pertussis ("DTaP"), inactivated polio ("IPV"), haemophilus influenzae ("HiB"), Pneumococcal Conjugate ("PCV"), and Rotavirus vaccinations on September 2, 2011, J.B. passed away from Sudden Infant Death Syndrome ("SIDS") on September 3, 2011. *See* Petition (ECF No. 1); Amended Petition (ECF No. 15).

After carefully analyzing and weighing all of the evidence and testimony presented in this case in accordance with the applicable legal standards, the undersigned finds that petitioners have met their legal burden. Petitioners have put forth preponderant evidence that the vaccines J.B. received on September 2, 2011 actually caused or substantially contributed to his death from Sudden Infant Death Syndrome. Furthermore, respondent has failed to put forth preponderant evidence that J.B.'s death was in fact caused by factors unrelated to the vaccines. Accordingly, petitioners are entitled to compensation.

I. BACKGROUND

A. Procedural History

Petitioners filed a petition for compensation pursuant to the Vaccine Act on behalf of their deceased minor son, J.B., on August 27, 2013. Petition (ECF No. 1). They filed an amended petition on February 6, 2014. Amended Petition (ECF No. 15). Petitioners filed the expert report of Dr. Douglas C. Miller, a neuropathologist, along with the medical literature referenced in his report, on May 20, 2014. Exhibit 13, 14 (ECF No. 21).[4]

On September 9, 2014, respondent filed a Rule 4(c) report advising against compensation. Rule 4(c) Report (ECF No. 28). That same day, he filed an expert report and medical literature referenced therein from Dr. Brent Harris, a pathologist. Exhibit A (ECF No. 29). Respondent also filed an expert report and medical literature from Dr. Christine T. McCusker. Exhibit C (ECF Nos. 30-32). Petitioners filed a supplemental report from Dr. Miller on November 10, 2014. Exhibit 16 (ECF No. 35). Extensive and detailed medical literature was submitted in support of all of the expert reports.[5]

At numerous stages of this case, the undersigned encouraged the parties to pursue the possibility of an informal resolution and/or to consider mediation. *See, e.g.*, Order filed December 9, 2014 (ECF No. 37). The parties ultimately did not settle the case. An entitlement hearing was held on Thursday, August 6, and Friday, August 7, 2015, in Washington, D.C. Dr. Miller testified on behalf of petitioners, and Dr. Harris and Dr. McCusker testified for respondent. The case was well tried and involved detailed expert testimony from both sides. *See*

[4] On October 14, 2014, petitioners refiled the medical literature cited in Dr. Miller's report, highlighting the specific portions being relied upon to support causation. Petitioners' Notice of Refiling Documents (ECF No. 34).

[5] I have read and digested all of the literature submitted in this case and will reference numerous but not all articles in the course of this opinion. However, all articles have been considered in coming to a conclusion in this case. More recent articles, particularly those by the same authors or groups, are referenced more frequently because they incorporate, build upon, and update the earlier literature. Petitioners and Dr. Miller filed Exhibits 13-A through 13-V and Exhibits 14 through 21. Respondent and Dr. Harris filed Exhibits A-1 through A-6. Respondent and Dr. McCusker submitted Exhibits C-1 through C-20 and Exhibits D through G.

Transcript filed on September 9, 2015 (ECF Nos. 50, 52). Petitioners filed their post-hearing brief on December 7, 2015. (ECF No. 61). Respondent filed his post-hearing brief on March 7, 2016. (ECF No. 63). Petitioners filed their reply to respondent's post-hearing brief on March 28, 2016. (ECF No. 64). This matter is now ripe for adjudication.

B. Standards for Adjudication

The Vaccine Act established the Program to compensate vaccine-related injuries and deaths. § 300aa-10(a). "Congress designed the Vaccine Program to supplement the state law civil tort system as a simple, fair and expeditious means for compensating vaccine-related injured persons. The Program was established to award 'vaccine-injured persons quickly, easily, and with certainty and generosity.'" *Rooks v. Sec'y of Health & Human Servs.*, 35 Fed. Cl. 1, 7 (1996) (quoting H.R. Rep. No. 908 at 3, reprinted in 1986 U.S.C.C.A.N. at 6287, 6344).

There are two avenues to compensation under the Program. The first is to demonstrate a "Table injury," that is, a specified injury within a specified period of time following administration of a vaccine listed on the Vaccine Injury Table. § 300aa-14(a). A Table injury creates a presumption of causation, which is only defeated if respondent shows that the injury was caused by a factor or factors unrelated to the vaccine. In the present case, petitioners allege that J.B. died suddenly of a cause that remained unexplained after a site investigation and autopsy, often referred to as SIDS, shortly after receiving various vaccines listed on the Table. The Table does not list SIDS occurring in any period of time after any vaccine.

Therefore, petitioners must take the second avenue towards compensation: they must establish an "off-Table injury," meaning that the vaccine(s) were the cause in fact of the vaccinee's injuries. In *Althen*, the Federal Circuit established a three-prong test: petitioners must establish (1) a medical theory causally connecting the vaccination and the injury; (2) a logical sequence of cause and effect showing that the vaccination was the reason for the injury; and (3) a proximate temporal relationship between vaccination and injury. *Althen v. Sec'y of Health & Human Servs.*, 418 F.3d 1274, 1278 (Fed. Cir. 2005).

The legal standard is by a preponderance of the evidence." §300aa-13(a)(1)(a). This does not require "conclusive scientific evidence" or "certainty." *Moberly v. Sec'y of Health & Human Servs.*, 592 F.3d 1315, 1322 (Fed. Cir. 2010). Instead, the standard has been interpreted to mean that a fact is more likely than not. *Id.* at 1322 n.2. The Federal Circuit has observed that this preponderance standard enables "the finding of causation in a field bereft of complete and direct proof of how the vaccines affect the human body." *Althen*, 418 F.3d at 1280. Petitioners must establish each *Althen* prong by the preponderance of the evidence. *Caves v. Sec'y of Health & Human Servs.*, 100 Fed. Cl. 119, 132 (2011), *aff. per curiam*, 463 Fed. Appx. 932 (Fed. Cir. 2012).

Each *Althen* prong may be satisfied by medical records or a medical opinion. *Althen*, 418 F.3d at 1279; *see also Capizzano v. Sec'y of Health & Human Servs.*, 440 F.3d 1317, 1326 (Fed. Cir. 2006) (noting that the same piece of evidence can support several *Althen* prongs). Petitioners are not required to provide "objective confirmation" by way of "medical

documentation." *Id.* at 1278. Such a requirement would "contravene the plain language of the statute." *Id.* at 1281.

In determining whether a petitioner is entitled to compensation, a special master must consider the entire record and is not bound by any particular piece of evidence. § 13(b)(1) (stating that a special master is not bound by any "diagnosis, conclusion, judgment, test result, report, or summary" contained in the record). Thus, a special master must weigh and evaluate opposing expert opinions, medical and scientific evidence, and the evidentiary record in deciding whether petitioners have met their burden of proof.

Epidemiological studies, or the lack thereof, are not dispositive of the causation in fact determination. *Grant v. Sec'y of Health & Human Servs.*, 956 F.2d 1144, 1149 (Fed. Cir. 1992). Indeed, petitioners are not required to present medical literature or epidemiological evidence to establish any *Althen* prong. *Andreu v. Sec'y of Health & Human Servs.*, 569 F.3d 1367, 1380 (Fed. Cir. 2009). However, the special master can consider [epidemiological evidence] in reaching an informed judgment as to whether a particular vaccination likely caused a particular injury…. Medical literature and epidemiological evidence must be viewed… not through the lens of the laboratorian, but instead from the vantage point of the Vaccine Act's preponderant evidence standard." *Andreu*, 569 F.3d at 1380.

Under the second *Althen* prong, petitioners need to show that the vaccine(s) was "not only a but-for cause of the injury but also a substantial factor in bringing about the injury." *Shyface v. Sec'y of Health & Human Servs.*, 165 F.3d 1344, 1352-53 (Fed. Cir. 1999). They do not need to show that the vaccine(s) was the "sole" or even the "predominant" cause. *Id.* at 1352. For example, in *Shyface*, the Federal Circuit affirmed that petitioners were entitled to compensation, based on their expert's testimony that the vaccine together with a bacterial infection caused the child's high fever and death (although the expert could not testify that the vaccine was the "sole" or "predominant" cause. 165 F.3d at 1353.

Showing a logical sequence of cause and effect between the vaccine(s) and the injury will tend to show that the injury was not caused by an alternative cause. However, a petitioner is not required to eliminate all possible alternative causes of the injury. *See Walter v. Sec'y of Health & Human Servs.*, 485 F.3d 1146, 1150 (Fed. Cir. 2007) ("the Vaccine Act does not require the petitioner to bear the burden of eliminating alternative causes where the other evidence on causation is sufficient to establish a *prima facie* case"). This standard permits the use of "circumstantial evidence" and accomplishes Congress's goal that "close calls regarding causation are resolved in favor of injured claimants." *Althen*, 165 F.3d at 1280.

Once a petitioner fulfills the *Althen* test, the burden of persuasion shifts to respondent to show that the alleged injury was caused by a factor unrelated to the vaccination. *Knudsen*, 35 F.3d 543 at 548; § 13(a)(1)(B). Respondent has the burden of demonstrating that "a factor unrelated to the vaccination is the more likely or principal cause of the injury alleged. Such a showing establishes that the factor unrelated, not the vaccination, was 'principally responsible' for the injury." *Deribeaux v. Sec'y of Health & Human Servs.*, 717 F.3d 1363, 1369 (Fed. Cir. 2013). Section 13(a)(2) specifies that factors unrelated "[do]not include any idiopathic, unexplained, unknown, hypothetical, or undocumented causal factor, injury, illness, or

condition." Close calls regarding causation must be resolved in favor of the petitioner. *Althen*, 418 F.3d at 1280; *Knudsen*, 35 F.3d at 551 ("If the evidence (on alternative cause) is seen in equipoise, then the government has failed in its burden of persuasion and compensation must be awarded.

C. Summary of Relevant Facts

J.B. was born on April 7, 2011, when his mother became pre-eclamptic and underwent a Caesarean section. Exhibit 1 at 10. J.B. was born 4 weeks prematurely at 36 weeks gestation. Exhibit 2 at 3. The mother's medical records report no history of tobacco, alcohol, or illicit drugs. Exhibit 1 at 3. At birth, J.B. was noted to be "well appearing, non-dysmorphic[,] alert and in no acute distress." Exhibit 2 at 9. His Apgar scores[6] were 8 at 1 minute and 9 at 5 minutes. Exhibit 2 at 9. J.B. and his mother are both noted to be African-American. Exhibit 2 at 3, 25.

On April 14, 2011, one week after birth, J.B. received his first Hep B vaccination. Exhibit 2 at 82.[7] At his two-week well baby visit on April 21, 2011, J.B. was "well appearing, alert . . . a healthy appearing 2 [week] old with normal growth and development." *Id.* at 79-81. On June 7, 2011, J.B. – exhibiting a cough and a runny nose – was brought to the emergency room. *Id.* at 73. He underwent a chest x-ray that revealed "no radiographic evidence of acute cardiopulmonary disease." *Id.*

J.B.'s subsequent well-baby visits were scheduled to account for the fact of his being born 4 weeks prematurely. On July 22, 2011, more than three months after J.B.'s birth, he had a two-month well baby visit with his pediatrician, Laura Wright, M.D. Exhibit 3 at 8-10. Dr. Wright's evaluation was thorough and well documented. *Id.* J.B. had no feeding difficulties, slept best at night, slept in his own room, and slept on his back. *Id.* at 8. He was noted to be a "well child, almost 4 months but behind on [vaccinations]" with "normal growth and development." *Id.* at 10. J.B. received DTaP, IPV, PCV, rotavirus, and Hep B vaccinations at this visit. *Id.* at 2, 8.

On September 2, 2011, almost five months after J.B.'s birth, he had his four-month well baby visit with Dr. Wright. Exhibit 3 at 5-7. He was nearly five months post-delivery, although his gestational age was about four months given his early delivery. J.B. was sleeping up to seven hours at a time, on his back, in a crib in his own room. *Id.* at 5. He was described as "healthy appearing and cooperative . . . well-nourished and well developed." *Id.* His chest and lungs were normal with no adventitious[8] sounds. *Id.* at 6.

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[6] Apgar score is defined as "a numerical expression of the condition of a newborn infant, usually determined at 60 seconds after birth, being the sum of points gained on assessment of the heart rate, respiratory effort, muscle tone, reflex irritability, and color." *Dorland's Illustrated Medical Dictionary* (32d ed. 2012) ("*Dorland's*") at 1682.

[7] Petitioners' expert, Dr. Miller, stated that normally an infant receives the first Hep B vaccination a day after delivery or just before going home. Exhibit 13 at 3. Dr. Miller characterized J.B. receiving the first Hep B vaccination one week after delivery as "a little unusual [but…] likely inconsequential." *Id.*

[8] Adventitious is defined as "accidental or acquired; not natural or hereditary." *Dorland's* at 34.

J.B.'s heart rate was regular with normal heart sounds and no pericardial friction rubs. *Id.* His reflexes were all 2/2 and his red reflex was normal. *Id.* His weight was 16 pounds, 8 ounces. *Id.* at 5. For infants of his age, his weight was stable at the 50th percentile, his height was up at the 50th percentile, and his head circumference was at the 75th percentile. *Id.* Nasal mucosa was normal, turbinates[9] were normal, and nares[10] were patent. Oropharynx was normal. *Id.* at 6. He was recorded as not having a fever, nasal congestion, or cough and history of wheezing. *Id.* at 5. He met numerous 4-month developmental milestones, including "head up 45 degrees, head up 90 degrees, sits – head steady." *Id.* During this visit, J.B. received DTaP, IPV, PCV, rotavirus, and Hep B vaccinations. *Id.* at 6; Exhibit 4 at 1. Dr. Wright completed her records from this visit on September 2, 2011, at 10:45 a.m., suggesting that the appointment had concluded by that time. Exhibit 3 at 7.

J.B.'s father attested that during the well-baby visit, J.B. was "smiling and cooing like normal." Exhibit 11 at 1. However, later that day after J.B. received the vaccinations, he "was not laughing or cooing like he normally did[,] he was not moving as much[, and] he seemed quiet and withdrawn." *Id.* That night, J.B. had a fever and he did not sleep well. *Id.* [11]

J.B.'s mother and father stated that on September 3, 2011, at 4:00 a.m., they gave J.B. Advil,[12] after which he went to bed in a supine position (on his back). Exhibit 8 at 2. When J.B. woke up a few hours later, he was distant, very quiet, and would not eat. Exhibit 11 at 2. He began running a fever again and was given another dose of Advil at approximately 8:00 a.m. *Id.*;

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[9] Turbinate is defined as "any of the nasal conchae.*" *Dorland's* at 1991.

[10] Nares is defined as "the external orifices of the nose; [also known as] nostrils." *Dorland's* at 1232.

[11] The following factual summary draws from:

- Exhibit 5 – Suffolk, Virginia Department of Fire & Rescue records of responding to the home on September 2, 2011.
- Exhibit 7 – Suffolk, Virginia Police Department records. This includes notes from the police's response to the home on September 3, 2011, and the police department's formal report on their response and a handwritten statement from J.B.'s mother, both completed on September 8, 2011.
- Exhibit 8 – Office of the Chief Medical Examiner, Tidewater District, Norfolk, Virginia, Records. This exhibit contains a summary of a child death reenactment with a doll, performed with J.B.'s parents in their home on September 8, 2011. Exhibit 8 at 3. The autopsy report was completed on November 2, 2011. Exhibit 8 at 1-2; 4-9.
- Exhibit 9 – Suffolk, Virginia Police Department records – photos of a bottle of Advil, taken on September 8, 2011; J.B. following the autopsy, undated; and the crib, bedroom, and exterior of the home, taken on September 3, 2011.

J.B.'s mother and father were not present to testify at the entitlement hearing.

[12] A bottle of children's Advil was taken into evidence. Exhibit 7 at 47. *But see* Exhibit 6 at 2, 5 ("aspirin"); Exhibit 8 at 2 ("infant Tylenol"); Exhibit 8 at 4-6 ("over-the-counter acetaminophen"). To the extent that it makes any difference it would seem most likely that it was the Advil that was given and the other notations were made subsequently without that same attention to this detail that the site investigation utilized.

Exhibit 7 at 11. J.B.'s mother said that J.B. sat up and played with her nephews during the morning. Exhibit 7 at 16.

In the early afternoon, J.B. became fussy and his father put him down for a nap in his bedroom, on the second floor of the house. Exhibit 7 at 3, 16; Exhibit 8 at 2. His father attested that he placed J.B. supine with his head to the right. Exhibit 7 at 5; Exhibit 8 at 3. J.B. seems to have had a pacifier in his mouth. Exhibit 7 at 16. He was placed in the middle of his crib, with a blanket across his midsection. Exhibit 8 at 3. The crib also contained a "little crib pillow – very flat," but no clutter or toys. Exhibit 7 at 5; Exhibit 8 at 3. J.B.'s mother attested that the air conditioning was always set at 76 degrees Fahrenheit. Exhibit 7 at 4. She indicated that J.B. slept on his back and that he could roll over on his own, lift his head, and pull or push himself up. Exhibit 7 at 5.

After putting J.B. down for his nap, his father left the home to get lunch. Exhibit 11 at 2. His mother remained in the home, but "heard [J.B.] fussing in crib" while she was cleaning and on the phone. Exhibit 7 at 16. After some period of time, J.B.'s mother went upstairs and put the pacifier in J.B.'s mouth. *Id.* (noting that J.B. "tend[ed] to cry when he spit the pacifier out"). When she returned, she found J.B. on his right side, with his head turned slightly, and unresponsive. Exhibit 7 at 17; Exhibit 8 at 2-3. She called J.B.'s father and said that J.B. was not breathing. Exhibit 7 at 17; Exhibit 11 at 2. The father told her to call 911 and he headed home. Exhibit 11 at 2.

J.B.'s mother said that "approximately 50 minutes passed" between his father placing J.B. down for a nap and when she found J.B. unresponsive. Exhibit 8 at 2. There was a "10-minute window" between when his mother checked on J.B. and replaced his pacifier, and when she returned to find him unresponsive. Exhibit 5 at 2. She informed the police that his nose and mouth were not covered. Pet. Ex 7 p 5.

J.B.'s mother called 911 at 2:39 p.m. Exhibit 7 at 35. She then attempted CPR. Exhibit 5 at 2; Exhibit 7 at 17. It appears that she removed him from the crib and placed him on his back on the floor. Exhibit 7 at 9-10. Officer Anderson was the first to arrive, at 2:42 p.m. – just 3 minutes and 21 seconds after the call. Exhibit 7 at 7, 9, 11, 35. Upon entering the home and going upstairs, the officer found J.B. lying on the bedroom floor, perpendicular to his crib. *Id.* at 9. J.B. was face up, with his eyes closed, and unresponsive. *Id.* He was still warm, but had no pulse or breath. *Id.* J.B.'s mother was kneeling over him. *Id.* The officer performed chest compressions until EMS arrived. *Id.*

The first responders left with J.B. at 3:02 p.m. and arrived at the emergency department of Harborview Medical Center at 3:08 p.m. Exhibit 7 at 36. J.B. was given oxygen under pressure during transport, but PEA (pulseless electrical activity) was noted on the monitor. Exhibit 5 at 1-2. Efforts at resuscitation were unsuccessful and J.B. was pronounced dead at the hospital, on September 3, 2011, at 4:01 p.m. Exhibit 7 at 10.

On September 5, 2011, a medical examiner, Dr. Jeffrey Gofton, completed an autopsy report for J.B. Exhibit 8 at 4-6. The medical examiner noted that the scene reenactment indicated that J.B. was placed to sleep on his back and was later found on his right side. *Id.* at 6. Scene photographs indicated a crib with soft blankets and a flat soft pillow, but no clutter or toys in the bed. *Id.* It was further noted that J.B. had no known medical problems, with regular infant care and immunizations. *Id.* He had a well-baby check-up on the day prior to his death, during which he received multiple vaccinations. *Id.* He had reportedly been fussy and had an intermittent temperature that seemed to be controlled with Tylenol. *Id.* J.B. was reportedly placed to sleep on his back and later found on his right side. *Id.* The medical examiner stated that J.B.'s lungs exhibited congestion and pulmonary edema.[13] *Id.* However, J.B. had no traumatic injury, congenital abnormalities, or viruses such as influenza. *Id.* Both a cerebral spinal fluid culture and a nasopharyngeal swab for viruses were negative. *Id.* J.B.'s brain weighed 876 grams (normal is 620 plus or minus 71 grams). *Id.* There was no evidence of epidural, subdural, or subarachnoid hemorrhage. *Id.* Serial sectioning showed normal configuration and infantile myelination of the cerebrum. *Id.* The brainstem was normally formed with no focal lesions. *Id.* at 5. Extensive drug testing was performed and was negative. *Id* at 6. The medical examiner, based on the "absence of findings and the reported sleeping position in a child with no anatomic or microscopic significant findings," stated that "the cause of death was SIDS and the manner was "natural." *Id.* The parties agree that the characterization of J.B.'s cause of death as SIDS is appropriate. Joint Prehearing Submission at 2.

The parties' experts in neuropathology – Dr. Miller for petitioners and Dr. Harris for respondent – reviewed 21 slides from J.B.'s autopsy, including two of J.B.'s brain. Exhibit 13 at 4-5; Exhibit A at 5. The first brain slide is a cross-section of pons at the level of the locus coeruleus (the upper pons), and the second slide is of two cingulate gyri with a portion of the adjacent corpus callosum. Exhibit 13 at 5. These brain sections demonstrated no abnormalities. *Id.* However, the medical examiner did not make slides from other parts of the brain, such as the medulla or hippocampus. *Id.* Furthermore, he did not take any photographs of the internal examination. *Id.* The parties' experts agreed that the medical examiner did not collect all of the data necessary to definitively analyze whether J.B. fit the Triple Risk Model of SIDS, introduced in the following section. Tr. 42-43 (testimony of Dr. Miller); Tr. 334 (testimony of Dr. Harris). The experts agreed that they would section considerably more of the brain in a possible SIDS autopsy than the two frontal lobes and one area of the pons that were sectioned in this case. Dr. Harris indicated that usually a SIDS autopsy should include samples of at least ten areas, including the medulla and hippocampus, which can help to show hypoxic ischemic changes as well as epilepsy related changes. Tr. 334. Both experts agreed, however, that in many SIDS cases, brains are not examined with the precision that they would recommend or that Dr. Kinney's group at Harvard did in their studies (introduced in the following section). Tr. 346.

[13] Dr. Miller and Dr. Harris agreed that congestion in the brain and lungs and other organs is a very common and non-specific finding at autopsy from which they would not draw any conclusion. Tr. 103 (Miller); Tr. 332-33 (Harris).

II. SUMMARY OF THE EVIDENCE

A. Medical Literature

The parties submitted voluminous literature to explain what is understood about sudden infant death syndrome ("SIDS"), the potential role of inflammatory cytokines generated by vaccines in acting as a necessary trigger, and the epidemiology of SIDS. Both parties submitted various studies from Hannah C. Kinney, M.D., a neuropathologist at Harvard, and others on her team which leads the research and current understanding of SIDS. The later articles tend to build upon and incorporate the earlier articles. Studies by other authors on SIDS and related subjects served to supplement and generally confirm that by Kinney et al.

A review of the literature is critical to the determination of whether petitioners have satisfied the *Althen* prongs (a reliable theory of how vaccines *can* cause death from SIDS, that the vaccines did in J.B.'s particular case, and that there was a medically acceptable temporal relationship between the vaccinations and J.B.'s death). This review is also necessary to determine whether respondent has sufficiently rebutted petitioners' theory by demonstrating that J.B.'s death was caused by factors unrelated to the vaccine.

SIDS is defined as "the sudden death of an infant under one year of age which remains unexplained after a thorough case investigation, including performance of a complete autopsy, death scene investigation, and review of the clinical history."[14] "Epidemiological studies link SIDS with sleep periods, leading to the premise that SIDS occurs during sleep or transitions between sleep and waking." *Id.*

SIDS is the leading cause of infant mortality in the United States, with an incidence of 0.53 per 1,000 infants.[15] Research has revealed that infants put to sleep in the prone position, i.e., with their heads facing downward, are twice as likely to experience SIDS. *Id.* Other risk factors for SIDS related to the "sleeping environment" have been recognized, including "[being] found face-down, head covered, sleeping on an adult mattress, couch or playpen, soft bedding, [and] bed-sharing." *Id.*

In 1994, Dr. Hannah C. Kinney, Dr. James Filiano, and their colleagues synthesized many neuropathological studies into their proposed Triple Risk Model.[16] This model posits that SIDS occurs when: (1) an infant in a critical development period; (2) possessing an underlying vulnerability; (3) encounters an exogenous stressor. *Id.* The following Venn diagram has been used to illustrate the Triple Risk Model:

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[14] Filiano, J.J. & H.C. Kinney, *Arcuate Nucleus Hypoplasia in the Sudden Infant Death Syndrome*, 51 J. Neuropathol. Exp. Neurol. 394 (1992), Exhibit 13-A at 394.

[15] Trachtenberg F.L., E.A. Haas, H.C. Kinney, C. Stanley & H.F. Krous, *Risk Factor Changes for Sudden Infant Death Syndrome After Initiation of Back-to-Sleep Campaign*, 129 Pediatrics 630 (2012), Exhibit C-11 at 631.

[16] Filiano, J.J. & H.C. Kinney, *A Perspective on Neuropathologic Findings in Victims of the Sudden Infant Death Syndrome*, 65 Biol. Neonate 194 (1994), Exhibit 13-B at 195 [also filed as Exhibit A-2].

Id. at 3, Figure 1. This model emphasizes the intersection of multiple factors in the pathogenesis of SIDS. According to this model, SIDS occurs only when components of all three factors are present simultaneously, which explains why all infants who are placed prone to sleep or who bed share do not die of SIDS.[17]

1. First Risk Factor: Critical Development Period

The first factor in the Triple Risk Model of SIDS is the critical development period, which Kinney et al. initially defined as the first year of life.[18] However, their more recent literature tends to define it as the first six months of life.[19] The peak incidence of SIDS deaths has historically occurred between two and four months of age. A study by Trachtenberg, Kinney, and others published in 2012 found slightly more younger and older infants succumbing to SIDS than had been seen in earlier studies. In the groups studied, the percentage of SIDS babies who were five months or more rose from 11.8% in the pre-Back-to-Sleep[20] era, to 17.6% in the 1996-2008 post-Back-to-Sleep era.[21] Kinney and Thach wrote, "Given the wide array of homeostatic functions modulated by the medullary 5-hydroxytrptamine system, sudden death may result from a convergence of defects in protective response to homeostatic stressors during sleep that are modulated by 5-hydroxytrptamine, probably in conjunction with related neurotransmitters."[22]

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[17] Kinney, H.C. et al., *The Brainstem and Serotonin in the Sudden Infant Death Syndrome*, 4 Annu. Rev. Pathol. Mech. Dis. 517 (2009), Exhibit 13-H at 521.

[18] Filiano & Kinney (1992), Exhibit 13-A at 394.

[19] *See, e.g.*, Kinney et al. (2009), Exhibit 13-H at 521.

[20] The "Back to Sleep" campaign refers to a major public health effort to encourage parents to place their infants on their backs to sleep, particularly during the first year of life as a means of reducing the incidence of SIDS.

[21] Trachtenberg, Kinney, et al. (2012), Exhibit C-11 at 634.

[22] Kinney, H.C. & B. Thach, *The Sudden Infant Death Syndrome*, 361 New England J. of Med. 795 (2009), Exhibit A-4 at 6.

2. Second Risk Factor: Vulnerable Infant

After Kinney et al. formulated the Triple Risk Model, the initial research was focused on determining why particular infants were "vulnerable", possibly because of environmental or genetic factors. Exhibit 13-H at 5. Intrinsic risk factors include "male gender, African-American race, poverty, adverse prenatal factors such as maternal smoking or alcohol use during pregnancy, and genetic polymorphisms." *Id.* It was also hypothesized as early as 1987 that most likely SIDS was related to a brainstem abnormality in the neuroregulation of cardiorespiratory control.[23] These intrinsic factors when combined with the vulnerable developmental period of the infant and a critical exogenous factor resulted in sudden infant death. As research progressed over the following decades, the above intrinsic risk factors remained but a significant emphasis was placed on the brainstem hypothesis, based upon the research of Dr. Kinney and others. In 2009, Dr. Kinney explained: "To date the most robust evidence for a neurochemical abnormality comes from research on the medullary 5-HT system,[24] in that approximately 50-70% of infants with SIDS appear to have abnormalities in this system. The medullary 5-HT system, which is considered critical for the modulation and integration of diverse homeostatic functions, is involved in ventilation and gasping, thermoregulation, autonomic control, response to carbon dioxide and oxygen, arousal from sleep, and hypoxia-induced plasticity.[25]

The 5-HT system refers to the serotonin system. "The caudal serotonergic (5-HT) system is a critical component of a medullary "homeostatic network" that regulates protective response to metabolic stressors such as hypoxia, hypercapnia and hyperthermia."[26] "Homeostasis refers to the ability of an organism to maintain a constant internal environment, thereby allowing survival over a wide range of external environmental conditions. It becomes self-sufficient at the moment of birth as the fetus takes the first breath in the extra-uterine world and begins to adjust instantaneously and independently to the myriad of changing metabolic demands. ... Receptor systems that sense deviations in any internal milieu (e.g., oxygen and carbon dioxide, glucose, and temperature levels) have been defined as well as the effector systems that are the final common pathway in mediating adjustments. Major focus has been placed upon the brain as the 'control center' which sets the range at which a particular parameter namely CO2 is maintained, and determines the protective response to deviations from this range namely hypercarbia."[27,28]

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[23] Kinney et al. (2009), Exhibit 13-H at 519.

[24] 5-HT (5-hydroxytryptamine), also called serotonin, is defined as "a monoamine vasoconstrictor, synthesized in the intestinal chromaffin cells or in central or peripheral neurons and found in high concentrations in many body tissues, including the intestinal mucosa, pineal body, and central nervous system." *Dorland's* at 1699.

[25] Kinney & Thach (2009), Exhibit A-4 at 6.

[26] Kinney, H.C. et al., *The Serotonergic Anatomy of the Developing Human Medulla Oblongata: Implications for Pediatric Disorders of Homeostasis*, 41 J. Chem. Neuroanat. 12 (2011), Exhibit 13-F at 182.

[27] Hypercarbia, also called hypercapnia, is defined as "excess of carbon dioxide in the blood." *Dorland's* at 887.

[28] Kinney et al. (2009), Exhibit 13-F at 183.

The serotonergic system, primarily concentrated in the medulla oblongata, which is called the caudal 5-HT system or the medullary 5-HT system, is now recognized as a key component of the brain's control system of homeostasis. *Id.* Dr. Kinney proposed that deficits in the caudal 5-HT system will lead to imbalances in respiratory, cardiovascular, and/or metabolic regulation – including in response to stress – in the pediatric age range, particularly in the first days and months following birth. *Id.* As noted by the Kinney group in a 2011 article on the serotonergic anatomy, "extensive experimental data implicate the caudal 5-HT system in homeostasis and respiratory and autonomic regulation, including upper airway control, respiration (including via modulation of the pre-Botzinger complex, the putative central rhythm generator of respiration), autoresuscitation, central chemoreceptor responses to hypercapnia and hypoxia, cardiovascular control, pain, motor function, and thermoregulation." *Id.* The article also notes that the medullary 5-HT system "interfaces with the cytokine system which is critical to homeostasis in its mediation of 'protective sickness' behaviors and cellular defenses against tissue damage." *Id.*

Dr. Kinney's team's research on the brainstem focused on a collection of neurons in the ventral medullary surface known as the arcuate nucleus "based upon cytological and positional homologies between the respiratory chemosensitive fields on the ventral medullary surface in cats. Structural underdevelopment of the arcuate nucleus was subsequently observed in SIDS cases."[29] As the research advanced, it was recognized that the "arcuate anomaly was similar to that reported in infants with clinical insensitivity to CO2 and sleep related sudden death." *Id.* By 2009, Dr. Kinney reported, "*Serotonergic neurons at the medullary ventral surface and in the midline (raphe) are now known to be preferentially chemosensitive to CO2* and although they are not the only central chemosensitive neurons they appear to play a critical potentially modulatory role…A small but important population of 5-HT neurons is embedded within the human arcuate nucleus suggesting that the putative dysfunction in chemosensitivity related to the arcuate anomaly specifically involved these embedded 5-HT neurons." *Id.* (emphasis added).

"Serotonergic neurons are well-suited to a role as central respiratory chemo-receptors, as they are closely associated with the basilar artery and its largest branches near the ventral surface of the medulla namely they are in a position to directly monitor arterial PCO_2.... 5-HT neurons respond intrinsically to increased PCO_2[30] with large increases in firing rate; this response is due to a decrease in intracellular pH induced by hypercapnia. On average these neurons increase their firing rate threefold in response to a decrease in pH of 7.4 to 7.2. Chemosensitivity increases during postnatal development, with a blunted response to pH before postnatal date 12 in rats. Physiological delay in chemosensitivity is potentially relevant to SIDS because it indicates that 5-HT neurons may be immature during the critical developmental period, throughout which all infants are susceptible to hypercapnia."[31] Harper and Kinney state the data now suggest that SIDS is associated with a brainstem (medullary) 5-HT deficiency rather than 5-

[29] Kinney et al. (2009), Exhibit 13-H at 522. Kinney defines chemosensitivity as "the ventilator response to a change in carbon dioxide/pH as sensed by tissue chemoreceptors, which are composed of neurons and/or astrocytes." *Id.*

[30] PCO_2 is defined as "the partial pressure of carbon dioxide." *Dorland's* at 2120.

[31] Kinney et al. (2009), Exhibit 13-H at 530.

HT overproduction.[32] Of note, the medullary 5-HT profile differed between infants dying of SIDS and those dying with known chronic oxygenation disorders, suggesting that chronic hypoxia does not necessarily play a major role in the pathogenesis of the impairments in the 5-HT tissue markers. *Id.*

Harper & Kinney explained that the insufficient function of the 5-HT system, which is necessary for breathing, leaves an infant vulnerable to a variety of crisis situations. These include external airway obstruction, upper airway obstruction resulting from loss of tone in the upper airway musculature in association with diaphragmatic movements, or importantly of central apnea, which has occupied a central focus of attention. These are also proposed mechanisms underlying the fatal event in SIDS. This failure can result from several components of the breathing process, including impaired sensory transduction or integration of either carbon dioxide or oxygen, or non-recruitment of gasping mechanisms, the final restorative mechanism to low oxygen. In SIDS, a principal concern is the "loss of the wakefulness drive to breathe." *Id.* at 5. The waking state activates processes which maintain breathing, while during sleep those influences are suppressed or not recruited. Thus, impaired central chemosensitivity to excess carbon dioxide or inadequate oxygen contributed to by defects in the medullary serotonin system, in addition to the normal reduction of the function of the 5-HT system during sleep, may play a central role in SIDS, which occurs primarily during sleep. *Id.* at 4-5.

Despite the emphasis on brainstem abnormality or underdevelopment, the other intrinsic risk factors are thought to continue to play an important role in the multi-factorial analysis of SIDS causation. Some of these factors may be related to the medullary 5-HT deficits described above. Several intrinsic risk factors are apparent in J.B.'s case. First, prematurity is defined as less than 37 weeks at birth[33] and J.B. was born at 36 weeks. Male gender, as boys exceed girls in SIDS deaths by a two-to-one ratio, and African-American race have also been called intrinsic risk factors because they are over-represented among SIDS victims.[34] Importantly, maternal smoking and alcohol consumption during pregnancy are considered important risk factors but are not relevant in this case, as J.B.'s mother did not smoke or drink during or after her pregnancy.

Dr. Kinney has hypothesized that males may predominate among SIDS deaths because males tend to be less responsive to the accumulation of carbon dioxide, and in the situation with a defective medullary 5-HT system may be particularly impaired from responding to excess carbon dioxide during sleep. *Id. T*he predominance of males in the occurrence of SIDS appears to be potentially related to the reduction of 5-HT binding in the medullary raphe compared to females dying of SIDS, as well as the report that plasma levels of testosterone, but not estradiol, are significantly higher in both male and female SIDS infants compared to age-matched controls. Several studies in knockout mice and piglets also "underscore gender differences in brainstem-mediated 5-HT function, with females' brains apparently relying less on 5-HT neurons in chemoreception and adapting more readily to the loss of 5-HT function. *Id.*

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[32] Harper, R.M. & H.C. Kinney, *Potential Mechanisms of Failure in the Sudden Infant Death Syndrome*, 6 Curr. Pediatr. Rev. 39 (2010), Exhibit C-12 at 7.

[33] Trachtenberg, Kinney, et al. (2012), Exhibit C-11 at 631.

[34] Kinney et al. (2009), Exhibit 13-H at 532.

The role of African-American race in SIDS is less defined, other than statistically. Most authors speculate that the statistical predominance of African-American children may represent lower socioeconomic status resulting in inadequate medical care. If that be the case however, J.B.'s race should not be an increased risk factor as he was receiving regular medical care with comprehensive and well-documented well baby visits occurring in July and September. His first set of vaccinations was somewhat late, but the second dose, those received on September 2, 2011, brought him up to date. His growth and functional milestones appeared to be normal. It is also reported that 75% of white infants are placed to sleep in the supine position, while only 53% of black infants are, and that there is greater incidence of bed sharing among black infants than in other groups.[35] J.B. was placed on his back and was in his own crib.

3. Third Risk Factor: Exogenous Stressor(s)

The third and last factor is referred to as exogenous stressor[s] present at the time of death.[36] These stressors identified in the literature include "prone sleep position, face-down position, covered face in the supine position, soft bedding, bed sharing, over-bundling, elevated room temperature, and minor infection at the time of death."[37] Virtually every SIDS case includes one or more exogenous stressors, implying that they act as "triggers" for SIDS.[38] Studies also show that often multiple risk factors are present in a given SIDS case. Trachtenberg et al. found that "at least 2 extrinsic risk factors" were present in a majority of 568 cases reviewed. *Id.* at 632.

Dr. Kinney has hypothesized that exogenous stressors "lead to asphyxia, hypoxia, hypercapnia, or thermal imbalance requiring intact brainstem defense systems to protect against lethal consequences."[39] Non-vulnerable infants are generally able to recover from these conditions, but vulnerable infants are less able to recover and succumb to SIDS. *Id.* at 521.

As a result of their research, Dr. Kinney and her team proposed the Triple Risk Model to explain the occurrence of SIDS. Dr. Kinney's group then proposed the "Back to Sleep Campaign" in the early 1990s in which they recommended that babies always be put to sleep on their backs (supine) on a firm mattress, without pillows, blankets, toys, bumpers or other items that could potentially obstruct breathing. The prone or face-down sleeping position was considered to make an infant particularly vulnerable because an infant in the first six months of life with one or more intrinsic defects may re-breathe excess carbon dioxide and lack the corrective arousal mechanisms during sleep that would prevent a fatal outcome. Generally, the accumulation of excess carbon dioxide in the body causes signaling to breathe, thereby exhaling

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[35] Moon R.Y. et al., American Academy of Pediatrics – Task Force on Sudden Infant Death Syndrome, *SIDS and Other Sleep Related Infant Deaths: Expansion of Recommendations for a Safe Infant Sleeping Environment,* 128 Pediatrics 1030 (2011), available at http://pediatrics.aappublications.org/content/128/5/1030.long.

[36] Trachtenberg, Kinney, et al. (2012), Exhibit C-11 at 631.

[37] Kinney et al. (2009), Exhibit 13-H at 521.

[38] Trachtenberg, Kinney, et al. (2012), Exhibit C-11 at 633.

[39] Kinney et al. (2009), Exhibit 13-H at 520.

carbon dioxide and inhaling room air containing oxygen. During sleep it is thought that excess carbon dioxide normally causes a person to turn the head toward fresh air and become aroused from sleep. When those mechanisms fail, the gasp reflex is triggered, which brings in oxygen and resets the rhythm of breathing. In SIDS, the dominant theory is that all of these mechanisms fail, leading to death.

The Back to Sleep Campaign has succeeded remarkably in reducing the number of SIDS deaths in the United States by approximately 50%.[40] In the U.S., the rate was reduced from more than 1 per 1,000 infants to 0.53 per 1,000, the current rate where it has plateaued. *Id.* However, SIDS remains the leading cause of post neo-natal infant death in the United States, raising some of the questions at issue in this case. *Id.* The emphasis has continued to be on the cardiorespiratory failure explanation of SIDS. Research has indicated that prone sleeping position increases the risk twofold or more. *Id.* They concluded that those not found prone sleeping were subject to alternative SIDS risk factors. *Id.* at 635.

The Trachtenberg article concluded that virtually all SIDS infants have at least one risk factor, and the majority have at least one intrinsic risk factor and two extrinsic factors. *Id.* The article also notes that the American Academy of Pediatrics risk reduction guidelines also include recommendations against side-sleeping and bed-sharing, and suggest a separate but proximate sleeping environment and pacifier use. *Id.* at 636. The data from the Trachtenberg study found a decline in prone position sleeping from 84% in the pre-Back-to-Sleep era to 48.5% in the post-era, but it also found that in the post-era 17.3% of SIDS infants were found on their sides while 22.6% were initially placed on their sides. *Id.* at 634, Table 2. Interestingly, 29% of the SIDS babies in that study were found supine while 41.7% were placed on their backs, suggesting that SIDS is not exclusively caused by prone sleeping. *Id.* at 632.

The Trachtenberg and Kinney articles emphasize the belief in the medical community that SIDS is multifactorial. As Trachtenberg noted, they were only able to evaluate which SIDS risk factors are most common, not which factors raise the odds of SIDS most significantly. *Id.* at 635. The authors suggest that the number of risks is probably underestimated and that "the majority of SIDS infants were subject to at least two extrinsic risk factors, suggesting that SIDS occurs from the simultaneous occurrence of multiple factors, rarely just one." *Id.* Additionally, Dr. Kinney has noted that under the Triple Risk Model, only infants with an underlying brainstem disease process die of SIDS, which explains why all infants who are placed prone to sleep or who bed share do not die of SIDS.[41] She states that SIDS essentially represents the occurrence of "the biologic version of the perfect storm in which the chance combination of multiple events is far more powerful than each individual event alone." *Id.* at 539. She suggests a possible scenario in which a child with the underlying brainstem deficit, during the critical developmental period, is exposed to excess carbon dioxide while he is sleeping. This may be based upon his sleeping position or he may have an issue with the laryngeal chemoreflex stimulated by reflux of gastric contents *or may have a mild infection with fever* causing the laryngeal chemoreflex induced apnea to be inordinately prolonged by mild hyperthermia" *Id.*

[40] Trachtenberg, Kinney, et al. (2012), Exhibit C-11 at 631.

[41] Kinney et al. (2009), Exhibit 13-H at 521.

(emphasis added). In this scenario, "if the infant's ventilator response to the progressive hypoxia and hypercapnia during the apnea is depressed, and if the hypoxic gasping and/or arousal mechanism is abnormal, oxygen lack from uninterrupted apnea results. Ultimately, death occurs *within minutes to hours*." *Id.* (emphasis added).

Respondent filed the article by Trachtenberg et al., which emphasized that they could find no positive correlations between risk factors or risk clusters but it appeared that any combination of risks together increased the odds of SIDS. The fact that most infants have at least two extrinsic risk factors suggests that SIDS occurs as a result of the occurrence of multiple factors and rarely just one.[42] The Kashiwagi article[43] filed by petitioners suggests that vaccines provoke an inflammatory cytokine response similar to that provoked by a mild infection. Petitioners theorize that these cytokines travel to the brainstem and further suppress the function of the already impaired medullary 5-HT system in a subset of SIDS infants.

a. Cytokines, Mild Infection and Vaccines

Relevant to this case, in a 2009 article in the New England Journal of Medicine, Kinney and Thach stated, "A causal role for mild infection in sudden infant death is suggested by reports that in approximately half of SIDS cases, the infants have a seemingly trivial infection around the time of death, as well as mild tracheobronchial inflammation, altered serum immunoglobulin or cytokine levels and the presence of microbial isolates at autopsy. In infants who die unexpectedly of infection, the given organism may precipitate a lethal cytokine cascade or toxic response."[44] The question arises as to whether the cytokine response stimulated by vaccination can have the same effect as a mild or trivial infection in a baby who presumably has a defect in the medullary 5-HT system.

The role of cytokines stimulated by either mild infection or by vaccination is central to petitioners' theory in this case. Approximately 50% of SIDS babies have been found in multiple studies to have had mild or even "trivial" infections, primarily of the upper respiratory tract at the time of death. In this case, J.B. was documented the prior day as being healthy with patent nares, normal turbinates, and clear chest, but during the 28 hours after the vaccine he was reported to have a fever, which is generated by cytokine signaling. He also was distant, quiet, and would not eat, according to his parents. The case raises the issue of whether inflammatory cytokines stimulated by the innate response to the vaccines triggered the fever and his fussiness, and ultimately suppressed his 5HT system sufficiently so that he could not process the carbon dioxide in his system. The question of whether inflammatory cytokines stimulated by the innate response to the vaccine could have been the trigger that led to his death was central to the testimony and much of the literature submitted by the parties particularly in light of the clear medical evaluation on the day of the vaccination and a fever within hours afterward.

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[42] Trachtenberg, Kinney, et al. (2012), Exhibit C-11 at 7.

[43] Kashiwagi Y et al., *Production of Inflammatory Cytokines in Response to Diphtheria-Pertussis-Tetanus (DPT), Haemophilus Influenzae Type B (Hib) and 7-Valent Pneumococcal (PC7) Vaccines*, 10 Hum. Vacc. Immunother. 677 (2014), Exhibit 17.

[44] Kinney & Thach (2009), Exhibit A-4 at 2.

As Dr. Kinney and her colleagues explained in 2011: "Cytokines orchestrate immune responses to microbial invasion and other insults and coordinate these responses with those of other physiological systems, including the autonomic nervous system, in the protection of the organism against tissue injury. They also mediate sickness behavior, including fever, anorexia, excessive sleepiness, blunted arousal, deep rest respiration, and lowered heart rate, which is thought to protect the organism during systemic illness by dampening excessive metabolic demands and thereby speeding repair and recovery - a form of homeostasis."[45] "Cytokines determine this sickness behavior by binding to endogenous cytokine receptors on neuronal populations in the hypothalamus and/or brainstem that mediate respiration, autonomic function, satiety, sleep, and arousal." *Id.* at 190. The cytokines which act within the brain in response to tissue injury are produced by astrocytes, and endothelial cells, microglia, *and/or peripheral immune cells* which enter the brain in response to binaural signals of tissue damage." *Id.* (emphasis added). During infection, peripherally produced IL-6 may cross the blood brain barrier and bind to IL-6 receptors on 5 HT neurons that mediate homeostasis in response to the infectious stressor and potentially mediate sickness behavior. *Id.* at 191. The role of pro-inflammatory cytokines in the pathology of SIDS is thought by multiple authors to be a potentially critical factor in tipping the molecular balance in the underdeveloped brainstem leading to death in infants in the vulnerable time period. IL-1β, IL-2, and IL-6 are pro-inflammatory cytokines that have been studied in connection with SIDS leading to theories about their potentially neuro-modulatory role in SIDS babies.

Kadhim et al. described a distinct cytokine profile in a SIDS brain in a study comparing SIDS brains with non-SIDS brains. The non-SIDS brains were from infants who died of known causes, including AIDS, cirrhosis of the liver, mononucleosis, purulent meningitis, and congenital heart disease with post-operative acidosis-shock. He found an over-expression of interleukin 1β in arcuate and dorsal vagal nuclei in all SIDS victims. In arcuate nuclei, high levels of interleukin 1β were detected in 17/17 SIDS brains vs. only 1 of 6 non-SIDS brains.[46] In dorsal vagal nuclei, interleukin 1β was also detected in high levels in 17 of 17 SIDS brains vs. only 2 of 7 non-SIDS brains. *Id.* Kadhim found a "region-specific pattern of cytokine expression in [the arcuate and dorsal vagal nuclei] of SIDS brains compared to non-SIDS brains." *Id.* at 1259. Kadhim theorized: "cytokines could exert neuromodulatory effects. Infectious inflammatory conditions and injury to the brain could up regulate pro inflammatory cytokines and produce functional alteration ... Cytokine/neurotransmitter interactions could therefore modify vital CNS functions." *Id.* Kadhim et al. further concluded that IL-1 causes prolonged apnea and depresses respiration, and that the brain appears to be less effective than the peripheral nervous system in inducing IL-1 antagonists to control IL-1 action.

[45] Kinney et al. (2011), Exhibit 13-F at 189.

[46] Kadhim, H. et al., *Distinct Cytokine Profile in SIDS Brain: A Common Denominator in a Multifactorial Syndrome?*, 61 Neurol. 1256 (2003), Exhibit 13-L at 1256.

In a second study from 2010, Kadhim focused on the expression of IL-2 in 28 autopsied infants who died at less than one year of age.[47] He described IL-2 as major immune-related cytokine that was originally thought to be a T-lymphocyte growth factor but is now recognized to have a wider spectrum of functions, targets and sources. *Id.* The study compared 18 SIDS brains to those of infants who died of diverse severe pathological conditions including infectious, hemodynamic, metabolic or other serious genetic conditions. In the severely ill children (non-SIDS), they found that IL-2 was preferentially expressed in specific neuronal centers within the brainstem (SNT-solitary nucleus tractus and TSNT-spinal trigeminal nucleus/tractus) in 10 of 10 cases of the fatally sick (non-SIDS) children and in the arcuate and dorsal vagal nuclei in 8 of 10. "Examination of the brainstem in the SIDS group showed a topographically similar profile with an equally intense immune reactivity within the very same neuronal circuits; precisely the strongly expressed cytokine labeling of IL-2 in SNTT and/or TSNT was observed in 17 out of 18 cases that constituted the 2nd study group (SIDS). IL-2 was also notable in the arcuate nucleus and dorsal vagus nucleus in 17 cases. These brainstem neuronal centers are known to be intricately implicated in autonomic control of vital homeostatic functions namely cardiorespiratory control mechanisms."[48] The authors concluded that it was not surprising to see the intense IL-2 expression in the infants who were severely ill before they died, but the SIDS victims are generally free from apparent potentially fatal conditions. "The SIDS victims often have preceding mild infectious/inflammatory conditions (like coryza[49]/mild upper respiratory infections, soft stools mild gastroenteritis, *postvaccinal fever*, etc.). Such trivial infections were found to induce a hypertuned immune/inflammatory response including high levels of immune inflammatory cytokines." *Id.* at 122. (emphasis added). Kadhim reviewed the Triple Risk Model, placing his study findings with regard to inflammatory cytokines in that framework; "Such mild infectious inflammatory conditions (extrinsic environmental stressors), if contracted in a vulnerable infant (intrinsic factors including prematurity and gene polymorphisms) during a critical developmental period whereby brainstem command centers undergo rapid maturation could provoke exaggerated immune responses with over expression of cytokines. We believe that this hypertuned immune response is behind the high IL-2 immune-reactivity we detected in situ in the brainstem of these victims." *Id.* at 125. Kadhim also noted that while pro-inflammatory cytokines have immune function, it is noteworthy here that cytokines have *neuro-modulatory effects* whereby they can modify neurotransmission. *Id.*

The role of mild infection was further discussed in an article by Rognum et al.[50] The Rognum group compared three groups of deceased infants. The group of 25 SIDS cases was selected from those subjects in whom no explanation for death was found. A second group died from known infectious causes and the third control group died primarily from violent causes

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[47] Kadhim, H. et al., *Interleukin-2 as a Neuromodulator Possibly Implicated in the Physiopathology of Sudden Infant Death Syndrome*, 480 Neurosci. Lett. 122 (2010), Exhibit 13-O at 123.

[48] Kadhim et al. (2010), Exhibit 13-O at 124.

[49] Coryza, also known as acute rhinitis, is defined as an "inflammation of the mucous membranes of the nose." *Dorland's* at 423, 1639.

[50] Rognum, I.J., R.L. Haynes, A. Vege, M. Yang, T.O. Rognum & H.C. Kinney, *Interleukin-6 and the Serotonergic System of the Medulla Oblongata in the Sudden Infant Death Syndrome*, 118 Acta Neuropathol. 519 (2009), Exhibit 13-N at 519-30.

such as drowning, suffocation or strangulation. *Id.* at 522. The IL-6 levels were significantly higher in SIDS subjects than in controls. The IL-6 levels in SIDS infants with minor infection were comparable to those infants who succumbed to severe infection. *Id.* at 520.

Rognum et al. wrote: "We previously showed that IL-6 is elevated in the cerebrospinal fluid of SIDS infants and that this elevation may be induced by a peripheral immune reaction. Approximately one half of the SIDS cases we have studied show signs of a mild infection, but IL-6 levels are comparable to those of infants succumbing to severe infection, suggesting an overreaction to the slight infection." *Id.*

According to Rognum: "In addition to its pro-inflammatory properties, IL-6 exerts effects outside the immune system. Non-immune cells including neurons can produce and secrete IL-6 and express its receptor. Of critical relevance to the premise that cytokines interact with central neurons to affect their function, IL-6 is shown to be important in neuronal development in the modulation of neuronal signaling." *Id.* "A major site of 5-HT cell bodies in the human infant brainstem is in the arcuate nucleus, the putative site for central carbon dioxide (CO2) sensitivity in humans and animal models. In this regard the synergistic effect of prone sleeping and infection on SIDS risks may be a set up for CO2 accumulation, as both rebreathing in the face down prone position and increased metabolism due to infection may increase CO2 levels. Death may be triggered if CO2 sensing regions in the brainstem, such as the arcuate nucleus, are compromised and cannot mount an arousal response to protect the infant from the dangerous situation. The arcuate nucleus is of particular interest in the study due to the previous finding by others of high neuronal IL-1β immunoreactivity at this site in SIDS cases compared to controls." *Id.*

Rognum et al. did identify one particular confounder to this theory in that they found that the mean IL-6R (receptor) intensity grade in the arcuate nucleus was significantly higher in the SIDS group than in the control group but the gp130 transducer was significantly higher in the infection group but less so in SIDS relative to the controls. While Rognum et al. acknowledged difficulty in grading the immunosensitivity of IL-6R and gp130 in this study due to its small size as a major limitation in the study, the result led the authors to hypothesize that the increased expression of IL-6R in the arcuate nucleus may be a compensatory mechanism as defective arcuate neurons may require excessive IL-6 stimulation in order to respond to altered carbon dioxide levels and there may be an inability in the SIDS babies to upregulate gp130 to mount an effective response.[51] *Id.* at 528. Nevertheless, the study concluded that abnormal IL-6R expression was found in the arcuate nucleus of SIDS babies 44% of whom had mild infections prior to death and thereby "provides evidence for aberrant interactions in SIDS infants between Il-6 and the arcuate nucleus, a key medullary 5-HT related region involved in protective responses to hypercapnia, potentially induced by the combined effect of prone position and mild infection." *Id.* at 529.

[51] Dr. Miller explained that gp130 is a second messenger in the cell that takes the message that the receptor has bound something and does something with it to take (tell) the cell to do something else. This is a very common mechanism in membrane signaling, that there's a second messenger system that then tells the cell to do something. Tr. p 32.

Rognum et al. concluded: "The key finding in this study is abnormal IL-6R expression in the arcuate nucleus in the SIDS cases, 44% of whom had signs of mild infection immediately prior to death. *Id.* at 528. Rognum further noted that the arcuate nucleus contains 5-HT and glutamatergic neurons that have been shown in animals to be critical to chemosensitivity. It is also the site for several neurotransmitter abnormalities in SIDS, including in 5-HT, muscarinic and kainite receptor binding. It is well documented that CO2 levels are elevated during severe neonatal infection and, interestingly, even mild upper respiratory infection may increase CO2 levels in infants over 3 months of age. Animal studies indicate that the *CO2 elevation can be attributed to a hyper metabolic state induced by proinflammatory cytokines*." *Id.* at 527-28 (emphasis added).

Kashiwagi studied the production of cytokines after vaccination in 61 vaccine recipients with fever and 18 without fever within 24 hours of vaccination. Blood samples were taken within 48 hours of vaccination in both groups. He reviewed the role of the innate immune system in responding to vaccination noting that the activation of the innate immune system including the enhanced production of inflammatory cytokines is indispensable for immunogenicity and these cytokines may be related to the occurrence of adverse events.[52] This group found that cytokine production began about 6 hours after the stimulation by a single or combination of vaccines and *increased for 24 hours, showing the same level afterward. Id.* at 679. They found that higher levels of IL-1β, IL-6, G-CSF[53] and TNF-α were produced with the concurrent stimulation by multiple vaccines than with the single vaccine in PBMC cultures (peripheral blood mononuclear cells - obtained from young infants in this study). *Id.* at 679. Higher levels of IL-6, IL-10, IL 12, G-CSF, IFNγ and TNFα in both the febrile and non-febrile groups were found after vaccination and G-CSF was significantly higher in the febrile group. *Id.* at 680. He noted that innate immune systems are not fully functional at the time of birth. Kashiwagi's group found that TLR (Toll-Like Receptors) stimulated the production of pro-inflammatory cytokines (specifically IL-β, IL-6, and IL-8) which was markedly higher in neonates than in adults. He also found that higher levels of IL-1β were produced in PBMC cultures stimulated with PCV7 than with DPT or Hib. Hib induced higher levels of IL-6 and TNF-α. IL-1β increased in PBMCs stimulated concurrently with Hib/PCV7 and DPT/Hib/PCV7 with similar patterns of TNF-α and G-CSF. However, when blood was drawn 48 hours post-vaccination, IL-1β was not found. *Id.* Dr. Miller theorized that IL-1β rises rapidly and then disappears by 48 hours whereas the other inflammatory cytokines have a longer half-life. Tr. 47

Kashiwagi noted: "All effective vaccines induce the production of cytokines or chemokines, which modulate immunogenicity and are also involved in inducing adverse events, such as systemic febrile illness and immunotoxicity. In this standpoint, IL-6, IL-10, IL-12, G-CSF, IFN-γ, and TNF-α were detected in both febrile and non-febrile groups after vaccination in comparison with those in normal subjects." *Id.* at 683. Inflammatory cytokine profiles after vaccination were similar to the outpatient group infected with the influenza virus. *Id.*

[52] Kashiwagi, et al. (2014), Exhibit 17 at 678.

[53] G-CSF is granulocyte colony stimulating factor *Dorlands* at 767- It is now classified as another cytokine. Tr. 47.

Vege and Rognum reviewed the literature and their own work and noted that "in 1995 they found that half of the SIDS victims had elevated levels of interleukin-6 (IL-6) in their cerebrospinal fluid (csf). The concentrations of IL-6 in SIDS infants were comparable to those we found in infants dying from infectious diseases like meningitis and septicaemia." They concluded that there were two groups of SIDS infants—one with IL-6 levels similar to infants dying of severe infections and another having low levels similar to those dying violent deaths. They hypothesized that one group of SIDS deaths may be attributable to sleep position and another to an uncontrolled inflammatory response to infection, predominantly occurring at night when cortisol levels, another mechanism for controlling inflammatory responses, are low.[54]

Others have studied cytokine expression in animals. Brambilla demonstrated in animal studies that Interleukin 1 (IL-1) inhibited firing of excitatory or wakefulness producing neurons in the dorsal raphe nucleus and enhanced activity of GABAergic or inhibitory neurons and, as such, induces enhancement of NREM sleep.[55]

Respondent submitted an article by Siljehav, Hofstetter et al. which sheds additional light on the possible mechanism involved with apnea in infants occurring in response to infection. These authors wrote: "Our data suggest that PGE2[56] induced by IL-1β as well as hypoxia selectively modulates respiration-related neurons in the rostral ventrolateral medulla, including the preBotzinger Complex via EP3R. Other neuromodulators, including PGE1, have been shown to inhibit preBotC neurons and slow respiration-related rhythm and preBotC lesions may disrupt anoxic gasping and evoke central apneas and ataxic breathing. Moreover, these respiration-related neurons were recently shown to be critical for adequate response to hypoxia, maintaining brainstem homeostasis with gasping and autoresuscitation and thus restoring oxygen levels. PGE2-induced depression of this vital brainstem neuronal network, e.g., during an infectious response, could result in gasping and autoresuscitation failure and ultimately death."[57] The model of the IL-1β induced respiratory depression and autoresuscitation failure via a PGE2 –mediated pathway was described. "During a systemic immune response, the proinflammatory cytokine IL-1ß is released into the peripheral blood stream. It binds to its receptor (IL-1R) located on endothelial cells of the blood brain barrier. Activation of IL-1R induces the synthesis of PGH2 from arachidonic acid via COX-2 and the synthesis of PGE2 from PGH2 via the rate limiting enzyme mPGES-1. PGE2 is released into the brain parenchyma and binds to the EP3R located in respiratory control regions of the brainstem, e.g., nucleus tractus solitarius and rostral ventrolateral medulla. This results in depression of central respiration-related neurons and

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[54] Vege, A & T. Rognum, *Sudden Infant Death Syndrome, Infection, and Inflammatory Responses*, 42 FEMS Immunol. Med. Microbiol. 3 (2004), Exhibit 13-Q at 5 and 8.

[55] Brambilla, D. et al., *Interleukin-1 Inhibits Firing of Serotonergic Neurons in the Dorsal Raphe Nucleus and Enhances GABAergic Inhibitory Post-Synaptic Potentials*, 26 Eur. J. Neurosci. 1862 (2007), Exhibit 13-M at 1862.

[56] PGE2 is a symbol for a prostaglandin. *Dorland's* at 1529. Prostaglandins are "any of a group of components derived from unsaturated 20-carbon fatty acids, primarily arachidonic acid, via the cyclooxygenase pathway; they are potent mediators of numerous different physiologic processes." *Dorland's* at 1528.

[57] Siljehav, V. et al., *mPGES-1 and Prostaglandin E2: Vital Role in Inflammation, Hypoxic Response, and Survival*, 72 Pediat. Res. 460 (2012), Exhibit C-9 at 9897.

breathing, which may fatally decrease the ability to gasp and autoresuscitation during hypoxic events." *Id.* at 9898.

Stoltenberg[58] experimented on piglets and concluded IL-1 stimulates the release of beta endorphin and indicated that his group had shown that the level of beta-endorphin in cerebral spinal fluid correlates strongly with the duration of apnea. Furthermore IL-1β stimulates GABA transmission and hence increases the inhibitory postsynaptic function by opening of chloride delective channels, and this will reduce the activity in the central respiratory neurons and may produce hypoxia. He concluded that intravenous and intrathecal injections of interleukin 1β in piglets' prolonged apnea and modified autoresuscitation. Such a mechanism may play a role in depressing respiration in some infants dying of sudden infant death syndrome. *Id.* at 427.

In a study looking at the role of vaccination in producing apnea, bradycardia and oxygen desaturations in pre-term infants who received first DPT (whether whole cell or acellular pertussis, inactivated polio and Haemophilus influenza B), Lee found elevations in apnea, bradycardia and desaturations defined as cessation of respiration for 20 seconds, with a heart rate less than 100 and oxygen saturation less than 85%. Almost half had adverse cardiorespiratory events in the 72 hours post-vaccination which was statistically significantly higher than the control group which did not receive a vaccination in the prior 72 hours.[59]

Schulzke also studied apnea and bradycardia in pre-term infants, not on oxygen or respiratory support but in the NICU when they received pentavalent or hexavalent vaccines. Rate of increased apnea and bradycardia (defined the same as by Lee) was 13% in otherwise stable infants. Infants received ventilatory support and recovered. Events occurred between 8 and 24 hours after vaccination with onset of fever between 6 and 24 hours post immunization. [60]

B. SIDS Epidemiology

Although epidemiology is not required to demonstrate entitlement to compensation in the Vaccine Program, the parties submitted multiple articles, primarily from European studies, which looked at the question of the possible relationship between vaccination and the incidence of SIDS, as well as several articles that reported on cases. Articles by Venneman[61], Jonville Bera, Traversa, VonKries, Goldman, and Kuhnert studied the question of vaccine causation in SIDS by various methodologies all of which described their own limitations. Others by Ottaviani and

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[58] Stoltenberg, L. et al., *Changes in Apnea and Autoresuscitation in Piglets After Intravenous and Intrathecal Interleukin-1β Injection*, 22 J. Perinat. Med. 421 (1994), Exhibit 13-J.

[59] Lee et al., *Frequency of apnea, bradycardia, and desaturations following first diphtheria-pertussis invactivated polio-Haemophilus influenzae type B immunization in hospitalized preterm infants,* BMC Pediatrics (2006), Exhibit 20 at 3-4.

[60] Schulzke, *Apnea and bradycardia in preterm infants following immunization with pentavalent or hexavalent vaccines,* European Journal of Pediatrics (2005), Exhibit 21 at 432-35.

[61] Vennemann M.M. et al., *Sudden Infant Death Syndrome: No Increased Risk After Immunization*, 25 Vaccine 336 (2007), Exhibit C-17.

Zinka discussed individual cases of unexplained deaths occurring in close temporal proximity to receipt of vaccinations.

Goldman looked at VAERS data from 1990 to 2010 for hospitalizations and deaths after vaccinations and found a statistically significant positive correlation between mortality and receipt of five to eight vaccines compared to one to four.[62] (J.B. received 7 counting DTaP as three as the study did). Traversa conducted a large study using data from the Italian health system where vaccines are offered for free and the belief is that 95% of children are vaccinated. The study found a statistically significant relative risk for death in the first seven days after vaccination for the first hexavalent vaccine (six vaccines) but not after subsequent doses.[63]

Kuhnert did a review of studies from Germany, England, and New Zealand and critiqued the case control methodology through the use of the self-controlled case series method (SCCS). It concluded that the re-analysis using the latter method showed that the risk of SIDS was neither increased or decreased in SIDS cases or controls during the early post-vaccination periods but did "provide more detailed insights into the methodological pitfalls of such analyses using conventional case control methods."[64] Dr. McCusker testified that the Kuhnert study looked at three different studies and applied 39 statistical tests to them. She read the study as concluding that despite the application of multiple statistical post hoc tests, they still did not see anything. Tr. 236.

Other papers submitted in evidence included Zinka[65] reporting on six deaths in Germany within 48 hours of receipt of hexavalent vaccines. Kries[66] reported on a slight elevation in day one in the first year of life after one particular hexavalent vaccine but a significant increase in deaths in the second year of life after receipt of that vaccine. Ottaviani[67] did a detailed case study of one young child who died three hours after receipt of a hexavalent vaccine at 3 months of age. He did a detailed autopsy identifying bilateral hypoplasia of the arcuate nucleus. He concluded that this death could be consistent with the Triple Risk Model or be one of the SIDS

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[62] Goldman, G.S. and N.Z. Miller, *Relative Trends in Hospitalizations and Mortality Among Infants by the Number of Vaccine Doses and Age, based on the Vaccine Adverse Event Reporting System (VAERS): 1990-2010*, 31 Hum. Exp. Toxicol. 1012 (2012), Exhibit 19 at 1016, Table 4.

[63] Traversa, G. et al., *Sudden Unexpected Deaths and Vaccinations During the First Two Years of Life in Italy: A Case Study*, 6 PLoS One 1 (2011), Exhibit 13-U at 4.

[64] Kuhnert R. et al., *Reanalyses of Case Control Studies Examining the Temporal Association Between Sudden Infant Death and Vaccination*, 30 Vaccine 2349 (2012), Exhibit C-20 at 2355.

[65] Zinka, B. et al., *Unexplained Cases of Sudden Infant Death Syndrome Shortly After Hexavalent Vaccination*, 24 Vaccines 5779 (2006), Exhibit 13-S.

[66] Kries, R. et al., *Sudden and Unexpected Deaths After the Administration of Hexavalent Vaccines (Diphtheria, Tetanus, Pertussis, Poliomyelitis, Hepatitis B, Haemophilus Influenza Type B): Is There a Signal?*, 164 Eur. J. Pediatr. 61 (2005), Exhibit 13-R.

[67] Ottoviani, G. et al., *Sudden Infant Death Syndrome (SIDS) Shortly After Hexavalent Vaccination: Another Pathology in Suspected SIDS?*, 448 Virchows Arch. 100 (2006), Exhibit 13-T at 4.

"grey zone" cases in which it is difficult to establish if the pathological findings were sufficient to cause death.

Each of the studies contained considerable acknowledgment of its own methodological deficiencies that may have affected the results. In different papers, these included inclusion without autopsies, small samples, comparing SIDS victims to living children rather than vaccinated SIDS to unvaccinated SIDS, as well as having no control group or having potential underreporting as in VAERS. The Kuhnert paper which analyzed three other case control studies including Venneman, said, "The small number of cases is a problem with the three case control studies, particularly in view of the short time periods under investigation. This problem is illustrated by the very broad confidence intervals of estimates that are only related to the events of the first few days."[68]

Dr. Miller criticized several of the studies for failing to use cases that were verified by autopsy, that the Vennemann study compared a new hexavalent vaccine to older vaccines rather than asking the question as to whether vaccines regardless of new or old could be associated with SIDS, and used data based on the number of vaccines sold rather than administered. Tr. 70-74. He noted that the IOM concluded that the evidence that it reviewed was insufficient to accept or reject causation. Tr. 387. In his report, Dr. Miller explained why it is difficult to do reliable epidemiological studies of SIDS. He said, "[I]f the risk for SIDS is present only in those infants who are already vulnerable because of a pre-existing brainstem abnormality, then no retrospective (or prospective) epidemiological study not grounded in a thorough neuropathological examination of all of the supposed SIDS cases would be likely to identify that putative causal relationship." Exhibit 13 at 5. He observed that J.B. would be one of those not counted as he did not have a complete neuropathological autopsy. *Id.* at 6.

Dr. McCusker criticized some studies as case reports or having no control group. She looked to Kuhnert which incorporated Vennemann to argue that there was no significant finding that SIDS occurred more often than chance. Tr. 228.

The Vaccine Program does not require epidemiological evidence and the studies presented contained multiple methodological flaws, and did not tend to shed much light on the question at issue, that is, whether the death of the child in this case was caused or triggered by the vaccinations received the day before. Thus the studies were read and considered and credited to show that vaccines are generally safe, but were specifically unpersuasive as to whether they are on rare occasions the exogenous factor resulting in the perfect storm in a child with a defective arcuate nucleus or other 5HT structure during the vulnerable period of life. They were also unpersuasive to reject causation as they frequently showed some temporal correlation to the receipt of vaccines even if those correlations were not found to be statistically significant.

[68] Kuhnert et al. (2012), Exhibit C-20 at 2355.

C. Expert Opinions

1. Petitioners' Expert Douglas C. Miller

Dr. Douglas C. Miller earned his bachelor's degree from Williams College and his medical degree from the University of Miami School of Medicine in 1978.[69] He received a Ph.D. in Physiology and Biophysics from the University of Miami in 1980. *Id.* Dr. Miller was a resident at Massachusetts General Hospital from 1980-1984, focusing in the areas of anatomic pathology and neuropathology. *Id.* He currently serves as a clinical professor of pathology and anatomical sciences, as well as the program director of pathology residency, at the University of Missouri School of Medicine. *Id.* at 3. He also serves as an associate medical examiner for Boone, Callaway, and Greene Counties in Missouri. *Id.*; Tr. 10. Dr. Miller has been a full-time faculty member at the medical schools at Robert Wood Johnson in New Jersey, New York University, and the University of Missouri. He has published over 150 articles in medical journals and is the author of a textbook on neuropathology.

i. *Althen* Prong One: Medical Theory

Dr. Miller, consistent with the dominant literature in the field, proposed the Triple Risk Model of SIDS as the framework for his theory of causation.[70] Tr. 19. As explained above, this model first provides that SIDS can occur only when an infant is in a critical developmental period (the first year of life). Tr. 20. Second, SIDS can occur only to an infant who is inherently vulnerable in some way. *Id.* Third, the infant must encounter an exogenous stressor. *Id.*

Dr. Miller explained the normal physiological process for handling carbon dioxide and stimulating breathing. He said if the carbon dioxide levels rise above a normal threshold to an abnormal threshold, a normal brainstem's response – in this age group – is mediated by the arcuate nuclei alone. The excess carbon dioxide stimulates other neuronal systems to alert the cervical spinal cord motor neurons to tell the diaphragm and other muscles of respiration to contract, at the same time signaling up through other mechanisms in the basal forebrain, underneath the lower part of the frontal lobes, to wake up. In general, there is arousal and there is deeper breathing to blow off the carbon dioxide, and if it is position-related, the infant would also move so that homeostasis is restored. Tr. 29. He explained that this process is dependent on serotonin, an excitatory neurotransmitter, which stimulates the cells to which it signals to fire more rapidly to increase breathing or arousal. Tr. 28. That is in contrast to GABA, which is inhibitory and balances the excitatory effect of serotonin. *Id.*

Dr. Miller explained that the majority opinion in the medical community is based principally but not exclusively on work done by Dr. Hannah C. Kinney, in a series of papers that stretch back more than 25 or 30 years and has been verified by other people. She has shown that "the medulla, the lowest part of the brainstem, in infants who have died of SIDS and have been autopsied and have had the appropriate examinations is defective. In particular, it has a defect in

[69] Curriculum Vitae of Dr. Douglas C. Miller, Exhibit 14 at 1.
[70] Kinney, H.C. et al., *Medullary Serotonergic Network Deficiency in the Sudden Infant Death Syndrome: Review of a 15-Year Study of a Single Dataset*, 60 J. Neuropathol. Exp. Neurol. 228 (2001), Exhibit 13-C.

a set of nuclei [or] groups of neurons, which use, as a neuro-transmitter a molecule called serotonin … which is also known as 5-hydroxytryptophan and which is abbreviated as 5-HT." Tr. 19. He further explained that Dr. Kinney and others have shown various deficits in infants, but the ones who die of SIDS have in common deficits in either the number of 5-HT neurons or in receptors for serotonin on those neurons or various other associated abnormalities. All of these suggest that the infants who die of SIDS usually die in their sleep and usually after an episode of apnea – that is, the cessation of breathing with elevated carbon dioxide in the blood to which they fail to respond normally. They fail to respond because the 5-HT system is the system which, in that age group, allows for arousal and increased breathing to respond to that kind of a danger. Since they fail to respond, they do not wake up, they do not breathe, and they die. Tr. 20.

Dr. Miller theorized, consistently with the research of Dr. Kinney and others, that many SIDS infants have "abnormalities of the medullary serotonergic synaptic systems governing respiration and arousal from apnea." *Id.* at 6. He said that "we have data that at least 70 percent of infants who ultimately die of SIDS have a defective 5-HT system which is way over half and thus statistically likely that [J.B.] was one of those." Tr. 62. Dr. Miller said, "It's really a neurochemical question. These molecules (cytokines) are provoked by an immune response, an innate immune response, originally in the periphery, but their effect in terms of SIDS is a neurochemical effect, affecting synaptic transmission and neuronal activity of the 5-HT system and maybe the GABA system in the medulla, and that's a neurochemical synaptic effect." Tr. 61. He stressed that the role of the cytokines in SIDS was in their capacity to modify normal neurologic function rather than being purely immune in nature. He assumed that J.B. was an immunologically normal child, who when given a vaccination would have had an appropriate immune response, including the production of cytokines such as the ones identified by Kashiwagi et al. Therefore, he would expect the level of cytokines to be transiently increased after vaccination. Tr. 62. "These cytokines would have been circulating in his body after vaccination and we have direct evidence that there was some cytokine-central nervous system interaction in that he had fever. Then there is a logical chain of events that says cytokines depressed the 5-HT system in a defective medulla leading to SIDS during sleep." Tr. 62-63.

Dr. Miller stated that research is still identifying all of the exogenous stressors for SIDS. Tr. 44. He opined that one very well-recognized exogenous stressor for SIDS is mild infection. Tr. 45. Some of the estimates indicate that 40 - 50% of SIDS victims have had a very recent or current mild upper respiratory infection (URI) at the time of death. Tr. 45. He said that it is explicit in the literature from Dr. Kinney's laboratory and others that what happens with mild infections is that the response to the infection involves the production of certain cytokines and that those cytokines can act on the central nervous system. He presented a theory: that a mild upper respiratory infection can act as a neurochemical stressor by prompting the upregulation of cytokines, which he theorizes are detrimental in two ways. He said that an infection could cause fever, an extrinsic risk factor, and can cause elevated IL-1β levels, which would further depress a defective medullary 5-HT system. The system would then be incapable of responding to excess carbon dioxide, resulting in death. Tr. 46.

Dr. Miller cited several studies, including ones discussed above by Rognum, Kashiwagi, Kadhim, Brambilla, Stoltenberg, and Froen, that addressed the issue of cytokine stimulation and the function of cytokines entering the central nervous system. From these studies, Dr. Miller concluded that either mild URIs or vaccinations upregulate the production of cytokines, and these inflammatory cytokines, can "shut down" a structurally vulnerable 5-HT system and completely prevent it from restoring an infant's normal breathing. Tr. 35. In other words, the cytokines and the structural defect in the serotonin system acting in concert during a vulnerable period have the cumulative effect of causing SIDS by making the baby incapable of responding to excess carbon dioxide.

Dr. Miller noted that Kashiwagi found similar cytokine profiles in the recently-vaccinated population and those suffering from influenza, and further that the cytokine profiles were similar in post-vaccination babies whether they had a fever or not. Tr. 49. He explained that cells that are injured by infection initially produce an innate immune response. The cells of the innate immune system release cytokines which signal further activation of the adaptive immune system to respond to the foreign antigen. He said that there is a wide range of things that the cytokines produce, but the initial production is certainly peripheral where there is infection. Tr. 50. He testified that there is a whole lot of evidence that cytokines, produced peripherally, interact with the central nervous system and the easiest one to understand is the way fever is produced. He explained that fever is mediated by the central nervous system and specifically by the hypothalamus in the brain. The hypothalamus sets our body temperatures. It causes us to shiver if we are in the cold and need to warm up, or to sweat when we are overheated. Tr. 50-51. He further explained that if the fever was generated in response to an infection outside of the brain, such as in the case of a URI, there would be no inflammation in the brain as the brain is not infected, but there is still an interaction with the hypothalamus in the brain caused by cytokine signaling that causes fever in response to an infection outside of the brain. Tr. 51-52. Dr. Miller stated that he was not aware of any literature describing URI as a *mechanical* exogenous stressor and that in his professional experience conducting autopsies, he had never seen a URI "obstruction of the airway" that would be sufficient on its own to cause death. Tr. 46.

Dr. Miller stated that vaccinations can be an extrinsic risk factor in SIDS, as they prompt the upregulation of cytokines that, among other things, produce fever. Tr. 62-63. He testified that, based on the literature, there is a scientifically-plausible mechanism for vaccinations acting as the extrinsic risk factor in SIDS in much the same way as a mild infection. He explained that when you get a vaccination or a whole group of them at once, as J.B. did, it evokes a response which includes the production of cytokines, and that among those cytokines are IL-6, TNFα, and IL-1β. The physiological studies have shown that these can raise body temperature by producing fever, which is a risk factor, and they can inhibit the activity of 5-HT neurons in the medulla causing prolonged apneas and interference with autoresuscitation. Tr. 54, 62-63. When the vaccines are administered in the presence of the defects in the medulla, during the critical developmental period, they are likely to have a similar effect as mild infection that may cause a failure of the medullary response system and ultimately a death. Tr. 54.

Dr. Miller stated that mild upper respiratory tract infection is widely recognized to be an exogenous stressor under the Triple Risk Model. However, he acknowledged that there is not wide recognition, or a generally accepted theory, that vaccinations are an exogenous stressor. He stated that the Institute of Medicine concluded "the evidence is insufficient to say that there is an effect or there isn't an effect." Tr. 55. The Kinney research team has not studied the relationship between vaccination and SIDS. Tr. 60. Dr. Miller pointed to "multiple reports of similar cases of SIDS following various neonatal or infant vaccinations, mostly stressing the close temporal relationships between vaccination, increased cytokine production, and death from apparent SIDS as seen with this case."[71] He said that these individual cases and small case series show a "suspicious association between the timing of vaccination and the timing of SIDS deaths." Tr. at 55.

Summarizing his theory and review of the literature, Dr, Miller testified that the papers cited, including Kadhim, Kashiwagi, Rognum, Stoltenberg, and Froen, "verify the importance of the 5-HT system and its interactions with the GABA system in the medulla in terms of response to apnea or other respiratory-related insults." Tr. 34. Second, "they showed that there's an altered cytokine profile in SIDS cases versus non-SIDS cases, dying of other things, like drowning or trauma." *Id.* Third is the specific information on IL-1β, in that it inhibits the 5-HT system. *Id.* Therefore, in the context of SIDS, this suggests that if there is an elevated level of IL-1β to which the 5-HT neurons are exposed in an infant who already has too little 5-HT activity because of a defective brainstem, this additional cytokine effect would shut down the system such that it would not respond to other external stressors such as prone sleeping, nicotine, infection or fever. Tr. 34-35.

Dr. Miller addressed this analysis in terms of the cytokine reaction generated by vaccines. He said that we know that when a child gets a vaccine or a whole group of vaccines all at once, as occurred in this case, it evokes a response which includes the production of cytokines; that among those cytokines are IL-6, TNFα, and IL-1β. Those levels go up in the blood. We know that IL-1β can inhibit the activity of the 5-HT neurons in the medulla. If you take an infant who has a defective medulla with a defective 5-HT system already, you put in a stress situation with elevated carbon dioxide or low oxygen, and there is a vaccination which further shuts down the 5-HT system, and you can get a complete failure of response and therefore a death. He concluded that the mechanism is plausible. Tr. 54.

ii. *Althen* Prong Two: Logical Sequence of Cause and Effect

Dr. Miller then applied his theory to J.B.'s specific case. As an initial matter, he agreed with the decision to classify J.B.'s death as SIDS. Exhibit 13 at 1. Under the Triple Risk Model, Dr. Miller opined that J.B. was in the critical developmental period. Tr. 44. Statistically, he was inherently vulnerable. Dr. Miller opined that Kinney et al. have found that a significant proportion – up to 70% – of SIDS infants have abnormalities in the arcuate nuclei and other sections of the medulla. Exhibit 13 at 3. Dr. Miller said that there is also a Japanese study in

[71] Vege & Rognum (2004), Exhibit 13-Q; Kries et al. (2005), Exhibit 13-R; Zinka et al. (2006), Exhibit 13-S; Ottoviani et al. (2006), Exhibit 13-T; Traversa et al. (2011), Exhibit 13-U; Institute of Medicine, *Adverse Effects of Pertussis and Rubella Vaccines* (1991), Exhibit 13-V.

which that number went as high as 90 percent. Tr. 38. He testified that it is statistically most likely that J.B. also had this medullary 5 HT defect based on the Kinney data and other studies, even though it was not confirmed because the medical examiner did not sample that section of the brain. Exhibit 13 at 4-6; Tr. 37-38. Dr. McCusker agreed that "according to the Triple Risk theory, the brain problem must exist [in J.B.'s case]." Tr. 206.

A great many autopsies of SIDS infants outside of the research context do not section all of the necessary areas of the brain or view them histopathologically, which is typical of medical examiner autopsies. Tr. 16. Respondent's expert pathologist, Dr. Harris, acknowledged that based on Dr. Kinney's research, the majority of SIDS babies and up to 70% in some of her studies had an abnormality of the 5-HT system. Tr. 346. However, "[d]etection of these abnormalities requires special immune-histochemical research techniques not generally available for a 'routine' autopsy." *Id.* Dr. Miller testified that even in some autopsies where no structural abnormality was found in Dr. Kinney's research, when the full histochemistry was performed, there were still receptor binding deficits, such as in the IL-6 and gp130 studies. Tr. 41-42. Unfortunately, the types of tools she used including autoradiography and immunohistochemistry are not generally available for autopsies. Tr. 42-43.

Dr. Miller discussed the logical sequence of cause and effect between vaccines administered on September 2, and J.B.'s death on September 3. He opined that the vaccines acted as a critical external stressor in this case. He noted that J.B. was a "healthy infant… developing normally." Exhibit 13 at 4. He was "immunologically normal." Tr. 62. Therefore, after receiving vaccinations, his body mounted an innate immune response including the production of cytokines. Exhibit 13 at 6; Exhibit 16 at 1; Tr. 63. Those cytokines circulated in J.B.'s body, specifically into the central nervous system. Exhibit 13 at 6; Tr. 63. These peripheral cytokines interacted with the hypothalamus to provoke fever the night after the vaccinations, and the following day (before J.B.'s death). Exhibit 13 at 6; Exhibit 16 at 1; Tr. 62-64. "Those cytokines then acted in the brainstem which was already deficient in serotoninergic drive for respiratory effort, leading to an apneic episode from which he did not recover, i.e., SIDS." Exhibit 13 at 6; *see also* Tr. 62 (the cytokines "depress[ed the] 5-HT system in a defective medulla, leading to SIDS during sleep").

He opined that there was "no other demonstrable inciting event" for J.B.'s death. Exhibit 13 at 1. There was no evidence of the fever being related to anything other than J.B.'s vaccinations. Tr. 66. The autopsy did not identify any other infectious processes. Tr. 66.[72]

Dr. Miller was asked whether the pillow in J.B.'s crib increased the risk of SIDS. Tr. 87. Dr. Miller was not sure whether J.B.'s head was on the pillow. *Id.* He said, "If the pillow was by his feet, I don't think it's a risk factor." *Id.* A review of the investigation files indicates that there was no evidence as to whether or not his head was on the pillow. The only relevant evidence was that it was "a little crib pillow-very flat" and that his mother told the police that his nose or mouth were not covered when she found him about ten minutes after replacing his pacifier. Exhibit 7 at 5.

[72] Dr. Miller noted that there was bacterial growth and food particles in J.B.'s lungs and epithelial cells in the upper airways. He opined that this was not evidence of a separate infectious process. He agreed with the medical examiner that these were terminal or resuscitative events. Tr. 17-18; 66; 352-53.

On cross-examination, Dr. Miller stated that J.B. was placed on his back but was found on his side, which demonstrates that he was able to "move around." Tr. 92. However, J.B. did not pass away until "something else intervened." Tr. 85. Based on his theory and the temporal association, Dr. Miller opined that the vaccines were the intervening factor that caused J.B.'s death. Tr. 85; Exhibit 7 at 5. He said that he looks at SIDS cases individually and that it was his diagnosis that the vaccines contributed substantially to the death of J.B. in this case. Tr. 106-08.

iii. *Althen* Prong Three: Timing

With regard to timing, Dr. Miller stated several reports "have noted an elevated risk for SIDS within the first 48 hours following immunization, although this is not statistically significant." Exhibit 13 at 5. He stated that J.B. died within this 48-hour "window of elevated risk" following vaccination. *Id.*

Dr. Miller also stated that the available evidence is that foreign antigens, like those contained in vaccinations, activate the production of cytokines "within hours" and that production "peaks within 2 to at most 4 days." Exhibit 16 at 1. Thus, a vulnerable infant who receives vaccinations is most likely to suffer a fatal event if one is to occur "within the first 48 hours to at most 4 days." *Id.* Dr. Miller opined that J.B.'s death was "well within this vulnerable period." *Id.*

2. Respondent's Expert Dr. Christine McCusker

Dr. Christine McCusker earned a Masters in Molecular Virology in 1988, followed by an M.D. in 1993, at McMaster University, in Hamilton, Ontario. Exhibit D at 1. Her residency training was in pediatrics, at Montreal Children's Hospital, McGill University, from 1993-1996. *Id.* at 2. She was then a clinical fellow in allergy and immunology at McGill University from 1996-1999. *Id.* Dr. McCusker is board certified in pediatrics. *Id.* She is currently the division director of pediatric allergy, immunology, and dermatology at the Montreal Children's Hospital at McGill University Health Center and is the director of the Clinical Immunology Lab. Tr. 122. She has a wet lab that studies developmental immunology, which has peer-reviewed funding. *Id.* She also runs a clinical research program that uses databases to follow patients with primary immunodeficiency. *Id.* In addition, she sees pediatric patients at McGill Children's emergency room and at several allergy, immunology, and general pediatrics clinics. Tr. 124. Dr. McCusker also teaches medical students in the areas of immunology, dermatology, and malignant hematology. *Id.*

i. *Althen* Prong One: Response to Petitioners' Medical Theory

Like petitioners' expert Dr. Miller, Dr. McCusker accepted Dr. Kinney's formulation of the Triple Risk Model. Dr. McCusker agreed with Dr. Miller on the critical development period, and that an infant may be "vulnerable" because of a brain defect, premature birth, male gender, and/ or African American race. Dr. McCusker disagreed with Dr. Miller's opinion that upper respiratory infection, and by extension, vaccines, act as *neurochemical* exogenous stressors within the Triple Risk Model.

Dr. McCusker spent considerable time explaining why upper respiratory infection and other exogenous stressors, such as "being placed or found in a prone/ side-sleep position, found face down, head covered, sleeping on an adult mattress, couch, or playpen, soft bedding, bed-sharing, and signs of upper respiratory tract infection," are *mechanical*. Specifically, each one impedes an infant's ability to exhale carbon dioxide and inhale fresh oxygen, thereby increasing the risk of SIDS. Tr. 127-28.[73]

She opined that the prone sleep position is more widely recognized as an exogenous stressor for SIDS, but that the side-sleep position poses just as much risk. Tr. 131. She stated that breathing depends on "drop[ping] the diaphragm down and creat[ing] a negative airspace, [in which] the air comes rushing in." Tr. 130. An infant's body is not fully developed, so it uses "more than just the diaphragm" and "a lot of abdominal muscle to breathe." *Id.* An infant lying supine with the head back breathes most easily. *Id.* In contrast, an infant in either the prone or side-sleep position has more difficulty dropping the diaphragm and exhaling carbon dioxide. *Id.* Dr. McCusker also opined that the side-sleep position compresses "at least half your rib cage." Tr. 132. She stated that an infant's rib cage is "soft" and "very pliable." Therefore, it does not take much to influence the infant's ability to exchange air. *Id.* She also noted that an infant's breath is much more shallow and rapid than an adult's, and therefore the diffusion of exhaled carbon dioxide is less than in adults and rebreathing is more likely. *Id.* Theoretically, this means that an infant is at greater risk of re-inhaling expelled carbon dioxide. *Id.* Dr. McCusker acknowledged that the Back to Sleep Campaign previously advised parents to avoid all risk factors for SIDS, and that early research emphasized avoiding prone sleeping. *Id.* at 132-33. However, she said more recent studies looking "a little bit more closely" indicate that "prone and side-sleeping have equal risk." Tr. 134. She also stated that an infant learns to roll from the supine position to the side or prone position, but "usually not until somewhere between four and six months." Tr. 134-35. She did acknowledge, however, that the American Academy of Pediatrics does say that once a child is able to roll from his back to his side or to prone, then the parent should not disturb them. They should just have nothing else in the crib that could obstruct breathing. Tr. 135.

She also stated that gastroesophageal reflux is an exogenous stressor. Tr. 137. Specifically, an infant's airway and esophagus are linked at the back of the throat. *Id.* An infant may regurgitate and inhale at the same time, and therefore stop breathing momentarily. *Id.* at 138. If the infant neither swallows nor expels the food, his breathing will become obstructed and he will not recover. *Id.*

Dr. McCusker stated that bundling is an exogenous stressor and suggested several possible reasons why. *Id.* at 135. First, she opined that bundling decreases an infant's arousal, which helps the infant go back to sleep, but may increase the incidence of SIDS. *Id.* at 136. Second, a bundled infant may be less able to roll out of the prone or side-sleeping position. *Id.* Third, bundling may be an exogenous risk factor by leading to hyperthermia. *Id.* It should be noted that there is no evidence of bundling in this case, as J.B.'s father said he placed him on his back with a blanket across the midsection, but there was no indication that he was wrapped or bundled.

[73] Trachtenberg, Kinney, et al. (2012), Exhibit C-11.

Dr. Miller stated that hyperthermia was a term encompassing both high ambient temperature and fever. But Dr. McCusker disagreed. She testified that hyperthermia was high ambient temperature, and *hyperpyrexia* was fever. She stated that older literature listed both hyperthermia and hyperpyrexia as exogenous risk factors for SIDS. Tr. at 201, 287. However, she opined that newer literature, such as an article by Trachtenberg, lists hyperthermia as a risk factor for SIDS, but not fever. Tr. at 201, 287, 290. She agreed with this distinction. She reasoned that an infant experiencing hyperthermia tries to cool himself down. Tr. 289. To do so, the infant takes short, shallow breaths, which increase CO2 levels, which trigger the pathway to SIDS. Tr. 288, 295. She cited an article by Harper and Kinney, which provides that "vasodilation associated with overheating makes compensation for low blood pressure more difficult."[74] Dr. McCusker opined that fever is *not* a risk factor for SIDS. Specifically, she said in fever the body fasciculates or shivers – it makes small muscle movements that create friction, which generates heat inside the body. *Id.* at 184. The body cannot make these movements during deep REM sleep. Therefore, it stays in NREM sleep. *Id.* at 184-85. She opined that an infant generating or maintaining a fever, who does not descend into REM sleep, is less susceptible to SIDS. *Id.* at 202. It should be noted that nowhere in the submitted literature was an explicit distinction made between hyperthermia and hyperpyrexia, including in Trachtenberg or the Harper & Kinney article. Dr. McCusker is correct that in a 1992 article by Dr. Kinney, she mentioned "infection, fever and hyperthermia" as exogenous stressors.[75] Later articles generally reference hyperthermia and overheating. However, in a 2009 article, Dr. Kinney described a SIDS scenario in which in part she describes "an infant may be slightly febrile due to an otherwise trivial upper respiratory tract infection (3) as a consequence, the apnea component of the LCR is inordinately prolonged by mild hyperthermia,"[76] This reference would appear to suggest that the term hyperthermia may be more broadly inclusive.

Unlike Dr. Miller, Dr. McCusker characterized mild upper respiratory infection as a purely mechanical extrinsic risk factor for SIDS. Tr. at 127-28. She opined that an infant is accustomed to breathing through the nose, which enables uninterrupted bottle or breast-feeding. *Id.* at 138-39. When the nose is congested, she said, the infant still exerts significant effort to breathe through the nose, which elevates carbon dioxide. *Id.* at 139. If and when the infant finally resorts to breathing through the mouth, that is less effective and also increases the risk of respiratory distress. *Id.* at 140-43.

Dr. McCusker then spoke about cytokines. She asserted that cytokines serve a variety of positive functions in the healthy human brain. *Id.* at 145-58.[77] Researchers initially theorized that cytokines found in the brain, including IL-6, IL-1β, and tumor necrosis factor-alpha (TNF-alpha), had traveled there through the cerebrospinal fluid, to respond to inflammation in the brain. *Id.* at 151-52. However, research beginning in the late 1990s indicates that the brain itself

[74] Harper & Kinney (2010), Exhibit C-12 at 3.

[75] Filiano & Kinney (1992), Exhibit 13-A at 401.

[76] Kinney et al. (2009), Exhibit 13-H at 539.

[77] Besedovsky, H.O. and A. del Ray, *Central and Peripheral Cytokines Mediate Immune-Brain Connectivity*, 36 Neurochem Res. 1 (2011), Exhibit C-3.

produces cytokines. *Id.* at 152. Dr. McCusker cited articles reporting that inflammatory cytokines such as IL-6 and IL-1β regulate pain sensitivity, memory consolidation, stress, fever, and sleep. *Id.* at 152-56.[78] Ron-Harel wrote, "Pro-inflammatory cytokines are abundantly expressed in healthy brain and are involved in the regulation of many physiological functions such as pain sensitivity, memory consolidation and neural plasticity. Elevation in brain cytokine levels is considered part of the adaptive response to external stimuli. Exposure to acute psychological stressors by induction of adrenalin, noradrenalin and dopamine induces an increase in brain proinflammatory cytokines which modulate the neuroendocrine and behavioral response to the stressor. *Id.* at 3. She also cited an article by Moidunny et al. suggesting that cytokines including IL-6 may play a neuroprotective role in the brain after stroke or head trauma. *Id.* at 157.[79] Moidunny was studying the role of IL-6 in reducing glutamate excitotoxicity in stroke and head trauma with the goal of further research to identify additional pharmacological protection with IL-6 from glutamate neurotoxicity in these patients. Moidunny does not discuss SIDS or the role of peripheral cytokines in this article.

Dr. McCusker also cited to an article by Chen Miller, which discusses the role of Tryptophan Hydroxylase 2 which is a rate limiting enzyme in 5-HT biosynthesis. The article discusses advances in understanding Tryptophan Hydroxylase TPH and TPH2 which are critical for the initiation of the synthesis of 5-HT (serotonin) which modulates the stress response by interacting with the hormonal hypothalamic pituitary adrenal axis and neuronal sympathetic nervous system. The TPH2 mRNA expression is abundant in the raphe nuclei or regions containing raphe nuclei such as the pons and medulla, while it is detectable in a number of other regions including the cortex, hypothalamus, thalamus, hippocampus, amygdala and cerebellum. TPH2 gene expression is sensitive to stressful events including hemorrhage and hypoxia and involves neuronal and hormonal mechanisms. The article hypothesizes about the role of TPH2 and serotonin in response to stimulating events such as hypotensive hemorrhage, hypoxia and adverse events experienced in early life or as an adult, and a possible role in such conditions as PTSD but it was not clear how this paper directly addresses the issue of respiratory depression in SIDS. [80]

Dr. McCusker argued that the various animal studies cited by Dr. Miller were not relevant to cytokines' effect in infant brains *in vivo*. *Id.* at 162-87. First, she stated that the Brambilla article,[81] which showed that IL-1β depressed serotonin in rats' brain tissue, was not

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[78] Ron-Harel, N. et al., *Brain Homeostasis is Maintained by "Danger" Signals Stimulating a Supportive Immune Response Within the Brain's Borders*, Brain Behav. Immun. (2011), Exhibit C-1; Su, Y. et al., *Predator Exposure-Induced Cerebral Interleukins are Modulated Heterogeneously in Behavioral Asymmetry*, 135 Immunol. Let. 158 (2011), Exhibit C-4; Kinney et al. (2011), Exhibit 13-F.

[79] Moidunny, S. et al., *Interleukin-6-Type Cytokines in Neuroprotection and Neuromodulation: Oncostatin M, but not Leukemia Inhibitory Factor, Requires Neuronal Adenosine A1 Receptor Function*, 114 J. Neurochem. 1667 (2010), Exhibit C-2.

[80] Chen, G.L. & G.M. Miller, *Advances in Tryptophan Hydroxylase-2 Gene Expression Regulation: New Insights into Serotonin-Stress Interaction and Clinical Implications*, 159B Am. J. Med. Genet. B. Neuropsychiatr. Genet. 152 (2012), Exhibit C-15.

[81] Brambilla, D. et al., *Interleukin-1 Inhibits Firing of Serotonergic Neurons in the Dorsal Raphe Nucleus and Enhances GABAergic Inhibitory Post-Synaptic Potentials*, 26 Eur. J. Neurosci. 1862 (2007), Exhibit 13-M.

relevant to sleeping infants. *Id.* at 185. Specifically, the Brambilla study submerged rats' brain tissue in "super-physiologic doses" of IL-1*β* for an extended period of time; and kept it isolated in petri dishes, which would not reflect what happens to a vulnerable infant in a "crisis situation." *Id.* at 186-87.

Similarly, Dr. McCusker opined that the Stoltenberg and Froen articles,[82] which reported that very young piglets did not recover from apnea as quickly when they received super-physiological doses of cytokines, had limited significance. *Id.* at 162-63. The articles reported this effect only in piglets younger than fifteen days old; in a previous study, cytokines did not have any effect on older piglets. *Id.* at 163. Dr. McCusker opined that pigs' and infants' respiratory systems develop at similar paces; therefore, piglets younger than fifteen days old could be compared only to infants under one month old. *Id.* at 164. Furthermore, she argued that Froen induced extremely high cytokine levels that would not occur naturally in infants. *Id.* at 171. On rebuttal, Dr. Miller responded to this criticism, by saying that pigs' *brains* are very different from human brains. Pigs are born with much more myelin than adult brains; they are much more mature than our brains. The piglets are walking and do things early in piglet life that humans take up to a year or more to do. Thus, this model is not an irrelevant model for a 4-month-old in terms of brain development. He noted correctly that what Stoltenberg and Froen were looking at was *brain physiolog*y or pathophysiology. They were not looking at respiratory development in terms of pulmonary or bronchial development or vascular or cardiac development. They were looking at the responsive neurons in the brain. Tr. 358.

Dr. McCusker also argued that studies of cytokine levels in human brains were only observational, and did not support Dr. Miller's theory. She stated that the Rognum article[83] found similar IL-6 levels in SIDS infants *with* and *without* minor infections. She argued that if infection upregulates cytokine levels, the data between these two groups should be different. *Id.* at 173-74.

Dr. McCusker opined that cytokines play a protective role. Specifically, they maintain homeostasis in the body. She stated that cytokines carry messages (e.g., that an infant's breathing is disrupted) to receptor cells, which contain gp130 molecules, which are supposed to respond to those messages (e.g., by prompting the infant to arouse or gasp). *Id.* at 174-77. Dr. McCusker noted that the Rognum article reported that SIDS brains showed increased binding of IL-6 to neurons in the arcuate nucleus, but no corresponding increase in expression of gp130 (a "signal transducer" for the 5-HT system).[84] She said that if the lack of a corresponding increase in gp130 is physiologically important, which "is a big if," it would imply that the increased IL-6 would not be doing anything. Tr. 175

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[82] Stoltenberg et al. (1994), Exhibit 13-J; Froen, J.F. et al., *Adverse Effects of Nicotine and Interleukin-1β on Autoresuscitation After Apnea in Piglets: Implications for Sudden Infant Death Syndrome*, Pediatrics (April 2000), Exhibit 13-K.

[83] Rognum, Kinney et al. (2009), Exhibit 13-N; Kadhim et al. (2010), Exhibit 13-O.

[84] Rognum, Kinney et al. (2009), Exhibit 13-N.

As Dr. Miller mentioned, Rognum suggested that IL-6 may have "aberrant interactions" with the arcuate nucleus, leading to SIDS. However, Rognum also suggested another theory: that the "increased expression of the IL-6R in the arcuate nucleus *may be a compensatory mechanism* as defective arcuate neurons may require excessive IL-6 stimulation in order to respond to altered CO2 levels." *Id.* at 528 (emphasis added). Kinney cited this theory, writing: "The expression of IL-6 is elevated in the arcuate nucleus in SIDS infants, which may reflect a compensatory mechanism whereby defective arcuate 5-HT neurons require excessive cytokine stimulation to respond to infection-induced hypercapnia."[85] Dr. McCusker adopted and elaborated on this theory suggesting that IL-6 mounts a protective response. Tr. 157. She cited an article by Moidunny, which states that some IL-6 cytokines have "neuroprotective properties" and that IL-6 requires gp130 receptor subunits to be activated for signaling.[86] When a stressor – such as inadequate oxygen or hypoxia - occurs, the cytokines bind to the 5-HT system, which expresses gp130 molecules to prompt a response – such as prompting the body to turn over or gasp. Tr. 155-56, 161, 175-77, 241. Dr. McCusker opined that these responses can be "quite rapid, within hours or days." Tr. 180-81. Based on these findings, Dr. McCusker suggested that SIDS infants have potentially protective IL-6 molecules in the brain, but in SIDS infants hey fail to prompt the upregulation of gp130 molecules. Thus the IL-6 is ineffective. Tr. 176

Dr. McCusker stated that neither the Kinney team nor the AAP lists vaccinations as a risk factor for SIDS. *Id.* at 144. Dr. Miller testified to a conversation that he had with Dr. Kinney who told him that she did not want to study vaccines because she did not want to testify and did not want to be involved in vaccine controversies. Tr. 60. Dr. McCusker acknowledged that medical literature has reported a temporal association between vaccination and infant death in certain cases. Specifically, the Ottaviani study reported that a three-month-old white female infant received a hexavalent vaccine, lost consciousness one hour later, did not recover upon resuscitation, and passed away a few hours later.[87] Dr. McCusker highlighted that Ottaviani suggested the case might fall into a "SIDS 'gray zone'" because it was "difficult to establish whether the pathological findings [were] sufficiently severe to have caused the death." *Id.* Dr. McCusker noted that Ottaviani published another study of five infants displaying those same pathological abnormalities; however, that study did not mention vaccinations.[88] Therefore, she suggested that the vaccination in the first Ottaviani case was temporally associated with, but did not cause, that infant's death despite the fact that the author stated that in this case the sudden death in a child with arcuate hypoplasia could have been triggered by the hexavalent vaccine or could have been a gray zone case where it is difficult to determine if the pathological findings were sufficient to cause the death. Tr. at 103. It should be noted that the gray zone study focused on the neuropathology and histopathology of five specific SIDS victims to identify the possible brainstem abnormalities. The victims were chosen for study with no reference to vaccines or other specific causal pattern. The case report involving the child who died three

[85] Kinney et al. (2011), Exhibit 13-F at 195.

[86] Moidunny et al. (2010), Exhibit C-2 at 1668.

[87] Ottoviani et al. (2006), Exhibit 13-T at 101-02.

[88] Ottoviani G. et al., *Sudden Infant Death Syndrome "Gray Zone" Disclosed Only by a Study of the Brainstem on Serial Sections*, 33 J. Perinat. Med. 165 (2005), Exhibit C-16 at 6.

hours after receipt of the hexavalent vaccine was published subsequently to the gray zone study and mentions it as the group's prior work. It does hypothesize that the death could have been triggered by the vaccination or fall into the gray zone category.[89]

Dr. McCusker's comments in her report about the literature submitted by petitioners caused some concern, in that they could be read as misleading. Exhibit C at 7-8. Dr. McCusker stated that in the study by Rognum et al., "although [in SIDS infants] there was increased intensity staining for IL-6R, it was not different from those dying of infectious causes." Exhibit C at 7 (discussing Exhibit 13-N). However, Dr. McCusker did not note that at most the SIDS infants had mild infections, which would not be expected to cause elevated cytokines in the brain, while the other group had severe infections which *would* be expected to cause elevated cytokines in the brain and that "the mean IL-6R intensity grade in the arcuate nucleus was significantly higher in the SIDS group than in the control group."[90] [the control group died of "primarily violent causes."] *Id.* at 521.

Of greater concern was Dr. McCusker's characterization of the article by Kadhim et al. Exhibit C at 7-8 (discussing Exhibit 13-O). She stated: Kadhim et al. "examined IL-2 levels in SIDS versus non-SIDS brains and showed no difference in expression in IL-2 and they hypothesize that IL-2, like the cytokines IL-1β, TNFα, and IL-6, may be expressed in normally functioning brains of infants." Exhibit C at 7-8. Kadhim et al. actually stated; "SIDS victims often have preceding mild infectious/ inflammatory conditions (like coryza/ mild upper respiratory infections, soft stools/ mild gastroenteritis, post-vaccinal fever, etc.)"[91] They compared the brains of SIDS infants to those of infants who died of *severe* infectious/ inflammatory conditions. *Id.* at 123. They found that IL-2 levels were unexpectedly comparable in the two groups. *Id.* Kadhim said, "the comparable (equally intense) expression of IL-2 in SIDS infants was rather unexpected as SIDS victims have no obvious or detectable serious health conditions before death and that autopsies show no obvious cause for their demise. (as per definition). However, this high expression in SIDS would corroborate the tenet that SIDS victims experience hyperimmune reactions with 'exaggerated cytokine response to the often reported preceding mild/trivial infectious/inflammatory conditions. Upregulated cytokines exert serious effects on many biological systems including the turnover, release, and transmission of neurotransmitters; cytokines therefore act as neuro-modulators that could modify neural, neuroimmune, and neuroendocrine functions, and can modify synaptic transmissions." *Id.* at 125. The authors further concluded, "Thus various biological stressors such as infectious inflammatory, ischemic or anoxic, and hyperimmune conditions, and metabolic disorders induce IL-2 which is preferentially expressed in vital brainstem neuronal centers. IL-2 and other subsequently triggered cytokines in downstream immune inflammatory mediators interact with neurotransmitters and/or their receptors and modify their function. The resulting neuronal molecular disequilibrium tips the delicate molecular balance causing dysfunction in those vital

[89] Ottoviani et al. (2006), Exhibit 13-T at 103.

[90] Rognum, Kinney et al. (2009), Exhibit 13-N at 521.

[91] Kadhim et al. (2010), Exhibit 13-O at 122.

brainstem centers in producing disturbed homeostasis with potentially drastic effects on target organs systems and eventual death." *Id.*

Dr. McCusker reviewed the epidemiological papers submitted and noted that an article by Kuhnert found a *decreased* incidence of SIDS in days 1-3 after vaccination, then *increased* incidences of SIDS in days 4-7, 8-14, and 15-21. Tr. 229-35.[92] Furthermore, she stated that other studies did not find *any* temporal association between vaccination and SIDS. First, an article by Jonville-Bera et al. did not find a heightened risk of SIDS in French infants vaccinated at three months old.[93] Second, Toro et al. found that the incidence of SIDS in two-month-old children in Hungary decreased when that country instituted vaccinations at that age. *Id.* at 7.[94] Third, Vennemann et al. did not find an increased risk of SIDS with vaccination.[95] In Dr. McCusker's opinion, "large studies, designed to unmask rare events, have shown no link between vaccination and SIDS and have at least in some studies demonstrated a vaccine protective effect for SIDS." Exhibit C at 7.

At trial, Dr. McCusker added that the Kries study cited by petitioners did not support their case. Specifically, SIDS is defined as a syndrome that only affects children "under one year of age."[96] However, Kries et al. did not find an association between vaccination and death in children under one year old. They found an increased incidence of SIDS only in children vaccinated during the *second* year of life. *Id.* Therefore, she said this study does not support petitioners' theory about vaccination and SIDS. Tr. at 257.

ii. *Althen* Prong Two: Response to Petitioners' Opinion of a Logical Sequence of Cause and Effect

Dr. McCusker stated that there was "no evidence" that vaccinations contributed to J.B.'s death from SIDS on September 3, 2011. Exhibit C at 8; Tr. 126. She did not dispute that J.B. was in the critical development period. She agreed that "according to the triple-risk theory, the brain problem must exist" for an infant to succumb to SIDS. Tr. 206.

She agreed that vaccines "increase cytokine circulation." Tr. 195. She also stated that Kashiwagi et al. showed that 24-48 hours after vaccination, a child will have elevated cytokines, whether or not he has a fever. Tr. 199. "Cytokine elevation in this model is independent of fever." *Id.* Dr. McCusker stated that J.B. had a fever, and because he was generally healthy and had no signs of upper respiratory infection, the fever could be attributed only to his vaccinations. Tr. 204-05. The fever was "an indication that [J.B.] was responding… to the vaccine." Tr. 238.

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[92] Kuhnert et al. (2012), Exhibit C-20.

[93] Jonville-Bera A., et al., *Sudden Unexpected Death in Infants Under 3 Months of Age and Vaccination Status – A Case Control Study*, 51 Br. J. Clin. Pharmacol. 271 (2001), Exhibit C-18.

[94] Toro K. et al., *Change in Immunization Schedule and Sudden Infant Death Syndrome in Hungary*, 42 FEMS Immunol. and Med. Microbiol. 119 (2004), Exhibit C-19.

[95] Vennemann et al. (2007), Exhibit C-17.

[96] Kries et al. (2005), Exhibit 13-R at 1.

She stated that J.B. had a fever on September 3, 2011, but after he was given Advil that morning at approximately 8:00 a.m., his fever resolved. Exhibit C at 4; Tr. 204-05, 237. She also stated that a non-steroidal would last for eight hours. Tr. 192. She stated that "if IL-1β mediated respiratory depression [occurred] in the case of J.B., the Advil he was given would have acted to counter this effect, suggesting that this mechanism was not involved in his death from SIDS." Exhibit C at 5, 8.

Her theory was that J.B. 'was put down for his nap, he rolled over, he started rebreathing, and he died of a sudden infant death due to hypercapnia… independent of any cytokines." Tr. 206. She opined that there were several recognized exogenous stressors in J.B.'s case: formula feeding, side sleeping, soft bedding, and a pillow under his head. Exhibit C at 5; *also* Tr. 128-29. In her report, Dr. McCusker stated that J.B. "was found on his side with his face down on a pillow." Exhibit C at 4 (citing Exhibit 7 at 6). (The sixth page of this exhibit is a confirmation of faxing the record.) However, the preceding page is a handwritten scene investigation form. It states that J.B.'s crib had a "little crib pillow." Exhibit 7 at 5. J.B. was found "on side with head downward." *Id.* The form also indicates that neither J.B.'s nose nor his mouth was covered. *Id.*

At the hearing, Dr. McCusker first testified that J.B.'s "face was downward according to the reports." Tr. 128. On cross-examination, she could not identify where in the record it said that his face was down on a pillow. Tr. 265. She thought "he was found with his head down. There was a pillow in the bed, which is clear from the photos. So, it would be easy to hypothesize that he was at least found face down in the general vicinity of a pillow, and one would wonder what the pillow was doing in the bed if it wasn't for under his head." Tr. 266. She noted that the photos of the crib showed a pillow on one end of the bed and diapers and wipes on the other end. Tr. 266 (discussing Exhibit 9 at 8-9). She opined that J.B.'s head would have been on the end of the bed where the pillow was. Tr. 266-67. Dr. McCusker acknowledged, however, that she did not know whether J.B. was actually found with his head on the pillow. Tr. 267. She also agreed that J.B.'s crib was taken down shortly after his death, after which law enforcement and J.B.'s parents participated in a death scene reenactment. Tr. 267-68. That reenactment does not mention the pillow or any other elements that were in the crib. Tr. 268.

The undersigned asked Dr. McCusker about the "mechanical effect" of the sleep position she assumed that J.B. was found in. Tr. 269. Dr. McCusker stated that side-sleeping, a pillow under the head, "the lack of tight bed sheets," and the "disarray" in the crib all together present "the same risk factors as prone" sleeping. Tr. 269-72. The undersigned commented that these facts were not completely clear from the record. Tr. 272.

iii. *Althen* Prong Three: Response to Petitioners' Timing Argument

Dr. McCusker stated that she understood Dr. Miller's testimony to be that "the upregulation of the serotonin through the TPH2 and 1433 system… would not be an instantaneous event and that it would take time and presumably more than 24 hours' time." Tr. 180. She stated that "the production of increasing cortisol that occurs following a stimulus and

upregulation through IL-6 is actually quite rapid, within hours, not days." Tr. 181.[97] But she also stated that Kashiwagi et al. showed that a child will have elevated cytokine levels in the blood 24-48 hours after vaccination. Tr. 198.

3. Respondent's Expert Dr. Brent Harris

Dr. Brent A. Harris earned a Masters in Biology from Hahnemann University in 1988. Exhibit A at 1. He then earned a M.D. and a Ph.D. in Pharmacology from Georgetown University in 1995. *Id.* He then obtained post-doctoral training at Stanford Medical School, where he was a resident in Anatomic Pathology from 1995-1999, chief resident from 1997-1998, and a neuropathology fellow from 1997-1999. *Id.* Dr. Harris is board certified in anatomic pathology and neuropathology and is a Fellow of the College of American Pathologists. *Id.* He is currently an Attending Pathologist, Associate Professor in Neurology and Pathology, and Director of Neuropathology at Georgetown University Medical Center. *Id.* He also serves as a Neuropathology Consultant for the Chief Medical Examiner, the National Institutes of Health, Howard University Hospital, the Washington, DC Veterans Administration Hospital, and the American International Pathology Laboratory. *Id.*

i. *Althen* Prong One: Response to Petitioners' Theory

Dr. Harris agreed with the other experts that the Triple Risk Model is a generally accepted and reliable model of SIDS. Tr. 345. He could not say whether all extrinsic risk factors are mechanical or whether some of them may be neurochemical. *Id.* at 346. However, he testified that he would want to see conclusive proof before he would list vaccines as a risk factor in a medical report that he wrote. Tr. 348. He was aware of studies finding that vaccinations induce the production of cytokines in the brain, but not of any studies finding that those cytokines have a detrimental effect. Exhibit A at 6.

ii. *Althen* Prong Two: Response to Petitioners' Opinion of a Logical Sequence of Cause and Effect

Dr. Harris agreed with the characterization of J.B.'s death as SIDS and that under the Triple Risk Model, J.B. was in the critical development period. Exhibit A at 6. It cannot be confirmed whether J.B. had a brain defect rendering him "vulnerable" because the autopsy did not sample that section of the brain. Exhibit A at 6.

Dr. Harris opined that if vaccinations are found to be an exogenous stressor, they "certainly cannot be proven in J.B.'s death." Exhibit A at 6. He stated that there were "no pathologic findings in the brain or other organs in this case that indicate a vaccine-related death." Exhibit A at 7; *see also* Tr. 328. J.B.'s brain was found to have metabolic glia, which are not fully understood. Exhibit A at 6-7. Dr. Harris also opined: Induction of cytokines after

[97] This may not be an accurate characterization of Dr. Miller's opinion. A review of the transcript did not find a clear statement from Dr. Miller about the timing of cytokine production. But in his expert report, Dr. Miller actually opined that cytokine production would *begin* "within hours" and would *peak* "within 2 to at most 4 days." *See* section above (citing Exhibit 16 at 1).

vaccination is a recognized physiological response involved in the immune process. The primary immune surveillance cells in the brain are microglia.These cells when activated by circulating molecules or direct invasion in the brain by organisms change their morphology and produce a host of cytokines in response. Over-activation of these cells in J.B.'s brain is a non-specific finding that could be related to the prior day's vaccination and/ or infection." Exhibit A at 6. Dr. Harris testified that the "circulating molecules" that activate microglia can be either lipopolysaccharides from bacteria or "circulating cytokines," although this is not completely understood. Tr. 342.

iii. *Althen* Prong Three: Response to Petitioners' Timing Argument

Dr. Harris agreed with Dr. McCusker's opinion that cytokine signaling "doesn't happen immediately but happens over a period of time." Tr. 343. He did not otherwise address the timing for the cytokine response or whether it fit the case of J.B.

III. ANALYSIS

A. Summary of the Arguments

The parties agree that the sole issue to be resolved is "whether the vaccines that J.B. received on September 2, 2011 caused or substantially contributed to his death." Joint Prehearing Submission at 2. Pursuant to *Althen,* petitioners must show by a preponderance of the evidence a reasonable theory as to how the vaccine could cause the harm at issue, a logical but not scientifically certain explanation of how it did, and show the timing was appropriate given the theory of causation. The Federal Circuit has observed that this preponderance standard enables "the finding of causation in a field bereft of complete and direct proof of how the vaccines affect the human body." *Althen v. Sec'y of Health & Human Servs.,* 418 F.3d 1274, 1280 (Fed. Cir. 2005). The standard permits the use of "circumstantial evidence" and accomplishes Congress's goal that "close calls regarding causation are resolved in favor of injured claimants." *Id.* (citing *Knudsen v. Sec'y of Health & Human Servs.*, 35 F.3d 543, 549 (Fed. Cir. 1994) ("to require identification and proof of specific biological mechanisms would be inconsistent with the purpose and nature of the vaccine compensation program")).

To address the issue in the case, several questions must be addressed. The specific questions for decision are whether inflammatory cytokines generated by a mild infection are likely the critical exogenous stressor in many cases of SIDS when mild infection is also present. The second question is whether the same cytokines are stimulated by the innate immune response to vaccines and whether they are likely to be the exogenous stressor in some SIDS cases, particularly, as in this case, when the child was thoroughly examined by a physician the day before he died and found to be completely healthy, and there was no evidence of viral infection by nasal swab at autopsy.

Petitioners' theory is essentially that a high percentage of SIDS infants, almost 50% in most studies, have no history of a serious illness in the days and weeks prior to death, but have a mild infection or fever at the time of death. In most instances, the mild infection was an upper

respiratory infection, although one author listed post-vaccinal fever among the conditions.[98] In this case, J.B., a nearly five-month-old African American boy, who had been born at 36 weeks, died of unknown causes while napping in the early afternoon one day after receiving his scheduled four-month vaccines. He had a well-documented physical examination the prior day, performed by an M.D. pediatrician who had performed a similar examination about five weeks prior. J.B. was documented to be healthy, with no signs or symptoms of illness. He had patent nasal passages and clear lungs, and he was progressing well in terms of growth and milestones. His pediatrician noted that he was able to raise his head, hold it steady and roll over. In the 28-hour period following vaccination, at 4 a.m. and again at 8 a.m., his mother noticed that he had a mild fever and gave him children's Advil. He seemed to be fine and playing normally during the morning, but was fussy and started running a fever again in the early afternoon. Exhibit 8 at 2. His father then put him in his crib for a nap. He was put in the crib on his back, with a blanket over his midsection. He was using a pacifier. There was a small, flat, crib pillow in the bed. The air conditioning in the house was set at 76 degrees. His mother checked on him and replaced his pacifier during his nap. She came back about ten minutes later, noticed that he had rolled onto his side with his head tilted slightly downward, and he was not breathing. There is no evidence that his breathing passages were in any way obstructed or that his face was down in the bed or pillow when his mother found him. She called 911. Police and emergency medical personnel responded within minutes. J.B. was transported to the hospital when he could not be revived on scene. He was pronounced dead at the hospital.

Under the first leg of the Triple Risk Model, petitioners theorize that J.B. likely had a defective or under-developed serotonin system in the arcuate nucleus or other medullary area, which unfortunately was not examined or sectioned at autopsy. He was clearly within the vulnerable risk period for SIDS in that he was between four and five months old and, given his pre-maturity, only about four months based on dates of conception. He had several intrinsic risk factors in that he was born at 36 weeks, he was male and he was African American, all of which groups are overrepresented among SIDS deaths – blacks more than whites and Hispanics, boys more than girls, and preterm babies more than term babies. As noted above, at birth, J.B. had Apgar scores of 8 at one minute and 9 at five minutes. He had grown to 16 pounds and was well within the average ranges for height, weight and head circumference. He appeared to be meeting expected milestones as documented by his pediatrician. He was receiving good medical care and did not appear to be affected by issues associated with poverty, which is often speculated to account for the overrepresentation of African American babies in the SIDS statistics. He was a boy and it has been suggested, as noted above, that boys are more dependent than girls on an effective serotonin system for sensing the accumulation of carbon dioxide and responding appropriately to clear it.

Also, J.B. was put to bed on his back. At J.B.'s two last appointments, Dr. Wright noted that he slept on his back. The available evidence indicates that he rolled onto his side but was not prone. His mother described in the police reenactment that he had turned to his right side and his head was turned slightly downward. Nothing in the notes of the reenactment indicated that the baby's mouth or nose were in or close to the bedding, and in her police interview his mother noted that his nose and mouth were not covered. His father indicated that he had a fever when he was put down for his nap.

[98] Kadhim et al. (2010), Exhibit 13-O at 122.

Thus, petitioners theorize that he did have a fever during the night, early morning and before his nap. Dr. Miller testified that the fever documents the effect of inflammatory cytokines, likely IL-1 and/or IL-6 signaling from the periphery to the hypothalamus to cause the fever. They also theorize that the fever elevates body temperature, which is another risk factor for SIDS. According to petitioners' theory, because J.B. had no evidence of illness or infection prior to vaccination, it is therefore highly likely that the fever was generated by the vaccines, which likely caused a cascade of cytokines to cross the blood brain barrier and further suppress the function of the already underdeveloped medullary serotonin system during sleep. This caused his death to occur within about 28 hours of the administration of the four-month vaccines.

Respondent disagrees, saying that J.B. was premature, an African American boy, and was side sleeping, all of which are risk factors for SIDS. Citing the principle of Occam's Razor, he argues that it is unnecessary to consider anything beside these known risk factors and that the proximate timing to the administration of the vaccines can be explained by coincidence given that the peak time period of the occurrence of SIDS deaths coincides with the timing of the two month and four month vaccine administration schedules. He further argues that there has not been epidemiology to substantiate a causal relationship between vaccines and SIDS. Dr. McCusker argued that the role of mild infection in relation to SIDS deaths is one of obstructing airways rather than one of chemosensitivity, and she discussed her experience of suctioning the noses of infants brought into the emergency room with upper respiratory infections.

Dr. Miller and Dr. Harris agreed that an ideal autopsy would have sectioned the ventral medulla and that that was not done in this case. They also agreed that the type of histological examination that was done by Dr. Kinney and others would be unlikely to be done in a standard autopsy. Tr. 339. They agreed that there is not definitive proof of defective medullary structures.

B. *Althen* Prong One

After extensive review of the literature in the field of SIDS causation and listening to the testimony of the experts in this case, I think it is clear that the Triple Risk Model is broadly accepted as the general structure for understanding SIDS, even if the lack of comprehensive autopsies do not allow the medical profession to say that SIDS always has a deficient medullary serotonin system, as demonstrated in up to 75% of the cases examined by Dr. Kinney and her group.[99] She has said that "the most compelling hypothesis is that SIDS is related to a brainstem abnormality in the neuroregulation of cardiorespiratory control."[100] She further observed, "according to the Triple Risk Model, *only* infants with an underlying brainstem disease process die of SIDS, which explains why all infants who are placed prone to sleep or who bed share do not die of SIDS. They do not have the underlying vulnerability." *Id.* at 521. Dr. Miller opined that it is likely that J.B. had this defect based on the data from these studies. Tr. 37. Dr. McCusker agreed, "according to the triple-risk theory that the brain problem must exist." Tr. 206. The "brain problem" described in the triple-risk literature is that in the respiratory control center in the medulla. As such, it is reasonable to conclude that the petitioners have shown by a

[99] Kinney & Thach (2009), Exhibit A-4 at 6.

[100] Kinney et al. (2009), Exhibit 13-H at 519.

preponderance of the evidence that an infant who has died of unknown causes, and in whom autopsy has ruled out other causes, had the inherent brainstem vulnerability. I do conclude that J.B. did.

There is also no disagreement that the Back to Sleep Campaign convincingly demonstrated the danger of prone sleeping. By persuading parents to place babies on their backs to sleep during the vulnerable risk period, the campaign brought about an approximate 50% reduction in the rate of SIDS. Side-sleeping has also been recognized as having an elevated relative risk for SIDS, but the reason for this is not entirely clear. Dr. McCusker stated at some length her understanding of the mechanics of breathing in an infant. Essentially, she explained that the diaphragm drops down creating negative pressure within the lung relative to the atmosphere, at which point air rushes in. She suggested that the stomach muscles which the baby uses to help drop the diaphragm are compressed, as are the soft ribs in infants who are prone or side-sleeping, which reduces the gas exchange. Tr. 129-32. Dr. Miller disagreed with her explanation of respiratory physiology in that he did not find persuasive the notion that side-sleeping in a four-month-old is going to inhibit the ability to have inspiratory motion in the diaphragm, which creates the negative pressure in the lungs. Rather, he said the literature in SIDS has emphasized the pocket of air and re-inhaled carbon dioxide. Tr. 354.

The policy statement by the American Academy of Pediatrics, which was repeatedly referenced by Dr. McCusker but not marked as an exhibit, says that the risk of side-sleeping is similar in magnitude to prone sleeping (2.0 vs. 2.6).[101] The statement appears to focus on the risk of turning if the infant is placed on his side. "The risk of SIDS is exceptionally high for infants who are placed on their sides and found on their stomach. The side sleep position is inherently unstable, and the probability of an infant rolling to the prone position from the side sleep position is significantly greater than rolling prone from the back." *Id.* at 7. Interestingly, the same report addresses the issue of children who are able to roll over, which it notes generally occurs at 4-to-6 months of age, and that as they age it is more likely that they will roll. The Academy recommends, "If the infant can roll from supine to prone and from prone to supine, the infant can then be allowed to remain in the sleep position that he or she assumes." *Id.* at 8.

In this case, J.B. was placed supine and he rolled to his side, but not prone. It would appear from this policy statement that the greatest concern with side sleeping is when the infant is placed on its side and can easily roll to the prone position. The fact that the Academy recommends allowing the baby to remain in the position to which he rolls after being placed supine suggests that it is likely that a baby who can roll probably also has developed the ability to raise and turn his head.

All of the experts in this case appeared to agree that at least the predominant thinking in medicine as to the cause of SIDS is explained by the Triple Risk Model. Although as Dr. Harris testified we do not know with certainty that the medullary serotonergic network deficiency is always present because a great many autopsies, such as the one in this case, are not adequate to

[101] Moon R.Y. et al., American Academy of Pediatrics – Task Force on Sudden Infant Death Syndrome, *SIDS and Other Sleep Related Infant Deaths: Expansion of Recommendations for a Safe Infant Sleeping Environment,* 128 Pediatrics 1030 (2011), available at http://pediatrics.aappublications.org/content/128/5/1030.long.

document that deficiency, it was also recognized that as Dr. Kinney stated in a 2009 paper, "only infants with an underlying brainstem disease process die of SIDS."[102] Dr. McCusker agreed that according to the triple risk theory the brain problem must exist. Tr. 206. There has also not been significant debate about the statistical relevance of the other intrinsic risk factors. The success of the Back to Sleep Campaign in educating the public about the danger of prone sleeping has been remarkable in reducing SIDS deaths by half. But the other half still occur. The question remains as to what extrinsic risk factors come to play at that "fatal intersection of vulnerability, critical period and stressor."[103] The literature strongly suggests that SIDS is likely to be multi-factorial. Some cases are likely to be caused by continued prone sleeping, but others are likely caused by other factors. Mild infections, often described as "trivial" infections, appear to be a factor as they have been reported to be present in nearly 50% of SIDS deaths, raising the question of what it is about mild, otherwise non-life threatening infections that appear to interact with the impaired medullary serotonin system during the vulnerable period to cause the "perfect storm" that results in an unexplained death of a child?

Dr. Miller, relying on multiple pieces of research described in the SIDS literature, opined that it is likely that the cytokine signaling triggered in the immune system by mild infection interacts with the underdeveloped 5-HT system in the brainstem, during sleep when the excitatory function of serotonin is reduced, to further suppress the function of the brainstem to cause a cardio-respiratory crisis. The further issue raised is whether, in the absence of a mild infection, can the multiple vaccines administered together – in this case the day before – trigger the same cytokines as does a mild infection with the same fatal result? Dr. Miller concluded that they do.

Petitioners refer to the significant number of SIDS deaths that document the co-occurrence of mild or trivial infections which appear to stimulate a cytokine response similar to that generated by severe infections with adverse or repressive effects on the 5-HT system for chemosensitive response to hypercarbia, leading to failure to arouse and failure to initiate a gasping reflex and ultimately death. Petitioners are not the first to suggest this theory. Dr. Kinney has written, "A causal role for mild infection in sudden infant death is suggested by reports that in approximately half of SIDS cases, the infants have a seemingly trivial infection around the time of death, as well as mild tracheobronchial inflammation and altered serum immunoglobulin or cytokine levels and the presence of microbial isolates at autopsy. In infants who die unexpectedly of infection, the given organism may precipitate *a lethal cytokine cascade or toxic response*."[104] Another article by her group explained the likely mechanism: "During infection, peripherally produced IL-6 may cross the blood brain barrier and bind to IL-6 receptors on 5-HT neurons that mediate homeostasis in response to the infectious stressor and potentially mediate sickness behavior. …We found ubiquitous expression of IL-6 receptors and gp130 neurons in all regions in the infant medulla, including those effector nuclei critical to respiratory and autonomic control, and those that contain 5-HT source neurons. Serotonergic

[102] Kinney et al. (2009), Exhibit 13-H at 521.

[103] Filiano & Kinney (1994), Exhibit 13-B at 197 [also filed as Exhibit A-2].

[104] Kinney & Thach (2009), Exhibit A-4 at 2 (emphasis added).

neurons in the caudal 5-HT system, including in the raphe obscurus and arcuate nucleus, express IL-6Rs on somata and processes, indicating the site of IL-6/5 HT interaction."[105]

Various authors have identified the presence of IL-1β, IL-6, and IL-2, which are all pro-inflammatory cytokines, in elevated levels in the infant medulla in SIDS. Stoltenberg studied the effects of injection of IL-1β in piglets, and theorizes that in addition to cytokines being transported to the brain by retrograde axonal transport, his findings suggested an equally important alternative route in the immune-stimulation of the brain, inducing hypoxia and sudden infant death. He said that it has been shown that IL-1β is internalized by blood brain barrier endothelial cells, which implies that this cytokine passes through the blood brain barrier at the endothelial rather than the ependymal or blood cerebrospinal fluid part of the brain barrier. He found in his experiments with piglets that IL-1 stimulates the release of β-endorphin and the level of β-endorphin in CSF correlates strongly with the duration of apnea. Further, he found that "IL-1β stimulates GABA-transmission and hence increases the inhibitory postsynaptic function by opening of chloride-delective channels, and this will reduce the activity in the central respiratory neurons and may produce hypoxia."[106] Dr. McCusker referred to an article by Besedovsky for the proposition that cytokines are produced in the brain, suggesting that cytokines active in the brain necessarily originate in the brain. However, on review of the article, Besedovsky also noted that some cytokines such as IL-1 and IL-6 are produced both peripherally and within the brain.[107] He postulated that tripartite synapses possess the cellular and molecular components to function as a "relay system" capable of receiving and integrating peripheral immune signals with central neural signals. *Id.* at 5.

One of the best understood functions of cytokines in the case of infection and vaccination is the triggering of fever. When this occurs, cytokines from the periphery at the site of the infection travel to the brain, in particular to the hypothalamus, which then causes fever. As J.B. had a fever in the day following vaccination after having a completely clear medical examination the day before, Dr. McCusker agreed with Dr. Miller that in order for fever to have occurred there had to be a hypothalamic signal, which is mediated by endogenous pyrogens, i.e. IL-6 or TNFα. Tr. 286. The literature also recognizes IL-1 and others which are known pyrogens as well. She also agreed that in the absence of an infection, the only thing we can attribute the fever to is the vaccine. Tr. 205.

After identifying a plausible mechanism for the means of activation of cytokines in the medullary brainstem from a peripheral source, the next key question is why does mild or trivial infection appear to occur in conjunction with SIDS? It is not the infection itself which causes death, as by its mild nature it is not life threatening. Whether the infection is mild or severe, it triggers the innate immune response, which in turn triggers the release of cytokines. As Dr. McCusker explained, cytokines are small molecules that are released by different cell types originally described in immune cells. They are viewed primarily as communication molecules,

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[105] Kinney et al. (2011), Exhibit 13-F at 191.

[106] Stoltenberg et al. (1994), Exhibit 13-J at 427.

[107] Besedovsky, H.O. and A. del Ray, *Central and Peripheral Cytokines Mediate Immune-Brain Connectivity*, 36 Neurochem Res. 1 (2011), Exhibit C-3 at 1.

because they are released by one cell and bind to another through a series of signaling steps. Tr. 145. Dr. Miller explained that cytokines are messenger molecules that have a lot of different effects which were first identified as products of the innate immune system, but are seen elsewhere as well, including the brain. IL-6 binds with 5-HT and IL-1 has been shown in animals to inhibit 5-HT firing. Tr. 30. There was no disagreement between the experts or in the literature that cytokines are released by the innate immune response to infection, whether it be mild or severe.

The Siljehav-Hofstetter article filed by respondent provides an additional theoretical basis for the role of cytokines in SIDS. The authors found that IL-1β stimulates a prostaglandin (PGE2) with receptors in the rostral ventrolateral medulla. They explained that once stimulated by IL-1β, PGE2 induced depression of this vital brainstem neuronal network, e.g., during an infectious response, that could result in gasping and autoresuscitation failure and ultimately death."[108]

Dr. Miller found further support in the work of Kadhim, who found overexpression of IL-1β in the arcuate nuclei in 17 of 17 SIDS brains studied, but only in 1 of 6 non-SIDS brains.[109] Kadhim noted that cytokines could exert neuromodulatory effects in the ascending reticular activating system, which is involved in the arousal reflex. He noted that IL-1 causes prolonged apneas and depresses respiration and the brain appears to be less effective than the periphery in inducing IL-1 antagonist to terminate IL-1β actions. He hypothesized that the particular pattern of neuronal cytokine he detected might therefore overturn a subtle equilibrium in a molecular chain involving vital brain centers, causing SIDS. *Id.* at 1259.

In a second study involving SIDS brains, Kadhim's group noted that SIDS victims often have preceding mild infections and that cytokines have neuromodulatory effects whereby they can modify neurotransmission. In this study, they compared the brainstems of SIDS victims to those of infants who died of diverse severe pathological conditions, mainly infectious, hemodynamic, metabolic, severe congenital, or other serious conditions. They found that IL-2, another inflammatory cytokine, was preferentially expressed in specific neuronal centers within the brainstem. In this study, they found equally intense immune reactivity within the arcuate and dorsal vagal nuclei in fatally sick infants, as with SIDS victims who had no obvious or detectable serious health condition before death. They hypothesized that a hyperimmune response to mild infection in the SIDS babies may result in a molecular disequilibrium which tips the delicate molecular balance, causing dysfunction in those vital brainstem centers and producing disturbed homeostasis with potentially drastic effects on target organs/systems and eventual death.[110]

[108] Siljehav (2012), Exhibit C-9 at 9897.

[109] Kadhim et al. (2003), Exhibit 13-L at 1256.

[110] Kadhim et al. (2010), Exhibit 13-O at 122-26.

Brambilla also provided some support for this theory by demonstrating in animals that IL-1 inhibited firing of neurons that promoted wakefulness in the dorsal raphe nucleus and enhanced activity of GABAergic neurons which are inhibitory and induce enhancement of NREM sleep.[111]

Rognum further compared brains of SIDS victims to those of babies who died of severe infections and to another group who died from drowning, suffocation, strangulation, or other violent causes. They found that the SIDS babies had higher cytokines in the medullary brainstem than did those who died of violent causes but their levels were not as high as those that died of infectious causes. In a small section of their study, the Rognum group found elevations of IL-6R in the arcuate nucleus in the SIDS and infection groups relative to the controls. However, they found that the gp130, which is necessary for IL-6 to function, did not rise as high above the controls as did the infection group, although it was higher than in those dying violent deaths. This caused them to speculate that the IL-6R might be reactive to an excess carbon dioxide crisis rather than its cause. Thus significant evidence has been produced to show that cytokines are abundantly present in the medullary brainstem of SIDS infants relative to those dying of other causes which strongly suggests a hyperimmune response to mild infection in these children well out of proportion to the mild or trivial infection that they had. The presence of these cytokines also appears likely to suppress the 5-HT response to the accumulate of carbon dioxide in the body and the ultimate failure of the respiratory response system.

The next important question is whether the vaccines can play the same cytokine generating role as mild infection in a child who does not have an infection. If, as his father described, the child developed symptoms such as a fever, crankiness and not being himself, signs of cytokine activation, and had no evidence of infection, could one or more of the seven vaccines he received the day before have generated a cytokine cascade that caused him to be unable to respond to elevated carbon dioxide in his system, whether it was produced by rebreathing or metabolically? Dr. Miller's thesis was that the main role for mild inflammation as a risk factor for SIDS is thought to be in elevating cytokines. He said that is explicit in multiple articles that have been submitted. Then, if vaccines produce the same cytokine responses as very mild upper respiratory infections, which is what is demonstrated by Kashiwagi, it would seem logical to impute both having the same effect on the central nervous system. Tr. 370.

Indeed, Kashiwagi conducted testing with multiple vaccines and studied the cytokine response. He found that there was a more significant response in children who received three or four vaccines at one time than in those who received fewer, and he found that higher IL-1β production was noted in young infants, but decreased at around 2 years or older.[112]

He also examined the cytokine profiles in 61 serum samples obtained from recipients who exhibited febrile illness within 24 hours of being vaccinated and 18 serum samples from recipients without febrile illness. The samples were taken within 48 hours of vaccination in both groups. These were compared to each other and to cytokine profiles of ten normal subjects

[111] Kinney et al. (2009), Exhibit 13-H.

[112] Kashiwagi et al. (2014), Exhibit 17 at 680.

without vaccination. "Higher levels of IL-6, IL-10, IL-12, G-CSF,[113] and IFN-α were detected in both the febrile and non-febrile vaccination subjects in comparison with those in normal subjects." *Id.* at 680.

The Lee and Schulzke studies of multiple vaccine administration to premature infants, referenced above, found an elevation in the rate of apnea, bradycardia, and, in the Lee study, oxygen desaturations (Schulzke did not look at desaturations). Both authors hypothesized that the adverse events may be related to the immune response to the vaccines, particularly as Lee found there was no difference in the rate of adverse events between whole cell pertussis and acellular pertussis. [114] Schulzke noted that the adverse events occurred within 6 to 24 hours of vaccination.[115] While not studying SIDS, these studies focused on premature infants in a controlled environment – a hospital – where the mechanism that is hypothesized to occur in SIDS could be rapidly recognized, addressed, and treated. It seems quite likely that the same sequence occurring post-administration of multiple vaccines may be what occurs in the uncontrolled environment of the home when the child and often the parents are sleeping, or at least not in the same room with the child when the combination of events leading to the fatal sequence occurs.

Dr. Miller's theory, consistent with many of the articles in the literature, is that SIDS is multifactorial. Multiple factors come together at the fatal moment that causes the perfect storm leading to death. He theorizes that the cytokines triggered by the vaccines in the initial innate immune response to the vaccines travel to their receptors in the arcuate nucleus and suppress the serotonin function in a child whose functionality in that area is already impaired by an underdeveloped or defective 5-HT system while he is asleep, which further reduces 5-HT function. The input of the cytokines stimulated by the vaccines causes the lack of response to elevation of carbon dioxide that converts a recoverable event to a fatal one. Whether the vaccine generated cytokines cause additional metabolic activity generating fever and additional production of carbon dioxide, or whether they caused the neurons in the brainstem to be unable to respond to rebreathed or accumulated carbon dioxide, it is probable that they played an important role in causing the death of this infant.

Dr. McCusker disagreed. She argued that the presence of the various intrinsic risk factors together with a flat pillow in the bed and side-sleeping to which the child turned after being placed supine was sufficient to explain the death. She argued that the role of mild infection was that it caused obstruction in the nasal passages in infants who are "obligate nose breathers" (Tr. 138) and mucous in the nose would obstruct the breathing of the child sufficient to cause death. She referred to infants she sees in the emergency room with upper respiratory tract infections who need to be suctioned which then brings down their carbon dioxide level. Tr. 139-40. Dr. Miller disagreed. He stated that he had never seen a SIDS autopsy where the death was

[113] G-CSF is an abbreviation for granulocyte colony stimulating factor. It is another cytokine which mobilizes and recruits neutrophils to the site of inflammation from the marginal pool. Kashiwagi et al. (2014), Exhibit 17 at 693.

[114] Lee, J. et al., *Frequency of Apnea, Bradycardia, and Desaturations Following First Diphtheria-Tetanus-Pertussis-Inactivated Polio-Haemophilus Influenzae Type B Immunization in Hospitalized Preterm Infants*, 6 BMC Pediatr. 20 (2006), Exhibit 20.

[115] Schulzke (2005), Exhibit 21 at 3.

attributed to nasal passage obstruction by mucous and that he had never seen any literature to support that concept. Tr. 355.

The literature certainly suggests that Dr. McCusker's interpretation of the role of mild infection was too limited in that she ignored the entire concept of brainstem chemosensitivity in response to carbon dioxide accumulation. Dr. Kinney wrote, "Serotonergic neurons at the medullary ventral surface and in the midline (raphe) are now known to be preferentially chemosensitive to CO2 and although they are not the only central chemosensitive neurons they appear to play a critical potentially modulatory role. … A small but important population of 5 HTE neurons is embedded within the human arcuate nucleus suggesting that the putative dysfunction in chemosensitivity related to the arcuate anomaly specifically involved these embedded 5 HT neurons."[116] In an article in the New England Journal of Medicine, Kinney wrote, "the arousal from sleep that is triggered by abnormal levels of carbon dioxide and oxygen is essential for the initiation of protective airway responses. … Arousal involves a progressive activation of specific subcortical to cortical brain structures and consists of ascending and descending components that mediate cortical and subcortical arousal respectively."[117] The importance of the chemosensitive role in the stimulation of breathing, arousal, and ultimately gasping in response to the accumulation of excess carbon dioxide appears critical to all of the triple risk hypotheses. A stuffy nose does not explain the inability of the neurons in the arcuate nucleus to modulate breathing rhythm and respond to excess carbon dioxide by initiating breathing, particularly when there was no evidence of mucous congestion in the nose the day before at the medical exam, in the report of the parents, or at the autopsy. The role of cytokines stimulated by vaccines administered approximately 28 hours before seems much more likely to play a critical role, similar to that of mild infection in causing the ultimate convergence of the multiple factors leading to death. The inhibition of the 5-HT response, beyond its initially impaired level with which the child had lived to that date, seems more likely to be caused by the cytokine response to the multiple vaccines than to a stuffy nose or the side-sleeping position to which he had turned, particularly when there was no evidence of nasal congestion or of the breathing passages being obstructed. Exhibit 7 at 5. In fact the evidence was to the contrary.

Dr. McCusker, citing to the Imeri article[118] on sleep in general, also testified that fever would tend to push the child out of REM sleep and into NREM, which she argued would make him more arousable. A review of the Imeri article, which discusses the immune system and sleep in general, and not specifically in infants, does indeed discuss the role of fever and the generation of shivering in NREM sleep and that during the course of most infections there is an increase in the amount of time spent in NREM sleep and a decrease in the amount of REM sleep. *Id.* However, it also discusses the role of IL-1 and the generation of GABAergic inhibitory cytokines. *Id.* at 205. Imeri also acknowledged the role of peripherally generated cytokines in the regulation of sleep. Imeri concluded that at present we know little about these mechanisms

[116] Kinney et al. (2009), Exhibit 13-H at 522.

[117] Kinney & Thach (2009), Exhibit A-4 at 5.

[118] Imeri L. & M.R. Opp, *How (and Why) the Immune System Makes Us Sleep*, 10 Nat. Rev. Neurosci. 199 (2009), Exhibit C-6 at 201.

by which cytokines inhibit REM sleep and argued that it is important because REM sleep is disrupted in many pathologies that involve altered cytokine concentrations. *Id.*

Dr. Miller hypothesized two roles for fever – overheating and travel of cytokines to the brain in the mechanism of SIDS. Dr. McCusker agreed with cytokine signaling as relevant to the production of fever but disagreed that fever was the equivalent of hyperthermia in the SIDS literature. On the witness stand she drew a sharp distinction between environmental hyperthermia and overheating secondary to fever, which she called hyperpyrexia. The literature was unclear on this point. But the significant importance of fever to this case was in demonstrating the travel of peripheral cytokines stimulated by the vaccines across the blood brain barrier to the hypothalamus. Fever is the most obvious manifestation of the signaling of cytokines from the peripheral location of the vaccinations to the brain. The SIDS literature suggests that production of inflammatory cytokines IL-6, IL-10, IL-12, and IFNγ in response to DPT, Hib, and PCV7 were detected in both febrile and non-febrile groups, with febrile illness appearing 12-16 hours post vaccination.[119] NREM sleep is also implicated in SIDS. A distinctive feature of 5-HT neurons is that they exhibit differential firing rates according to the level of arousal, with increased firing during waking, decreased firing during NREM, and almost complete absence of firing during REM. Given the relationship of the firing of raphe 5-HT neurons to arousal, the medullary 5-HT system is postulated to modulate and integrate homeostatic function according to the level of arousal.[120] Thus, particularly in the deeper levels of NREM sleep, the 5-HT system is also functioning at lower levels, potentially contributing to the multi-factorial causal picture.

After review of all of the above, I have concluded that petitioners have presented a reasonable and reliable theory of vaccine causation involving the role of inflammatory cytokines acting as an extrinsic stressor in a baby with a brainstem deficit during the vulnerable time period. It is particularly important to note that the literature indicates that SIDS is likely caused by a multi-factorial process. Dr. Kinney wrote in the New England Journal of Medicine in 2009, "Current evidence suggests that SIDS involves a convergence of stressors that probably results in the asphyxia of a vulnerable infant who has defective cardiorespiratory or arousal defense systems during a critical developmental period when immature defense mechanisms are not fully integrated. Thus our current understanding of the pathogenesis of SIDS reflects the simultaneous juxtaposition of multiple events that, when taken individually, are far less powerful than the result of their chance combination."[121] In another 2009 article she wrote; "We now conceptualize SIDS as the biologic version of the perfect storm, in which the simultaneous and chance combination of multiple events is far more powerful than any individual event alone."[122]

[119] Kashiwagi et al. (2014), Exhibit 17 at 680.

[120] Kinney, H.C., *Brainstem Mechanisms Underlying the Sudden Infant Death Syndrome: Evidence from Human Pathologic Studies*, 51 Dev. Psychobiol. 223 (2009), Exhibit 13-E at 226.

[121] Kinney & Thach (2009), Exhibit A-4 at 7.

[122] Kinney et al. (2009), Exhibit 13-H at 539.

I have concluded that the petitioners have demonstrated by a preponderance of the evidence that the vaccines can and likely did play a critical role in this child's death by stimulating the production of inflammatory cytokines that suppressed the respiratory response system and caused the vulnerable infant to be unable to respond in the normal way to the accumulation of carbon dioxide in his system. Accordingly, petitioners have satisfied the requirement of *Althen* Prong One by presenting a reasonable explanation of how the vaccine could cause or substantially contribute to the child's death.

C. *Althen* Prong Two

Althen Prong Two requires the demonstration of a logical cause and effect as to how the vaccine caused the harm, in this case the sudden unexplained death of J.B. Under *Althen* Prong Two, petitioners must prove that there is a "logical sequence of cause and effect showing that the vaccination was the reason for the injury." *Capizzano*, 440 F.3d at 1324 (quoting *Althen*, 418 F.3d at 1278).

Dr. Miller testified that it was his diagnosis that J.B. died of SIDS and that the vaccines were a substantial contributing factor to his death. Tr. 126. Having accepted the theory of a causal role of vaccine stimulated cytokines as an exogenous factor converging with the first two prongs of the Triple Risk Model, the question of logical cause and effect requires a review of the likely mechanism and comparing it to the operative facts of the case. Kashiwagi in particular found that cytokines began to be produced 6 hours after stimulation and increased until 24 hours, showing the same level thereafter. Higher levels of IL-1B, IL-6, G-CSF, and TNFα were produced in that study by the concurrent stimulation of three vaccines than by one alone.[123] J.B. received seven vaccines at his 4 to 5 month well baby visit with his pediatrician on September 2, 2011. He was carefully examined and documented to be in entirely good health the day before. Overnight, he developed a mild fever, consistent with cytokine signaling from the vaccination site to the brain. In the early afternoon of September 3, he died during his nap.

Dr. Miller discussed the logical sequence of cause and effect explaining how he believed the vaccines acted as an exogenous stressor which caused J.B. to succumb to SIDS. He noted that J.B. was a "healthy infant… developing normally." Exhibit 13 at 4. He was "immunologically normal." Tr. 61. Therefore, after receiving vaccinations, his body mounted an innate immune response including the production of cytokines. Exhibit 13 at 6; Exhibit 16 at 1; Tr. 62. Those cytokines circulated in J.B.'s body, going to the central nervous system. Exhibit 13 at 6; Tr. 62. These peripheral cytokines interacted with the hypothalamus to provoke fever the night after the vaccinations and during the following day (before J.B.'s death). Exhibit 13 at 6; Exhibit 16 at 1; Tr. 62-64. "Those cytokines then acted in the brainstem which was already deficient in serotoninergic drive for respiratory effort, leading to an apneic episode from which he did not recover, i.e., SIDS." Exhibit 13 at 6; *see also* Tr. 62 (the cytokines "depress[ed the] 5-HT system in a defective medulla, leading to SIDS during sleep").

[123] Exhibit 17 at 679.

He opined that there was "no other demonstrable inciting event" for J.B.'s death. Exhibit 13 at 1. There was no evidence of the fever being related to anything other than J.B.'s vaccinations. Tr. 66. The autopsy did not identify any other infectious processes. Tr. 66.[124]

On cross-examination, Dr. Miller stated that J.B. was placed on his back but was found on his side, which demonstrates that he was able to "move around." Tr. 92. However, J.B. did not pass away until "something else intervened." Tr. 85. Based on his theory and the temporal association, Dr. Miller opined that the vaccines were the intervening factor that caused J.B.'s death. Tr. 85.

An innate immune response to either mild infection or to a vaccine is likely to be fast and begins the process of immune attack of a foreign antigen. Part of that response is the triggering of cytokines to signal further response in the immune system. The triggering of the innate immune system by vaccination is necessary and fundamental to producing the adaptive response and immune memory which vaccines are designed to produce. After review and consideration of all of the testimony and the literature submitted, I have concluded that Dr. Miller has presented a reasonable and persuasive theory that the cytokine cascade triggered by the innate response to the vaccine antigens is similar to the cytokine response to a mild infection, and that the inflammatory cytokines had an immune modulatory effect on J.B.'s impaired medullary 5-HT system causing a prolonged apneic event resulting in his death. As such, the progression from vaccination to an unexplained death within approximately 28 hours is logical.

This logical progression is also consistent with reports of at least mildly elevated SIDS deaths in some studies such as Traversa, which found a 2.0 relationship in the first 7 days.[125] Goldman reported a statistically significant increase in deaths when 5 to 8 vaccines were administered simultaneously as opposed to 1 to 4.[126] Ottaviani[127] and Zinka[128] reported on SIDS deaths within 48 hours of receiving vaccinations. Other studies, such as Kuhnert[129], found neither a protective effect nor elevated risk, but Kuhnert noted that the small number of cases is a problem with the three case control studies he reviewed, particularly in view of the short time periods under investigation. According to Kuhnert, this problem was illustrated by the very broad confidence intervals of estimates that were related to the first few days. *Id.* at 2355.

[124] Dr. Miller noted that there were bacterial growth and food particles in J.B.'s lungs and epithelial cells in the upper airways. He opined that this was not evidence of a separate infectious process. He agreed with the medical examiner that these were terminal or resuscitative sequelae. Tr. 17-18; 66; 352-53.

[125] Traversa et al. (2011), Exhibit 13-U at 8.

[126] Goldman & Miller (2012), Exhibit 19 at 1016.

[127]Ottoviani et al. (2006), Exhibit 13-T.

[128] Zinka et al. (2006), Exhibit 13-S.

[129] Kuhnert et al. (2012), Exhibit C-20.

The statistical prevalence of boys, African Americans and premature babies among the victims of SIDS also seems to be clear and causes their inclusion as intrinsic risk factors. I think it is reasonable to question in this case whether the influence of prematurity would still be a likely factor, given that he had nearly reached the age of five months and appeared to be developing very well. It is also reasonable to question whether the statistical prevalence of African Americans should be a significant factor, as it is often speculated that this may be a function of socioeconomic status and poor medical care. This child appeared to have been living in a two-parent household, with attentive parents, was well-nourished, and was receiving good medical care. The role of his male gender may well have been important, as Dr. Kinney has reported a greater reduction in 5-HT-1A in the medullary raphe in males compared to females dying of SIDS.[130]

Given that Dr. Miller's thesis and that of much of the literature for the Triple Risk Model is that SIDS results from the convergence of multiple factors, it seems likely that his male gender may well have been a contributing intrinsic factor that may have amplified the effect of the cytokine response to the vaccines on the day that he died. But, his gender, his race, and his prematurity – all intrinsic factors – do not explain his death without the interaction with a critical extrinsic factor, which I have concluded was likely the cytokines triggered by the vaccines which depressed his 5-HT system sufficiently that he did not respond when carbon dioxide became elevated in his system.

The evidence for J.B.'s death occurring as a result of his having turned to his side without a causal input from another significant extrinsic factor such as the vaccine stimulated cytokines suppressing his response system is weak in this case. As noted above, the Academy of Pediatrics recommends leaving a child in the assumed position when he has rolled from his back presumably because it is also likely that he can push up and lift his head by the time he can roll. This capability was documented in J.B's case by his pediatrician. Although there was a flat pillow and a light blanket in the bed, J.B.'s mother told the police investigators that his head was not covered and that his head was turned downward only slightly. The scene investigation noted her report that J.B.'s mouth and nose were not covered. Exhibit 7 at 5. It was described that he had been put to sleep in the middle of the bed. Thus, there is no evidence in this case that the baby's breathing passages were obstructed or that he was breathing into an air pocket. The possibility of rebreathing carbon dioxide in that position cannot be ruled out, but seems less likely based upon this evidence derived from the extensive interviews and the site re-enactment performed by the responding police. Thus, even if the side- sleeping position did cause some rebreathing of carbon dioxide, I have concluded from the evidence that it is most likely that the cytokines stimulated by the vaccines caused suppression of the already impaired medullary serotonin system with the consequent failure to chemically sense elevated carbon dioxide, which caused the ultimate failure to arouse and to breathe normally thus substantially contributing to the death of J.B

The emphasis of the Triple Risk Model on prone sleeping has had a powerful impact in reducing SIDS deaths by approximately 50%. But there remains a significant number of SIDS deaths each year, some of which are likely related to continued prone-sleeping and some to side-sleeping. But the co-occurrence of mild infection in the statistics in nearly 50% of cases raises a

[130] Kinney et al. (2009), Exhibit 13-H at 532.

significant issue about the operative extrinsic risk factor or factors in the remaining cases, including many that are found supine. In this case, an apparently perfectly healthy child was found dead a day after vaccination, having had a mild fever in the interim without evidence of infection. He was not prone sleeping but had turned to his side, with no evidence that his breathing passages were in any way impaired. Significant literature introduced demonstrates that the triggering of inflammatory cytokines in response to vaccines is similar to that raised in response to mild infection. J.B.'s post-vaccinal fever provided confirmation of responsive cytokine activity. The cause and effect between the vaccines, the cytokines triggered by the vaccines, and their co-occurrence with other intrinsic and/or extrinsic risk factors in the presence of a defective or underdeveloped brainstem seems likely to have produced the perfect storm that resulted in J.B.'s death. Thus, I am persuaded that petitioners have proved prong two.

D. *Althen* Prong Three

Under *Althen* prong three, petitioners must provide "preponderant proof that the onset of symptoms occurred within a timeframe for which, given the understanding of the disorder's etiology, it is medically acceptable to infer causation-in-fact." *De Bazan*, 539 F.3d at 1352. The acceptable temporal association will vary according to the particular medical theory advanced in the case. *See Pafford*, 451 F.3d at 1358. A temporal relationship between a vaccine and an injury, standing alone, does not constitute preponderant evidence of vaccine causation. *See, e.g.*, *Veryzer v. Sec'y of Health & Human Servs.*, 100 Fed. Cl. 344, 356 (2011) (explaining that "a temporal relationship alone will not demonstrate the requisite causal link and that petitioner must posit a medical theory causally connecting the vaccine and injury").

Dr. Miller stated that the available evidence is that foreign antigens, like those contained in vaccinations, activate the production of cytokines "within hours" and that production "peaks within 2 to at most 4 days." Exhibit 16 at 1. Thus, a vulnerable infant who receives vaccinations is most likely to suffer a fatal event if one is to occur "within the first 48 hours to at most 4 days." Exhibit 13 at 5. Dr. Miller opined that J.B.'s death was "well within this vulnerable period." *Id.*

In this case, the timing of the innate immune response to the multiple scheduled vaccinations that J.B. received on September 2, to his death the following afternoon appears entirely appropriate for an innate immune response in the vulnerable risk period for SIDS. It is also consistent with reports of at least mildly elevated SIDS deaths in some studies and reports of deaths that occur within the first several days after the vaccination. In this case, one day post-vaccination is appropriate timing, in that inflammatory cytokines stimulated during the innate immune response to the vaccine antigens are likely to be active in close proximity to the stimulating event. As Dr. Miller stated, an adverse event that can be caused by the inflammatory cytokine response to vaccine antigens would be likely to occur within a few days of the vaccination. The cytokine response has been shown by Kashiwagi[131] to occur within 6 to 24 hours of the vaccination, and the very essence of the innate immune response is one that occurs rapidly after the invasion by a foreign antigen. As noted above, that rapid innate immune response is necessary to initiate the ultimate adaptive immune response necessary to achieve the

[131] Kashiwagi et al. (2014), Exhibit 17 at 679.

design purpose of vaccination. The close temporal relationship of the child's death to the receipt of seven vaccines is reasonable and consistent with the theory of neuro-modulation in the arcuate nucleus by the cytokine response to the vaccines. Accordingly, I am persuaded that prong three of *Althen* has been satisfied.

IV. CONCLUSION

In this case, I have concluded that petitioners have presented sufficient evidence and testimony to entitle them to compensation in the Vaccine Program. I have not concluded that vaccines present a substantial risk of SIDS. In fact, the evidence is to the contrary. The vast majority of vaccine recipients do not succumb to SIDS. Under the multi-factorial analysis of the Triple Risk Model, it is theorized that the ultimate fatal event may occur when multiple factors converge during this vulnerable period to cause death when one stressor acting alone may not have. As Dr. Kinney wrote, "Current evidence suggests that SIDS involves a convergence of stressors that probably results in the asphyxia of a vulnerable infant who has defective cardiorespiratory or arousal defense systems during a critical developmental period when immature defense mechanisms are not fully integrated. The convergence of these factors appears to be far more powerful than any one taken individually."[132] Thus, even if J.B. were rebreathing some carbon dioxide on this occasion, it was likely the combination with the cytokines that caused depression of the 5-HT system that caused his death by blunting the normal chemosensitive response to excess carbon dioxide. The multi-factorial analysis, including vaccines as an extrinsic risk factor, meets the *Shyface* standard that the vaccine need not be the sole or even predominant factor but must be a "but for cause" and a substantial factor in causing the death. *Shyface*, 165 F.3d at 1352. In this case, I have concluded, after review of the evidence, that it is more likely than not that the vaccines played a substantial causal role in the death of J.B. without the effect of which he would not have died. The role of inflammatory cytokines as neuro-modulators in the infant medulla has been well described and is likely the reason for a significant number of SIDS deaths occurring in conjunction with mild infection. I have concluded that it is more likely than not that the vaccine-stimulated cytokines had the same effect in this vulnerable infant during sleep.

Accordingly, petitioners are entitled to compensation. A separate damages order will issue.

IT IS SO ORDERED.

s/ Thomas L. Gowen
Thomas L. Gowen
Special Master

[132] Kinney et al. (2009), Exhibit 13-H at 539.

MISCONDUCT & FINANCIAL INCENTIVES

Centers for Disease Control and Prevention (CDC)

SPIDER Bites CDC

Concerns about the inner workings of the U.S. Centers for Disease Control and Prevention (CDC) have been mounting in recent months amid disclosures of cozy corporate alliances.

By
Carey Gillam, Contributor

I am a veteran journalist and research director for U.S. Right to Know, a non-profit consumer education group.

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Concerns about the inner workings of the U.S. Centers for Disease Control and Prevention (CDC) have been mounting in recent months amid disclosures of cozy corporate alliances. Now a group of more than a dozen senior scientists have reportedly lodged an ethics complaint alleging the federal agency is being influenced by corporate and political interests in ways that short-change taxpayers.

A group calling itself CDC Scientists Preserving Integrity, Diligence and Ethics in Research, or CDC SPIDER, put a list of complaints in writing in a letter to the CDC Chief of Staff and provided a copy of the letter to the public watchdog organization U.S. Right to Know (USRTK). The members of the group have elected to file the complaint anonymously for fear of retribution.

"It appears that our mission is being influenced and shaped by outside parties and rogue interests... and Congressional intent for our agency is being circumvented by some of our leaders. What concerns us most, is that it is becoming the norm and not the rare exception," the letter states. "These questionable and unethical practices threaten to undermine our credibility and reputation as a trusted leader in public health."

The complaint cites among other things a "cover up" of the poor performance of a women's health program called the Well-Integrated Screening and Evaluation for Woman Across the Nation, or WISEWOMAN. The program provides standard preventive services to help 40- to 64-year-old women reduce their risks for heart disease, and promote healthy lifestyles. CDC currently funds 21 WISEWOMAN programs through states and tribal organizations. The complaint alleges there was a coordinated effort within the CDC to misrepresent data given to Congress so that it appeared the program was involving more women than it actually was.

"Definitions were changed and data 'cooked' to make the results look better than they were," the complaint states. "An 'internal review' that involved staff across CDC occurred and its findings were essentially suppressed so media and/or Congressional staff would not become aware of the problems."

The letter mentions that Congresswoman Rosa DeLauro, a Democrat from Connecticut, who has been a proponent of the program, has made inquiries to CDC regarding the data. A spokesman for her office, confirmed as much.

The complaint also alleges that staff resources that are supposed to be dedicated to domestic programs for Americans are instead being directed to work on global health and research issues.

And the complaint cites as "troubling" the ties between soft drink giant Coca-Cola Co., an advocacy group backed by Coca-Cola, and two high-ranking CDC officials - Dr. Barbara Bowman who directed the CDC's Division for Heart Disease and Stroke Prevention until retiring in June, and Dr. Michael Pratt, senior Advisor for Global Health in the National Center for Chronic Disease Prevention and Health Promotion (NCCDPHP) at the CDC.

Bowman, retired after revelations of what the complaint called an "irregular" relationship with Coca-Cola and the nonprofit corporate interest group set up by Coca-Cola called the International Life Sciences Institute (ILSI). Email communications obtained through Freedom of Information Act (FOIA) requests by USRTK revealed that in her CDC role, Bowman had been communicating regularly with - and offering guidance to - a leading Coca-Cola advocate seeking to influence world health authorities on sugar and beverage policy matters.

Emails also suggested that Pratt has a history of promoting and helping lead research funded by Coca-Cola while being employed by the CDC. Pratt also has been working closely with ILSI, which advocates for the agenda of beverage and food industries, emails obtained through FOIA showed. Several research papers co-written by Pratt were at least partly funded by Coca-Cola, and Pratt has received industry funding to attend industry-sponsored events and conferences.

Last month, Pratt took a position as Director of the University of California San Diego Institute for Public Health. Next month, ILSI is partnering with the UCSD to hold a forum related to "energy balance behavior," planned for November 30 to December 1 of this year. One of the moderators is another CDC scientist, Janet Fulton, Chief of the CDC's Physical Activity and Health Branch. Pratt is on annual leave from the CDC during his stint in San Diego, according to the CDC.

The forum fits into the messaging of "energy balance" that Coca-Cola has been pushing. Consumption of sugar-laden foods and beverages is not to blame for obesity or other health problems; a lack of exercise is the primary culprit, the theory goes.

Experts in the nutrition arena have said that the relationships are troubling because the mission of the CDC is protecting public health, and yet certain CDC officials appear to be close with an industry that, studies say, is linked to about 180,000 deaths per year worldwide, including 25,000 in the United States. The CDC is supposed to be addressing rising obesity rates among children, not advancing beverage industry interests.

CDC spokeswoman Kathy Harben would not address what the agency might be doing, if anything, in response to the SPIDER complaint, but she said the agency makes use of a "full range of federal ethics statutes, regulations, and policies" that apply to all federal employees."

"CDC takes seriously its responsibility to comply with the ethics rules, inform employees about them, and take steps to make it right any time we learn that employees aren't in compliance," Harben said. "We provide regular training to and communicate with staff on how to comply with ethics requirements and avoid violations."

The SPIDER group complaint ends with a plea for CDC management to address the allegations; to "do the right thing."

Let's hope someone is listening.

Carey Gillam, Contributor
I am a veteran journalist and research director for U.S. Right to Know, a non-profit consumer education group.

CDC Members Own More Than 50 Patents Connected to Vaccinations

The CDC Immunization Safety Office is responsible for investigating the safety and effectiveness of all new vaccinations; once an investigation is considered complete, a recommendation is then made to the CDC's Advisory Committee on Immunization Practices (ACIP) who then determines whether the new vaccine will be added to the current vaccination schedule. Members of the ACIP committee include physicians such as Dr. Paul Offit, who also serves as the chief of infectious diseases at the Children's Hospital of Philadelphia. Offit and other CDC members own numerous patents associated with vaccinations and regularly receive funding for their research work from the very same pharmaceutical companies who manufacture vaccinations which are ultimately sold to the public. This situation creates an obvious conflict of interest, as members of the ACIP committee benefit financially every time a new vaccination is released to the market.

Members of the ACIP Committee Directly In uence Public Health

Each of the 12 members of the CDC's ACIP Committee has a significant influence on the health of nearly every member of the American population. Because they are responsible for adding to and/or altering the national vaccine schedule, it is of critical importance that they remain objective and unbiased before determining whether a new vaccination is appropriate for use, particularly in the bodies of vulnerable young children. Unfortunately, a significant number of ACIP committee members receive direct financial returns when more vaccinations are added to the current schedule. Many own vaccination related patent(s) and/or stock shares of the pharmaceutical companies responsible for supplying new vaccines to the public. Others receive research grant money, funding for their academic departments, or payments for the oversight of vaccine safety trials.

A Long List of Patents Owned by ACIP and Other CDC Members

The following is a partial list of some of the patents that are owned or shared by members of the CDC and/or ACIP committee, including Dr. Paul Offit:

- "**Nucleic acid vaccines for prevention of flavivirus infection"** - This patent comes into play during the manufacturing process of vaccines for yellow fever, Zika, Dengue, West Nile virus and more.
- **Various vaccination testing methods** - When pharmaceutical companies need to test aspects of a new vaccine, they may utilize one of the CDC's patented testing methods including an artificial lung system for aerosol vaccines and a process that screens new vaccines for human rhinoviruses.
- **Adjuvant patents** - Adjuvants are components within vaccinations intended to create an intensified immune reaction; members of the ACIP own patents on adjuvants used specifically in vaccinations created for premature babies and full term newborns.
- **Assays that assist vaccine development** - During the vaccine development process, manufacturers will often observe biological samples for specific antibodies; the CDC owns a patent on an assay that facilitates this monitoring system.
- **Vaccine quality control** - patents on various aspects of quality control for vaccinations are utilized by pharmaceutical companies on a large scale once a new vaccine is actively distributed to the public.

In total, 56 individual patents were found to be owned or shared by one or more members of the ACIP committee or other committees within the CDC.

Members Claim They are Unbiased

When prompted with questions pertaining to their financial connections with pharmaceutical companies, most ACIP members claim they are able to remain unbiased despite the rewards they receive every time a new vaccination is recommended to the public. In numerous instances, vaccines released to the market are later removed after serious side effects are documented. The rotavirus vaccine was one such example; it was pulled from the market in 1999, a year after its initial approval. In 2001, the House Government Reform Committee found that four out of the eight ACIP members who voted to approve the vaccine had direct financial ties to one or more of the pharmaceutical companies who produced the vaccine for public use. Similar situations involving many other vaccinations have been independently documented over the course of nearly 20 years.

A Multi Billion Dollar Industry

The vaccination industry currently generates $30 billion in profit each year, some of which reaches the hands of the very people who create the vaccine schedule. Despite concerns connecting vaccinations to the increase in autism and a host of other disorders, the number of recommended vaccines continues to grow each year. With a new federal administration interested in uncovering the dirty secrets hidden within alliances between CDC members and vaccine manufacturers, we may begin to see a wave of personal injury and wrongful death lawsuits related directly to unethical behaviors which have led to numerous unsafe vaccines being pushed on an unknowing public. If the National Vaccine Injury Compensation Program (NVICP) is amended or repealed, victims of vaccine damage will be legally permitted to file claims directly against vaccine manufacturers and members of the ACIP committee who often have had knowledge of vaccine risks yet continue to recommend their widespread use.

Food and Drug Administration (FDA)

BACK TO ALL NEWS

FDA's revolving door: Companies often hire agency staffers who managed their successful drug reviews

Job changes raise conflict of interest questions

5 JUL 2018 · BY CHARLES PILLER

The Food and Drug Administration (FDA) says its rules, along with federal laws, stop employees from improperly cashing in on their government service. But how adequate are those revolving door controls? *Science* has found that much like outside advisers, regular employees at the agency, headquartered in Silver Spring, Maryland, often reap later rewards—jobs or consulting work—from the makers of the drugs they previously regulated.

FDA staffers play a pivotal role in drug approvals, presenting evidence to the agency's advisory panels and influencing or making approval decisions. They are free to move to jobs in pharma, and many do; in a 2016 study in *The BMJ*, researchers examined the job histories of 55 FDA staff who had conducted drug reviews over a 9-year period in the hematologyoncology field. They found that 15 of the 26 employees who left the agency later worked or consulted for the biopharmaceutical industry.

FDA's safeguards are supposed to keep the prospect of industry employment from affecting employees' decisions while at the agency, and to discourage them from exploiting relationships with former colleagues after they depart. For example, former high-level employees can't appear before the agency on the precise issues they regulated—sometimes permanently, in other cases for a year or two.

Through web searches and online services such as LinkedIn, however, *Science* has discovered that 11 of 16 FDA medical examiners who worked on 28 drug approvals and then left the agency for new jobs are now employed by or consult for the companies they recently regulated. This can create at least the appearance of conflicts of interest.

In 2009, for example, an FDA panel weighed whether the agency should approve AstraZeneca's widely prescribed antipsychotic drug quetiapine (Seroquel) for a wider range of conditions. The panel heard from health policy expert Wayne Ray of Vanderbilt University in Nashville, who described his research linking the drug to sudden cardiac death when used with certain other medications. Ray recalls "an FDA staff member who gave a very negative presentation on our paper." And according to the meeting transcript, the agency's then-Director of Psychiatric Products Thomas Laughren, who was instrumental in shepherding Seroquel and similar drugs through the review process and personally signed their FDA approvals, also challenged Ray's results and defended AstraZeneca's clinical trial findings in the discussion that followed. The company's "analysis should have been able to pick up a difference in sudden cardiac death, and they didn't find any difference between drug and placebo," he said.

Ray told Laughren and the panel that AstraZeneca had pooled data from all its trials as though the data were one data set, causing a well-known statistical error called Simpson's paradox. To take the company's conclusion "as definitive" would be "very dangerous," Ray said, according to the transcript. Laughren responded by calling sudden death "a pretty definitive event."

Ultimately, the committee voted overwhelmingly to advise approval of the drug for new indications and made no recommendation on labeling it to warn about sudden cardiac death. Later evidence showed that the cardiac problems Ray described are real, and in 2011, FDA required adding a warning on Seroquel's label.

Soon after, Laughren left the agency and formed a consultancy to help psychiatric drug makers, including AstraZeneca, navigate FDA approvals. He did not respond to repeated requests for comment.

In 2012 and 2013, data expert Joan Buenconsejo led FDA's analysis of medical statistics in drug reviews, including offerings from AstraZeneca. In 2014, she joined the company as a director and biometrics team leader. By 2015, Buenconsejo had already represented AstraZeneca before her former FDA colleagues as the company sought a drug's approval. In an email, Buenconsejo wrote that she strictly adhered to FDA's recusal rules "when considering employment with AstraZeneca." She added, "I do not believe there was any conflict of interest around my transition."

Former FDA employees, AstraZeneca spokesperson Karen Birmingham wrote in an email, "bring the perspective of seasoned regulators" who can assist current regulators with the "challenging decisions in approving innovative medicines to meet unmet medical needs."

Jeffrey Siegel, who was an FDA staff member specializing in reviews for arthritis drugs, oversaw the 2010 approval of Genentech's arthritis drug tocilizumab (Actemra). Months later, he left the agency to join the company and its parent, Roche, as director of the division that includes Actemra and related offerings. Siegel represented Roche before his former FDA colleagues when the company sought approval to promote Actemra for new conditions. Last year, he told *STAT* that the timing of his decision to join Roche and Genentech was coincidental.

Laughren, Buenconsejo, and Siegel apparently complied with existing federal laws and FDA requirements. And David Kessler, who led FDA under former Presidents George H. W. Bush and Bill Clinton, says such moves to industry by former FDA experts, steeped in "a culture of drug regulation," can benefit the public if they improve pharma practices. But "revolving door" rules need a fresh look, he adds, to ensure that "the tipping point, where that balance is," serves the public interest.

Vinay Prasad, a hematologist-oncologist at Oregon Health & Science University in Portland who co-wrote the 2016 study in *The BMJ*, contends that weak federal restrictions, plus an expectation of future employment, inevitably bias how FDA staffers conduct drug reviews.

"When your No. 1, major employer after you leave your job is sitting across the table from you, you're not going to be a hard-ass when you regulate. That's just human nature."

***Correction, 10 July, 6:10 p.m.:** An earlier version of this story stated that Thomas Laughren gave a negative presentation on Wayne Ray's paper. He was among FDA staff who critiqued that work at the advisory meeting, but he did not give the detailed presentation.*

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How a New Policy Led to Seven Deadly Drugs

BY DAVID WILLMAN

TIMES STAFF WRITER

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WASHINGTON — For most of its history, the United States Food and Drug Administration approved new prescription medicines at a grudging pace, paying daily homage to the physician's creed, "First, do no harm."

Then in the early 1990s, the demand for AIDS drugs changed the political climate. Congress told the FDA to work closely with pharmaceutical firms in getting new medicines to market more swiftly. President Clinton urged FDA leaders to trust industry as "partners, not adversaries."

The FDA achieved its new goals, but now the human cost is becoming clear.

Seven drugs approved since 1993 have been withdrawn after reports of deaths and severe side effects. A two-year Los Angeles Times investigation has found that the FDA approved each of those drugs while disregarding danger signs or blunt warnings from its own specialists. Then, after receiving reports of significant harm to patients, the agency was slow to seek withdrawals.

According to "adverse-event" reports filed with the FDA, the seven drugs were cited as suspects in 1,002 deaths. Because the deaths are reported by doctors, hospitals and others on a voluntary basis, the true number of fatalities could be far higher, according to epidemiologists.

An adverse-event report does not prove that a drug caused a death; other factors, such as preexisting disease, could play a role. But the reports are regarded by public health officials as the most reliable early warnings of danger.

The FDA's performance was tracked through an examination of thousands of pages of government documents, other data obtained under the Freedom of Information Act and interviews with more than 60 present and former agency officials.

The seven drugs were not needed to save lives. One was for heartburn. Another was a diet pill. A third was a painkiller. All told, six of the medicines were never proved to offer lifesaving benefits, and the seventh, an antibiotic, was ultimately judged unnecessary because other, safer antibiotics were available.

The seven are among hundreds of new drugs approved since 1993, a period during which the FDA has become known more for its speed than its caution. In 1988, only 4% of new drugs introduced into the world market were approved first by the FDA. In 1998, the FDA's first-in-the-world approvals spiked to 66%. The drug companies' batting average in getting new drugs approved also climbed. By the end of the 1990s, the FDA was approving more than 80% of the industry's applications for new products, compared with about 60% at the beginning of the decade.

And the companies have prospered: The seven unsuccessful drugs alone generated U.S. sales exceeding $5 billion before they were withdrawn.

Once the world's unrivaled safety leader, the FDA was the last to withdraw several new drugs in the late 1990s that were banned by health authorities in Europe.

"This track record is totally unacceptable," said Dr. Curt D. Furberg, a professor of public health sciences at Wake Forest University. "The patients are the ones paying the price. They're the ones developing all the side effects, fatal and non-fatal. Someone has to speak up for them."

The FDA's faster and more lenient approach helped supply pharmacy shelves with scores of new remedies. But it has also yielded these fatal missteps, according to the documents and interviews:

* Only 10 months ago, FDA administrators dismissed one of its medical officer's emphatic warnings and approved Lotronex, a drug for treating irritable bowel syndrome. Lotronex has been linked to five deaths, the removal of a patient's colon and other bowel surgeries. It was pulled off the market on Nov. 28.

* The diet pill Redux, approved in April 1996 despite an advisory committee's vote against it, was withdrawn in September 1997 after heart-valve damage was detected in patients put on the drug. The FDA later received reports identifying Redux as a suspect in 123 deaths.

* The antibiotic Raxar was approved in November 1997 in the face of evidence that it may have caused several fatal heart-rhythm disruptions in clinical studies. FDA officials chose to exclude any mention of the deaths from the drug's label. The maker of the pill withdrew it in October 1999. Raxar was cited as a suspect in the deaths of 13 patients.

* The blood pressure medication Posicor was approved in June 1997 despite findings by FDA specialists that it might fatally disrupt heart rhythm and interact with certain other drugs, posing potentially severe risk. Posicor was withdrawn one year later; reports cited it as a suspect in 100 deaths.

* The painkiller Duract was approved in July 1997 after FDA medical officers warned repeatedly of the drug's liver toxicity. Senior officials sided with the manufacturer in softening the label's warning of the liver threat. The drug was withdrawn 11 months later. By late 1998, the FDA had received voluntary reports citing Duract as a suspect in 68 deaths, including 17 that involved liver failure.

* The diabetes drug Rezulin was approved in January 1997 over a medical officer's detailed opposition and was withdrawn this March after the agency had linked 91 liver failures to the pill. Reports cite Rezulin as a suspect in 391 deaths.

* The nighttime heartburn drug Propulsid was approved in 1993 despite evidence that it caused heart-rhythm disorders. The officials who approved the drug failed to consult the agency's own cardiac specialists about the signs of danger. The drug was taken out of pharmacies in July after scores of confirmed heart-rhythm deaths. Overall, Propulsid has been cited as a suspect in 302 deaths.

The FDA's handling of Propulsid put children at risk.

The agency never warned doctors not to administer the drug to infants or other children even though eight youngsters given Propulsid in clinical studies had died. Pediatricians prescribed it widely for infants afflicted with gastric reflux, a common digestive disorder.

Parents and their doctors had no way of knowing that the FDA, in August 1996, had found Propulsid to be "not approvable" for children.

"We never knew that," said Jeffrey A. Englebrick, a heavy-equipment welder in Shawnee, Kan., whose 3-month-old son, Scott, died on Oct. 28, 1997, after taking Propulsid. "To me, that means they took my kid as a guinea pig to see if it would work."

By the time the drug was pulled, the FDA had received reports of 24 deaths of children under age 6 who were given Propulsid. By then the drug had generated U.S. sales of $2.5 billion for Johnson & Johnson Co.

Questions also surround the recent approvals of other compounds that remain on the market, including a new flu drug called Relenza. In February of 1999, an FDA advisory committee concluded that Relenza had not been proved safe and effective. The agency nevertheless approved it. Following the deaths of seven patients, the FDA in January issued a "public health advisory" to doctors.

A 'Lost Compass'

A total of 10 drugs have been pulled from the market in just the past three years for safety reasons, including three pills that were approved before the shift that took hold in 1993. Never before has the FDA overseen the withdrawals of so many drugs in such a short time. More than 22 million Americans--about 10% of the nation's adult population--took those drugs.

With many of the drugs, the FDA used tiny-print warnings or recommendations in package labeling as a way to justify approvals or stave off withdrawals. In other instances, the agency has withheld safety information from labels that physicians say would call into question the use of the product.

Present and former FDA specialists said the regulatory decisions of senior officials have clashed with the agency's central obligation, under law, to "protect the public health by ensuring . . . that drugs are safe and effective."

"They've lost their compass and they forget who it is that they are ultimately serving," said Dr. Lemuel A. Moye, a University of Texas School of Public Health physician who served from 1995 to 1999 on an FDA advisory committee. "Unfortunately the public pays for this, because the public believes that the FDA is watching the door, that they are the sentry."

The FDA's shift is felt directly in the private practice of medicine, said Dr. William L. Isley, a Kansas City, Mo., diabetes specialist. He implored the agency to reassess Rezulin three years ago after a patient he treated suffered liver failure taking the pill.

"FDA used to serve a purpose," Isley said. "A doctor could feel sure that a drug he was prescribing was as safe as possible. Now you wonder what kind of evaluation has been done, and what's been swept under the rug."

FDA officials said that they have tried conscientiously to weigh benefits versus risks in deciding whether to approve new drugs. They noted that many doctors and patients complain when a drug is withdrawn.

"All drugs have risks; most of them have serious risks," said Dr. Janet Woodcock, director of the FDA's drug review center. She added that some of the withdrawn drugs were "very valuable, even if not lifesaving, and their removal from the market represents a loss, even if a necessary one."

Once a drug is proved effective and safe, Woodcock said, the FDA depends on doctors "to take into account the risks, to read the label. . . . We have to rely on the practitioner community to be the learned intermediary. That's why drugs are prescription drugs."

In a May 12, 1999, article co-authored with FDA colleagues and published by the Journal of the American Medical Assn., Woodcock said, “The FDA and the community are willing to take greater safety risks due to the serious nature of the [illnesses] being treated.”

Compared to the volume of new drugs approved, they wrote, the number of recent withdrawals “is particularly reassuring.”

However, agency specialists point out that both approvals and withdrawals are controlled by Woodcock and her administrators. When they consider a withdrawal, they face the unpleasant prospect of repudiating their original decision to approve.

Woodcock, 52, received her medical degree at Northwestern University and is a board-certified internist. She alluded in a recent interview to the difficulty she feels in rejecting a proposed drug that might cost a company $150 million or more to develop. She also acknowledged the commercial pressures in a March 1997 article.

“Consumer protection advocates want to have drugs worked up well and thoroughly evaluated for safety and efficacy before getting on the market,” Woodcock wrote in the Food and Drug Law Journal. “On the other hand, there are economic pressures to get drugs on the market as soon as possible, and these are highly valid.”

But this summer--following the eighth and ninth drug withdrawals--Woodcock said the FDA cannot rely on labeling precautions, alone, to resolve safety concerns.

“As medical practice has changed . . . it’s just much more difficult for [doctors] to manage” the expanded drug supply, Woodcock said in an interview. “They rely upon us much more to make sure the drugs are safe.”

Another FDA administrator, Dr. Florence Houn, voiced similar concern in remarks six months ago to industry officials: “I think the lessons learned from the drug withdrawals make us leery.”

Yet the imperative to move swiftly, cooperatively, remains.

“We are now making decisions more quickly and more predictably while maintaining the same high standards for product safety and efficacy,” FDA Commissioner Jane E. Henney said in a National Press Club speech on Dec. 12.

Motivated by AIDS

The impetus for change at the FDA emerged in 1988, when AIDS activists paralyzed operations for a day at the agency's 18-story headquarters in Rockville, Md. They demanded immediate approval of experimental drugs that offered at least a ray of hope to those otherwise facing death.

The FDA often was taking more than two years to review new drug applications. The pharmaceutical industry saw a chance to loosen the regulatory brakes and expedite an array of new products to market. The companies and their Capitol Hill lobbyists pressed for advantage: If unshackled, they said, the companies could invent and develop more remedies faster.

The political pressure mounted, and the FDA began to bow. By 1991, agency officials told Congress they were making significant progress in speeding the approval process.

The emboldened companies pushed for more. They proposed that drugs intended for either life-threatening or "serious" disorders receive a quicker review.

"The pharmaceutical companies came back and lobbied the agency and the Hill for that word, 'serious,' " recalled Jeffrey A. Nesbit, who in 1991 was chief of staff to FDA Commissioner David A. Kessler. "Their argument was, 'Well, OK, there's AIDS and cancer. But there are drugs [being developed] for Alzheimer's. And that's a serious illness.' They started naming other diseases. They began to push that envelope."

The wielding of this single, flexible adjective--"serious"--swung wide the regulatory door knocked ajar by the AIDS crisis.

New Order Takes Hold

In 1992, Kessler issued regulations giving the FDA discretion to "accelerate approval of certain new drugs" for serious or life-threatening conditions. That same year a Democrat-controlled Congress approved and President Bush signed the Prescription Drug User Fee Act. It established goals that call for the FDA to review drugs within six months or a year; the pharmaceutical companies pay a user fee to the FDA, now $309,647, with the filing of each new drug application.

The newly elected Clinton administration climbed aboard with its "reinventing government" project. Headed by Vice President Al Gore, the project called for the FDA, by January 2000, to reduce "by an

average of one year the time required to bring important new drugs to the American public." As Clinton put it in a speech on March 16, 1995, the objective was to "get rid of yesterday's government."

For the FDA's medical reviewers--the physicians, pharmacologists, chemists and biostatisticians who scrutinize the safety and effectiveness of emerging drugs--a new order had taken hold.

The reviewers work out of public view in secure office buildings clustered along Maryland's Route 355. At the jet-black headquarters building, the decor is institutional, the corridors and third-floor cafeteria without windows. The reviewers examine truckloads of scientific documents. They are well-educated; some are highly motivated to do their best for a nation of patients who unknowingly count on their expertise.

One of these reviewers was Michael Elashoff, a biostatistician who arrived at the FDA in 1995 after earning degrees from UC Berkeley and the Harvard School of Public Health.

"From the first drug I reviewed, I really got the sense that I was doing something worthwhile. I saw what a difference a single reviewer can make," said Elashoff, the son and grandson of statisticians.

Last year he was assigned to review Relenza, the new flu drug developed by Glaxo Wellcome. He recommended against approval.

"The drug has no proven efficacy for the treatment of influenza in the U.S. population, no proven effect on reducing person-to-person transmissibility, and no proven impact on preventing influenza," Elashoff wrote, adding that many patients would be exposed to risks "while deriving no benefit."

An agency advisory committee agreed and on Feb. 24 voted 13 to 4 against approving Relenza.

After the vote, senior FDA officials upbraided Elashoff. They stripped him of his review of another flu drug. They told him he would no longer make presentations to the advisory committee. And they approved Relenza as a safe and effective flu drug.

Lost Faith in the System

Elashoff and other FDA reviewers discern a powerful message.

"People are aware that turning something down is going to cause problems with [officials] higher up in FDA, maybe more problems than it's worth," he said. "Before I came to the FDA I guess I always

assumed things were done properly. I've lost a lot of faith in taking a prescription medicine."

Elashoff left the FDA four months ago.

"Either you play games or you're going to be put off limits . . . a pariah," said Dr. John L. Gueriguian, a 19-year FDA medical officer who opposed the approval of Rezulin, the ill-fated diabetes drug. "The people in charge don't say, 'Should we approve this drug?' They say, 'Hey, how can we get this drug approved?'"

Said Dr. Rudolph M. Widmark, who retired in 1997 after 11 years as a medical officer: "If you raise concern about a drug, it triggers a whole internal process that is difficult and painful. You have to defend why you are holding up the drug to your bosses. . . . You cannot imagine how much pressure is put on the reviewers."

The pressure is such that when a union representative negotiated a new employment contract for the reviewers last year, one of his top priorities was to defend what he called the "scientific integrity" of their work.

"People feel swamped. People are pressured to go along with what the agency wants," said Dr. Robert S.K. Young, an FDA medical officer who in 1998 formed a union chapter to represent the reviewers. "You're paying for these highly educated, trained people, and they're not being allowed to do their job."

Each new drug application is accompanied by voluminous medical data, enough at times to fill 1,000 or more phone books. The reviewers must master this material in less than six months or a year, while juggling other tasks.

"The devil is in the details, and detail is something we no longer have the time to go into," said Gurston D. Turner, a veteran pharmacologist with the FDA's scientific investigations division who retired this year. "If you know you must have your report done by a certain date, you get something done. That's what they [top FDA officials] count, that's all they count. And that is really, to me, a worrisome thing."

The FDA did spur reviewers to move at record speed.

In 1994, the FDA's goal was to finish 55% of its new drug reviews on time; the agency achieved 95%. In 1995, the goal was 70%; the FDA achieved 98%. In 1996, the goal was 80%; the FDA achieved 100%. In both 1997 and 1998, the goal was 90% and the FDA achieved 100%.

From 1993 to 1999 the agency approved 232 drugs regarded as “new molecular entities,” compared with 163 during the previous seven years, a 42% increase.

The time-limit goals quickly were treated as deadlines within the FDA--imposing relentless pressure on reviewers and their bosses to quickly conclude their work and approve the drugs.

“The goals were to be taken seriously. I don’t think anybody expected the agency to make them all,” said William B. Schultz, a deputy FDA commissioner from 1995 to 1999.

Schultz, who helped craft the 1992 user-fee act as a congressional staff lawyer, added: “You can meet the goal by either approving the drug or denying the approval. But there are some who argue that what Congress really wanted was not just decisions, but approvals. That is what really gets dangerous.”

Indeed, the FDA drug center’s 1999 annual report referred to the review goals as “the law’s deadlines.” And, Dr. Woodcock, the center director, elaborated in a subsequent agency newsletter:

“In exchange [for the user fees], FDA makes a commitment to meet certain goals for review times. [The agency] has exceeded almost all of the goals, and it expects to continue to exceed them. Basically, the number of new approved drugs has doubled, and the review times have been cut in half.”

The user fees have enabled the FDA to hire more medical reviewers. Last year, 236 medical officers examined new drugs compared with 162 officers on duty in 1992, the year before the user fees took effect.

Even so, Woodcock acknowledged in an FDA publication this fall that the workloads and tight performance goals “create a sweatshop environment that’s causing high staffing turnover.”

An FDA progress report in 1998, describing the work of agency chemists, said that “too many reviews are coming ‘down to the wire’ against the goal date. . . . This suggests a system in stress.”

Said Nesbit, the former aide to Commissioner Kessler: “The clock is always running, whereas before the clock was never running. And that changes people’s behavior.”

Dozens of officials interviewed by The Times made similar observations.

“The pressure to meet deadlines is enormous,” said Dr. Solomon Sobel, 65, director of the FDA’s metabolic and endocrine drugs division throughout the 1990s. And the pressure is not merely to

complete the reviews, he said. “The basic message is to approve.”

Over the last seven years, “there has been a huge shift,” said Kathleen Holcombe, a former FDA legislative affairs staffer and congressional aide who now is a drug industry consultant. “FDA, historically, had an approach of, ‘Regulate, be tough, enforce the law [and] don’t let one thing go wrong,’ ” Holcombe said, adding that now, “the FDA sees itself much more in a cooperative role.”

The perception of coziness with drug makers is perpetuated by potential conflicts of interest within the FDA’s 18 advisory committees, the influential panels that recommend which drugs deserve approval or should remain on the market. The FDA allows some appointees to double as consultants or researchers for the same companies whose products they are evaluating on the public’s behalf. Such was the case during committee appraisals of several of the recently withdrawn drugs, including Lotronex and Posicor, The Times found.

Few doubt the $100-billion pharmaceutical industry’s clout. Over the last decade, the drug companies have steered $44 million in contributions to the major political parties and to candidates for the White House and both houses of Congress.

The FDA reviewers said they and their bosses fear that unless the new drugs are approved, companies will erupt and Congress will retaliate by refusing to renew the user fees. This would cripple FDA operations--and jeopardize jobs.

The companies’ money now covers about 50% of the FDA’s costs for reviewing proposed drugs--and agency officials say that persuading Congress to renew the user fees into 2007 is now a top priority.

Yet even if the user fees remain, the FDA is prohibited from spending the revenue for anything other than reviewing new drugs. So while the budget for pre-approval reviews has soared, the agency has gotten no similar increase of resources to evaluate the safety of the drugs after they are prescribed.

“It’s shocking,” said Dr. Brian L. Strom, chairman of epidemiology at the University of Pennsylvania. “How can you say, ‘Release drugs to the market sooner,’ and not know if they’re killing people? . . . It really is a dramatic statement of public priorities.”

More than 250,000 side effects linked to prescription drugs, including injuries and deaths, are reported each year. And those “adverse-event” reports by doctors and others are only filed voluntarily. Experts, including Strom, believe the reports represent as few as 1% to 10% of all such events.

"There's no incentive at all for a physician to report [an adverse drug reaction]," said Strom, who has documented the phenomenon. "The underreporting is vast."

Even when deaths are reported, records and interviews show that companies consistently dispute that their product has caused a given death by pointing to other factors, including preexisting disease or use of another medicine.

To be sure, a chain of events affects the safe use of a prescription drug: The companies' conduct of clinical studies; the FDA's regulatory actions; the doctor's decision to prescribe; the pharmacist's filling of a handwritten prescription; the patient's ability to take the drug as directed. A lapse at any link could prove fatal.

And once a pill is approved by the FDA, the manufacturer often spends heavily on promotion to seize the largest possible market share. This can exacerbate the risk to public health, according to experts.

"Aggressive promotion increases exposure--and doesn't give you the time to find the problem before patients get hurt," said Dr. Raymond L. Woosley, pharmacology department chairman at Georgetown University and a former FDA advisory committee member.

When serious side effects emerge, the FDA officials have championed using package labeling as a way to, in their words, "manage" risks. Yet the agency typically has no way to know if the labeling precautions--dense, lengthy and in tiny print--are read or followed by doctors and their patients.

The FDA often addresses unresolved safety questions by asking companies to conduct studies after the product is approved. But the research frequently is not performed--prompting the inspector general of the Department of Health and Human Services to say in 1996 that "FDA can move to withdraw drugs from the market if the post-marketing studies are not completed with due diligence."

Since that report was issued, the FDA has not withdrawn any drug due to a company's failure to complete a post-approval safety study. Officials conceded this week that they still do not know how often the studies are performed.

One consequence is that greater risk is shifted to doctors and patients.

For example, Woodcock and her senior aides allowed Rezulin to remain on the U.S. market nearly 2½ years after it was withdrawn in Britain in December 1997. The FDA recommended frequent laboratory

testing of patients using the drug but had no scientific assurance that the tests would prevent Rezulin-induced liver failure.

“They kept increasing the number of liver-function tests you should have,” noted Dr. Alastair J.J. Wood, a former FDA advisory committee member who is a professor of medicine at Vanderbilt University. “That was clearly designed to protect the FDA, to protect the manufacturer, and to dump the responsibility on the patient and the physician. If the patient developed liver disease and he hadn’t had his [tests] done, somebody was to blame and it wasn’t the manufacturer and it wasn’t the FDA.”

Industry Assurances

Leading industry officials say Americans have nothing to fear from the wave of drug approvals.

“Do unsafe drugs enter and remain in the marketplace? Absolutely not,” said Dr. Bert A. Spilker, senior vice president for scientific and regulatory affairs for the Pharmaceutical Research and Manufacturers of America, in remarks last year to industry and FDA scientists.

But during interviews over the last two years, current and former FDA specialists cited repeated instances when drugs were approved with less than compelling evidence of safety or effectiveness. They also said that important information has been excluded from the labels on some medications.

Elashoff, for instance, was surprised at the labeling for a drug called Prograf, approved in 1997 to prevent rejection of transplanted kidneys. The drug first had been approved in 1994 for use among liver-transplant patients.

The new label notes that Prograf was proved effective in a study of 412 U.S. kidney transplant patients. But no mention is made of the company’s 448-patient European study, in which 7% of the patients who took Prograf died--double the 3.5% death rate among those who received a different anti-rejection drug, documents show.

An auditor from the FDA’s scientific investigations unit, Antoine El-Hage, examined the European study results and concluded the “data are reliable.” Elashoff agreed in his review.

Yet the only way for doctors or patients to find that data is to search the medical literature or seek the FDA’s review documents.

Excluding the European study from the Prograf label, Elashoff said, “was just a total whitewash. . . . I think any rational person would reconsider taking this drug if they knew what happened in Europe.”

A spokesman for the manufacturer of Prograf said the company had no objection to including the European study results in the labeling. William E. Fitzsimmons, a vice president of drug development for Fujisawa Healthcare Inc., said the decision to exclude the results was entirely the FDA’s.

“We submitted that data,” he said. “It came down to what the FDA was comfortable putting in the label. . . . We certainly have no interest in trying to hide that information. We presented it at major meetings on transplantation. . . . We’re comfortable with that information being out in the public domain.”

But if the FDA had included the European results in the label, it would have impugned the agency’s basis for approving the new, expanded use for Prograf, according to Elashoff and others.

Asked why the agency excluded the information, Woodcock said the European results were “unreliable . . . and could be potentially misleading to doctors and patients in the U.S. if these were included in the label.”

David Willman

David Willman is a former investigative reporter for the Los Angeles Times.

SCIENCE

Are Your Medications Safe?

The FDA buries evidence of fraud in medical trials. My students and I dug it up.

BY CHARLES SEIFE

FEB 09, 2015 • 11:16 AM

Agents of the Food and Drug Administration know better than anyone else just how bad scientific misbehavior can get. Reading the FDA's inspection files feels almost like watching a highlights reel from a *Scientists Gone Wild* video. It's a seemingly endless stream of lurid vignettes—each of which catches a medical researcher in an unguarded moment, succumbing to the temptation to do things he knows he really shouldn't be doing. Faked X-ray reports. Forged retinal scans. Phony lab tests. Secretly amputated limbs. All done in the name of science when researchers thought that nobody was watching.

That misconduct happens isn't shocking. What is: When the FDA finds scientific fraud or misconduct, the agency doesn't notify the public, the medical establishment, or even the scientific community that the results of a medical experiment are not to be trusted. On the contrary. For more than a decade, the FDA has shown a pattern of burying the details of misconduct. As a result, nobody ever finds out which data is bogus, which experiments are tainted, and which drugs might be on the market under false pretenses. The FDA has repeatedly hidden evidence of scientific fraud not just from the public, but also from its most trusted scientific advisers, even as they were deciding whether or not a new drug should be allowed on the market. Even a congressional panel investigating a case of fraud regarding a dangerous drug couldn't get forthright answers. For an agency devoted to protecting the public from bogus medical science, the FDA seems to be spending an awful lot of effort protecting the perpetrators of bogus science from the public.

Much of my research has to do with follies, foibles, and fraud in science, and I knew that the FDA wasn't exactly bending over backward to correct the scientific record when its inspectors found problems during clinical trials. So as part of my investigative reporting class at New York University, my students and I set out to find out just how bad the problem was—and how much important information the FDA was keeping under wraps.

We didn't have to search very hard to find FDA burying evidence of research misconduct. Just look at any document related to an FDA inspection. As part of the new drug application process, or, more rarely, when the agency gets a tipoff of wrongdoing, the FDA sends a bunch of inspectors out to clinical sites to make sure that everything is done by the book. When there are problems, the FDA generates a lot of paperwork—what are called form 483s, Establishment Inspection Reports, and in the worst cases, what are known as Warning Letters. If you manage to get your hands on these documents, you'll see that, most of the time, key portions are redacted: information that describes what drug the researcher was studying, the name of the study, and precisely how the misconduct affected the quality of the data are all blacked out. These redactions make it all but impossible to figure out which study is tainted. My students and I looked at FDA documents relating to roughly 600 clinical trials in which one of the researchers running the trial failed an FDA inspection. In only roughly 100 cases were we able to figure out which study, which drug, and which pharmaceutical company were involved. (We cracked a bunch of the redactions by cross-referencing the documents with clinical trials data, checking various other databases, and using carefully crafted Google

searches.) For the other 500, the FDA was successfully able to shield the drugmaker (and the study sponsor) from public exposure.

It's not just the public that's in the dark. It's researchers, too. And your doctor. As I describe in the current issue of *JAMA Internal Medicine*, my students and I were able to track down some 78 scientific publications resulting from a tainted study—a clinical trial in which FDA inspectors found significant problems with the conduct of the trial, up to and including fraud. In only three cases did we find any hint in the peer-reviewed literature of problems found by the FDA inspection. The other publications were not retracted, corrected, or highlighted in any way. In other words, the FDA knows about dozens of scientific papers floating about whose data are questionable—and has said nothing, leaving physicians and medical researchers completely unaware. The silence is unbroken even when the FDA itself seems shocked at the degree of fraud and misconduct in a clinical trial.

Such was the case with the so-called RECORD 4 study. RECORD 4 was one of four large clinical trials that involved thousands of patients who were recruited at scores of clinical sites in more than a dozen countries around the world. The trial was used as evidence that a new anti-blood-clotting agent, rivaroxaban, was safe and effective. The FDA inspected or had access to external audits of 16 of the RECORD 4 sites. The trial was a fiasco. At Dr. Craig Loucks' site in Colorado, the FDA found falsified data. At Dr. Ricardo Esquivel's site in Mexico, there was "systematic discarding of medical records" that made it impossible to tell whether the study drug was given to the patients. At half of the sites that drew FDA scrutiny—eight out of 16—there was misconduct, fraud, fishy behavior, or other practices so objectionable that the data had to be thrown out. The problems were so bad and so widespread that, contrary to its usual practice, the FDA declared the entire study to be "unreliable." Yet if you look in the medical journals, the results from RECORD 4 sit quietly in *The Lancet* without any hint in the literature about falsification, misconduct, or chaos behind the scenes. This means that physicians around the world are basing life-and-death medical decisions on a study that the FDA knows is simply not credible.

It's not just one study, either. The FDA found major problems with sites involved in the other three clinical trials that were used to demonstrate rivaroxaban's safety and effectiveness. RECORD 2, for example, was nearly as awful as RECORD 4: Four out of 10 sites that the FDA inspected showed evidence of misconduct, or other issues grave enough to render the site's data worthless—including clear evidence of data falsification at one site. In aggregate, these problems raise serious doubts about the quality of all four key rivaroxaban studies—and, by extension, doubts about how seriously we should take the claim that rivaroxaban is safe and effective. The FDA is keeping mum, even as wrongful-death lawsuits begin to multiply.

The FDA's failure to notify the public is not merely a sin of omission. In March 2009, the FDA convened a committee of outside scientific experts to mull the "robustness and meaningfulness" of the results from the four rivaroxaban trials, RECORDs 1, 2, 3, and 4. (The agency regularly calls in advisers to get advice, or, more cynically, to get cover, about a decision the agency has to make.) When the agency briefed the committee, it was (to put it mildly) coy about the problems it was finding. It said only that inspectors had found "significant issues" at two clinical sites involved in the RECORD 4 study—and that data from one of them was included in the analysis. Inspections were still ongoing, so it's not easy to say precisely what the agency knew at that point, but it's clear that the FDA wasn't admitting to everything it knew. A bunch of inspections had been completed a month prior to the meeting, and we know for certain that the agency was fully aware of major issues beyond the two it revealed to the advisory committee. In a memo dated three days before

the advisory committee meeting convened, the FDA detailed "falsification of data by a subinvestigator" at a RECORD 2 site. The advisory committee was not told.

By itself, this might seem like a miscommunication or an oversight, but the FDA has a history of not notifying the public about the misconduct it finds. About a decade ago, the agency got into trouble over a newly approved antibiotic, Ketek. Inspectors had found extensive problems (including fraud) affecting key clinical trials of the drug. Yet the agency did its best to hide the problems from even its most trusted advisers. As David Ross, the FDA official in charge of reviewing Ketek's safety, put it, "In January 2003, over reviewers' protests, FDA managers hid the evidence of fraud and misconduct from the advisory committee, which was fooled into voting for approval." However, when the reports of misconduct at one clinical site began appearing in the press—along with stories of liver damage and blurred vision associated with the new drug—Congress stepped in, demanding information from the agency about the fraud.

But even the Senate couldn't wring key information about the misconduct out of the FDA. "Every excuse under the sun has been used to create roadblocks," complained an indignant Sen. Charles Grassley, "even in the face of congressional subpoenas requesting information and access to FDA employees." The head of the FDA, Andrew von Eschenbach, attempted to explain to Congress why the agency didn't tell its advisory committee about the problems in the Ketek study: "After considering the fact that the investigation results were preliminary … FDA decided to hold the Advisory Committee meeting as planned …" without notifying the committee of the potential problems. But Rep. Bart Stupak quickly pointed to an email, which, he argued, contradicted von Eschenbach's testimony. "So either you are not being forthright with us, when I believe you are, but whoever is doing your work is trying to lead this committee down the wrong path." And the correct path showed that site after site involved in study 3014, as well as other key Ketek studies, were tainted as well.

In the decade since the Ketek affair, it's hard to see any change in behavior by the agency. On occasion, the FDA has even actively approved and promoted statements about drugs that, according to its own inspectors, are based upon falsehoods. At the end of 2011, the FDA learned that an audit of a Chinese site involved in a key clinical trial of a different anti-clotting agent, apixaban, had turned up evidence of fraud: Personnel had apparently been fiddling with patient records. Worse yet, the fraud appeared to invalidate one key finding of the study. Just three months earlier, the researchers running the trial proudly announced in the *New England Journal of Medicine* that there was a "significant reduction in mortality" among patients who took apixaban compared with those who took the old standby, warfarin. Alas, the moment you exclude the data from the Chinese fraud site, as per standard FDA procedure, that statement went out the window. Yet look at the label for apixaban—the one approved by the FDA after the fraud was discovered—and you read that "treatment resulted in a significantly lower rate of all-cause death … than did treatment with warfarin," backed up by the data set with the Chinese site included. In other words, the label is carrying a claim that the FDA knows is based upon fraud. In a written response to my questions on this subject, the FDA stated that, "The FDA extended the drug's review period to address the concerns. However, the review team did conclude concluded [sic] that the data at that site and other sites in China did reflect meaningful clinical information; that was not what was considered unreliable."

Again, this isn't an isolated incident. I had previously encountered bogus data on FDA-approved labels when a colleague and I were looking into a massive case of scientific misconduct —a research firm named Cetero had been caught faking data from more than 1,400 drug trials. That suddenly worthless data had been used

to establish the safety or effectiveness of roughly 100 drugs, mostly generics, that were being sold in the United States. But even after the agency exposed the problem, we found fraud-tainted data on FDA-approved drug labels. (The FDA still maintains its silence about the Cetero affair. To this day, the agency refuses to release the names of the 100-odd drugs whose approval data were undermined by fraud.)

And the FDA covers up drug-related misconduct in other, more subtle ways, too. For example, the agency publishes the canonical listing of generic drugs in the United States, known as the "Orange Book." Prescription drugs in this book are often given what's called a "therapeutic equivalence code." This code is a two-letter designation that signals the quality of the scientific evidence that a generic is "bioequivalent" to the name-brand drug. The code "AB," for example, tells pharmacists and physicians that there are solid scientific studies proving that bioequivalence. Another code, "BX," signals that there isn't sufficient data to prove the generic is bioequivalent to the name brand.

When the Cetero misconduct was uncovered, key bioequivalence studies for scores of generic drugs turned out to be worthless. By rights, some of those drugs should have had their designation downgraded from AB to BX. But even though the FDA updates the Orange Book monthly, there was no rash of drugs losing their AB rating in the months after the Cetero affair broke. In the year and a half after the Cetero fraud was first announced, I was able to identify a grand total of four generic drugs (in various dosages) that were downgraded to BX, none of which appeared to be linked to the Cetero problem. On the other hand, the one prescription generic drug that I knew for sure had been hit hard by the Cetero fraud—both key studies supporting its bioequivalence to the name brand were declared worthless—had no change in its designation. The FDA apparently allowed the drug to keep its AB badge for months without any valid data backing the drug's bioequivalence. When asked, point blank, whether the agency had downgraded the bioequivalence code of any products due to the Cetero affair, officials promptly dodged the question. A written statement issued by the agency's press office in response to my queries noted that the FDA requested additional data from the companies whose drugs were implicated in the Cetero affair and that "If the data were not provided within 6 months or the data provided did not support a finding of bioequivalence, FDA said it would consider changing the generic product's therapeutic equivalence rating in the Orange Book from AB to BX." Not a word about a single bioequivalence rating *actually* being changed.

This, too, is a pattern of behavior rather than a one-off. In the past few weeks, another major Cetero-type case began to emerge—this time, having to do with GVK Biosciences, a firm in Hyderabad, India. The European Medicines Agency, the European equivalent of the FDA, examined more than 1,000 drugs in various dosages affected by GVK's "data manipulations" and has suggested pulling 700 off the market. You can find the full list on the EMA website; to their credit, the Europeans are being relatively transparent as the crisis develops. Not so much on this side of the pond, alas. So far from the FDA, we've heard precious little, even though there are drugs on the U.S. market that rely entirely on GVK's tests. In a written statement, the FDA admitted that there were some 40-odd drugs whose approval depended upon GVK-run studies. Which ones? The agency is keeping mum, as it did with Cetero and with other similar cases. However, the agency assures us that it inspected GVK's facility and found nothing to be concerned about; if the situation changes, "FDA will take swift and appropriate action to ensure that the drug products available to American consumers are safe and effective."

Why does the FDA stay silent about fraud and misconduct in scientific studies of pharmaceuticals? Why would the agency allow claims that have been undermined by fraud to appear on drug labels? And why on

earth would it throw up roadblocks to prevent the public, the medical community, its advisory panels, and even Congress from finding out about the extent of medical misconduct? The answers the FDA gives are fascinating—they show how an agency full of well-meaning people can do intellectual backflips to try to justify secrecy.

The most common excuse the agency gives is that exposing the details about scientific wrongdoing—naming the trials that were undermined by research misconduct, or revealing which drugs' approvals relied upon tainted data—would compromise "confidential commercial information" that would hurt drug companies if revealed. This claim falls apart under scrutiny. The courts have ruled that when information is provided by companies involuntarily, such as the information that an FDA inspector finds, "commercial confidential information" refers to proprietary material that causes substantial, specific harm when it falls into the hands of a competitor. It doesn't cover embarrassing peccadilloes—or misconduct that might cause bad publicity when word gets out.

Another excuse I've heard from the FDA is that it doesn't want to confuse the public by telling us about problems, especially when, in the FDA's judgment, the misconduct doesn't pose an immediate risk to public health. For example, when my colleague and I asked the director of FDA's Center for Drug Evaluation and Research why the agency wouldn't name the drugs affected by the Cetero fraud, she told us that the matter "did not rise to the level where the public should be notified. We felt it would result in misunderstanding and inappropriate actions." But even the most paternalistic philosophy of public health can't explain why the FDA would allow drug companies to put data on its labels that the agency knows are worthless, or to fail to flag bioequivalence problems in a publication that is specifically designed for the purpose of flagging those very problems.

The sworn purpose of the FDA is to protect the public health, to assure us that all the drugs on the market are proven safe and effective by reputable scientific trials. Yet, over and over again, the agency has proven itself willing to keep scientists, doctors, and the public in the dark about incidents when those scientific trials turn out to be less than reputable. It does so not only by passive silence, but by active deception. And despite being called out numerous times over the years for its bad behavior, including from some very pissed-off members of Congress, the agency is stubbornly resistant to change. It's a sign that the FDA is deeply captured, drawn firmly into the orbit of the pharmaceutical industry that it's supposed to regulate. We can no longer hope that the situation will get better without firm action from the legislature.

The FDA wants you to take it on faith that its officials have the public's best interest at heart. Justification through faith alone might be just fine as a religious doctrine, but it's not a good foundation for ensuring the safety and effectiveness of our drugs. After all, the whole point of science-based medicine is to keep us from having to make a leap of faith every time we swallow a pill.

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Hidden conflicts? Pharma payments to FDA advisers after drug approvals spark ethical concerns

Science investigation of journal disclosures and pharmaceutical funding records shows potential influence on physician gatekeepers

5 JUL 2018 • BY CHARLES PILLER, JIA YOU

On a sweltering July day in 2010, seven medical researchers and one patient advocate gathered in a plush Marriott hotel in College Park, Maryland, to review a promising drug designed to prevent heart attacks and strokes by limiting blood clotting. The panel is one of dozens of advisory committees that vote each year on whether the Food and Drug Administration (FDA) should approve a therapy for the U.S. market. That day, panel members heard presentations on the drug's preclinical and clinical data from agency staff and AstraZeneca in Cambridge, U.K., its maker and one of the world's largest pharmaceutical companies. The occasion sparked little drama. In the cool refuge of the conference room, advisers politely questioned company scientists and complimented their work. By day's end, the panel voted seven to one to approve. FDA, as usual, later signed off. The drug, ticagrelor, marketed under the name Brilinta, sold rapidly, emerging as a billion-dollar blockbuster. It cuts risk of death from vascular causes, heart attacks, and strokes modestly more than its chief competitor—and currently costs 25 times as much.

FDA, headquartered in Silver Spring, Maryland, uses a well-established system to identify possible conflicts of interest before such advisory panels meet. Before the Brilinta vote, the agency mentioned no financial conflicts among the voting panelists, who included four physicians. As Brilinta's sales took off later, however, AstraZeneca and firms selling or developing similar cardiovascular therapies showered the four with money for travel and advice. For example, those companies paid or reimbursed cardiologist Jonathan Halperin of the Icahn School of Medicine at Mount Sinai in New York City more than $200,000 for accommodations, honoraria, and consulting from 2013 to 2016. During that period, for example, AstraZeneca says it paid Halperin more than $11,000 in expenses and fees for work on an advisory board, service on a data monitoring committee for a clinical trial of Brilinta led by the University of California, San Francisco, and for his service chairing the data monitoring committee for an AstraZeneca-sponsored multimillion-dollar clinical trial of Brilinta led by Duke University.

Brilinta fits a pattern of what might be called pay-later conflicts of interest, which have gone largely unnoticed—and entirely unpoliced. In examining compensation records from drug companies to physicians who advised FDA on whether to approve 28 psychopharmacologic, arthritis, and cardiac or renal drugs between 2008 and 2014, *Science* found widespread after-the-fact payments or research support to panel members. The agency's safeguards against potential conflicts of interest are not designed to prevent such future financial ties.

Other apparent conflicts may have also slipped by: *Science* found that at the time of or in the year leading up to the advisory meetings, many of those panel members—including Halperin—received payments or other financial support from the drugmaker or key competitors for consulting, travel, lectures, or research. FDA did not publicly note those financial ties.

The analysis, which used physician disclosures in freely available publications and Centers for Medicare & Medicaid Services records for 2013 to 2016 on the federal Open Payments website, examined direct payments to physicians from firms whose drugs were voted on. It also considered payments from competitors selling or researching drugs of the same class or intended for the same condition—because competing drugs might be affected positively or negatively by the market entry of a new contender or by restrictions or warnings placed on a new drug's label. *Science* further looked at research funding from a company to an FDA adviser, directly or through their institution. Such

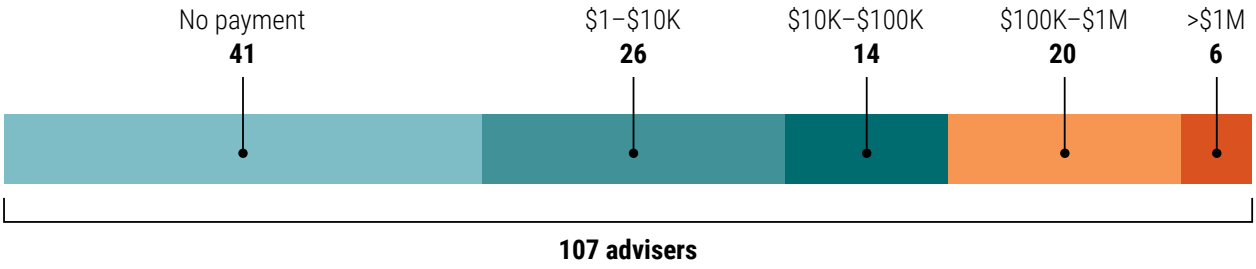
money—including "associated research" funding that nearly always supports principal investigators—affects a scientist's career advancement, compensation, or professional influence. (Check out an interactive that details all of these payments.)

Among the investigation's key findings:

- Of 107 physician advisers who voted on the committees *Science* examined, 40 over a nearly 4-year period received more than $10,000 in post hoc earnings or research support from the makers of drugs that the panels voted to approve, or from competing firms; 26 of those gained more than $100,000; and six more than $1 million.
- Of the more than $24 million in personal payments or research support from industry to the 16 top-earning advisers—who received more than $300,000 each—93% came from the makers of drugs those advisers previously reviewed or from competitors.
- Most of those top earners—and many others—received other funds from those same companies, concurrent with or in the year before their advisory service. Those payments were disclosed in scholarly journals but not by FDA.

Varying sums

An analysis of pharma payments to 107 physicians who advised FDA on 28 drugs approved from 2008 to 2014 found that a majority later got money for travel or consulting, or received research subsidies from the makers of the drugs on which they voted or from competing firms.



Corporate payments and other support given to advisers before a drug review are widely acknowledged as troubling. When "a voting member of a committee demonstrably had financial associations with the company or the competitor prior to the meeting, and the FDA doesn't flag it, then somebody's dropping the ball on due diligence," says Yale University physician Robert Steinbrook, editor at large for *JAMA Internal Medicine*.

Yet benefits that come later, even years after a drug approval vote—jobs, money, professional prestige, and influence—are also fraught, ethicists say. They are a way of "postponing your reward," says Carl Elliott, a medical ethicist at the University of Minnesota in Minneapolis who has persistently criticized the financial inducements pharma gives to researchers. "You do something positive for a company that you feel confident is going to pay you back for it later on. And they do."

Vinay Prasad, a hematologist-oncologist at Oregon Health & Science University in Portland who has studied financial conflicts in drug approvals, is similarly troubled. "The people who are asked to weigh this evidence impartially often stand to gain tremendously in their further professional careers from a positive relationship with the company," he says. It might not be a "quid pro quo," according to Prasad, "but you don't have to evoke that to be very concerned. It's in their best interest to play nice with these companies."

FDA declined interview requests about *Science*'s findings. A spokesperson provided a statement saying people serving on drug approval advisory panels must disclose any "prospective employer," but not anticipated payments. The statement further notes that "FDA also screens potential participants for relationships and situations that do not create a financial conflict of interest but that may create the appearance that a committee member lacks impartiality."

AstraZeneca spokesperson Karen Birmingham says "we are not aware" of any effort to support advisers after they serve on FDA panels reviewing the company's drugs, "other than the routine involvement in clinical trials or expert panels for which that [adviser] may have been sought independently because of their expertise."

Halperin says a direct payment from a drug company for a lecture or consulting "isn't really very much different than having an insurance company giving you a check for seeing a patient one day. It's the same thing." His 2009 recommendation for Brilinta's approval, he says, was not influenced by anticipation of large payments or research funding from AstraZeneca or its competitors. And Halperin argues that such relationships may be the price of expertise. "It's probably better to have someone who has some experience in [the specialized topics considered] than a bunch of unconflicted high school students," he says.

But the cardiologist agrees that expectations of future rewards can promote bias. "I share [the] concern that this could lead to people acting in ways that you would not want them to do," Halperin says. "We don't want incentives that are not serving the public interest. In my case, it's the patient's interest." And he notes that some medical organizations have begun to address delayed incentives. They ask members who write clinical practice guidelines to avoid financial relationships with affected companies for a period afterward—a tougher standard than what FDA requires for its advisers.

That solution and others should be up for debate, say ethicists and regulatory experts, including one prominent former FDA employee. "The idea of banning future payments is likely to have a lot of thorny aspects, but it's worth discussing," even at the risk of losing some experts to government service, says David Kessler, FDA commissioner under former Presidents George H. W. Bush and Bill Clinton. "It's a balancing act, but public trust is paramount."

After the Brilinta vote

In 2010, FDA advisers voted to recommend approval for Brilinta, which helps prevent blood clots in heart-disease patients. Four physicians who voted later received funds from AstraZeneca, its maker, and competing firms for consulting and travel, or worked on research underwritten by those companies.

Jacob Sitko enlisted in the U.S. Army in January 2008 and gave his heart and soul to it for more than 3 years—for a time serving in Iraq as a Humvee gunner in the infantry. In 2011, the private died in bed at his barracks at Fort Carson in Colorado, where he was being treated for posttraumatic stress disorder (PTSD). Months later, the Army finally gave Sitko's heart back to his mom.

Lois Vinall cries softly as she recounts her son's story. Right after his death, the Army told her that Sitko, who was 21 and in good health other than his PTSD, had been killed by "mixed-drug intoxication." Army doctors had been giving him a cocktail of medicines that included quetiapine, a top-selling antipsychotic from AstraZeneca sold under the name Seroquel. The particular mixture had been linked for years to sudden cardiac death, though no evidence has been made public that Sitko was told that.

"They sent his body home without his heart" and didn't say why, Vinall says. "They returned it in a baby coffin to me 3 months later, wrapped in green felt." Vinall recently learned that after removing her son's heart, the Army decided not to examine it further. She says a military medical examiner told her Sitko's autopsy hadn't been correctly "certified" and that her son might have suffered cardiac death. Vinall had cremated his body but buried his heart in a veterans' cemetery in Redding, California, close to family.

Two years earlier, two panels of FDA advisers had considered whether to approve Seroquel for new conditions—schizophrenia and bipolar disorder in children, and depression in adults who are taking other medicines. Seroquel was then known to be associated with sudden cardiac death when used with certain drugs, and several antipsychotics similar to Seroquel also had a record of cardiac fatalities. But AstraZeneca presented results from its clinical studies, which company representatives said showed, at worst, minimal risks.

In 2009, both panels voted by wide margins to approve Seroquel for the additional conditions. In the years afterward, several FDA advisers received significant financial support from AstraZeneca and the makers of competing drugs. The biggest payments went to Duke cardiologist Christopher Granger, who sat on one of the two groups. From 2013 to 2016, the period recorded by Open Payments, he or Duke on his behalf received more than $63,000 from AstraZeneca and $1.3 million from competitors. According to conflict-of-interest disclosures in journal articles on which Granger was an author, he received additional, unspecified amounts from those companies between 2010 and 2012.

Granger says the industry funds solely underwrote research on cardiovascular topics and did not augment his salary. But according to the federal data, more than $400,000—including all of AstraZeneca's portion—went to him for travel, consulting, and honoraria.

"I fully realize that when I'm paid by somebody, like every other human being, that may affect the way that I think about things. So I'm not naïve," Granger says. But the expectation of future support from the makers of antipsychotics, he adds, did not influence his assessments of Seroquel or similar drugs. Granger says he recommended the drug's conditional approval after becoming convinced—as were nearly all others on his panel—that Seroquel's value outweighed its risks for some people with severe psychiatric disabilities.

The next year, in 2010, AstraZeneca paid the government $520 million to settle lawsuits involving alleged improprieties in the company's clinical trials and improper marketing of Seroquel for unapproved conditions. The company, which denied wrongdoing, pulled in more than $5 billion in revenues from the drug that year. In 2011, after mounting evidence of sudden cardiac deaths, FDA forced AstraZeneca to add a warning to Seroquel's label that the drug posed risks of fatal cardiac events when combined with certain other drugs. Sitko died 3 weeks later.

In recent years, FDA has fielded thousands of complaints about cardiac problems, including many deaths, tied to Seroquel. Granger calls the drug's widespread use for unapproved conditions, such as insomnia, a "public health tragedy." Sitko and many others were given the drug, in part, to treat insomnia. The company has said repeatedly that Seroquel is acceptably safe and effective to treat conditions for which FDA approved it.

Policing future drug industry payments received by FDA advisory committee members would be challenging even for an agency adept at limiting conflicts of interest. Yet *Science*'s investigation raises questions about how well FDA enforces more traditional conflict rules.

FDA asks panel members who vote on recommending drug approvals to disclose in advance details of investments, contracts, or other payments from drugmakers. The agency uses those disclosures to determine whether pharma backing during or before a meeting should disqualify an adviser. Each adviser must "certify to the truth and completeness of any information provided," according to the FDA statement to *Science*. The agency can issue a waiver to permit participation despite an active conflict or one that ended during the 12 months preceding a meeting if special expertise cannot readily be obtained otherwise. That system helps secure researchers with "deep scientific and medical expertise," Kessler, a pediatrician and lawyer now at the University of California, San Francisco, says.

But the agency's financial review process is primarily an honor system and seems to miss obvious conflicts. For the 17 physicians receiving the most compensation after a drug advisory vote, *Science* examined whether they also received industry compensation concurrent with or shortly before their FDA service. Evidence of such payments came from conflict-of-interest statements in journal articles that those authors published near the time of their

advisory role. Eleven physicians acknowledged support from competing companies on one or more drugs they reviewed. Five of those also received such funding from the makers of one or more of the drugs. Yet FDA publicly noted none of those apparent conflicts and issued no conflict waivers.

Science found that AstraZeneca and makers of rival drugs made payments to, or funded research by, several FDA advisers—including Granger—in the year leading up to the 2009 meetings on Seroquel. Granger calls full financial disclosure "crucially important" in order for FDA to assemble the best committee. "I certainly hope that I disclosed everything," he says. "If I hadn't, I would be horrified because that's antithetical to everything I believe in." After initially offering to share his disclosure forms, Granger did not respond to repeated requests for copies. In response to a Freedom of Information Act (FOIA) request, FDA says it could not locate his documents.

Halperin has a similar history. In addition to receiving funds from AstraZeneca and its competitors after he voted to approve the anticlotting drug Brilinta, Halperin was receiving unspecified payments or research support from rival firms during the 12 months before the meeting. He says he disclosed the payments to FDA and that it did not flag them as conflicts. *Science* requested copies of his disclosure materials, but Halperin did not provide them. Again, FDA says it could not locate Halperin's disclosures.

"The system is dependent on the truthfulness of the self-reporting of disclosures," says Genevieve Kanter, a University of Pennsylvania economist who has studied conflicts of interest in FDA drug evaluations. She calls *Science*'s findings of payments to advisers during the year before a committee meeting "significant." And she added that such payments would be "stunning" if consistently large.

After the Seroquel vote

In 2009, FDA advisers voted to recommend approval of the antipsychotic Seroquel for new indications, despite data linking the drug and similar offerings to sudden cardiac death. Four physicians who voted later received funds for consulting, travel, or research from AstraZeneca, Seroquel's maker, and its competitors.

The journal disclosures don't specify payment amounts, and the Open Payments data cover only a few years, making such a pattern impossible to show. But an FDA advisory committee that in 2016 voted unanimously to recommend approval of adalimumabatto (Amjevita), Amgen's immune-altering drug for rheumatoid arthritis, serves as one striking example. Amjevita, which FDA then greenlighted, is similar to AbbVie's blockbuster adalimumab (Humira), and experts believe Amjevita will be a big seller.

Rheumatologist Daniel Solomon of Harvard Medical School in Boston chaired the Amjevita panel. Neither FDA nor Solomon disclosed publicly that about 3 months before that meeting, Amgen provided $232,000 for his study of etanercept (Enbrel), another arthritis drug made by Amgen, and 1 month before the meeting AbbVie provided $819,000 for a Solomon study of Humira.

That support was for "in-kind donations" of drugs "evaluated as part of a NIH-funded research study for which I am one of the principal investigators," Solomon wrote in an email. He does not regard them as a conflict with Amjevita's approval. Drug donations, a common practice, benefit both parties. Donated drugs help ensure that leading academic specialists will prioritize a company's product in major studies that also enhance the researcher's professional standing and influence. Solomon says he described the payments in an FDA disclosure, but he hadn't kept a copy. The agency rejected a FOIA request for the document, calling its release "a clearly unwarranted invasion of personal privacy."

From such responses, it's not clear whether the agency knew about those potential conflicts and, if so, whether officials decided they didn't warrant a waiver. FDA would not discuss any individual adviser or detail what, if anything, the agency does to validate advisers' disclosures.

Kanter says she favors more research to learn how commonly payments are not disclosed by advisers, or by FDA, to "give us a sense of whether the agency should do some independent verification."

Kessler suggests that greater FDA transparency also could help. "Maybe we need to think about whether the process for reviewing conflicts of interest should be done in a more open, independent manner than the current black box the agency uses," he says. But the former agency head warns that FDA still must find and retain the relatively few specialists "who really can contribute to the issues at hand with exquisite, detailed experience." When so many of them take pharma money, Kessler adds, the agency has to be flexible.

Halperin—a national leader in cardiology research and practice—puts it bluntly: "The key is disclosure, not squeaky cleanness."

Yet some ethicists say such arguments are unconvincing, if not self-serving. The 107 advisers that *Science* reviewed, combined with 11 federal scientists who served on at least one of the 28 review panels and remain with the government, suggest that potential conflicts can be avoided and often are. Among that group, 47 took less than $800 from pharma after their service on the advisory panel. Thirty-four took no money at all. (Regular federal employees can almost never accept outside compensation.) Elliott argues that the prestige and importance of serving on an FDA advisory committee would outweigh the lure of industry financial favors for many more discipline experts if FDA forced them to choose.

The European Medicines Agency in London, the closest analog to FDA, does force such choices. It has no policy on payments to advisers after serving on a drug advisory panel. However, it bars advisers who have concurrent financial ties to drug companies whose products are under consideration, and it prohibits or strictly limits the participation of advisers whose connections to a company go back at least 3 years before an advisory meeting. Disqualifying factors can include speaking fees, consulting contracts, and research grants—both for scientists conducting industry-sponsored studies and for those, like Halperin, who work on data monitoring committees. The agency investigates financial disclosures on its own initiative or after tips from whistleblowers.

Given the apparent gaps *Science* found, Kanter says the FDA system for evaluating possible conflicts of interest—hidden from the public and based primarily or completely on adviser disclosures—might be strengthened to guard against the clearest causes of potential bias. For example, she found that advisory committee members are more likely to vote for a drug's approval if their financial ties were exclusively to that drug's maker rather than to several companies.

Elliott suggests a more radical solution. "Even in the best of circumstances, disclosure is a remarkably weak way of controlling conflicts of interest," he says. "A better way would simply be for the FDA to say, 'We are not taking anybody with any kind of conflict on an advisory committee.'"

Story written by Charles Piller. Data analysis by Charles Piller and Jia You. The methodology and data for this story are available online. Meagan Weiland and Katie Langin contributed reporting. The story was supported by the Science *Fund for Investigative Reporting.*

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ABOUT THE AUTHOR

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Department of Health and Human Services (HHS)

UNITED STATES DISTRICT COURT
SOUTHERN DISTRICT OF NEW YORK

INFORMED CONSENT ACTION NETWORK,

Plaintiff,

-against-

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES

Defendant.

STIPULATION

18-cv-03215 (JMF)

WHEREAS, 42 U.S.C. § 300aa-27, entitled "Mandate for safer childhood vaccines," provides as follows:

> (a) General rule
>
> In the administration of this part and other pertinent laws under the jurisdiction of the Secretary [of the Department of Health and Human Services], the Secretary shall—
>
> > (1) promote the development of childhood vaccines that result in fewer and less serious adverse reactions than those vaccines on the market on December 22, 1987, and promote the refinement of such vaccines, and
> >
> > (2) make or assure improvements in, and otherwise use the authorities of the Secretary with respect to, the licensing, manufacturing, processing, testing, labeling, warning, use instructions, distribution, storage, administration, field surveillance, adverse reaction reporting, and recall of reactogenic lots or batches, of vaccines, and research on vaccines, in order to reduce the risks of adverse reactions to vaccines.
>
> . . .
>
> (c) Report
>
> Within 2 years after December 22, 1987, and periodically thereafter, the Secretary shall prepare and transmit to the Committee on Energy and Commerce of the House of Representatives and the Committee on Labor and Human Resources of the Senate a report describing the

> actions taken pursuant to subsection (a) of this section during the preceding 2-year period.

WHEREAS, on August 25, 2017, Informed Consent Action Network ("ICAN") submitted a Freedom of Information Act request (the "FOIA Request") to the Department of Health and Human Services ("HHS" or the "Department"), which was assigned control number 2017-01119-FOIA-OS, that sought the following records:

> **Any and all reports transmitted to the Committee on Energy and Commerce of the House of Representatives and the Committee on Labor and Human Resources of the Senate by the Secretary of HHS pursuant to 42 U.S.C. §300aa-27(c).**

WHEREAS, on April 12, 2018, ICAN filed a Complaint for Declaratory and Injunctive Relief in the United States District Court, Southern District of New York against HHS seeking records, if any, responsive to the FOIA Request;

WHEREAS, the HHS Immediate Office of the Secretary ("IOS") maintains the official correspondence file of the Secretary of HHS, including reports to Congress by the Secretary of HHS, and therefore those files were most likely to contain records responsive to the FOIA Request;

WHEREAS, on June 27, 2018, HHS sent ICAN the following response to the FOIA Request:

> The [Department]'s searches for records did not locate any records responsive to your request. The Department of Health and Human Services (HHS) Immediate Office of the Secretary (IOS) conducted a thorough search of its document tracking systems. The Department also conducted a comprehensive review of all relevant indexes of HHS Secretarial Correspondence records maintained at Federal Records Centers that remain in the custody of HHS. These searches did not locate records responsive to your request, or indications that records responsive to your request and in the custody of HHS are located at Federal Records Centers.

WHEREAS, ICAN believes the foregoing response from HHS now resolves all claims asserted in this action;

IT IS HEREBY STIPULATED AND AGREED, by and between the parties by and through their respective counsel:

1. That the above-captioned action is voluntarily dismissed, with prejudice, pursuant to Federal Rule of Civil Procedure 41(a)(1)(A)(ii), each side to bear its own costs, attorney fees, and expenses; and

2. That this stipulation may be signed in counterparts, and that electronic (PDF) signatures may be deemed originals for all purposes.

Dated: July 6, 2018
New York, New York

KENNEDY & MODONNA LLP
Attorney for Plaintiff

By: _____
Robert F. Kennedy, Jr.
48 Dewitt Mills Road
Hurley, NY 12443
(845) 481-2622

Dated: July 6, 2018
New York, New York

GEOFFREY S. BERMAN
United States Attorney
Attorney for Defendant

By: _____
ANTHONY J. SUN
Assistant United States Attorney
86 Chambers Street, Third Floor
New York, New York 10007
(212) 637-2810
anthony.sun@usdoj.gov

SO ORDERED:

_____
HON. JESSE M. FURMAN, U.S.D.J.

Dated: New York, New York
July 6, 2018

Any pending motions are moot. All conferences are vacated. The Clerk of Court is directed to close the case.

Informed Consent Action Network

For Immediate Release: July 13, 2018

US District Court Judge signs order granting Plaintiff, Informed Consent Action Network (ICAN) and counsel, Robert F. Kennedy, Jr., the relief sought in a lawsuit against the US Department of Health and Human Services (HHS)

On Monday, June 9th, the United States District Court for the Southern District of New York signed an order granting Plaintiff, the nonprofit Informed Consent Action Network (ICAN), the relief it sought against the Defendant, the United States Department of Health and Human Services, HHS. ICAN was represented by Robert F. Kennedy, Jr.

In May 2017, ICAN Founder, Del Bigtree, Robert F. Kennedy, Jr.. and a handful of other individuals concerned about vaccine safety were selected by the White House to participate in a seminal meeting with the Counselor to the Secretary of HHS, the heads of the National Institute of Health, NIH, the Center for Disease Control, CDC, and Food and the Drug Administration, FDA. Del Bigtree and Robert F. Kennedy, Jr. suspected that HHS was not fulfilling its critical vaccine safety obligations as required by Congress in The National Childhood Vaccine Injury Act of 1986.

The 1986 Act granted unprecedented, economic immunity to pharmaceutical companies for injuries caused by their products and eviscerated economic incentive for them to manufacture safe vaccine products or improve the safety of existing vaccine products. Congress therefore charged the Secretary of HHS with the explicit responsibility to assure vaccine safety.

Hence, since 1986, HHS has had the primary and virtually sole responsibility to make and assure improvements in the licensing, manufacturing, adverse reaction reporting, research, safety and efficacy testing of vaccines in order to reduce the risk of adverse vaccine reactions. In order to assure HHS meets its vaccine safety obligations, Congress required as part of the 1986 Act that the Secretary of HHS submit a biennial reports to Congress detailing the improvements in vaccine safety made by HHS in the preceding two years.

ICAN therefore filed a Freedom of Information Act, FOIA, request on August 25th, 2017 to HHS seeking copies of the biennial reports that HHS was supposed to submit to Congress, starting in 1988, detailing the improvements it made every two years to vaccine safety. HHS stonewalled ICAN for eight months refusing to provide any substantive response to this request.

ICAN
Informed Consent Action Network

ICAN was therefore forced to file a lawsuit to force HHS to either provide copies of its biennial vaccine safety reports to Congress or admit it never filed these reports. The result of the lawsuit is that HHS had to finally and shockingly admit that it never, not even once, submitted a single biennial report to Congress detailing the improvements in vaccine safety. This speaks volumes to the seriousness by which vaccine safety is treated at HHS and heightens the concern that HHS doesn't have a clue as to the actual safety profile of the now 29 doses, and growing, of vaccines given by one year of age.

In contrast, HHS takes the other portions of the 1986 Act, which require promoting vaccine uptake, very seriously, spending billions annually and generating a steady stream of reports on how to improve vaccine uptake. Regrettably, HHS has chosen to focus on its obligation to increase vaccine uptake and defend against any claim vaccines cause harm in the National Injury Vaccine Compensation Program (aka, the Vaccine Court) to such a degree that it has abandoned its vaccine safety responsibilities. If HHS is not, as confirmed in Court this week, even fulfilling the simple task of filing a biennial report on vaccine safety improvements, there is little hope that HHS is actually tackling the much harder job of actually improving vaccine safety.

For additional information or interviews please contact:
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National Institutes of Health (NIH)

Judicial Watch
Because no one is above the law!

JUNE 16, 2020 | JUDICIAL WATCH

U.S. Medical Research Agency Fires Dozens of Scientists with Financial Ties to China

Dozens of scientists at the National Institutes of Health (NIH), the U.S. government's handsomely funded medical research agency, have been fired over their secret financial ties to Communist China. It is not clear how long they went undetected or how much taxpayer-funded research they stole, but so far 54 scientists have been booted for failing to disclose a troubling financial arrangement with a foreign government. In the overwhelming majority of cases—93%—the cash came from China, according to an ongoing **NIH investigation** that started two years ago. Also, in most of the probes the targets were Asian men in their 50s. The bulk of the ousted researchers received generous grants from the NIH, a federal agency with a **$41.7 billion** annual budget.

The probe, launched in the summer of 2018, is being conducted by Dr. Michael S. Lauer, Deputy Director for Extramural Research at the NIH. It mainly focuses on 285 active grants distributed to institutions in 27 states and 59 cities, totaling $164 million. Investigators singled out 399 "scientists of possible concern" and found that 133 (70%) had an "undisclosed grant" from a foreign government and 102 (54%) had an "undisclosed talents award." More than 150 committed other NIH violations. Nine percent of the researchers concealed ties to a foreign company and 4% had an undisclosed foreign patent. Around three quarters of those investigated had active NIH grants and almost half of the scientists had at least two grants funded by American taxpayers. Every year the NIH invests **tens of billions of dollars** in medical research by giving around 50,000 grants to more than 300,000 researchers at more than 2,500 universities, medical schools and other institutions throughout the country. Only 10% of the agency's budget supports projects conducted by scientists in its own lab in Bethesda Maryland.

Less than a year ago, a congressional investigation found that the NIH is among the government agencies that have long permitted Communists working in the U.S. to steal billions in taxpayer-funded scientific research. Others include the National Science Foundation (NSF) and the Department of Energy's (DOE) national laboratories. For years all have been deeply impacted by Chinese infiltrators stealing valuable research, according to a scathing U.S. Senate **report** that describes the probe's findings. Investigators determined that billions of dollars in scientific research funded by American taxpayers has been stolen by China right under our noses and the U.S. government has no viable plan to stop the ongoing theft of the highly valued intellectual property. In the meantime, the publicly funded work is helping the Communist nation meet its goal of becoming a world leader in science and technology. "This report exposes how American taxpayer funded research has contributed to China's global rise over the last 20 years," the document states. "During that time, China openly recruited U.S.-

based researchers, scientists, and experts in the public and private sector to provide China with knowledge and intellectual capital in exchange for monetary gain and other benefits."

While the Chinese Communists run their illicit operation on our own soil, the federal government's grant-making and law enforcement agencies do little to stop it, which makes the NIH's probe long overdue though it only considers a tiny portion of its multi-billion-dollar grants. Besides the FBI practically ignoring the violations, the government research agencies impacted by the costly crimes have failed to develop a coordinated response to mitigate the ongoing threat, Senate investigators found. "These failures continue to undermine the integrity of the American research enterprise and endanger our national security," Senate investigators determined. China uses hundreds of government-funded talent recruitment plans—specifically mentioned in the new NIH probe—to incentivize individuals engaged in research and development in the U.S, transmit information in exchange for salaries, research funding, lab space and other perks. The Communists then use the American research for their own economic and military gain. An example is Chinese talent recruitment members who downloaded sensitive electronic research files before returning to China, submitted false information when applying for grant funds and willfully failed to disclose receiving money from the Chinese government on U.S. grant applications. One Chinese talent recruitment member removed 30,000 electronic files before heading back home. Another filed a patent based on U.S. government-funded research and hired other Chinese recruitment plan members to work on American national security projects. The NIH has not revealed specifics on the recently fired scientists with ties to China nor have the culprits been identified.

Chinese infiltrators have been stealing valuable research from the U.S. government for decades. In fact, more than 20 years ago Judicial Watch helped expose a Chinese Communist scientist (Wen Ho Lee), who stole nuclear secrets from the **Los Alamos National Laboratory** in New Mexico, among the world's largest science institutions and the nation's key nuclear weapons research facility. The Bill Clinton Justice Department refused to prosecute Lee because then Attorney General Janet Reno claimed the accusations against him were racist. Judicial Watch **represented the whistleblower**, Notra Trulock, responsible for launching an investigation into Lee's actions. Trulock was the DOE's intelligence operations chief and Clinton administration officials defamed him by accusing him of being a racist to cover up Lee's repeated and embarrassing security violations.

Did Fauci and Collins Receive Royalty Payments from Drug Companies?

By **WESLEY J. SMITH**
May 11, 2022 10:44 AM

Open the Books is a nonprofit government watchdog organization dedicated to investigating and disclosing the many ways in which government spends — and wastes — our money.

It has a new report out that should raise eyebrows. According to information garnered from Freedom of Information Act Requests, between 2009-2014, both Anthony Fauci and former NIH director Francis Collins received royalty payments from pharmaceutical companies. This may present a conflict of interest since they had a great deal of influence in deciding what research the government funds. From the report:

> Last year, the National Institutes of Health – Anthony Fauci's employer – doled out $30 billion in government grants to roughly 56,000 recipients. That largess of taxpayer money buys a lot of favor and clout within the scientific, research, and healthcare industries.
>
> However, in our breaking investigation, we found hundreds of millions of dollars in payments also flow the other way. These are royalty payments from third-party payers (think pharmaceutical companies) back to the NIH and individual NIH scientists.
>
> We estimate that between fiscal years 2010 and 2020, more than $350 million in royalties were paid by third-parties to the agency and NIH scientists – who are credited as co-inventors.
>
> Because those payments enrich the agency and its scientists, each and every royalty payment could be a potential conflict of interest and needs disclosure.

When bench scientists' research leads to monetized benefit in the private sector, I suppose royalties are in order. And certainly, government funding should reap benefits for the government when that investments leads to the development of profitable products.

But Collins and Fauci, as far as I know, were administrators, not researchers. Yet OTB found that they received royalties from drug companies:

> Since the NIH documents are heavily redacted, we can only see how many payments each scientist received, and, separately, the aggregate dollars per NIH agency. This is a gatekeeping at odds with the spirit and perhaps the letter of open-records laws.
>
> We found agency leadership and top scientists at NIH receiving royalty payments. Well-known scientists receiving payments during the period included:

- **Anthony Fauci**, director of the National Institute of Allergy and Infectious Diseases (NIAID) and the highest-paid federal bureaucrat, received 23 royalty payments. (Fauci's 2021 taxpayer-funded salary: $456,028).
- **Francis Collins**, NIH director from 2009-2021, received 14 payments. (Collins' 2021 taxpayer-funded salary: $203,500)
- **Clifford Lane**, Fauci's deputy at NIAID, received 8 payments. (Lane's 2021 taxpayer-funded salary: $325,287)

In the above examples, although we know the number of payments to each scientist, we still don't know how much money was paid – because the dollar figure was deleted (redacted) from the disclosures.

It's been a struggle to get any useful information out of the agency on its royalty payments. NIH is acting like royalty payments are a state secret. (They're not, or shouldn't be!)

Did Collins and Fauci earn these royalties from work performed before their government service or as bench researchers? Are they partial patent owners? If so, what did they contribute to the product's development? If they were rewarded for acting as administrators and not researchers, is it akin to a kickback?

Unfortunately, the NIH is keeping the matter as opaque as they can:

Consider how NIH is using taxpayer money to try and keep taxpayers ignorant and in the dark:

1. NIH defied the federal Freedom of Information Act law and refused to even acknowledge our open records request for the royalty payments. We filed our FOIA last September.

2. NIH used expensive taxpayer-funded litigation to slow-walk royalty disclosures (releasing the oldest royalties first). Although the agency admits to holding 3,000 pages, it will take ten months to produce them (300 pages per month). With Judicial Watch as our lawyers, we sued NIH in federal court last October.

3. NIH is heavily redacting key information on the royalty payments. For example, the agency erased 1. the payment amount, and, 2. who paid it! This makes the court-mandated production virtually worthless, despite our use of the latest forensic auditing tools

NIH is essentially telling you, the taxpayer, to pay up and shut up. They'll run things.

To say the least, congressional oversight is warranted over these questions. It's time for Fauci and Collins to answer some pointed questions in open hearings.

Vaccine Manufacturers

Merck Has Some Explaining To Do Over Its MMR Vaccine Claims

Merck now faces federal charges of fraud from the whistleblowers, a vaccine competitor and doctors in New Jersey and New York. Merck could also need to defend itself in Congress: The staff of representative Bill Posey (R-Fla) -- a longstanding critic of the CDC interested in an alleged link between vaccines and autism -- is now reviewing some 1,000 documents that the CDC whistleblower turned over to them.

By Lawrence Solomon, Contributor

Columnist

Sep 25, 2014, 05:29 AM EDT | **Updated** Nov 27, 2014

Merck, the pharmaceutical giant, is facing a slew of controversies over its Measles-Mumps-Rubella (MMR) vaccine following numerous allegations of wrongdoing from different parties in the medical field, including two former Merck scientists-turned-whistleblowers. A third whistleblower, this one a scientist at the Centers for Disease Control, also promises to bring Merck grief following his confession of misconduct involving the same MMR vaccine.

The controversies will find Merck defending itself and its vaccine in at least two federal court cases after a U.S. District judge earlier this month threw out Merck's attempts at dismissal. Merck now faces federal charges of fraud from the whistleblowers, a vaccine competitor and doctors in New Jersey and New York. Merck could also need to defend itself in Congress: The staff of representative Bill Posey (R-Fla) --a longstanding critic of the CDC interested in an alleged link between vaccines and autism -- is now reviewing some 1,000 documents that the CDC whistleblower turned over to them.

The first court case, United States v. Merck & Co., stems from claims by two former Merck scientists that Merck "fraudulently misled the government and omitted, concealed, and adulterated material information regarding the efficacy of its mumps vaccine in violation of the FCA [False Claims Act]."

According to the whistleblowers' court documents, Merck's misconduct was far-ranging: It "failed to disclose that its mumps vaccine was not as effective as Merck represented, (ii) used improper testing techniques, (iii) manipulated testing methodology, (iv) abandoned undesirable test results, (v) falsified test data, (vi) failed to adequately investigate and report the diminished efficacy of its mumps vaccine, (vii) falsely verified that each manufacturing lot of mumps vaccine would be as effective as identified in the labeling, (viii) falsely certified the accuracy of applications filed with the FDA, (ix) falsely certified compliance with the terms of the CDC purchase contract, (x) engaged in the fraud and concealment describe herein for the purpose of illegally monopolizing the U.S. market for mumps vaccine, (xi) mislabeled, misbranded, and falsely certified its mumps vaccine, and (xii) engaged in the other acts described herein to conceal the diminished efficacy of the vaccine the government was purchasing."

These fraudulent activities, say the whistleblowers, were designed to produce test results that would meet the FDA's requirement that the mumps vaccine was 95 per cent effective. To the whistleblowers' delight, the judge dismissed Merck's objections to the case proceeding, finding the whistleblowers had plausible grounds on all of the claims lodged against Merck.

If the whistleblowers win, it would represent more than a moral victory (they repeatedly tried to stop Merck while still in its employ). Under the False Claims Act, the whistleblowers would receive a share -- likely 25 per cent to 30 per cent -- of the amount the government recovers. Previous settlements involving extensive fraud by pharmaceutical companies under the False Claims Act have run into the hundreds of millions of dollars, and in some cases such as against GlaxoSmithKline and Pfizer, into the billions.

The second court case, Chatom Primary Care v. Merck & Co. relies on the same whistleblower evidence. This class action suit claims damages because Merck had fraudulently monopolized the mumps market. Doctors and medical practices in the suit would be able to obtain compensation for having been sold an overpriced monopolized product, and a defective one to boot, in that the mumps vaccine wasn't effective (indeed, the suit alleged that Merck expected outbreaks to occur and, as predicted, they did -- mumps epidemics occurred in 2006 in a highly vaccinated population and again in 2009-2010).

"Plaintiffs have argued sufficient facts to sustain a claim for proximate causation, detailing the significant barriers that other companies would face to enter the mumps vaccine market," the court ruled.

The third whistleblower -- a senior CDC scientist named William Thompson -- only indirectly blew the whistle on Merck. He more blew it on himself and colleagues at the CDC who participated in a 2004 study involving the MMR vaccine. Here, the allegations involve a cover-up of data pointing to high rates of autism in African-American boys after they were vaccinated with MMR. In what could be high-profile House hearings before Congressman Posey's Science Committee -- hearings made all the more explosive given the introduction of race into the mix -- Merck could find itself under unprecedented scrutiny. The CDC still stands by its study although Frank DeStefano, the CDC's Director of Immunization Safety and a co-author in the CDC study, also stated that he plans to review his notes with an eye to reanalyzing the data.

Some say all publicity is good. In Merck's case, regardless of the ultimate merits, the publicity will be all bad.

AUTISM | FALSE VACCINE CLAIMS | MERCK | MERCK PHARMACEUTICALS | MMR

Lawrence Solomon, Contributor
Columnist

BUSINESS DAY

Glaxo Agrees to Pay $3 Billion in Fraud Settlement

By KATIE THOMAS and MICHAEL S. SCHMIDT JULY 2, 2012

In the largest settlement involving a pharmaceutical company, the British drugmaker GlaxoSmithKline agreed to plead guilty to criminal charges and pay $3 billion in fines for promoting its best-selling antidepressants for unapproved uses and failing to report safety data about a top diabetes drug, federal prosecutors announced Monday. The agreement also includes civil penalties for improper marketing of a half-dozen other drugs.

The fine against GlaxoSmithKline over Paxil, Wellbutrin, Avandia and the other drugs makes this year a record for money recovered by the federal government under its so-called whistle-blower law, according to a group that tracks such numbers.

In May, Abbott Laboratories settled for $1.6 billion over its marketing of the antiseizure drug Depakote. And an agreement with Johnson & Johnson that could result in a fine of as much as $2 billion is said to be imminent over its off-label promotion of an antipsychotic drug, Risperdal.

No individuals have been charged in any of the cases. Even so, the Justice Department contends the prosecutions are well worth the effort — reaping more than $15 in recoveries for every $1 it spends, by one estimate.

But critics argue that even large fines are not enough to deter drug companies from unlawful behavior. Only when prosecutors single out individual executives for punishment, they say, will practices begin to change.

“What we’re learning is that money doesn’t deter corporate malfeasance,” said Eliot Spitzer, who, as New York’s attorney general, sued GlaxoSmithKline in 2004 over similar accusations involving Paxil. “The only thing that will work in my view is C.E.O.’s and officials being forced to resign and individual culpability being enforced.”

The federal whistle-blower law, officially the False Claims Act, dates to 1863 and was originally envisioned as a check on war profiteering after the Civil War.

Whistle-blowers get a share of any money recovered by the federal government. So far, according to Patrick Burns, spokesman for the whistle-blower advocacy group Taxpayers Against Fraud, at least $10 billion has been agreed to in settlements this fiscal year, which ends in September.

The settlement, which requires court approval, stems from claims made by four employees of GlaxoSmithKline, including a former senior marketing development manager for the company and a regional vice president, who tipped off the government about a range of improper practices from the late 1990s to the mid-2000s.

Prosecutors said the company had tried to win over doctors by paying for trips to Jamaica and Bermuda, as well as spa treatments and hunting excursions. In the case of Paxil, prosecutors claim GlaxoSmithKline employed several tactics aimed at promoting the use of the drug in children, including helping to publish a medical journal article that misreported data from a clinical trial.

A warning was later added to the drug that Paxil, like other antidepressants, might increase the risk of suicidal thoughts in teenagers. Prosecutors said the company had marketed Wellbutrin for conditions like weight loss and sexual dysfunction when it was approved only to treat major depressive disorder.

They said that in the case of Avandia, whose use was severely restricted in 2010 after it was linked to heart risks, the company had failed to report data from studies detailing the safety risks to the F.D.A.

"Today's multibillion-dollar settlement is unprecedented in both size and scope," said James M. Cole, the deputy attorney general. "It underscores the administration's firm commitment to protecting the American people and holding accountable those who commit health care fraud."

The initial terms of the settlement were announced in November, and Glaxo had already set aside cash for the settlement. In a statement Monday, the company said it has since changed many of its policies, including no longer rewarding sales representatives for the number of drug prescriptions sold.

Andrew Witty, the chief executive, sought to portray the illegal actions as part of the company's past.

"Whilst these originate in a different era for the company, they cannot and will not be ignored," he said in the statement. "On behalf of GSK, I want to express our regret and reiterate that we have learned from the mistakes that were made."

The three criminal charges involved Paxil, Wellbutrin and Avandia and included a criminal fine of $1 billion. The remaining $2 billion involves fines in connection with a civil settlement over the sales and marketing practices of the blockbuster asthma drug Advair and several other drugs.

Part of the civil settlement also includes claims that the company overcharged the government for drugs. Glaxo did not admit any wrongdoing in the civil settlement.

Despite the large amount, $3 billion represents only a portion of what Glaxo made on the drugs. Avandia, for example, racked up $10.4 billion in sales, Paxil brought in $11.6 billion, and Wellbutrin sales were $5.9 billion during the years covered by the settlement, according to IMS Health, a data group that consults for drugmakers.

"So a $3 billion settlement for half a dozen drugs over 10 years can be rationalized as the cost of doing business," Mr. Burns said.

Mr. Burns and others have said that to institute real change, executives must be prosecuted criminally or barred from participating in the Medicare and Medicaid programs, an action known as "exclusion."

This has occurred in only a handful of cases, and rarely in a case involving a major pharmaceutical company. In 2011, four executives of the medical device company Synthes were sentenced to less than a year in prison for conducting clinical trials that were not authorized by the Food and Drug Administration.

That same year, the former chief executive of K.V. Pharmaceutical was sentenced to 30 days in jail and fined $1 million for selling misbranded morphine tablets. The previous year, the Department of Health and Human Services excluded him from doing business with the federal government.

Those in the pharmaceutical industry have stressed that the activities revealed in the recent settlements occurred many years ago, and practices have changed radically since then. The Glaxo settlement includes an agreement by the company to withdraw bonuses from top executives if they engaged in or supervised illegal behavior, believed to be a first.

“That creates pressure and it creates an element of responsibility,” said Erika Kelton, who represented two of the four whistle-blowers in the Glaxo case. “I think it’s a good step in the right direction.”

Correction: July 6, 2012

An article on Tuesday about a fine levied on the British drug maker GlaxoSmithKline for illegal marketing of some of its drugs misstated the use of Depakote, an Abbott Laboratories drug involved in a similar case. It is an antiseizure drug, not an antipsychotic.

A version of this article appears in print on July 3, 2012, on Page A1 of the New York edition with the headline: Drug Firm Guilty In Criminal Case.

Medical Research

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PLoS one

How Many Scientists Fabricate and Falsify Research? A Systematic Review and Meta-Analysis of Survey Data

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Abstract

The frequency with which scientists fabricate and falsify data, or commit other forms of scientific misconduct is a matter of controversy. Many surveys have asked scientists directly whether they have committed or know of a colleague who committed research misconduct, but their results appeared difficult to compare and synthesize. This is the first meta-analysis of these surveys. To standardize outcomes, the number of respondents who recalled at least one incident of misconduct was calculated for each question, and the analysis was limited to behaviours that distort scientific knowledge: fabrication, falsification, "cooking" of data, etc… Survey questions on plagiarism and other forms of professional misconduct were excluded. The final sample consisted of 21 surveys that were included in the systematic review, and 18 in the meta-analysis. A pooled weighted average of 1.97% (N = 7, 95%CI: 0.86–4.45) of scientists admitted to have fabricated, falsified or modified data or results at least once –a serious form of misconduct by any standard– and up to 33.7% admitted other questionable research practices. In surveys asking about the behaviour of colleagues, admission rates were 14.12% (N = 12, 95% CI: 9.91–19.72) for falsification, and up to 72% for other questionable research practices. Meta-regression showed that self reports surveys, surveys using the words "falsification" or "fabrication", and mailed surveys yielded lower percentages of misconduct. When these factors were controlled for, misconduct was reported more frequently by medical/pharmacological researchers than others. Considering that these surveys ask sensitive questions and have other limitations, it appears likely that this is a conservative estimate of the true prevalence of scientific misconduct.

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Introduction

The image of scientists as objective seekers of truth is periodically jeopardized by the discovery of a major scientific fraud. Recent scandals like Hwang Woo-Suk's fake stem-cell lines [1] or Jan Hendrik Schön's duplicated graphs [2] showed how easy it can be for a scientist to publish fabricated data in the most prestigious journals, and how this can cause a waste of financial and human resources and might pose a risk to human health. How frequent are scientific frauds? The question is obviously crucial, yet the answer is a matter of great debate [3,4].

A popular view propagated by the media [5] and by many scientists (e.g. [6]) sees fraudsters as just a "few bad apples" [7]. This pristine image of science is based on the theory that the scientific community is guided by norms including disinterestedness and organized scepticism, which are incompatible with misconduct [8,9]. Increasing evidence, however, suggests that known frauds are just the "tip of the iceberg", and that many cases are never discovered. The debate, therefore, has moved on to defining the forms, causes and frequency of scientific misconduct [4].

What constitutes scientific misconduct? Different definitions are adopted by different institutions, but they all agree that fabrication (invention of data or cases), falsification (wilful distortion of data or results) and plagiarism (copying of ideas, data, or words without attribution) are serious forms of scientific misconduct [7,10]. Plagiarism is qualitatively different from the other two because it does not distort scientific knowledge, although it has important consequences for the careers of the people involved, and thus for the whole scientific enterprise [11].

There can be little doubt about the fraudulent nature of fabrication, but falsification is a more problematic category. Scientific results can be distorted in several ways, which can often be very subtle and/or elude researchers' conscious control. Data, for example, can be "cooked" (a process which mathematician Charles Babbage in 1830 defined as "an art of various forms, the object of which is to give to ordinary observations the appearance and character of those of the highest degree of accuracy"[12]); it can be "mined" to find a statistically significant relationship that is then presented as the original target of the study; it can be selectively published only when it supports one's expectations; it can conceal conflicts of interest, etc… [10,11,13,14,15]. Depending on factors specific to each case, these misbehaviours lie somewhere on a continuum between scientific fraud, bias, and simple carelessness, so their direct inclusion in the "falsification" category is debatable, although their negative impact on research can be dramatic [11,14,16]. Henceforth, these misbehaviours will be indicated as "questionable research practices" (QRP, but for a technical definition of the term see [11]).

Ultimately, it is impossible to draw clear boundaries for scientific misconduct, just as it is impossible to give a universal definition of professional malpractice [10]. However, the intention to deceive is a key element. Unwilling errors or honest differences

in designing or interpreting a research are currently not considered scientific misconduct [10].

To measure the frequency of misconduct, different approaches have been employed, and they have produced a corresponding variety of estimates. Based on the number of government confirmed cases in the US, fraud is documented in about 1 every 100.000 scientists [11], or 1 every 10.000 according to a different counting [3]. Paper retractions from the PubMed library due to misconduct, on the other hand, have a frequency of 0.02%, which led to speculation that between 0.02 and 0.2% of papers in the literature are fraudulent [17]. Eight out of 800 papers submitted to *The Journal of Cell Biology* had digital images that had been improperly manipulated, suggesting a 1% frequency [11]. Finally, routine data audits conducted by the US Food and Drug Administration between 1977 and 1990 found deficiencies and flaws in 10–20% of studies, and led to 2% of clinical investigators being judged guilty of serious scientific misconduct [18].

All the above estimates are calculated on the number of frauds that have been discovered and have reached the public domain. This significantly underestimates the real frequency of misconduct, because data fabrication and falsification are rarely reported by whistleblowers (see Results), and are very hard to detect in the data [10]. Even when detected, misconduct is hard to prove, because the accused scientists could claim to have committed an innocent mistake. Distinguishing intentional bias from error is obviously difficult, particularly when the falsification has been subtle, or the original data destroyed. In many cases, therefore, only researchers know if they or their colleagues have wilfully distorted their data.

Over the years, a number of surveys have asked scientists directly about their behaviour. However, these studies have used different methods and asked different questions, so their results have been deemed inconclusive and/or difficult to compare (e.g. [19,20]). A non-systematic review based on survey and non-survey data led to estimate that the frequency of "serious misconduct", including plagiarism, is near 1% [11].

This study provides the first systematic review and meta-analysis of survey data on scientific misconduct. Direct comparison between studies was made possible by calculating, for each survey question, the percentage of respondents that admitted or observed misconduct at least once, and by limiting the analysis to qualitatively similar forms of misconduct -specifically on fabrication, falsification and any behaviour that can distort scientific data. Meta-analysis yielded mean pooled estimates that are higher than most previous estimates. Meta-regression analysis identified key methodological variables that might affect the accuracy of results, and suggests that misconduct is reported more frequently in medical research.

Methods

Searching

Electronic resources were searched during the first two weeks of August 2008. Publication and journal databases were searched in English, while the Internet and resources for unpublished and "grey" literature were searched using English, Italian, French and Spanish words.

Citation databases. The Boolean string "research misconduct" OR "research integrity" OR "research malpractice" OR "scientific fraud" OR "fabrication, falsification" OR "falsification, fabrication" was used to search: Science Citation Index Expanded (SCI-EXPANDED), Social Sciences Citation Index (SSCI), Arts & Humanities Citation Index (A&HCI), Conference Proceedings Citation Index- Science (CPCI-S), BIOSIS Previews, MEDLINE, Business Source Premier, CINAHL Plus, SPORTDiscus, Library, Information Science & Technology Abstracts, International Bibliography of the Social Sciences, America: History & Life, Teacher Reference Center, Applied Social Sciences Index And Abstracts (ASSIA), ERIC, Index Islamicus, CSA linguistics and language behaviour, Physical Education Index, PILOTS, Social Services Abstracts, Sociological Abstracts, Proquest Dissertation & Theses, ECONLIT, Educational Research Abstracts (ERA) Online, Article First, Economic and Social Data Service, Francis, Geobase, Georefs, Global Health (CABI), Index to Theses, International Bibliography of the Social Sciences (IBSS), IEEE Xplore, INSPEC, JSTOR, Mathematical Sciences Net (MathSciNet), PubMEd, Russian Academy of Sciences bibliographies, Sciencedirect, Teacher Reference Center, EMBASE, EMBASE Classics, PSYCHINFO.

Scientific journals. The Boolean string "research misconduct" OR "research integrity" OR "research malpractice" OR "scientific fraud" OR "fabrication, falsification" OR "falsification, fabrication" was used to search: Interdisciplinary Science Reviews, American Journal of Sociology, Annual Review of Sociology, PNAS, Issues in Science & Technology, Journal of Medical Ethics, PLoSONE, Science and Engineering Ethics, Sociology of Health & Illness, Minerva, The Scientific World Journal, Social Science Research, Social Studies of Science, Science in Context.

Grey literature databases. The Boolean string "research misconduct" OR "research integrity" OR "research malpractice" OR "scientific fraud" OR "fabrication, falsification" OR "falsification, fabrication" was used to search: SIGLE, National Technical Information Service, British Library Collections, British Library Direct, Canadian Evaluation Society, Bioethics Literature Database.

The Italian string "etica AND ricerca" was used in: CNR database.

The French string "scientifique AND "ethique" OR "fraude" OR "faute" OR "enquete" OR "sondage" was used in: LARA - Libre acces aux rapports scientifiques et techiques

Internet search engines. The Boolean string "research misconduct" OR "research integrity" OR "research malpractice" OR "scientific fraud" OR "fabrication, falsification" OR "falsification, fabrication", the Spanish Boolean string "ética cientifica" OR "faltas éticas" the French Boolean string "faute scientifique" OR "éthique scientifique" were used to search: ScienceResearch.com, Scirus.

Titles and available abstracts of all records were examined, and the full text of all potentially relevant studies was retrieved. The references list of the retrieved studies and of other documents was also examined in search of potentially relevant papers.

Selection

Only quantitative survey data assessing how many researchers have committed or observed colleagues committing scientific misconduct in the past were included in this review. Surveys asking only opinions or perceptions about the frequency of misconduct were not included.

To allow direct quantitative comparison across data sets, studies were included only if they presented data in frequency or percentage categories, one of which was a "never" or "none" or "nobody" category - indicating that the respondent had never committed or observed the behaviour in question. Studies lacking such a category, or presenting results in statistical formats that prevented the retrieval of this information (e.g. mean and standard deviation) were excluded. Respondents of any professional position and scientific discipline were included, as long as they were actively conducting publishable research, or directly involved in it

(e.g. research administrators). Surveys addressing misconduct in undergraduate students were excluded, because it was unclear if the misconduct affected publishable scientific data or only scholastic results.

This review focused on all and only behaviours that can falsify or bias scientific knowledge through the unjustified alteration of data, results or their interpretation (e.g. any form of fabrication and falsification, intentional non-publication of results, biased methodology, misleading reporting, etc…). Plagiarism and professional misconduct (e.g. withholding information from colleagues, guest authorship, exploitation of subordinates etc…) were excluded from this review. Surveys that made no clear distinction between the former and latter types of misconduct (e.g. that asked about fabrication, falsification and plagiarism in the same question) were excluded.

Any available data on scientists' reaction to alleged cases of misconduct was extracted from included studies. Since these data provided only additional information that was not the focus of the review, survey questions that did not distinguish between data manipulation and plagiarism were included in this section of the results, but clearly identified.

Validity assessment

Surveys that did not sample respondents at random, or that did not provide sufficient information on the sampling methods employed where given a quality score of zero and excluded from the meta-analysis. All remaining papers were included, and were not graded on a quality scale, because the validity and use of quality measures in meta-analysis is controversial [21,22]. Instead of using an arbitrary measure of quality, the actual effect of methodological characteristics on results was tested and then controlled for with regression analysis. In the tables listing study characteristics, the actual words reported in the paper by the authors are quoted directly whenever possible. The few cases where a direct quotation could not be retrieved are clearly indicated.

Data abstraction

For each question, the percentage of respondents who recalled committing or who observed (i.e. had direct knowledge of) a colleague who committed one or more times the specified behaviour was calculated. In the majority of cases, this required summing up the responses in all categories except the "none" or "never" category, and the "don't know" category.

Some studies subdivided the sample of respondents according to a variety of demographic characteristics (e.g. gender, career level, professional position, academic discipline, etc…) and disaggregated the response data accordingly. In all these cases, the data was re-aggregated.

Given the objectivity of the information collected and the fact that all details affecting the quality of studies are reported in this paper, it was not necessary to have the data extracted/verified by more than one person.

Quantitative data synthesis

The main outcome of the meta-analysis was the percentage (proportion) of respondents that recalled committing or that knew of a colleague committing the specified behaviour at least once in the given recall period. This measure was not normally distributed (Kolmogorov-Smirnov test: 0.240, df = 19, P = 0.005) so it was *logit* transformed [23], and weighted by inverse variance of logit transformed proportion using the following equations for effect size, standard error and weight, respectively:

$$\mathrm{ES} = \mathrm{Log_e}\left[\frac{p}{(1-p)}\right]$$

$$\mathrm{SE} = \sqrt{\frac{1}{np} + \frac{1}{n(1-p)}}$$

$$\mathrm{W} = \frac{1}{SE^2} = np(1-p)$$

Where p is the proportion of respondents recalling at least one case of the specified behaviour, and n is the total number of respondents. The distribution of the logit-transformed effect sizes was not significantly different from normal (K-S: 0.109, df = 19, P = 0.2). To facilitate their interpretation, the final logit results (ES and 95%CI) were back-transformed in percentages using the following equations for proportion and percentages, respectively:

$$p = \frac{e^x}{e^x + 1}$$

$$\% = 100p$$

Where x is either ES or each of the corresponding 95%CI values.

Mean pooled effect size was calculated assuming a random effects model, and homogeneity was tested with Chochran's Q test. Differences between groups of studies were tested using inverse variance weighted one-way ANOVA. The combined effect of independent variables on effect sizes was tested with inverse variance weighted regression assuming a random effects model and estimated via iterative maximum likelihood.

To avoid the biasing effect of multiple outcomes within the same study, all meta-analyses on the main outcome of interest (i.e. the prevalence of data fabrication, falsification and alteration) were conducted using only one outcome per study. For the same reason, in the regression analysis, which combined all available effect sizes on data fabrication, falsification and alteration, studies that had data both on self- and on non self- where used only for the former.

The regression model first tested the combined effect of three methodological factors measured by binary variables (self- vs non-self- reports, handed vs mailed questionnaire, questions using the word "falsification" or "fabrication" vs questions using "alteration", "modification" etc…). Then, the effect of several study characteristics was tested (year when the survey was conducted, surveys conducted in the USA vs anywhere else, surveys conducted exclusively on researchers vs any other, biomedical vs other types of research, social sciences vs natural sciences, medical consultants and practitioners vs other). To avoid over-fitting, each study characteristic was tested independently of the others.

Questions on behaviours of secondary interest (questionable research practices) where too diverse to allow meaningful meta-analysis, so they were combined in broad categories for which only crude unweighted parameters were calculated. All statistical analyses were run on SPSS software package. Meta-analyses were conducted using the "MeanES", "MetaF" and "MetaReg" macros by David B. Wilson [24].

Publication bias-Sensitivity analysis

The popular funnel-plot-based methods to test for publication bias in meta-analysis are inappropriate and potentially misleading

when the number of included studies is small and heterogeneity is large [25,26]. However, the robustness of results was assessed with a sensitivity analysis. Pooled weighted estimates for effect size and regression parameters were calculated leaving out one study at a time, and then compared to identify influential studies. In addition, to further assess the robustness of conclusions, meta-analyses and meta-regression were run without logit transformation.

Results

Flow of included studies

Electronic search produced an initial list of 3276 references. Examination of titles and abstracts, and further examination of the references lists in the retrieved papers and in other sources led to a preliminary list of 69 potentially relevant studies. Of these, 61 were published in peer-reviewed journals, three were dissertations theses, three were published in non-peer reviewed popular science magazines, one was published in a book chapter, and one was published in a report. All studies were published in English except for one in Spanish.

After examination of full text, 33 studies were excluded because they did not have any relevant or original data, two because they presented data exclusively in a format that could not be used in this review (e.g. means and standard deviations), eight because their sample included non-researchers (e.g. students) and/or because they addressed forms of academic misconduct not directly related to research (e.g. cheating on school projects), five because they do not distinguish fabrication and falsification from types of misconduct not relevant to the scopes of this review (Table S1). Therefore, 21 studies were included in the review. Three of these did not match the quality requirements to be included in the meta-analysis. Data from these three studies was only used to estimate crude unweighted means for QRP and more generic questions, and not for analyzing the main outcome of interest (data fabrication, falsification, modification). Therefore, the meta-analysis was conducted on 18 studies (Figure 1).

Study characteristics

Table 1 lists the characteristics of included studies and their quality score for inclusion in meta-analysis. Included surveys were published between 1987 and 2008, but had been conducted between 1986 ca and 2005. Respondents were based in the United States in 15 studies (71% ca of total), in the United Kingdom in 3 studies (14% ca), two studies had a multi-national sample (10% ca) and one study was based in Australia. Six studies had been conducted among biomedical researchers, eight were more specifically targeted at researchers holding various positions in the medical/clinical sciences (including pharmacology, nursing, health education, clinical biostatistics, and addiction-studies), six surveys had multi-disciplinary samples, one surveyed economists.

Quantitative data analysis

Scientists admitting misconduct. When explicitly asked if they ever fabricated or falsified research data, or if they altered or modified results to improve the outcome (see Table S2, questions 1, 4, 6, 8, 10, 17, 26), between 0.3% and 4.9% of scientists replied affirmatively (N = 7, crude unweighted mean: 2.59%, 95%CI = 1.06–4.13). Meta-analysis yielded a pooled weighted

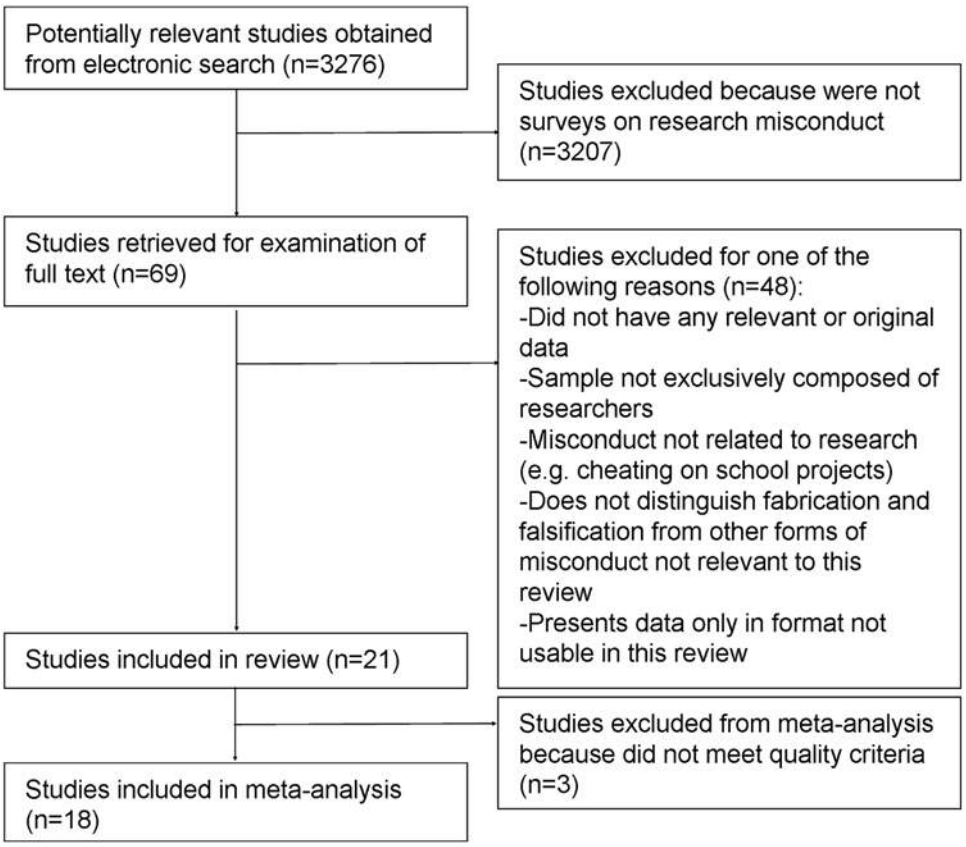

Figure 1. Study selection flow diagram.
doi:10.1371/journal.pone.0005738.g001

Table 1. Characteristics of studies included in the review.

ID	Date Country	Sample	Method	N (%)	Self-/Non self-	Quality
Tangney, 1987 [32]	n.s US	Researchers in a "highly ranked American university".	Distributed within department	245 (22)	n	1
Lock, 1988 [29]	1988 UK	Professors of medicine or surgery, other academics, doctors, research managers, editors of medical journals non-randomly contacted by the author	Mailed+pre-paid return	79 (98.7)	n	0
Simmons, 1991 [54]	1989 US	Active members of the Society of University Surgeons	n.s.	202 (82)	n	0
Kalichman, 1992 [35]	1990 US	Research trainees in the clinical and basic biomedical sciences at the University of California, San Diego	Distributed through the department	549 (27)	s+n	1
Swazey, 1993 [53]	1990 US	Doctoral students and faculty, from 99 of the largest graduate departments in chemistry, civil engineering, microbiology and sociology	Mailed+prepaid return+postcard to confirm response	2620 (65.5)	n	1
Glick, 1993 [55]	1992 US	Biotechnology companies' executives known by the author	Administered orally, on the phone	15* (n.s)	n	0
Greenberg, 1994 [20]	1991 US	Members of the Society for Risk Analysis, Association of Environmental and Resource Economists, American Industrial Hygiene Association	Mailed	478 (32)	n	1
Glick, 1994 [30]	1993 US	Attendees at the Third Conference on Research Policies and Quality Assurance	Handed out, personally returned by respondents on the same	36 (34)	n	1
Eastwood, 1996 [56]	1993 US	All postdoctoral fellows registered with the Office of Research Affairs of the University of California, San Francisco	Mailed+follow-up letter	324 (32.8)	s+n	1
Bebeau, 1996 [33]	1995 US	Program chairs and officers of the American Association for Dental Research	Mailed+prepaid return+postcard to confirm response	76 (78)	n	1
Rankin, 1997 [57]	1995 US	Research coordinators or directors of master's and doctoral nursing programs	Mailed	88 (43)	n	1
May, 1998 [34]	1997 UK	Randomly selected authors of papers published in the past 3 years on addiction-related subjects	Mailed	36 (51)	n	1
Ranstam, 2000 [46]	1998 Various	Members of the International Society of Clinical Biostatistics	Mailed+online electronic version	163 (37)	n	1
List, 2001 [28]	1998 US	Participants to the January 1998 meetings of the American Economic Association	Hand-delivered, Direct Response+Random Response method, drop box for returning responses	94 (23.5)	s	1
Geggie, 2001 [58]	2000 UK	Medical consultants appointed between Jan 1995 and Jan 2000 working in 7 hospital trusts in the Mersey region	Mailed+pre-paid return	194 (63.6)	s+n	1
Meyer, 2004 [59]	n.s US	Members of editorial boards of American Accounting Association journals, and participants at the 1998, 1999, and 2000 American Accounting Association New Faculty Consortia	Email asking to reply if unwilling to participate, mailed+pre-paid return	176 (48.5)	n	1
Martinson, 2005 [19]	2002 US	Researchers funded by the National Institutes of Health	Mailed, pre-paid return, 2$	3247 (47.2)	s	1
Henry, 2005 [60]	2002 Australia	Medical specialists, from the 2002 edition of the Medical directory of Australia, involved in pharmaceutical industry-sponsored research	Mailed	338* (n.a.)	s	1
Gardner, 2005 [27]	2002 Various	Authors of pharmaceutical clinical trials published in the Cochrane Database of Systematic Reviews, equally selected between first, middle and last author.	Mailed+10$ check+second survey to non-respondents+follow-up call or email	322 (64)	s+n	1
Kattenbraker 2007 [61]	2005 US	Health education professors at every rank, teaching at 94 institution of higher education	Email+web-based survey+follow up email+final reminder	153 (25.8)	n	1
Titus, 2008 [31]	2005 US	Researchers funded by the National Institutes of Health, one per department	Pre-notification+mailed+reminder postcard+additional survey packet+follow-up letter	2212 (52)	n	1

Abbreviations: "Date" is the year when the survey was actually conducted, "N" is the number of respondents who returned the questionnaire, "%" is the response rate of the survey.
*Number of respondents who ad engaged in industry-sponsored research in the previous 12 months, out of a total sample of 2253, with 39% response rate.
doi:10.1371/journal.pone.0005738.t001

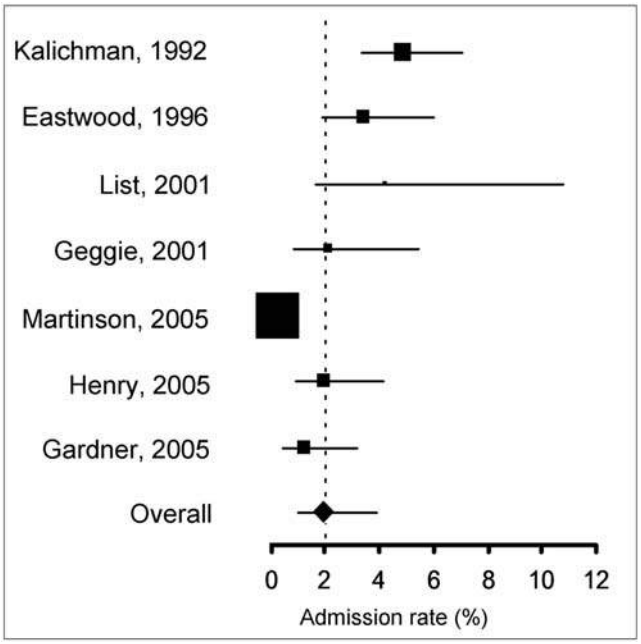

Figure 2. Forrest plot of admission rates of data fabrication, falsification and alteration in self reports. Area of squares represents sample size, horizontal lines are 95% confidence interval, diamond and vertical dotted line show the pooled weighted estimate.
doi:10.1371/journal.pone.0005738.g002

estimate of 1.97% (95%CI: 0.86–4.45), with significant heterogeneity (Cochran's $Q = 61.7777$, $df = 6$, $P < 0.0001$) (Figure 2). If only questions explicitly using the words "fabrication" or "falsification" were included (Table S2, questions 3, 6, 10, 26), the pooled weighted estimate was 1.06% (N = 4, 95%CI: 0.31–3.51)

Other questionable practices were admitted by up to 33.7% of respondents (Table S2) (Figure 3, N = 20 (six studies), crude unweighted mean: 9.54%, 95%CI = 5.15–13.94).

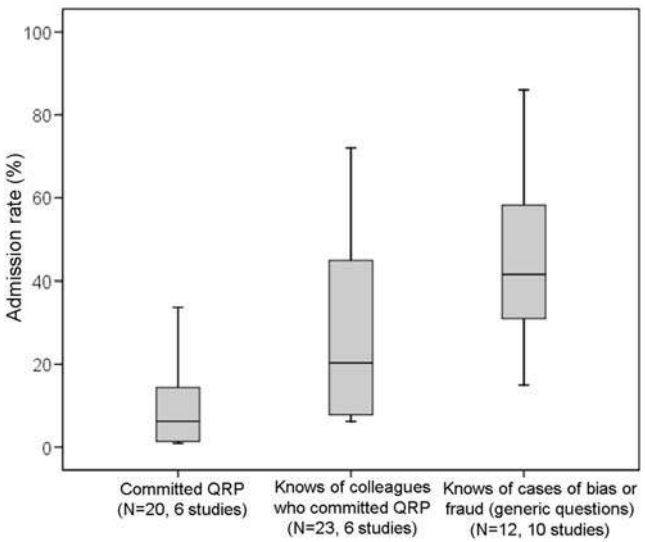

Figure 3. Admission rates of Questionable Research Practices (QRP) in self- and non-self-reports. N indicates the number of survey questions. Boxplots show median and interquartiles.
doi:10.1371/journal.pone.0005738.g003

Consistently across studies, scientists admitted more frequently to have "modified research results" to improve the outcome than to have reported results they "knew to be untrue" (Inverse Variance Weighted Oneway ANOVA $Q(1,4) = 14.8627$, $P = 0.011$)

In discussing limitations of results, two studies [19,27] suggested that their results were very conservative with respect to the actual occurrence of misconduct, while the other studies made no clear statement. Non-response bias was recognized as a limitation by most surveys. One study employed a Random-Response technique on part of its sample to control for non-response bias, and found no evidence for it [28] (see Discussion for further details).

Scientists observing misconduct. When asked if they had personal knowledge of a colleague who fabricated or falsified research data, or who altered or modified research data (Table S3, questions, 1, 6, 7, 10, 20, 21, 29, 32, 34, 37, 45, 54) between 5.2% and 33.3% of respondents replied affirmatively (N = 12, crude unweighted mean: 16.66%, 95%CI = 9.91–23.40). Meta-analysis yielded a pooled weighted estimate of 14.12% (95% CI: 9.91–19.72) (Figure 4). If only questions explicitly using the words "fabrication" or "falsification" were included (Table S3, questions 1, 6, 7, 10, 17, 21, 29, 32, 37, 45, 54), the pooled weighted estimate was 12.34% (N = 11, 95%CI: 8.43–17.71)

Between 6.2% and 72% of respondents had knowledge of various questionable research practices (Table S3) (Figure 3,

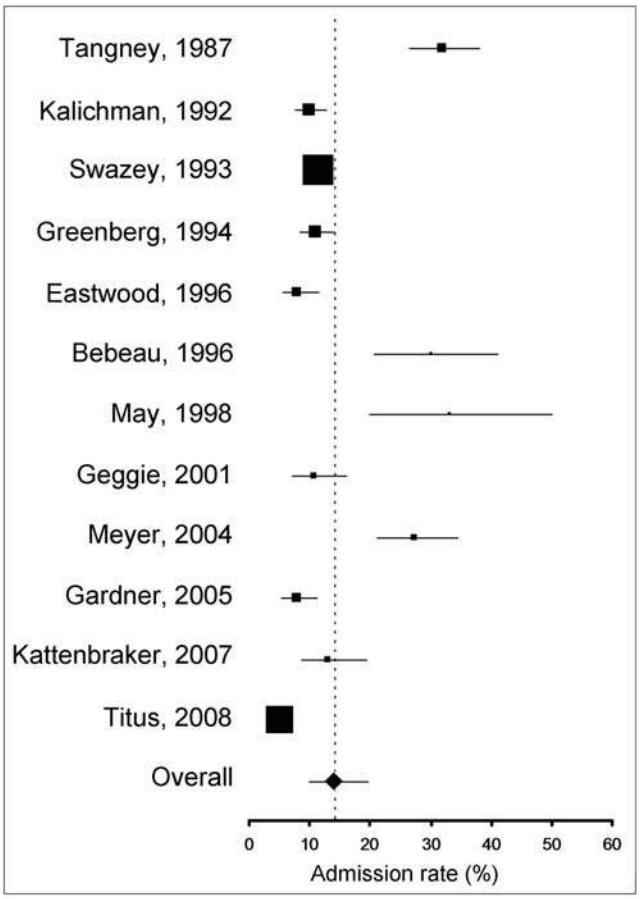

Figure 4. Forrest plot of admission rates of data fabrication, falsification and alteration in non-self reports. Area of squares represents sample size, horizontal lines are 95% confidence interval, diamond and vertical dotted line show the pooled weighted estimate.
doi:10.1371/journal.pone.0005738.g004

Table 2. Actions taken against misconduct.

ID	N cases	Action taken	%
Tangney, 1987 [32]	78	Took some action to verify their suspicions of fraud or to remedy the situation	46
Rankin, 1997 [57]	31 [ffp]	In alleged cases of scientific misconduct a disciplinary action was taken by the dean	32.4
		Some authority was involved in a disciplinary action	20.5
Ranstam, 2000 [46]	49	I interfered to prevent it from happening	28.6
		I reported it to a relevant person or organization	22.4
Kattenbraker, 2007 [61]	33	Confronted individual	55.5
		Reported to supervisor	36.4
		Reported to Institutional Review Board	12.1
		Discussed with colleagues	36.4
Titus, 2008 [31]	115 [ffp]	The suspected misconduct was reported by the survey respondent	24.4
		The suspected misconduct was reported by someone else	33.3

Abbreviations: "N cases" is the total number of cases of misconduct observed by respondents, [ffp] indicates that the number includes cases of plagiarism, "%" is the percentage of cases that had the specified action taken against them. All responses are mutually exclusive except in Kattenbraker 2007.
doi:10.1371/journal.pone.0005738.t002

N = 23 (6 studies), crude unweighted mean: 28.53%, 95%CI = 18.85–38.2). When surveys asked about more generic questions (e.g. "do you have knowledge of any cases of fraud?" [29,30]) or defined misconduct in more comprehensive ways (e.g. "experimental deficiencies, reporting deficiencies, misrepresentation of data, falsification of data" [30]) between 12% and 92% replied affirmatively (Table S3) (N = 10 (seven studies), crude unweighted mean: 46.24, 95%CI = 16.53–75.95).

In discussing their results, three studies [27,29,31] considered them to be conservative, four [30,32,33,34] suggested that they overestimated the actual occurrence of misconduct, and the remaining 13 made no clear statement.

Scientists reporting misconduct. Five of the included studies asked respondents what they had done to correct or prevent the act of misconduct they had witnessed. Around half of the alleged cases of misconduct had any action taken against them (Table 2). No study asked if these actions had the expected outcome. One survey [27] found that 29% of the cases of misconduct known by respondents were never discovered.

Factors influencing responses. Methodological differences between studies explained a large portion of the variance among effect sizes (N = 15, one outcome per study, Table 3). Lower percentages of misconduct were reported in self reports, in surveys using the words "falsification" or "fabrication", and in mailed surveys. Mailed surveys had also higher response rates than handed-out surveys (Mean: 26.63%±2.67SE and 48.53%±4.02SE respectively, t-test: t = −2.812, df = 16, P = 0.013), while no difference in response rates was observed between self- and non-self-reports (Mean: 42.44±6.24SE and 44.44±5.1SE respectively, t = −0.246, P = 0.809) and between surveys using or not "fabrication or falsification" (Mean: 42.98%±6.0SE and 44.51±4.76SE respectively, t = −0.19, P = 0.85). Excluding all surveys that were not mailed, were not self-reports and that did not use the words "falsification" or "fabrication" yielded a maximally conservative pooled weighted estimate of 0.64% (N = 3, 95%CI: 0.25–1.63).

When the three methodological factors above where controlled for, a significant effect was found for surveys targeted at medical and clinical researchers, who reported higher percentages of misconduct than respondents in biomedical research and other fields (Table 3). The effect of this parameter would remain significant if Bonferroni-corrected for multiple comparisons. If self-

Table 3. Inverse variance-weighted regression on admission rates.

	Variable	B±SE	P	Stand. Coeff.	Model R^2
Base Model	Constant	−4.53±0.81	<0.0001	0	0.82
	Self-/Non-self	−3.02±0.38	<0.0001	−1.04	
	Mailed/Handed	−1.17±0.4	0.0032	−0.33	
	"Fabricated, Falsified"/"Modified"	−1.02±0.39	0.0086	−0.34	
Candidate co-variables	Year	−0.03±0.03	0.3	−0.14	0.83
	USA/other	−0.71±0.4	0.08	−0.2	0.85
	Researcher/other	−0.33±0.33	0.32	−0.11	0.83
	Biomedical/other	0.17±0.39	0.66	0.06	0.82
	Medical/other	0.85±0.28	0.0022	0.29	0.89
	Social Sc./other	−0.03±0.37	0.94	−0.01	0.82

The table shows model parameters of an initial model including three methodological factors (top four rows) and the parameter values for each sample characteristic, entered one at a time in the basic model. All variables are binary. Regression slopes measure the change in admission rates when respondents fall in the first category.
doi:10.1371/journal.pone.0005738.t003

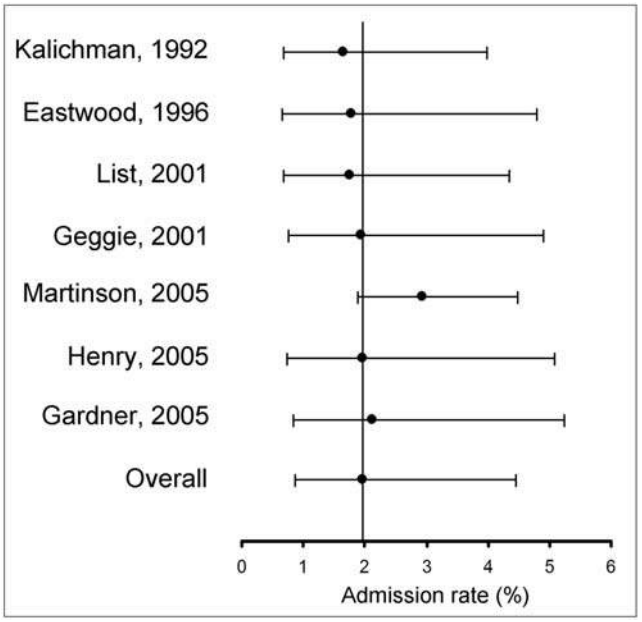

Figure 5. Sensitivity analysis of admission rates of data fabrication, falsification and alteration in self reports. Plots show the weighted pooled estimate and 95% confidence interval obtained when the corresponding study was left out of the analysis.
doi:10.1371/journal.pone.0005738.g005

and non-self- reports were tested separately for the effect of study characteristics (one characteristic at a time), a significant effect was found only in self-reports for year when survey was conducted ($k = 7$, $b = -0.1425 \pm 0.0519$, $P = 0.006$) and a nearly significant effect was found again in self-reports for survey delivery method ($k = 7$, $b = -1.2496 \pm 0.6382$, $P = 0.0502$)

Sensitivity analysis

Self-report admission rates varied between 1.65% -following the removal of Kalichman and Friedman (1992) [35]- and 2.93% -following the removal of Martinson et al. (2005) [19] (Figure 5). Reports on colleagues' misconduct varied between 12.85% (when Tangney (1987) [32] was removed) and 15.41% (when Titus et al. (2008) [31] was removed (Figure 6). Weighted pooled estimates on non-logit-trasformed data yielded self- and non-self- admission rates of 2.33% (95%CI 0.94–3.73%) and 14.48% (95%CI: 11.14–17.81%) respectively, showing that the results are robust and conservative.

Results of the regression analysis were robust to the leave-one-study-out test: the four significant variables remained statistically significant when anyone of the studies was excluded (Table S4). The largest portion of variance was explained when Titus et al. (2008) [31] was removed ($R^2 = 0.9202$). Meta-regression on non-transformed data showed similar trends to that on transformed data for all four parameters, but only two parameters remained statistically significant (self-/non-self- and delivery method, $P < 0.0001$ and $p = 0.0083$ respectively), and the overall portion of variance explained by the model was lower ($R^2 = 0.6904$).

Discussion

This is the first meta-analysis of surveys asking scientists about their experiences of misconduct. It found that, on average, about 2% of scientists admitted to have fabricated, falsified or modified data or results at least once –a serious form of misconduct my any standard [10,36,37]– and up to one third admitted a variety of other questionable research practices including "dropping data points based on a gut feeling", and "changing the design, methodology or results of a study in response to pressures from a funding source". In surveys asking about the behaviour of colleagues, fabrication, falsification and modification had been observed, on average, by over 14% of respondents, and other questionable practices by up to 72%. Over the years, the rate of admissions declined significantly in self-reports, but not in non-self-reports.

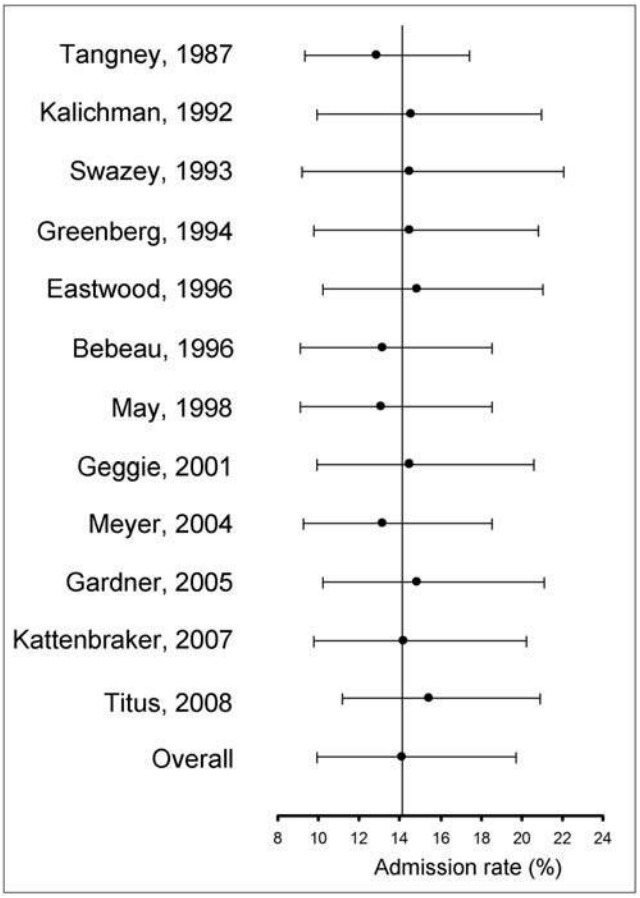

Figure 6. Sensitivity analysis of admission rates of data fabrication, falsification and alteration in non-self reports. Plots show the weighted pooled estimate obtained when the corresponding study was left out of the analysis.
doi:10.1371/journal.pone.0005738.g006

A large portion of the between-studies variance in effect size was explained by three basic methodological factors: whether the survey asked about self or not, whether it was mailed or handed out to respondents, and whether it explicitly used the words "fabrication" and "falsification". Once these factors were controlled for, surveys conducted among clinical, medical and pharmacological researchers appeared to yield higher rates of misconduct than surveys in other fields or in mixed samples.

All the above results were robust with respect to inclusion or exclusion of any particular study, with perhaps one exception: Martinson et al. (2005) [19], which is one of the largest and most frequently cited surveys on misconduct published to date. This study appears to be rather conservative, because without it the pooled average frequency with which scientists admit they have committed misconduct would jump to nearly 3%.

How reliable are these numbers? And what can they tell us on the actual frequency of research misconduct? Below it will be argued that, while surveys asking about colleagues are hard to interpret conclusively, self-reports systematically underestimate the real frequency of scientific misconduct. Therefore, it can be safely concluded that data fabrication and falsification –let alone other questionable research practices- are more prevalent than most previous estimates have suggested.

The procedure adopted to standardize data in the review clearly has limitations that affect the interpretation of results. In particular, the percentage of respondents that recall at least one incident of misconduct is a very rough measure of the frequency of misconduct, because some of the respondents might have committed several frauds, but others might have "sinned" only once. In this latter case, the frequencies reported in surveys would tend to overestimate the prevalence of biased or falsified data in the literature. The history of science, however, shows that those responsible of misconduct have usually committed it more than once [38,39], so the latter case might not be as likely as the former. In any case, many of the included studies asked to recall at least one incident, so this limitation is intrinsic to large part of the raw data.

The distinction made in this review between "fabrication, falsification and alteration" of results and QRP is somewhat arbitrary. Not all alterations of data are acts of falsification, while "dropping data points based on a gut feeling" or "failing to publish data that contradicts one's previous research" (e.g. [19]) might often be. As explained in the introduction, any boundary defining misconduct will be arbitrary, but intention to deceive is the key aspect. Scientists who answered "yes" to questions asking if they ever fabricated or falsified data are clearly admitting their intention to misrepresent results. Questions about altering and modifying data "to improve the outcome" might be more ambiguously interpreted, which might explain why these questions yield higher admission rates. However, even if we limited the meta-analysis to the most restrictive types of questions in self-reports, we would still have an average admission rate above 1%, which is higher than previous estimates (e.g. [11]).

The accuracy of self-reports on scientific misconduct might be biased by the effect of social expectations. In self-reports on criminal behaviour, social expectations make many respondents less likely to admit a crime they committed (typically, females and older people) and make others likely to report a crime they have not really committed (typically, young males) [40]. In the case of scientists, however, social expectations should always lead to underreporting, because a reputation of honesty and objectivity is fundamental in any stage of a scientific career. Anyone who has ever falsified research is probably unwilling to reveal it and/or to respond to the survey despite all guarantees of anonymity [41]. The opposite (scientists admitting misconduct they didn't do) appears very unlikely. Indeed, there seems to be a large discrepancy between what researchers are willing to do and what they admit in a survey. In a sample of postdoctoral fellows at the University of California San Francisco, USA, only 3.4% said they had modified data in the past, but 17% said they were "willing to select or omit data to improve their results" [42]. Among research trainees in biomedical sciences at the University of California San Diego, 4.9% said they had modified research results in the past, but 81% were "willing to select, omit or fabricate data to win a grant or publish a paper" [35].

Mailed surveys yielded lower frequencies of misconduct than handed out surveys. Which of the two is more accurate? Mailed surveys were often combined with follow-up letters and other means of encouraging responses, which ensured higher response rates. However, the accuracy of responses to sensitive questions is often independent of response rates, and depends strongly on respondents' perception of anonymity and confidentiality [43,44]. Questionnaires that are handed to, and returned directly by respondents might better entrust anonymity than surveys that need to be mailed or emailed. Therefore, we cannot rule out the possibility that handed out surveys are more accurate despite the lower response rates. This latter interpretation would be supported by one of the included studies: a handed out survey that attempted to measure non-response bias using a Random-Response (RR) technique on part of its sample [28]. Differently from the usual Direct Response technique, in RR, respondents toss coins to determine whether they will respond to the question or just mark "yes". This still allows admission rates to be calculated, yet it guarantees full anonymity to respondents because no one can tell whether an individual respondent answered "yes" to the question or because of chance. Contrary to author's expectations, response and admission rates were not higher with RR compared to DR, suggesting that in this handed out survey non-response bias was absent.

The effect of social expectations in surveys asking about colleagues is less clear, and could depend on the particular interests of respondents. In general, scientists might tend to protect the reputation of their field, by minimizing their knowledge of misconduct [27]. On the other hand, certain categories of respondents (e.g. participants at a Conference on Research Policies and Quality Assurance [30]) might have particular experience with misconduct and might be very motivated to report it.

Surveys on colleagues' behaviour might tend to inflate estimates of misconduct also because the same incident might be reported by many respondents. One study controlled for this factor by asking only one researcher per department to recall cases that he had observed in that department in the past three years [31]. It found that falsification and fabrication had been observed by 5.2% of respondents, which is lower than all previous non-self reports. However, since one individual will not be aware of all cases occurring around him/her, this is a conservative estimate [31]. In the sensitivity analysis run on the regression model, exclusion of this study caused the single largest increase in explained variance, which further suggests that findings of this study are unusual.

Another critical factor in interpreting survey results is the respondents' perception of what does and does not constitute research misconduct. As mentioned before, scientists were less likely to reply affirmatively to questions using the words "fabrication" and "falsification" rather than "alteration" or "modification". Moreover, three surveys found that scientists admitted more frequently to have "modified" or "altered" research to "improve the outcome" than to have reported results they "knew to be untrue". In other words, many did not think that the data they "improved" were falsified. To some extent, they were arguably right. But the fuzzy boundary between removing noise from results and biasing them towards a desired outcome might be unknowingly crossed by many researchers [10,14,45]. In a sample of biostatisticians, who are particularly well trained to see this boundary, more than half said they had personally witnessed false or deceptive research in the preceding 10 years [46].

The grey area between licit, questionable, and fraudulent practices is fertile ground for the "Mohammed Ali effect", in which people perceive themselves as more honest than their peers. This effect was empirically proven in academic economists [28] and in a large sample of biomedical researchers (in a survey assessing their adherence to Mertonian norms [47]), and may help to explain the lower frequency with which misconduct is admitted in self-reports: researchers might be overindulgent with their behaviour and overzealous in judging their colleagues. In support of this, one study

found that 24% of cases observed by respondents did not meet the US federal definition of research misconduct [31].

The decrease in admission rates observed over the years in self-reports but not in non-self-reports could be explained by a combination of the Mohammed Ali effect and social expectations. The level and quality of research and training in scientific integrity has expanded in the last decades, raising awareness among scientists and the public [11]. However, there is little evidence that researchers trained in recognizing and dealing with scientific misconduct have a lower propensity to commit it [47,48,49]. Therefore, these trends might suggest that scientists are no less likely to commit misconduct or to report what they see their colleagues doing, but have become less likely to admit it for themselves.

Once methodological differences were controlled for, cross-study comparisons indicated that samples drawn exclusively from medical (including clinical and pharmacological) research reported misconduct more frequently than respondents in other fields or in mixed samples. To the author's knowledge, this is the first cross-disciplinary evidence of this kind, and it suggests that misconduct in clinical, pharmacological and medical research is more widespread than in other fields. This would support growing fears that the large financial interests that often drive medical research are severely biasing it [50,51,52]. However, as all survey-based data, this finding is open to the alternative interpretation that respondents in the medical profession are simply more aware of the problem and more willing to report it. This could indeed be the case, because medical research is a preferred target of research and training programs in scientific integrity, and because the severe social and legal consequences of misconduct in medical research might motivate respondents to report it. However, the effect of this parameter was not robust to one of the sensitivity analyses, so it would need to be confirmed by independent studies before being conclusively accepted.

The lack of statistical significance for the effect of country, professional position and other sample characteristics is not strong evidence against their relevance, because the high between-study variance caused by methodological factors limited the power of the analysis (the regression had to control for three methodological factors before testing any other effect). However, it suggests that such differences need to be explored at the study level, with large surveys designed specifically to compare groups. A few of the included studies had done so and found, for example, that admission rates tend to be higher in males compared to females [42] and in mid-career compared to early career scientists [19], and that they tend to differ between disciplines [41,53]. If more studies attempted to replicate these results, possibly using standardized methodologies, then a meta-analysis could reveal important correlates of scientific misconduct.

In conclusion, several surveys asking scientists about misconduct have been conducted to date, and the differences in their results are largely due to differences in methods. Only by controlling for these latter can the effects of country, discipline, and other demographic characteristics be studied in detail. Therefore, there appears to be little scope for conducting more small descriptive surveys, unless they adopted standard methodologies. On the other hand, there is ample scope for surveys aimed at identifying sociological factors associated with scientific misconduct. Overall, admission rates are consistent with the highest estimates of misconduct obtained using other sources of data, in particular FDA data audits [11,18]. However, it is likely that, if on average 2% of scientists admit to have falsified research at least once and up to 34% admit other questionable research practices, the actual frequencies of misconduct could be higher than this.

Supporting Information

Table S1 Studies excluded from the review.
Found at: doi:10.1371/journal.pone.0005738.s001 (0.14 MB DOC)

Table S2 Self-report questions included in review, and responses.
Found at: doi:10.1371/journal.pone.0005738.s002 (0.07 MB DOC)

Table S3 Non-self report questions included in the review, and responses.
Found at: doi:10.1371/journal.pone.0005738.s003 (0.11 MB DOC)

Table S4 Sensitivity analysis for meta-regression model.
Found at: doi:10.1371/journal.pone.0005738.s004 (0.07 MB DOC)

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Author Contributions

Conceived and designed the experiments: DF. Performed the experiments: DF. Analyzed the data: DF. Wrote the paper: DF.

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Editorial

Mistaken identity: seasonal influenza versus influenza-like illness

Readers of *Clinical Evidence* who are interested in influenza will have been struck by the disparity between policy recommendations and the clinical evidence of the performance of inactivated influenza vaccines.[1][2] For example, there are few RCTs assessing the effectiveness of inactivated vaccines in children and the elderly. Only five RCTs have been carried out in elderly people, of which only one was carried out in the past 2 decades using vaccines available today.[3] Although the evidence is more robust in healthy adults, and partly supports the use of vaccines, this is the population who are universally considered to need them least.[1][2]

The reasons for the contradictions between policy and evidence, and the dearth of corroborating evidence on vaccine performance, are complex and include: the relative rarity of influenza; the current confusion between influenza-like illness and influenza (a simplistic aetiopathogenic model hide-bound by Henle-Koch's postulates of one germ, one disease, one solution); the inability of vaccines to protect populations from an ever-mutating agent; and the difficulty of conducting meaningful prospective studies to assess vaccine efficacy. In addition, the powerful image of influenza depicted by the media is not proportional to the actual threat. The "monster at your door" fame of influenza helps to create preventive expectations that are unachievable with today's technology and with only partial reading of the evidence. For example, we know that in the past 2 decades influenza vaccine studies have risen in prominence in the scientific media, possibly as a result of pharmaceutical sponsorship and the need of larger journals to boost their revenue by selling bulk reprints and subscriptions to offset the decline in print-based returns.[4][5] This rise in prominence is, however, in contrast to the threat from influenza. In the US, the influenza-related mortality rate of the past 20 years has not increased, but plateaued.

Here, I examine the evidence for and the impact of the first two factors listed above — the incidence of influenza, and the masking of its rarity by the systematic failure to distinguish between influenza (a disease) and influenza-like illness (a syndrome, caused also in minor part by influenza viruses).

The causal relationship between the two is scarcely investigated and is frequently overlooked, perhaps because of technical difficulties in quantifying the incidence of "seasonal" influenza and its complications. I must confess that I realised the importance of incidence only after having carried out scores of Cochrane reviews and updates on influenza vaccines and antivirals. I started from the end (the interventions) instead of concentrating on the beginning (the epidemiology of influenza and the other respiratory viruses).

The incidence statistic for influenza, which is often taken for granted, is estimated from virological testing of symptomatic people (so-called viral circulation). What is often poorly understood is that the patient presenting to a physician typically has a syndrome (influenza-like illness, or ILI) that can be caused by various agents. Only a proportion of these syndromes is caused by influenza A and B viruses, but differential diagnosis on clinical grounds alone is not possible.[6][7] Google's near real-time instrument, Flu Trends, provides an excellent example of the confusion generated from following the inaccurate equation "influenza = ILI".[7] Users of Flu Trends think they are following the spread of influenza, while in reality the site depicts the spread of ILI.

To determine (not estimate) the incidence of influenza at any one time, virological testing of a truly random sample of people with ILI is needed. At the same time, testing for all other major causal agents should be carried out, but this is not typically done. In addition, it is not known, or cannot be estimated accurately, how many people have ILI at a given time, which further complicates calculation of incidence. The consequence of this is biased estimates of incidence, where attention is focused on testing for influenza viruses in non-randomly identified people with ILI. Ignorance of the presence of other causal agents has made us blind to the complex ecology of respiratory viruses. How can systematic reviews obviate such tunnel vision?

At present, the only method of determining influenza incidence with a high level of accuracy is to use the control arms of influenza vaccines and antiviral studies. From these, reliable denominators (i.e., number of people with ILI) and numerators (i.e., number of people with influenza and its complications) can be calculated. This is simpler than it sounds. The Cochrane Vaccines Field group has a database of all identifiable studies from 1948 to 2007 that assess the effectiveness of inactivated influenza vaccines and report clinical outcomes (as opposed to surrogate outcomes, such as antibody responses). These are the studies that populate our Cochrane reviews and their updates. The database also comprises studies excluded from the reviews, provided they are comparative and report clinical outcomes. Data available in these studies are collected during the active follow-up of formal studies (often prospectively), in which participant controls with ILI are typically tested (figure 1). As such, they are the optimum data available on influenza incidence. However, high loss to follow-up detracts from the reliability of the data. The data depicted in figure 1 come from the control arms of 95 vaccine comparative studies published between 1965 and 2005 that report, between them, several million observations on incidence.

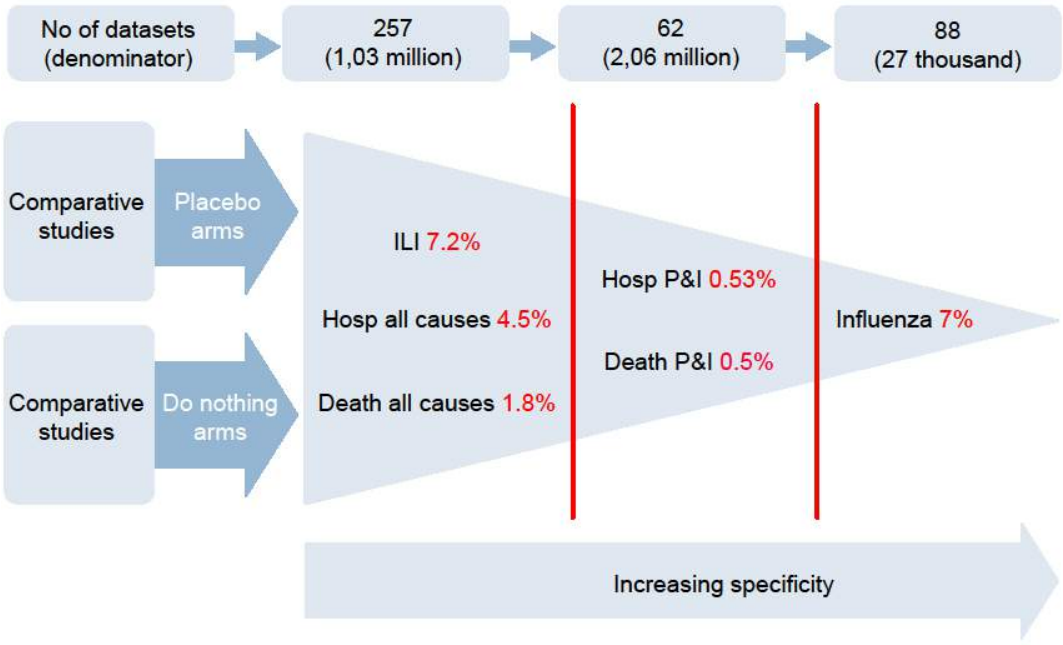

Figure 1. Graphic representation of funnel data in the general population. ILI, influenza-like illness; P&I, pneumonia and influenza (includes ICD 9 codes 480–488).

The availability of a considerable body of data, as figure 1 illustrates, does not always generate a strong evidence base on which to judge efficacy. In the case of inactivated influenza vaccines, the key issue in interpreting the data is over-reliance on non-specific outcomes, such as death from all causes, which may have little to do with influenza-related death. Studies with such non-specific outcomes have been purported to show the effectiveness of influenza vaccines, but actually they only introduce confounding. The funnel in figure 1 exemplifies the richness of data on non-specific outcomes, and the paucity of data on laboratory confirmed influenza A or B. However, this is only part of the story, as data from control arms of comparative vaccine studies seldom look for other viral agents among the samples. Control arms show what is certainly influenza (as is their objective), but do not identify other agents. One of the subliminal effects of this is that observers focus exclusively on one agent, ignoring the rest.

In addition, the data allow a best guess as to how prevalent influenza is, but not its complications. Based on studies in the Cochrane database, incidence of influenza is estimated at around 7%. However, the control arms of the 95 studies identified evaluate people with ILI. Therefore, 7% is not the absolute incidence of influenza in the general population, but is rather the portion of ILI that is caused by influenza, making the incidence of influenza itself in the general population much smaller (approximately 0.5%). Studies of influenza vaccines do not serve well for apportioning slices of the ILI "pie" to non-influenza agents, as they seek only influenza. To do this, we must turn to pie studies, which are a systematic assembling of data from the few studies that followed a defined population, and swabbed ILI symptomatic people for all major agents.

A brief review of pie studies published in the past decade and available in the Cochrane database paints a remarkably similar picture to that of control arms, with an incidence of influenza of 0.5% to 1% of ILIs. Figure 2 shows how the systematically assembled evidence from control arms fits with that from pie studies. Surprisingly, most ILIs cannot be attributed to a specific causal agent. Although many other conclusions can be drawn from observations of pie slices, our aim here is to discuss why influenza inactivated vaccine performance is poor, and why most studies rely on non-specific outcomes, such as death from all causes, and hospitalisations for pneumonia and influenza (which are not usually based on virological testing). One possible answer is that seasonal influenza is a relatively rare and benign condition, with an incidence not exceeding 1% in the general population during autumn and winter months.

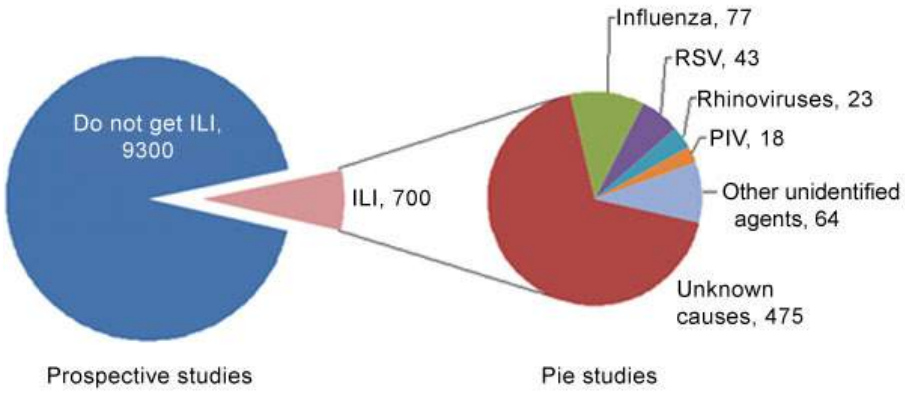

Figure 2. Incidence of influenza-like illnesses (ILI) per 10,000 people (calculated from prospective studies), with breakdown by agent, based on information in pie studies.

Vaccine effectiveness (expressed as a percentage) is calculated by subtracting the ratio of incidence in vaccinated and unvaccinated populations from 1. Therefore, if the incidence in the unvaccinated population is low, then the ratio will be close to 1 and effectiveness will be low. So, vaccines seem to be less effective in illnesses with low incidence. A systematic approach to best evidence completes the picture, and explains what is observed in trials and other comparative studies. In summary, evidence presented here points to influenza being a relatively rare cause of ILI and a relatively rare disease. It follows that vaccines may not be appropriate preventive interventions for either influenza or ILI.

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Why have three long-running Cochrane Reviews on influenza vaccines been stabilised?

Three Cochrane Reviews focussing on the prevention of influenza in healthy adults, healthy children, and in the elderly are long-running reviews under the same senior author team. The protocol for the oldest review was first published 20 years ago.

Over the years the reviews have progressively accumulated evidence leading to ever greater stability in their conclusions. 'Stable' is a publication flag that usually indicates that the results are unlikely to change with the inclusion of new studies, such is the certainty of the results. The influenza vaccine reviews present us with a partly different situation. Readers will notice important outcomes where we have little or no data. They may also see that for some measures of influenza and 'influenza-like illness' (ILI), we have low-certainty evidence. We have reached a point where the evidence is not showing anything different to what it has done for a number of years. We know with varying degrees of certainty about vaccination effects on influenza and ILI, but the gap in our understanding of how vaccines affect the consequences of influenza persist. For each review, the impact of single studies is documented in the summary table 1 "Studies included in the various versions of this review and their impact on our conclusions". This month the three reviews (https://www.cochrane.org/news/featured-review-three-updated-cochrane-reviews-assessing-effectiveness-influenza-vaccines) appear in their latest updated and stabilised format.[1,2,3] Whilst we do not believe that periodic updating will complete the picture, our decision to stabilise is conditional. The three reviews will not be updated again unless certain criteria are met.

First, a new trial that meets inclusion criteria becomes available. Few trials of interest have been conducted recently, as a comparison with an inactive control is considered by some to be unethical. In the elderly, the latest completed trial dates from nearly two decades ago. Our searches have failed to find relevant ongoing trials.

A second condition is the introduction of a new generation of vaccines, based on new technology. This is possible given that several new technologies are being developed, such as vaccines containing fragments of the haemagglutinin antigen "stalk" on the viral surface (so called stalk-specific vaccines).[4]

The third condition is more complex: the development and testing of a new causal paradigm for ILI and influenza. Currently, massive worldwide machinery is needed to produce new vaccines every year to address viral antigenic changes, and to address the poor persistence of the antibody response in individuals. However, the vaccination selection and production programmes are based on aetiological assumptions which are neither explanatory nor predictive, as shown in our reviews. Overall the largest dataset to have accumulated to date is from trials conducted in the population least likely to benefit from vaccines but most likely to produce immunity: healthy adults. In healthy adult trials a high serological response is matched by a very small clinical effect (71 healthy adults need to be vaccinated to prevent one of them experiencing influenza). This weak effect cannot be explained simply by the mismatch of vaccine antigens with wild virus ones. A larger effect is observed in children over the age of two (five children need to be vaccinated to prevent one case of influenza, although there is huge uncertainty around these estimates). There is little evidence on prevention of complications, transmission, or time off work. Other reviews have drawn similar conclusions.[5]

During stabilisation we updated the randomised evidence, but for the first time have decided against updating the large observational evidence base. The observational dataset still appears in the reviews, but only as a historical record of earlier versions. Observational studies were included in the reviews over a decade ago in the hope they could provide long-term and rare harms data and improve the external validity of the trial evidence. They turned out to be of such low quality that their conclusions were inconclusive or unreliable. The most important example is the case-negative study to assess influenza vaccine effectiveness *post hoc* (i.e. after an influenza season) by harvesting data from a surveillance programme. This study design, which is similar to a case-control study, selects influenza cases (cases of ILI which have tested positive for influenza) and controls (cases of ILI which have tested negative) and calculates the relevant odds ratio (OR) of exposure to that season's vaccine. An estimate of vaccine effectiveness is derived from this OR using a standard formula (vaccine effectiveness = 1 - OR%). However, despite their institutional popularity,[6,7] case-negative designs have limited public health significance because the design does not test field effectiveness, but, rather, laboratory efficacy of the vaccine (the capacity of the vaccine to generate a negative polymerase chain reaction (PCR) result). Both cases and controls are symptomatic, so any prevention is solely focused on PCR negativity. In addition, no useful public health absolute measures of effect can be derived (such as absolute risk reduction (ARR) and its reciprocal number needed to vaccinate to prevent one case (NNV)) because the background rates of infection and viral circulation are not part of the calculation of the estimates of effect. There are also problems with the mathematical assumptions made in this design (for details see the reviews). Case-negative studies are an illustration of the narrow and retrospective focus on influenza viruses at the expense of overall ILI - the

illness cluster of interest to patients and their clinicians. Retrospective calculation of relative estimates of laboratory efficacy can be of interest for future decisions on composition of vaccines, but their relevance to everyday decisions seems questionable.

The underlying assumption that influenza vaccination does not affect the risk of non-influenza is contradicted by a recent report from the follow up of a trial by Cowling et al.[8] In 115 participants, those who received trivalent influenza vaccines had higher risk of acute respiratory infection associated with confirmed non-influenza respiratory virus infection (RR, 4.40; 95% CI, 1.31–14.8) compared to placebo recipients. The agents were mainly rhinoviruses and coxsackie/echoviruses; ILI episodes occurred shortly after a peak of influenza activity.

Current yearly registration of candidate influenza vaccines is based on their ability to trigger a good antibody response. But antibody responses are poor predictors of field protection. This is another example of the use of surrogate outcomes in biomedicine, where effects on clinically important outcomes remain unmeasured or unproven from randomised trials: complications and death by influenza.

The simple answer is that we do not understand what the target is. What is the threat of influenza, and what can we ever expect of the vaccines?

The WHO Global Influenza Programme (http://www.who.int/influenza/surveillance_monitoring/en/) (GIP) with its backbone Global Influenza Surveillance and Response System (http://www.who.int/influenza/gisrs_laboratory/en/) (GISRS) is a complex network of 143 national reference centres and specialist laboratories in 113 states carrying out surveillance of circulating influenza viruses. GISRS was devised and developed to guide annual influenza vaccine production, and the emphasis is mainly on influenza viruses, their variants, and emerging strains.

However there is no reliable system to monitor and quantify the epidemiology and impact of ILI, the syndrome that presents clinically. Few states produce reliable data on the number of physician contacts or hospitalised cases due to ILI, and none tie these data to the proportion of ILI caused by influenza. We do not know for certain what the impact of ILI is, nor the impact of the proportion of ILI caused by influenza. Prospective studies apportioning positivity to the scores of viruses probably causing ILI are rare, as interest is focused on influenza. The standard quoted figure of 36,000 yearly deaths in the US is based on the “respiratory and circulatory deaths” category including all types of pneumonia, including secondary to meconium ingestion or bacterial causes. More recently, the US Centers for Disease Control and Prevention (CDC) have proposed estimates of impact ranging between 3,000 and 49,000 yearly deaths. When actual death certificates are tallied, influenza deaths on average are little more than 1,000 yearly (http://dspace.mit.edu/handle/1721.1/69811) . So, the actual threat is unknown (but likely to be small) and so is the estimation of the impact of vaccination.

The uncertainty over the aetiology of ILI, its capricious nature and the weak correlation between immunity and protection, point to possible causal or concurrent factors in the genesis of both ILI and influenza. In other words, virus positivity may only be one of the factors necessary for a case of influenza or ILI to manifest itself.

We await to see whether anyone has the interest or the courage to develop effective ways to control upper respiratory viral syndromes. Meanwhile our reviews will remain as a testimonial to the scientific failure of industry and governments to address the most important clinical outcomes for patients.

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TJ was a recipient of a UK National Institute for Health Research grant for a Cochrane Review of neuraminidase inhibitors for influenza. In addition, TJ receives royalties from his books published by Il Pensiero Scientifico Editore, Rome and Blackwells. TJ is occasionally interviewed by market research companies about phase I or II pharmaceutical products. In 2011-13, TJ acted as an expert witness in litigation related to the antiviral oseltamivir, in two litigation cases on potential vaccine-related damage and in a labour case on influenza vaccines in healthcare workers in Canada. He has acted as a consultant for Roche (1997-99), GSK (2001-2), Sanofi-Synthelabo (2003), and IMS Health (2013). In 2014 he was retained as a scientific adviser to a legal team acting on oseltamivir. TJ has a potential financial conflict of interest in the drug oseltamivir. In 2014-16, TJ was a member of three advisory boards for Boerhinger Ingelheim. He is holder of a Cochrane Methods Innovations Fund grant to develop guidance on the use of regulatory data in Cochrane Reviews. TJ was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine. Between 1994 and 2013, TJ was the coordinator of the Cochrane Vaccines Field. TJ is a co-signatory of the Nordic Cochrane Centre Complaint to the European Medicines Agency (EMA) over maladministration at the EMA in relation to the investigation of alleged harms of HPV vaccines and consequent complaints to the European Ombudsman. TJ is co-holder of a John and Laura Arnold Foundation grant for development of a RIAT support centre (2017-2020) and Jean Monnet Network Grant, 2017-2020 for The Jean Monnet Health Law and Policy Network.

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The Cochrane HPV vaccine review was incomplete and ignored important evidence of bias

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Introduction

The Cochrane Review conducted trial searches up until June 2017 and included 26 randomised trials with 73 428 women.[1] In January 2018, we published an index of the study programmes of the HPV vaccines that included 206 comparative studies.[2] As of June 2017, about a third of the 206 studies were not published and half of the completed studies listed on ClinicalTrials.gov had no results posted.[2] Although we sent our index to the Cochrane group handling the Cochrane Review, the review stated that, 'nearly all end-of-study reports have been published in the peer-reviewed literature'. When we applied the Cochrane Review's inclusion criteria to the 206 studies, we identified 46 completed and eligible trials. The number of randomised participants could be assessed for 42 of the 46 trials and was 121 704. With nearly half of the trials and half of the participants missing, the Cochrane authors' conclusion, 'that the risk of reporting bias may be small', was inappropriate. Fifteen of the 20 additional trials were listed on ClinicalTrials.gov; the Cochrane authors would therefore have identified more trials if they had searched ClinicalTrials.gov in more depth and searched additional trial registers (we searched 45 trial registers[2]).

The Cochrane authors stated that they 'did not include the nine-valent vaccine [Gardasil 9] ... since the randomised trials ... did not incorporate an arm with a non-HPV vaccine control'. This is not correct. The only saline placebo trial of approved HPV vaccines is a Gardasil 9 trial (V503-006; NCT01047345) that was published in 2015.[3] Its participants had previously been vaccinated with four-valent Gardasil, but according to the Cochrane Review protocol,[4] this was not an exclusion criterion. Since many countries are shifting to Gardasil 9,[5] it is unfortunate that the Gardasil 9 trial was not included in the Cochrane Review.

No included trial in the Cochrane Review used a placebo comparator

All 26 trials included in the Cochrane Review used active comparators: adjuvants (aluminium hydroxide ($Al(OH)_3$) or amorphous aluminium hydroxyphosphate sulphate) or hepatitis vaccines.

Adjuvants are not regulated separately from their vaccine antigens. According to the Food and Drug Administration, adjuvants are unreliable comparators.[6] One HPV vaccine manufacturer (GlaxoSmithKline that produces Cervarix) states that its aluminium-based comparator induces harms: 'higher incidences of myalgia might namely be attributable to the higher content of aluminium in the HPV vaccine [450 micrograms $Al(OH)_3$] than the content of aluminium in the HAV (hepatitis A) vaccine [225 micrograms $Al(OH)_3$]'.[7] The comparator hepatitis vaccines also used the HPV vaccines' aluminium-based adjuvant.

The Cochrane authors mistakenly used the term placebo to describe the active comparators. They acknowledged that 'The comparison of the risks of adverse events was compromised by the use of different products (adjuvants and hepatitis vaccines) administered to participants in the control group'. Nevertheless, this statement can easily be overlooked, as it comes after 7500 words about other issues in the discussion and under the heading 'Potential biases in the review process'. Active comparators was not a bias in the review process but a bias in the design of the HPV vaccine trials.

The use of active comparators probably increased the occurrence of harms in the comparator groups and thereby masked harms caused by the HPV vaccines. It is noteworthy that many women were excluded from the trials if they had received the adjuvants before or had a history of immunological or nervous system disorders; for example, in the PATRICIA Trial with 18 644 women[8] and the FUTURE II trial with 12 167 women.[9] These exclusion criteria lowered the external validity of the trials and suggest that the vaccine manufacturers were worried about harms caused by the adjuvants. The criteria are not listed as warnings on the package inserts of the HPV vaccines,[10–12] which may have led to more vaccine-related harms in clinical practice than in the trials.

The included HPV vaccine trials used composite surrogate outcomes for cervical cancer

In line with WHO recommendations,[13] the Cochrane Review was based on composite surrogate outcomes: 'cervical intraepithelial neoplasia grade two and above [$CIN2^+$], CIN grade three and above [$CIN3^+$], and adenocarcinoma-in-situ (AIS)'.[1] The use of such outcomes seemed reasonable for a preliminary assessment of HPV vaccine benefits, but the outcomes can be difficult to interpret. If there were clinically important differences in the severity of the cervical lesions in the two compared groups, they may not have been apparent in the composite outcomes of $CIN2^+$ and $CIN3^+$. The Cochrane authors did not describe any cervical cancers in the 26 trials, although cancers

did occur in the trials; for example, in the ClinicalTrials.gov entry for the VIVIANE Trial, one case of 'Adenocarcinoma of the cervix' and one case of 'Cervix cancer metastatic' are listed in the HPV vaccine group (see 'Results: Serious Adverse Events').[14] Furthermore, the relationship between CIN2 and cervical cancer is not clear-cut. Most CIN2 lesions in women below age 30 years regress spontaneously; an active surveillance approach has therefore been recommended for this group.[15] The Cochrane Review's 26 trials mainly included women below age 30 years and used frequent cervical screening (often every 6 months) that did not reflect real life practice (often every 3–5 years[5]).

The Cochrane Review incompletely assessed serious and systemic adverse events

The Cochrane authors reported that they made a 'Particular effort' to assess serious adverse events and performed a sensitivity analysis that gave them 'confidence that published and registry or website-sourced data are similar for the same study'.[1] This seems unlikely. As an example, the PATRICIA Trial publication only included two-thirds (1400/2028) of the serious adverse events listed on ClinicalTrials.gov. The Cochrane authors included 701 vs 699 serious adverse events (1400) from the PATRICIA Trial publication (see the Cochrane Reviews' 'Figure 10, Analysis 7.6.2') and 835 vs 829 serious adverse events from its ClinicalTrials.gov entry (see 'Comparison 7, Analysis 6: 7.6.2'; both analyses were called '7.6.2'). We found 1046 vs 982 serious adverse events (2028) when we summarised the data from ClinicalTrials.gov (see 'Results: Serious Adverse Events').[16]

The Cochrane authors concluded with 'high certainty' that the risk of serious adverse events was similar in the HPV vaccine groups and the comparator groups. However, the authors failed to mention that several of the included trials did not report serious adverse events for the whole trial period. For example, FUTURE I,[17] FUTURE II[9] and FUTURE III[18]—which in total included 21 441 women with up to 4 years follow-up—only reported serious adverse events occurring within 14 days postvaccination. Furthermore, the Cochrane authors did not explain what the serious adverse events consisted of or whether some of them were more common in the HPV vaccine groups.

The Cochrane authors found more deaths in the HPV vaccine groups than in the comparator groups. The death rate was significantly increased in women older than 25 years (risk ratio (RR) 2.36, 95% CI 1.10 to 5.03; no absolute numbers were provided for this subgroup analysis, but the total numbers of deaths were 51 in the HPV vaccine groups and 39 in the comparator groups). The Cochrane authors suggested that this was a chance occurrence since there was no pattern in the causes of death or in the time between vaccine administration and date of death. However, as the Cochrane Review only included randomised trials, the authors cannot rule out that the increase could be caused by the HPV vaccines. A death may be coded in a way that does not raise suspicion that the vaccine caused it; for example, a 'traumatic head injury' or 'drowning' could have been caused by a 'syncope,' which is a recognised harm.[10–12] As of May 2018, WHO's pharmacovigilance database—VigiBase, managed by the Uppsala Monitoring Centre (UMC)—contained 499 deaths reported as related to HPV vaccination.[19]

The Cochrane authors concluded that, 'Systemic events with general mild symptoms were similarly frequent in vaccinated recipients and placebo or control vaccine recipients'. Their Analysis 7.5 showed a non-significant increase in systemic events: RR 1.02 (95% CI 0.98 to 1.07) with a total of 9137 vs 9054 events. The Cochrane authors did not include all of their trials that were eligible for systemic events in Analysis 7.5; for example, the PATRICIA Trial was not included. On ClinicalTrials.gov, PATRICIA has 7129 vs 6557 systemic events listed under 'Results: Other Adverse Events (General disorders)', which in itself is a significantly increased risk: RR 1.09 (95% CI 1.07 to 1.11).[16]

The Cochrane authors 'planned requesting data from data owners, to fill in gaps with available unpublished data', but 'due to constraints in time and other resources' they were unable to do so.[1] Considering that 7 years passed from the publication of the Cochrane protocol in 2011[4] to the Cochrane Review in 2018,[1] lack of time seems a poor excuse for not trying to obtain unpublished trial documents and data. More importantly, harms cannot be assessed reliably in published trial documents—especially in journal publications of industry funded trials where even serious harms often are missing.[20] One reason may be the space restrictions that most medical journals have. As an example, the journal publication for the PATRICIA Trial is 14 pages long[8] while its publicly available corresponding clinical study report is over 7000 pages long;[21] although it is an interim report that has been shortened. Clinical study reports are usually confidential documents, but they can be requested from the European Medicines Agency (EMA) and ClinicalStudyDataRequest.com.

Despite the mentioned examples of reporting bias, the Cochrane authors judged all trials at low risk of reporting bias (see the Cochrane Review's 'figure 4: 'Risk of bias' summary').

The Cochrane Review did not assess HPV vaccine-related safety signals

The Cochrane authors referred to many observational studies in their discussion that found no safety signals of harms associated with the HPV vaccines.[1] They cited the WHO's Global Advisory Committee on Vaccine Safety that expressed 'concerns about unjustified claims of harms'. The Cochrane authors did not mention a study from 2017 by the WHO UMC that found serious harms following HPV vaccination overlapping with two syndromes: postural orthostatic tachycardia syndrome (POTS) and complex regional pain syndrome (CRPS).[22] The WHO UMC provided part of the rationale for EMA's investigation of POTS and CRPS in 2016.[23] As of May 2018, the WHO UMC VigiBase contained 526 cases of POTS and 168 cases of CRPS reported related to HPV vaccination.[19]

The Cochrane authors did not investigate whether the included trial data reported cases of POTS, CRPS or other safety signals. Instead, the authors cited EMA, which concluded that 'No causal relation could be established' between POTS or CRPS and the HPV vaccines.[1] The EMA's conclusion was based on the HPV vaccine manufacturers' own unverified assessments[23] that only included half of the eligible trials.[2] Furthermore, the HPV vaccine manufacturers search strategies for POTS and CRPS were inadequate and led to cases being overlooked.[24] As an example, in 2014, the Danish Medicines Agency (DMA) asked the HPV vaccine co-manufacturer Sanofi-Pasteur-MSD to search for specific POTS-related symptoms in its database (including dizziness, palpitations, rapid heart rate, tremor, fatigue and fainting). The manufacturer only searched for 'postural dizziness', 'orthostatic intolerance' and 'palpitations and dizziness'. The DMA discovered this because only 3 of 26 Danish reports of POTS showed up in Sanofi's searches.[24] As another example, EMA identified six possible cases of POTS and CRPS related to Gardasil 9 that Merck had not identified.[25]

Industry trial funding and other conflicts of interest

The Cochrane authors assessed the impact of industry funding 'by meta-regression. No significant effects were observed'.[1] They stated that, 'All but one of the trials was funded by the vaccine manufacturers', which is not correct. According to ClinicalTrials.gov, this particular trial ('CVT' or 'Costa Rica trial'[1]) was sponsored by GlaxoSmithKline.[26] Therefore, all included trials were funded by the HPV vaccine manufacturers and the meta-regression was meaningless.

The Cochrane Collaboration aims to be free from conflicts of interest related to the manufacturers of the reviewed products.[27] Most of the 14 Cochrane authors on the first published protocol for the Cochrane Review had major conflicts of interest related to the HPV vaccine manufacturers.[28] The Cochrane Review only has four authors; three of whom had such conflicts of interest a decade ago. The review's first author currently leads EMA's 'post-marketing surveillance of HPV vaccination effects in non-Nordic member states of the European Union', which is funded by Sanofi-Pasteur-MSD that was the co-manufacturer of Gardasil.

Cochrane's public relations of the review were uncritical

The announcement of the Cochrane Review on Cochrane.org under 'News' included a 'Science Media Centre roundup of third-party expert reaction to this review'.[29] Six experts were cited—all from the UK; although the Cochrane Collaboration is an international organisation. Two of the experts had financial conflicts of interest with the HPV vaccine manufactures. A third expert was responsible for vaccinations in Public Health England that promotes the HPV vaccines. The experts highlighted the 'intensive and rigorous Cochrane analysis', 'that the HPV vaccine is the most effective way for young girls to protect themselves against cervical cancer', and that, 'the vaccine causes no serious side-effects'. No expert criticised the review. In our view, this is not balanced and people with conflicts of interest in relation to the manufacturers should not be quoted in relation to a Cochrane review. Richard Smith—the former editor of the *British Medical Journal*—described medical journals as an extension of the marketing arm of the drug industry.[30] We are concerned that some observers may see Cochrane Reviews in the same light when Cochrane publishes such public relations messages.

Conclusion

Part of the Cochrane Collaboration's motto is 'Trusted evidence'. We do not find the Cochrane HPV vaccine review to be 'Trusted evidence', as it was influenced by reporting bias and biassed trial designs. We believe that the Cochrane Review does not meet the standards for Cochrane Reviews or the needs of the citizens or healthcare providers that consult Cochrane Reviews to make 'Informed decisions', which also is part of Cochrane's motto. We recommend that authors of Cochrane Reviews make every effort to identify all trials and their limitations and conduct reviews accordingly.

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Contributors LJ wrote the first draft. LJ, PCG and TJ contributed to the conception, drafting, critical revision for important intellectual content and the final approval of the article.

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Competing interests TJ was a co-recipient of a UK National Institute for Health Research grant (HTA – 10/80/01 Update and amalgamation of two Cochrane Reviews: neuraminidase inhibitors for preventing and treating influenza in healthy adults and children– https://www.journalslibrary.nihr.ac.uk/programmes/hta/108001#/). TJ is also in receipt of a Cochrane Methods Innovations Fund grant to develop guidance on the use of regulatory data in Cochrane reviews. TJ is occasionally interviewed by market research companies about phase I or II pharmaceutical products. In 2011–2014, TJ acted as an expert witness in a litigation case related to the antiviral oseltamivir, in two litigation cases on potential vaccine-related damage and in a labour case on influenza vaccines in healthcare workers in Canada. He has acted as a consultant for Roche (1997–1999), GSK (2001–2002), Sanofi-Synthelabo (2003) and IMS Health (2013). In 2014–2016, TJ was a member of three advisory boards for Boehringer Ingelheim. TJ was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine. TJ and PCG were co-signatories of a complaint to the European Ombudsman on maladminstration in relation to the EMA investigation of possible harms from HPV vaccines. PCG does not regard this as a competing interest.

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Correction: *The Cochrane HPV vaccine review was incomplete and ignored important evidence of bias*

Jørgensen L, Gøtzsche PC, Jefferson T. The Cochrane HPV vaccine review was incomplete and ignored important evidence of bias. *BMJ Evidence-Based Med* 2018;23:165–8. doi:10.1136/bmjebm-2018-111012

This article (https://ebm.bmj.com/content/23/5/165) has a correction. The changes are clarificatory, and for this reason, the editors have issued a correction and not a retraction. A linked Editor's note provides more background to this decision, and a marked copy is available to view (online supplementary appendix 1) .

The corrections are outlined below.

An additional table outlining the authors' reassessment of 20 studies identified as additionally eligible for the Cochrane HPV vaccine review has been included in the article (online supplementary table 1). Sixteen additional trials were eligible for inclusion in the Cochrane HPV Review (not 20 as stated in the article). Additional data from four trials already included in the Cochrane HPV Review (NCT00929526; NCT00518336; NCT00652938; NCT00578227) are potentially eligible for inclusion.

The number of randomised participants could be assessed for 42 of the 46 trials, and the authors found an additional 25 550 females (and possibly up to 30 195 for the Cochrane HPV Review's serious adverse events meta-analyses) who are eligible for the Cochrane HPV Review's meta-analyses. In the analysis, the authors did not originally subtract the male participants that were included in three of the studies.

The PATRICIA trial publication only included two thirds (1400/2028) of the serious adverse events listed on ClinicalTrials.Gov". The PATRICIA trial registry reports the total number of women with serious adverse events within each MedDRA preferred term category,[1] which yields a different total number of women than the total described in the PATRICIA published report and also in the Cochrane HPV Review.[2] The NCT entry reports the number of women with serious adverse events for each MedDRA term. The final total number of serious adverse events as a proportion of total events remains unknown.

Industry trial funding and other conflicts of interest:

"The Costa Rican Vaccine Trial is a longstanding collaboration between investigators in Costa Rica and NCI. The trial is sponsored and funded by NCI (N01-CP-11005) with support from the NIH Office of Research on Women's Health and conducted in agreement with the Ministry of Health of Costa Rica." The trial publication reports that the "Vaccine was provided for our trial by GSK (GlaxoSmithKline) Biologicals, under a Clinical Trials Agreement with NCI. GSK also provided support for aspects of the trial associated with regulatory submission needs of the company under FDA (Food and Drug Administration) BB-IND 7920. D R Lowy and J T Schiller are named inventors on the US government-owned HPV vaccine patents that are licensed to GSK and Merck, and so are entitled to limited royalties as specified by federal law."

In the context of FDA regulations, the trial may have been sponsored by GSK, but it is not clear if the trial received any funding from GSK. We consider it is reasonable to accept that GSK provided funding, at least in some kind, since it provided vaccines and support related to the regulatory submission. Therefore, all included trials were funded or sponsored by the HPV vaccine manufacturers.

BMJ Evidence-Based Medicine 2020;**25**:e3. doi:10.1136/bmjebm-2018-111012corr1

Editors' Note: *"The Cochrane HPV vaccine review was incomplete and ignored important evidence of bias"*

Carl Heneghan, Igho Onakpoya

A Cochrane systematic review of human papillomavirus (HPV) vaccine (hereafter referred to as the Cochrane HPV Review) was published on 9th May 2018.

The article, 'The Cochrane HPV vaccine review was incomplete and ignored important evidence of bias' was submitted to BMJ EBM on 24th May 2018 for the 'Debate, analysis and opinion' section of the journal. The handling editor, Dr Igho Onakpoya (Research Editor, BMJ EBM) sent it for external peer review to an expert in HPV vaccines and for internal peer review to Professor Carl Heneghan, Editor in Chief of BMJ EBM. Peer reviewers' reports were returned by 19th June and were sent to the authors, who were invited to make revisions. The revised version was submitted on third July, accepted by the handling editor on seventh July and published online on 27th July 2018.[1]

The analysis of the Cochrane HPV review stated there were missing eligible trials, reporting bias, and biased trial designs and conflicts of interest.[2 3]

Cochrane initiated an investigation in response to the criticism and published a response to the article on third September authored by Cochrane's then Editor in Chief (EiC) David Tovey and deputy EiC Karla Soares-Weiser. This response defended the Cochrane HPV Review and outlined key findings from the Cochrane investigation:

- The Cochrane Review did not miss "nearly half of the eligible trials". A small number of studies were missed due to the primary focus on peer-reviewed reports in scientific journals, but the addition of these data makes little or no difference to the results of the review for the main outcomes;
- The trials comparators were unambiguously, transparently, and accurately described;
- The selection of outcomes for benefits was appropriate and was consistent with WHO guidance;
- The review included published and unpublished data on serious harms, and the findings on mortality were reported transparently and responsibly;
- The review was compliant with Cochrane's current conflict of interest policy;
- Cochrane's media coverage was cautious and balanced, but we recognise that there could be improvements in relation to transparency where external experts are quoted;
- The BMJ Evidence-Based Medicine article "substantially overstated its criticisms"[4]

The response also criticised the peer review process of the journal and whether the conclusions were justified and proportionate. When these criticisms were raised with the journal, we embarked on a lengthy clarification process with the authors and Cochrane.

September 2018

We wrote to the authors of the BMJ EBM analysis article and received a response that asserted *'(their) analysis was appropriate and that the Cochrane editors substantially ignored several of (their) criticisms'*.[5] We also contacted Cochrane's EiC and the Cochrane corresponding author to ask for further details of their criticisms of the BMJ EBM peer review process and how the article was overstated.

We set out the contentious questions arising from the Cochrane Editors' response and considered whether the BMJ EBM analysis[6] required further editorial comments or corrections. We sent a copy of this review to all parties concerned and posted it on BMJ EBM Spotlight on 16th of October 2018. Our review concluded that the BMJ EBM analysis required several corrections and that the overall article did not overstate its claims, nor did it warrant retraction.

The authors of the BMJ EBM analysis article responded to the conclusions of the Cochrane investigation in a rapid response to the original article. They acknowledged and approved the conclusions of the BMJ EBM Editors' Review and provided further details and clarification.Jørgensen et al. 2018

October 2018

The Cochrane HPV review corresponding author contacted The BMJ to enquire whether The BMJ might provide a forum to reply. The BMJ EiC suggested sending a detailed response to the BMJ EBM publication, a rapid response to The BMJ and potentially an opinion piece about the broader issues.

November 2018

Cochrane EiC emailed the BMJ EBM EiC to say that there remained uncertainties with identifying studies for inclusion. Six studies had now been identified and will be added to the Cochrane HPV Review. Their re-analysis of the data incorporating the missing data suggested that their results did not change with the inclusion of this data. The email also reiterated concerns with the BMJ EBM peer review process for that article, specifically that there was only one external peer reviewer.

2019

Since then BMJ EBM has updated its editorial policy to ensure EBM Analysis pieces are reviewed by two external peer reviewers at a minimum. Debate, Insights and opinion pieces are sent to external

or internal review, and EBM verdicts as commentaries are subject to internal review. The journal's peer review policy is stated on the BMJ EBM website to increase transparency.

We have published a correction to the BMJ EBM HPV analysis article,[1] alongside this linked Editor's Note.

The BMJ EBM analysis article by Jørgensen, Gøtzsche and Jefferson defined methods for securing the available evidence and we consider the article title, and conclusions, are justified. Until the updated review is published we remain unclear about the actual number of trials that require inclusion in the Cochrane Review, and whether the missing trials impact on the Cochrane results.

Competing interests

CH has co-authored 16 peer-reviewed articles with Tom Jefferson (two of which are Cochrane reviews) and holds grant funding jointly with Tom Jefferson from Cochrane on methods for deciding when to prioritise the use of clinical study reports in systematic reviews. CH is a member of Cochrane and a contact editor for the Cochrane Acute Respiratory Infection group and has been an author 21 Cochrane reviews including updates. He is a founder of AllTrials and an advisor to the WHO International Clinical Trial Registry Platform. and He has received expenses and fees for his media work (including payments from BBC Radio 4 Inside Health). He has received expenses from the WHO and holds grant funding from the NIHR, the NIHR School of Primary Care Research, NIHR BRC Oxford and Cochrane. He has received financial remuneration from an asbestos case and given free legal advice on mesh cases. He has also received income from the publication of a series of toolkit books published by Blackwells. On occasion, he receives expenses for teaching EBM and is also paid for his NHS GP work in urgent care (contract with Oxford Health NHS Foundation Trust). He is Director of CEBM at the University of Oxford, which jointly runs the EvidenceLive Conference with the BMJ and the Overdiagnosis Conference with international partners, based on a non-profit making model. He is Editor in Chief of BMJ Evidence-Based Medicine. IO is Research Fellow in Evidence Synthesis at the CEBM, University of Oxford, a clinician who works across several NHS Trusts and is funded by the NIHR School of Primary Care Research. IO has co-authored eight peer-reviewed articles jointly with Tom Jefferson (one of these is a Cochrane review).

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Association of Tdap Vaccination With Acute Events and Adverse Birth Outcomes Among Pregnant Women With Prior Tetanus-Containing Immunizations

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Abstract

IMPORTANCE—The Advisory Committee on Immunization Practices (ACIP) recommends the tetanus, diphtheria, and acellular pertussis (Tdap) vaccine for pregnant women during each pregnancy, regardless of prior immunization status. However, safety data on repeated Tdap vaccination in pregnancy is lacking.

OBJECTIVE—To determine whether receipt of Tdap vaccine during pregnancy administered in close intervals from prior tetanus-containing vaccinations is associated with acute adverse events in mothers and adverse birth outcomes in neonates.

DESIGN, SETTING, AND PARTICIPANTS—A retrospective cohort study in 29 155 pregnant women aged 14 through 49 years from January 1,2007, through November 15,2013, using data from 7 Vaccine Safety Datalink sites in California, Colorado, Minnesota, Oregon, Washington, and Wisconsin.

EXPOSURES—Women who received Tdap in pregnancy following a prior tetanus-containing vaccine less than 2 years before, 2 to 5 years before, and more than 5 years before.

MAIN OUTCOMES AND MEASURES—Acute adverse events (fever, allergy, and local reactions) and adverse birth outcomes (small for gestational age, preterm delivery, and low birth weight) were evaluated. Women who were vaccinated with Tdap in pregnancy and had a prior tetanus-containing vaccine more than 5 years before served as controls.

RESULTS—There were no statistically significant differences in rates of medically attended acute adverse events or adverse birth outcomes related to timing since prior tetanus-containing vaccination.*

*Check METHODS, Study Design, and EXCLUSIONS. Page 5.

	Time Since Prior Tetanus-Containing Vaccination, y		
Outcome	**<2**	**2–5**	**>5 (Control)**
Local reactions, rate/10 000 women	4.2	7.0	11.2
Adjusted risk ratio (95% CI)	0.49 (0.11–2.20)	0.77 (0.31–1.95)	1 [Reference]
P value	.35	.59	
Preterm delivery, %	6.6	6.4	6.8
Adjusted risk ratio (95% CI)	1.15 (0.98–1.34)	1.06 (0.94–1.19)	1 [Reference]
P value	.08	.33	
Small for gestational age, %	9.0	8.7	9.1
Adjusted risk ratio (95% CI)	0.99 (0.87–1.13)	0.96 (0.87–1.06)	1 [Reference]
P value	.88	.45	

CONCLUSIONS AND RELEVANCE—Among women who received Tdap vaccination during pregnancy, there was no increased risk of acute adverse events or adverse birth outcomes for those who had been previously vaccinated less than 2 years before or 2 to 5 years before compared with those who had been vaccinated more than 5 years before. These findings suggest that relatively recent receipt of a prior tetanus-containing vaccination does not increase risk after Tdap vaccination in pregnancy.

Pertussis (whooping cough) is a vaccine-preventable illness that has been increasing in incidence over the past decade in the United States.[1–3] Neonates and infants are at increased risk of pertussis-related hospitalization and death compared with older children and adults. Many public health strategies have been recommended to decrease the burden of pertussis in neonates and infants.[4–6] Most recently, in 2012, the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommended tetanus, diphtheria, and acellular pertussis (Tdap) vaccination for all pregnant women during each pregnancy regardless of prior immunization status.[6]

However, few published studies have evaluated the safety of Tdap vaccine in pregnant women.[7–11] In these studies, Tdap vaccination during pregnancy has not been associated with an increased risk of spontaneous abortion, stillbirth, preterm delivery, low birth weight, neonatal complications, or congenital anomalies compared with unvaccinated pregnant controls. Additionally, 1 retrospective study showed that pregnant women vaccinated with Tdap who had received a prior Tdap vaccine within 5 years had no difference in neonatal outcomes compared with women receiving their first Tdap vaccine in pregnancy.[11]

Most safety studies on administering repeated doses of tetanus-containing vaccines are limited to nonpregnant individuals.[12–14] These studies have shown that intervals less than 5 years between tetanus-containing vaccines can be associated with increased local reactions and fever. Although these studies did not find an increased risk of Arthus reactions (severe dermal inflammation, endothelial damage, and vascular necrosis), this has been a concern with shortened intervals between tetanus vaccine doses.[12–14]

This study focused on determining whether there is association between receipt of Tdap vaccine during pregnancy administered in close intervals from prior tetanus-containing vaccinations and acute adverse events in mothers and adverse birth outcomes in neonates.

Methods

Study Population

The study protocol was reviewed and approved by institutional review boards at Emory University, the CDC, and the 7 Vaccine Safety Datalink (VSD) sites and was determined exempt from requiring participant consent. The study cohort included pregnant women enrolled in the VSD (Figure). The VSD is a collaborative project between the CDC and 9 integrated health care organizations.[15] The VSD includes data on more than 9 million individuals annually (approximately 3% of the US population), with an annual birth cohort of approximately 90 000. Data are collected from standardized files prepared at each site that contain individual demographic, enrollment, immunization, hospitalization, emergency department visits, and outpatient visits. For this study, 7 VSD sites contributed data: Group Health Cooperative (Washington), Kaiser Permanente Northwest (Oregon and Washington), Kaiser Permanente Northern California, Southern California Kaiser Permanente, HealthPartners (Minnesota), Marshfield Clinic (Wisconsin), and Kaiser Permanente Colorado. These sites were chosen because they contribute pregnancy data on a yearly basis. Although the majority of the VSD data comes from the 2 California sites, and thus the western United States, the demographic characteristics of the VSD population have been shown to be generally comparable with that of the entire US population.[16]

Study Design

We conducted a retrospective cohort study among pregnant women vaccinated with Tdap by evaluating medically attended acute adverse events (occurring in outpatient, inpatient, and emergency department settings) in mothers and adverse birth outcomes in their neonates. We compared adverse events between women receiving a prior tetanus-containing vaccine less than 2 years before and 2 to 5 years before with women who had received a prior tetanus-containing vaccine more than 5 years before (controls). We chose these comparisons based on intervals used in prior studies comparing acute adverse events following multiple tetanus-containing vaccines in nonpregnant individuals.[12–14] Prior vaccination status was irrespective of pregnancy status at the time of vaccination.

We identified pregnancies ending between January 1,2007, and November 15, 2013, in automated data using a validated pregnancy algorithm[17] that has been used in prior VSD pregnancy studies.[18,19] This pregnancy episode algorithm uses claims, administrative, and

birth data from the electronic medical record to identify pregnancies, pregnancy outcomes, and gestational age at pregnancy outcome, and has been shown to be accurate within 28 days in confirming the estimated pregnancy start date for 99% of live births and in confirming the pregnancy outcome date for 96% of live births.[17]

We included women aged 14 through 49 years who received Tdap vaccine during pregnancy and had continuous insurance coverage from 6 months prior to pregnancy to 6 weeks postpartum with no more than a 30-day gap in enrollment. We excluded women who had no documentation of prior tetanus-containing vaccines, women who received live vaccines during pregnancy, and women with a multiple gestation pregnancy. We also excluded pregnancies with non-live birth outcomes (stillborn, spontaneous abortion, therapeutic abortion, trophoblastic disease, and ectopic pregnancy) because we did not have the resources to access medical records to confirm the timing of these outcomes in relation to vaccination, which could result in inaccurate findings. Finally, we excluded all women who received non-Tdap tetanus-containing vaccines during pregnancy (ie, tetanus diphtheria [Td]).

We identified vaccinations using electronic medical record and insurance claims data that are captured in the standardized VSD vaccine file. We defined a vaccine administered during pregnancy as one given from 7 days after the woman's last menstrual period through 7 days before the date the pregnancy ended. We used these cutoffs to avoid misclassification of vaccines that might have been given prior to pregnancy or postpartum.[10,19]

Outcome Measures

We compared *International Classification of Diseases, Ninth Revision, Clinical Modification* (*ICD-9-CM*) codes for fever, local reactions (limb pain, limb swelling, cellulitis, lymphadenitis, and Arthus reaction), and allergic reactions (allergy, urti-caria, and anaphylaxis) occurring in intervals of 0 through 3 days and 0 through 7 days following Tdap vaccine, excluding duplicate diagnoses that had been given in the previous 30 days to capture incident cases associated with a health care visit. The day of vaccination was considered day 0, and we excluded any diagnoses on day 0 occurring in the outpatient setting, as they were likely present before the vaccination. As some allergic reactions may occur on day 0 in the outpatient setting, we performed a sensitivity analysis including diagnoses of allergic reactions in the outpatient setting on the day of vaccination. We compared the risk of incident cases of Guillain-Barré syndrome in the inpatient setting, using a 1-through 42-day time window following vaccination. We also examined the following adverse birth outcomes: preterm delivery (defined as gestational age <37 weeks), low birth weight (birth weight <2500 g), and small for gestational age (weight <10th percentile for gestational age and sex).[20]

Statistical Methods

We compared baseline characteristics between the 3 groups of pregnant women who received Tdap vaccine. We used χ^2 tests to compare categorical variables, and analysis of variance to compare continuous variables. We identified all adverse events using *ICD-9-CM* codes. We used log-binomial regression analysis to calculate the relative risks (RRs) for both

rare and non-rare events. Akaike Information Criterion measurements are included as assessments of model fit (eTable1 in the Supplement). We adjusted for differences in gestational age at time of vaccination and VSD site (Kaiser Permanente Northern California, Southern California Kaiser Permanente, or other site) when comparing acute events. When comparing birth outcomes, we also adjusted for maternal age, length of enrollment (in months) in the health plan prior to pregnancy, risk factors for pregnancy adverse events, pregnancy complications, and prenatal care utilization, because these are likely to independently affect birth outcomes. Prenatal care utilization was assessed using the Kotelchuck Adequacy of Prenatal Care Utilization Index, which takes into account the number of prenatal care visits from the time of the first prenatal care visit until delivery.[21] Comorbidities (asthma, diabetes, hypertension, and cardiovascular disease) and pregnancy complications were identified using *ICD-9-CM* codes. Only records that contained information on the neonate (ie, weight and gestational age) were used when analyzing birth outcomes. In addition, only vaccinations given prior to 37 weeks of gestation were included, so as to not bias the results for preterm delivery and low birth weight. All analyses were performed using SAS (SAS Institute), version 9.3.

We performed a priori power calculations based on an expected sample size of 24 000 women and determined that we had 80% or higher power to detect an RR greater than 2 for all of our birth outcomes. However, analyses for medically attended acute adverse outcomes, which are rare, were under-powered. To detect an RR greater than 2 for local reactions, 10 000 participants would be needed in each cohort; for fever, 37 000 participants; and for allergic reactions, 75 000 participants. We considered results to be statistically significant at an a error less than .05 using 2-tailed tests.

Results

From January 1, 2007, through November 15,2013, there were a total of 633 542 singleton pregnancies recorded in the VSD sites (Figure). After applying exclusion criteria, we identified 61 311 pregnancies in which a single Tdap vaccine was given. We excluded 32 156 pregnancies (52%) because there was no prior history of a tetanus-containing vaccine documented. Our final analytic data set included 29 155 pregnancies. Of these pregnancies, 4812 women (17%) had a prior tetanus-containing vaccine less than 2 years before, 9999 women (34%) 2 to 5 years before, and 14 344 women (49%) more than 5 years before (controls).

Among the 29 155 pregnancies ending from 2007 through 2013, the majority of Tdap vaccinations were administered from 2010 through 2013 (98.1%), and most were administered in 2013 (54.0%). In the overall cohort, Tdap was most often administered in the third trimester (67.4%). Fewer women received the vaccine in the second trimester (27.5%) and the first trimester (5.1%). Maternal age, length of enrollment, and gestational age at Tdap vaccination were significantly different in the 3 study groups ($P = <.001$) (Table 1). In addition, most pregnant women who received a prior tetanus-containing vaccine less than 2 years before (94%) and 2 to 5 years before (85%) their current Tdap vaccine had previously received Tdap (as opposed to a non-Tdap tetanus-containing vaccine) vs only 17% of controls ($P < .001$).

Acute Outcomes

Overall, acute adverse events after vaccination were rare (eTables 2–3 in the Supplement). There were no statistically significant differences in fever, allergic reactions, or local reactions among women who had received their prior tetanus-containing vaccine less than 2 years before and 2 to 5 years before compared with controls (Table 2). Fever beginning 0 through 3 days after vaccination occurred at a rate (per 10 000 women) of 2.1 in those who received Tdap and had a prior tetanus-containing vaccine less than 2 years before compared with 3.5 among controls (adjusted RR, 0.66 [95% CI, 0.07–5.77]; $P = .70$). Allergic reactions beginning 0 through 3 days after vaccination occurred at a rate (per 10 000 women) of 2.1 in women who received Tdap and had a prior tetanus-containing vaccine less than 2 years before (adjusted RR, 1.55 [95% CI, 0.13–18.45]; $P = .73$) and 1.0 in those receiving it 2 to 5 years before (adjusted RR, 0.71 [95% CI, 0.06–8.13]; $P = .78$) compared with 1.4 among controls. Local reactions beginning 0 through 3 days after vaccination occurred at a rate (per 10 000 women) of 4.2 in women who received Tdap and had a prior tetanus-containing vaccine less than 2 years before (adjusted RR, 0.49 [95% CI, 0.112.20]; $P = .35$) and 7.0 in those receiving it 2 to 5 years before (adjusted RR, 0.77 [95% CI, 0.31–1.95]; $P = .59$) compared with 11.2 among controls. There was no increased risk of allergic reactions based on the sensitivity analysis including outpatient diagnoses occurring on day 0 (eTable 4 in the Supplement). There were no cases of anaphylaxis, Arthus reactions, or Guillain-Barré syndrome following vaccination.

Birth Outcomes

There were no statistically significant differences in adverse birth outcomes among women who had received their prior tetanus-containing vaccine less than 2 years before and 2 to 5 years before compared with controls (Table 3). Preterm delivery occurred in 6.6% of women who received Tdap and had a prior tetanus-containing vaccine less than 2 years before (adjusted RR, 1.15 [95% CI, 0.98–1.34]; $P = .08$) and 6.4% of those receiving it 2 to 5 years before (adjusted RR, 1.06 [95% CI, 0.94–1.19]; $P = .33$) compared with 6.8% of controls. Low-birth-weight delivery occurred in 4.7% of women who received Tdap and had a prior tetanus-containing vaccine less than 2 years before (adjusted RR, 1.10 [95% CI, 0.92–1.32]; $P = .31$) and4.7% of those receiving it2to5 years before (adjusted RR, 1.03 [95% CI, 0.89–1.18]; $P = .72$) compared with 5.1% of controls. Small for gestational age delivery occurred in 9.0% of women who received Tdap and had a prior tetanus-containing vaccine less than 2 years before (adjusted RR, 0.99 [95% CI, 0.87–1.13]; $P = .88$) and 8.7% of those receiving it2to5 years before (adjusted RR, 0.96 [95% CI, 0.87–1.06]; $P = .45$) compared with 9.1% of controls.

Discussion

To our knowledge, this is the first study to evaluate medically attended acute adverse outcomes in mothers following Tdap vaccine in pregnancy looking specifically at intervals since receipt of prior tetanus-containing vaccinations. We did not find any differences in acute events in the mothers or adverse birth outcomes in neonates when comparing women who were vaccinated with Tdap during pregnancy regardless of the length of time since a prior tetanus-containing vaccine. Our findings should reassure patients and clinicians who

might be hesitant to give Tdap vaccine to pregnant women who recently received a Tdap or other tetanus-containing vaccination.

Our findings are similar to another retrospective cohort study evaluating women receiving Tdap in pregnancy who had a prior pregnancy with Tdap vaccine administered within 5 years compared with multiparous women with no prior Tdap vaccine in pregnancy.[11] This study found no difference in gestational age at delivery, stillbirth, major malformations, neonatal care admissions, ventilation requirements, and neonatal death, whereas ours focused on preterm delivery, small for gestational age, and low birth weight.* The prior study did note a small increase in average birth weight of neonates of women receiving multiple Tdap vaccines. Our study did not compare actual birth weights, but rather compared the presence of low-birth-weight (<2500 g) delivery, and did not find a statistically significant difference.

*This was a much smaller study and did not consider the fact that pregnant women in the group declining Tdap vaccination may have chosen to do so because they had high risk pregnancies.

"...the cohort of women who declined vaccination suggests that Tdap vaccine nonacceptance may identify a high-risk group..."

Our findings contrast with some studies in other populations that suggest an increased risk of adverse events when tetanus-containing vaccines are given at short intervals, most of which evaluated differences in solicited adverse events.[12–14] In 2006, a clinical trial of 7156 children found that Tdap vaccine was well tolerated when given at intervals as short as 18 months since prior tetanus-containing vaccines; however, there was an increase in solicited injection site swelling and erythema in participants who received a tetanus-containing vaccine more recently.[12] A VSD retrospective cohort study of 436 828 Td vaccinations demonstrated that medically attended local reactions, including cellulitis, were more common among persons who received a Td-containing vaccine within the last 5 years compared with a longer interval.[13] Another study assessed safety in 4524 Tdap-vaccinated health care workers during a pertussis outbreak in New England.[14] Overall, there was no difference in the rates of solicited moderate or severe injection site reactions, but there was an increase in redness, swelling, and subjective fever among patients who had received their prior Td-containing vaccine less than 2 years earlier. Among 20 pregnant women included in that study, only 1 person reported severe swelling and 2 reported feeling feverish without documented fever. All symptoms in these pregnant women resolved without treatment, and all neonates were born at term with normal newborn evaluations. None of the pregnant women had received a prior tetanus-containing vaccine 2 years before their Tdap vaccination.

One explanation of the apparent paucity of acute adverse events with short tetanus vaccination intervals in our study could be related to shifts in immunological responses that occur during pregnancy.[22] These include shifts in humoraland cellular-mediated immunity and natural killer cells that occur to protect the fetus from harm. Among other changes, there may be less inflammation that occurs in response to vaccinations, which may result in fewer adverse events following multiple tetanus-containing vaccinations given in close proximity. Another explanation could be that we relied exclusively on medically attended adverse events, which are rare, whereas the majority of prior studies included solicited adverse events. Therefore, milder reactions that do not come to medical care might not have been included.

Our study has some limitations. We had limited power for the acute adverse events analysis. However, the rates of acute adverse events were generally not more common in pregnant women who had more recent tetanus-containing vaccinations. We also excluded women with no prior documented tetanus-containing vaccination, which comprised 52% of the Tdap-vaccinated cohort, to reduce misclassification. Although it is unlikely that these women never received a tetanus-containing vaccine in the past, this exclusion allowed for more conservative estimates of risk as the cohorts were not diluted with women that were potentially previously unvaccinated. There is the potential for some confounding due to differences in the type of vaccine received because the majority of the women in our study who were vaccinated with tetanus-containing vaccines less than 2 years before received Tdap and those vaccinated more than 5 years before had previously received Td. Additionally, we did not review medical charts to validate the adverse events, which would correct for any potential overestimation of the rates of acute reactions following Tdap in pregnancy. Although this is important, we would expect any resulting misclassification bias to be nondifferential, and not to affect our overall results. Finally, the VSD population is an insured population, and these findings may not be generalizable to the entire US population. However, demographic characteristics of the VSD population, including race, ethnicity, income, and education, have been shown to be generally comparable with the population of the United States.[16]

Future studies are needed to determine if there are differences in other important adverse pregnancy outcomes, such as stillbirth and spontaneous abortion, when Tdap is given in pregnancy in close intervals from prior tetanus-containing vaccines.

Conclusions

Among women who received Tdap vaccination during pregnancy, there was no increased risk of acute adverse events or adverse birth outcomes for those who had been previously vaccinated less than 2 years before or 2 to 5 years before compared with those who had been vaccinated more than 5 years before. These findings suggest that relatively recent receipt of a prior tetanus-containing vaccination does not increase risk after Tdap vaccination in pregnancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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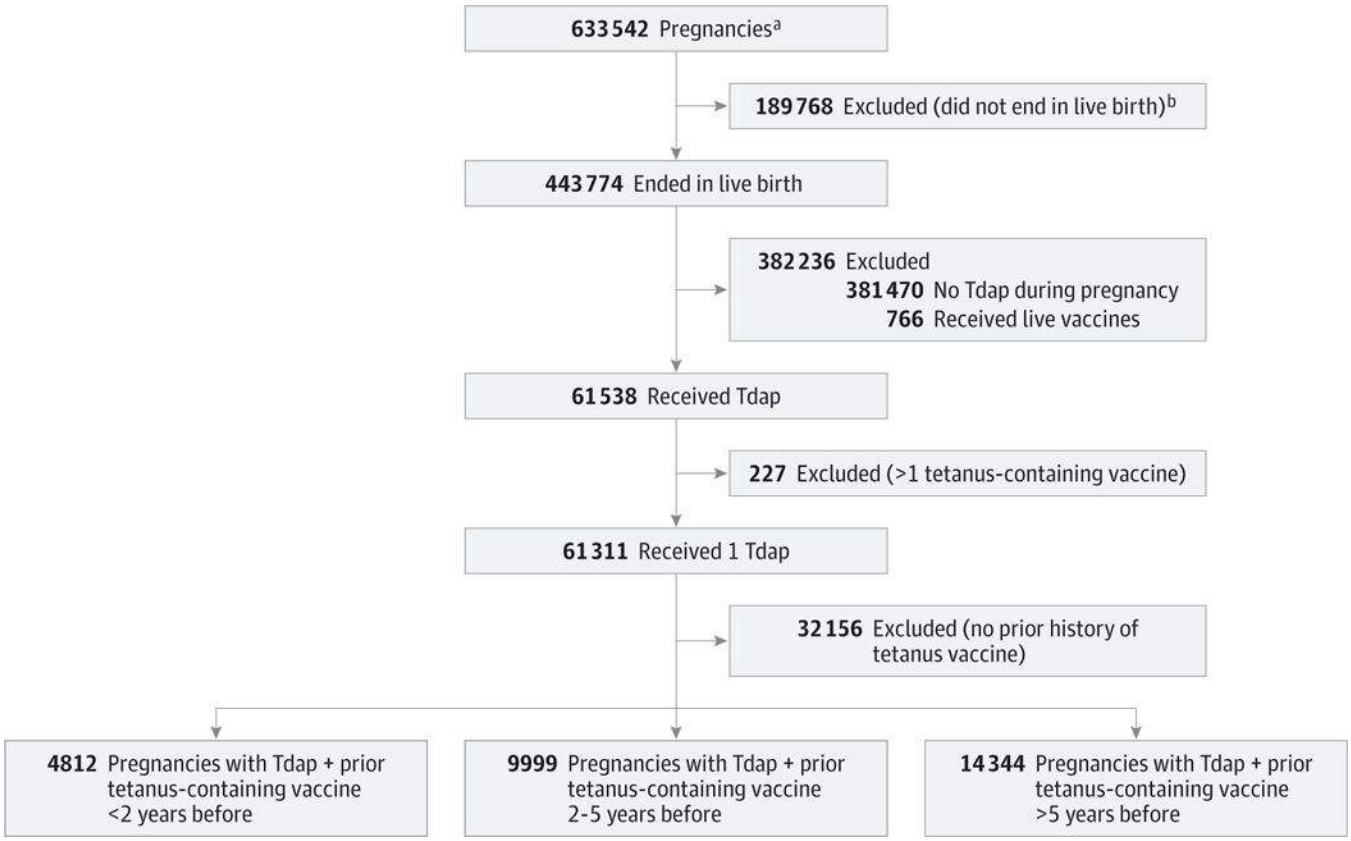

Figure. Tdap Vaccinations Received During Pregnancy From January 1, 2007, Through November 15, 2013, Recorded in 7 Vaccine Safety Datalink Sites

Tdap indicates tetanus, diphtheria, and acellular pertussis.

[a] Singleton pregnancies.

[b] Not live birth includes stillbirth, spontaneous abortion, therapeutic abortion, trophoblastic disease, ectopic pregnancy, and unknown outcomes.

Table 1.

Selected Demographic Characteristics of Pregnant Women Who Received Tdap During Pregnancy by Vaccination Status Recorded in the Vaccine Safety Datalink Sites From January 1, 2007, Through November 15, 2013

Variable	No. (%) Time Since Prior Tetanus-Containing Vaccination, y <2 (n = 4812)	2–5 (n = 9999)	>5 (Control) (n = 14 344)	*P* Value
Maternal age, mean (range), y	30.5 (15–49)	30.7 (14–49)	28.8 (14–48)	<.001
Enrollment prior to pregnancy, mean (range), mo	49.8 (6.0–99.9)	62.4 (6.0–100.5)	63.9 (6.0–99.8)	<.001
Gestational age at Tdap, mean (range), wk	30 (1–39)	30 (1–41)	27 (1–40)	<.001
Adequate prenatal care[a]	3629 (75)	7324 (73)	10 542 (73)	.01
Other vaccines in pregnancy	3012 (63)	6179 (62)	8996 (63)	.33
Maternal comorbidity[b]	1383 (29)	2956 (30)	4394 (31)	.03
Pregnancy complication[c]	2514(52)	5230 (52)	7565 (53)	.74
Prior tetanus vaccine Tdap[d]	4542 (94)	8511 (85)	2477 (17)	<.001

Abbreviation: Tdap, tetanus, diphtheria, and acellular pertussis.

[a]Adequate or adequate plus prenatal care based on Kotelchuck Adequacy of Prenatal Care Utilization Index.

[b]Presence of hypertension in pregnancy, diabetes, cardiovascular disease, or asthma.

[c]Includes any of the following diagnoses: fetal abnormality affecting maternal management, fetal or placental problems affecting maternal management, polyhydramnios, oligohydramnios, premature rupture of membranes, amnionitis, antepartum hemorrhage, placental abruption, placenta previa, or antepartum complications.

[d]Compared with non-Tdap tetanus vaccines (ie, tetanus diphtheria; tetanus toxoid; diphtheria and tetanus toxoids and acellular pertussis, etc).

Table 2.

Acute Outcomes Following Tdap Vaccination in Pregnancy By Interval Since Prior Tetanus-Containing Vaccination

	Time Since Prior Tetanus-Containing Vaccination, y						Relative Risk (95% CI)			
	<2 (n = 4812)		2–5 (n = 9999)		>5 (Control) (n = 14 344)		<2 y vs Control		2–5 y vs Control	
Outcome	No. of Patients	Rate[a]	No. of Patients	Rate[a]	No. of Patients	Rate[a]	Unadjusted[b]	Adjusted[b,c]	Unadjusted[d]	Adjusted[c,d]
Fever										
0–3 d	1	2.1	0	0	5	3.5	0.60 (0.07–5.10)	0.66 (0.07–5.77)		
0–7 d	3	6.2	1	1.0	6	4.2	1.49 (0.37–5.96)	1.61 (0.39–6.66)	0.24 (0.03–1.99)	0.21 (0.03–1.80)
Allergic Reaction										
0–3 d	1	2.1	1	1.0	2	1.4	1.49 (0.14–16.4)	1.55 (0.13–18.45)	0.72 (0.07–7.91)	0.71 (0.06–8.13)
0–7 d	2	4.2	4	4.0	5	3.5	1.19 (0.23–6.14)	1.32 (0.24–7.17)	1.15 (0.31–4.27)	1.23 (0.32–4.80)
Local Reaction										
0–3 d	2	4.2	7	7.0	16	11.2	0.37 (0.09–1.62)	0.49 (0.11–2.20)	0.63 (0.26–1.53)	0.77 (0.31–1.95)
0–7 d	6	12.5	17	17.0	22	15.3	0.81 (0.33–2.00)	1.01 (0.40–2.56)	1.11 (0.59–2.09)	1.28 (0.66–2.47)

Abbreviation: Tdap, tetanus, diphtheria, and acellular pertussis.

[a] Rate per10 000 women.

[b] Tdap + prior tetanus less than 2years before compared with Tdap + prior tetanus more than 5years before.

[c] Adjusting for Vaccine Safety Datalink site and gestational age at vaccination in weeks.

[d] Tdap + prior tetanus 2 to 5years before compared with Tdap + prior tetanus more than 5 years before.

Table 3.

Adverse Birth Outcomes Following Tdap Vaccination in Pregnancy by Interval Since Prior Tetanus-Containing Vaccination

	No. (%)			Relative Risk (95% CI)			
	Time Since Prior Tetanus-Containing Vaccination, y			<2 y vs Control		2–5 y vs Contro	
Outcome	<2 (n = 3313)	2–5 (n = 7226)	>5 (Control) (n = 10 633)	Unadjusted[a]	Adjusted[a,b]	Unadjusted[c]	Adjusted[b,c]
Preterm delivery[d]	218 (6.6)	460 (6.4)	723 (6.8)	0.97 (0.84–1.12)	1.15 (0.98–1.34)	0.94 (0.84–1.05)	1.06 (0.94–1.19)
Low birth weight[e]	156 (4.7)	342 (4.7)	543 (5.1)	0.92 (0.78–1.10)	1.10 (0.92–1.32)	0.93 (0.81–1.06)	1.03 (0.89–1.18)
Small for gestational age[f]	298 (9.0)	629 (8.7)	996 (9.1)	0.99 (0.87–1.12)	0.99 (0.87–1.13)	0.96 (0.87–1.05)	0.96 (0.87–1.06)

Abbreviation: Tdap, tetanus, diphtheria, and acellular pertussis.

[a] Tdap + prior tetanus less than 2 years before compared with Tdap + prior tetanus more than 5 years before.

[b] Adjusting for gestational age at Tdap vaccination in weeks, Vaccine Safety Datalink site, length of enrollment (in months), prenatal care utilization index, maternal comorbidity, pregnancy complication, and maternal age.

[c] Tdap + prior tetanus 2 to 5 years before compared with Tdap + prior tetanus more than 5 years before.

[d] Gestational age of less than 37 weeks.

[e] Birth weight of less than 2500 g.

[f] Weight of less than the 10th percentile for gestational age and sex.

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Infant Hospitalizations and Mortality After Maternal Vaccination

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Abstract

BACKGROUND: The Advisory Committee on Immunization Practices currently recommends pregnant women receive influenza and tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccines. There are limited studies of the long-term safety in infants for vaccines administered during pregnancy. We evaluate whether maternal receipt of influenza and Tdap vaccines increases the risk of infant hospitalization or death in the first 6 months of life.

METHODS: We included singleton, live birth pregnancies in the Vaccine Safety Datalink between 2004 and 2014. Outcomes were infant hospitalizations and mortality in the first 6 months of life. We performed a case-control study matching case patients and controls 1:1 and used

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conditional logistic regression to estimate odds ratios for maternal exposure to influenza and/or Tdap vaccines in pregnancy.

RESULTS: There were 413 034 live births in our population. Of these, 25 222 infants had hospitalizations and 157 infants died in the first 6 months of life. We found no association between infant hospitalization and maternal influenza (adjusted odds ratio: 1.00; 95% confidence interval [CI]: 0.96–1.04) or Tdap (adjusted odds ratio: 0.94; 95% CI: 0.88–1.01) vaccinations. We found no association between infant mortality and maternal influenza (adjusted odds ratio: 0.96; 95% CI: 0.54–1.69) or Tdap (adjusted odds ratio: 0.44; 95% CI: 0.17–1.13) vaccinations.

CONCLUSIONS: We found no association between vaccination during pregnancy and risk of infant hospitalization or death in the first 6 months of life. These findings support the safety of current recommendations for influenza and Tdap vaccination during pregnancy.

The Advisory Committee on Immunization Practices currently recommends 2 vaccines to be given during each pregnancy; influenza vaccine has been recommended at any time during pregnancy since 2004 to prevent maternal influenza disease and complications[1] and tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine has been recommended during each pregnancy since 2012, with a preference for administration between 27 and 36 weeks' gestation, to protect infants from pertussis disease.[2] Given the relative proximity of an immunization administered during pregnancy to a potential infant hospitalization or death, an observed temporal association with maternal influenza or Tdap vaccine during pregnancy and infant death or hospitalization may raise concerns about a possible causal relationship.

Both pertussis and influenza infections are associated with hospitalizations and fatalities in infants, and severity is highest before infants are eligible for the respective vaccines. Approximately half of infants <4 months of age with pertussis require hospitalization, and the majority of deaths from pertussis occur in these infants.[3] In 2014, the US pertussis case rate in infants <6 months of age was 169 per 100 000 infants.[4] Furthermore, there were 8 deaths in infants <3 months of age and 1 death in infants 3 to 11 months of age out of 13 total deaths from pertussis in all age groups in 2014. Similarly, infants are at high risk of hospitalization and death from influenza. The US influenza hospitalization rate ranges from 1.8 to 7.2 per 1000 in infants <6 months of age.[5] For the 2013–2014 influenza season, there were 96 laboratory-confirmed, influenza-associated pediatric deaths, 18 of which occurred in children aged <6 months.[6] Maternal immunization with influenza and Tdap vaccines allows for passive antibody transfer and protection to infants for the respective diseases when they are most vulnerable.[1,2,7]

In 2015, the infant (≤12 months) mortality rate in the United States was 589.5 per 100 000 live births,[8] and the leading causes of infant deaths were (1) congenital malformations, deformations, and chromosomal abnormalities; (2) disorders related to low birth weight and short gestation; and (3) sudden infant death syndrome. In 2010, the leading causes of hospitalizations in infants ≤12 months were (1) acute bronchitis (238 per 10 000 population), (2) jaundice (104 per 10 000 population), and (3) pneumonia (56 per 10 000 population).[9] Although there have been reassuring safety data for influenza and Tdap vaccines in which maternal acute events, pregnancy complications, and birth outcomes were

evaluated,[10–19] there have been limited safety studies beyond the immediate neonatal period.[20–24] Vaccine safety continues to be a primary reason why providers and patients choose not to vaccinate during pregnancy.[25–27] Although the biologic plausibility is unclear for the association of maternal vaccination and infant hospitalization or death, there may be concerns of long-term effects on infants after any pregnancy exposure. In this study, we evaluate whether maternal receipt of influenza and Tdap vaccines increases the risk of hospitalization or death in US infants in the first 6 months of life.

METHODS

Study Population

The Vaccine Safety Datalink (VSD) is a collaboration between the Centers for Disease Control and Prevention and 8 integrated health care systems (sites) and includes vaccination and health care data on ~10 million persons per year.[28] In addition, the VSD includes data on ~ 125 000 pregnant women annually.

We used data on pregnant women from 5 VSD sites with available data that comprise over 90% of the VSD population: Kaiser Permanente Northern California (Oakland, CA), Kaiser Permanente Southern California (Pasadena, CA), Kaiser Permanente Colorado (Denver, CO), Marshfield Clinic Research Foundation (Marshfield, WI), and Kaiser Permanente Northwest (Portland, OR).

We used the validated VSD Pregnancy Episode Algorithm to identify pregnant women.[29] The Pregnancy Episode Algorithm uses comprehensive electronic medical record and administrative databases (including diagnosis and procedure codes, laboratory tests, pharmacy records, and imaging procedures) to identify pregnancies, pregnancy outcomes, and pregnancy start and end dates, and it is able to link pregnant women to their infants. We included women from the VSD with pregnancies ending in a live birth between January 1, 2004, and June 30, 2014. We required pregnant women to be enrolled at a VSD site for the duration of the pregnancy episode and to have at least 1 prenatal care visit. To increase completeness of data, infants of these pregnant women were required to have a birth record and to have VSD site enrollment until 6 months of life or until the time of death. We excluded pregnancies in which a live vaccine was administered because live vaccines are contraindicated in pregnancy. We also excluded infants of multiple gestation pregnancies, infants born before 34 weeks' gestation, and infants with major birth defects because these infants are at a higher risk of hospitalization and death. Furthermore, we excluded all infants who died during their delivery hospitalization because cause of death in these infants is often a perinatal complication (such as placental abruption) that would likely be unrelated to maternal vaccination. Additionally, infants who die during the birth hospitalization may be less likely to be enrolled in the VSD and captured in our data.* We also excluded infants with external causes of death (*International Classification of Diseases, 10th Revision* [ICD-10] codes S00-T98 and V00-Y98) and infants with external causes of hospitalizations (*International Classification of Diseases, Ninth Revision* [ICD-9] codes 800–999, E800-E999) due to injury and poisonings because these are unlikely to result from a maternal vaccination. ICD-10 coding was not available for hospitalization diagnoses in the United States during the time of this study.

***They are admitting that the Vaccine Safety Datalink is not capturing data on infants who die during the birth hospitalization.**

Case-Control Matching

Among infants meeting inclusion criteria, those infants with hospitalizations or deaths within the first 6 months of life were included in this analysis. Respiratory hospitalization case patients were a subset of hospitalization case patients defined by any respiratory ICD-9 code (033, 460–488, 491–496, 510–519) associated with a hospitalization in the first 6 months of life. For infants with >1 hospitalization, the first hospitalization was selected for each category (ie, first all-cause hospitalization, first respiratory hospitalization). Furthermore, an infant could be included as a death case patient and hospitalization case patient if the infant was hospitalized and later died. In the VSD, deaths are identified from state death records, electronic medical records, and administrative sources, and there is approximately a 1-year lag from the time of death to the availability of state death records. Because of lag time in the death data, we evaluated deaths occurring from January 1, 2004, to December 31, 2013, and hospitalizations from January 1, 2004, to December 31, 2014. Matched controls for the infant mortality analysis were selected among infants in the study who survived the first 6 months of life. Matched controls for the infant hospitalization and respiratory hospitalization analyses were selected from infants without death or hospitalization in the first 6 months of life. All infant controls were required to have at least 1 diphtheria-tetanus-acellular pertussis (DTaP) vaccine recorded between 6 weeks and 6 months of age to ensure infants were accessing the health care system. We matched case patients and controls 1:1 using optimal matching.[30] Case patients and controls were matched on the basis of VSD site, birth month and year (within 1 month), and gestational age groups of late preterm (34–36 weeks), term (37–41 weeks), and postterm (42–44 weeks). With our optimal matching, we successfully found controls for 100% of our case patients by using these parameters.

Vaccinations

The exposure of interest was maternal vaccination with any influenza and/or Tdap vaccines during pregnancy. A vaccine during pregnancy was defined as one given from 7 days after the pregnancy start date to 7 days before the pregnancy end date. These time windows were chosen to avoid including exposures to vaccinations given before or immediately after pregnancy. We stratified vaccine exposures as any influenza vaccine (with or without Tdap), any Tdap vaccine (with or without influenza), and both influenza and Tdap vaccines in the same pregnancy. In our evaluation of maternal influenza vaccine, we also repeated our analysis limiting outcomes to events occurring during the influenza season (October through May), to ascertain any protective findings that may be more evident when influenza virus is circulating. We also did a sensitivity analysis stratifying our exposure by influenza vaccine only and Tdap vaccine only to see if our results would differ by limiting our exposure groups.

Statistical Analysis

We measured rates of influenza and Tdap maternal vaccination in our study cohort from 2004 to 2013. We also measured trends of infant deaths and hospitalizations during this same time period to look for any ecological associations between maternal vaccination and our infant outcomes. For our main analysis, we performed a conditional logistic regression

analysis to estimate the odds of maternal vaccination in matched case patients and controls. In our analysis, we determined a priori to include the following potential confounders from electronic VSD data sources[28]: Kotelchuck Adequacy of Prenatal Care Index,[31] race and ethnicity (non-Hispanic African American or American Indian versus other races and ethnicities), maternal age, pregnancy complications and maternal comorbidities (hemorrhage, hypertensive disorders, renal disease, diabetes, thyroid disease, cardiovascular disease, epilepsy), smoking during pregnancy (yes, no, or unknown), infant DTaP exposure before outcome (or index date in matched controls), duration of birth hospitalization in days, and gestational age at delivery in weeks.

We also reviewed medical records of infants with respiratory related deaths (ICD-10 codes: A37, J00–J99). We reviewed clinical information relating to a potential influenza- or pertussis-related cause of death and laboratory data in the 2-week period preceding death. For influenza laboratory data, we looked for positive influenza A or B rapid antigen, polymerase chain reaction (PCR), viral culture, and direct fluorescent antibody test results in all respiratory death case patients. For pertussis, we looked for positive *Bordetella pertussis* PCR and culture test results for any death case patient with the ICD-10 code A37 (whooping cough).

We determined a priori that with an expected average exposure rate of 15% for both vaccines throughout the study period,[32–34] we would need at least 840 case patients to have 80% power to detect an odds ratio of 1.5. The protocol for this study was approved by the Centers for Disease Control and Prevention Institutional Review Board and institutional review boards at each of the participating VSD sites. All analyses were conducted by using SAS version 9.3 (SAS Institute, Inc, Cary, NC).

RESULTS

During our study period, we identified 500 447 pregnancies ending in a live birth that met enrollment criteria. We excluded 87 413 (17.5%) because of maternal or infant factors (Fig 1). Of the remaining 413 034 infants, 25 222 infants had 1 or more hospitalizations and 157 infants died. Of the hospitalized infants, 4644 (18.4%) had a respiratory cause for their hospitalization; 105 (2.2%) of these infants had an influenza ICD-9 code (487, 488), and 137 (3%) had a pertussis ICD-9 code (033.0, 033.9). Of the deaths, 14 (9%) had a respiratory cause of death; however, none of these deaths were considered to have been caused by influenza or pertussis infections on the basis of our laboratory and medical record review. Of the 157 infants that died, the age at death ranged from 1 to 180 days with a mean of 61 days and a median of 51 days. The most common causes of death were unknown causes (32%), sudden infant death syndrome (21%), and certain conditions originating in the perinatal period (17%).

We analyzed overall trends of influenza and/or pertussis vaccination in pregnancy and trends of infant hospitalization and mortality in our study population from 2004 to 2013 (Fig 2). From 2004, there was an increase in maternal influenza vaccination, which became more dramatic in 2009 after the H1N1 influenza pandemic. Maternal Tdap vaccination increased starting in 2010 when California recommended pregnant women to receive Tdap in

pregnancy in response to the 2010 statewide pertussis epidemic.[35] There was another increase in Tdap vaccination in 2012 after the most recent Advisory Committee on Immunization Practices recommendation to administer Tdap vaccination in every pregnancy.[2] We observed no increase in the infant hospitalization rate or infant mortality rate during the same time period.

We matched case patients with eligible controls and compared characteristics between these groups (Table 1, Supplemental Table 3). Infants who were hospitalized were more likely to have mothers with pregnancy complications, less likely to be delivered by cesarean delivery, and less likely to be of African American non-Hispanic or American Indian race. Mean maternal age, gestational age at delivery, and length of birth hospitalization were statistically significantly different between the groups but not clinically different. Infants who died were similar to matched controls.

In our adjusted analysis, we found no significant association between infant hospitalization or death in the first 6 months of life and receipt of maternal influenza and/ or Tdap vaccines and no significant association between infant hospitalization from respiratory causes and maternal influenza vaccine (Table 2). However, the odds of maternal Tdap vaccination was significantly lower among infants with hospitalizations because of respiratory causes (adjusted odds ratio: 0.79; 95% confidence interval [CI]: 0.67–0.94; $P = .007$) compared with controls without hospitalization. Furthermore, when evaluating infant hospitalizations and death occurring during periods of influenza virus circulation (October through May) and peak influenza virus circulation (November through February), we found no association with maternal influenza vaccine exposure (data not shown). When limiting our exposure groups to women receiving influenza vaccine without Tdap vaccine and Tdap vaccine without influenza vaccine, our results were similar to our main analysis (Supplemental Table 4).

DiSCUSSiON

In our study of maternal influenza and Tdap vaccines, we found no increased risk of infant all-cause hospitalizations, hospitalizations from respiratory causes, or all-cause mortality in the first 6 months of life. Our study helps strengthen the growing evidence of long-term safety of vaccination in pregnancy for infants.

Our findings are similar to other studies that have evaluated infant mortality and morbidity after maternal vaccination in pregnancy, most of which have evaluated the safety of adjuvanted H1N1 influenza-containing vaccines. Studies of short-term infant mortality in the first 7 days of life,[20] growth and development and health care visits for infections in the first year of life,[23] early neonatal or childhood death,[22] and childhood hospitalization rates,[21] have not found an increased risk of these outcomes in children of women who received adjuvanted H1N1 influenza–containing vaccines in pregnancy. Unlike these previous studies, however, our study included women who received any type of influenza vaccine, none of which contain adjuvants in the United States, and we found similar results.

Our findings are also consistent with studies in which researchers have evaluated infant mortality and morbidity after Tdap vaccination in pregnancy. These researchers have

evaluated neonatal mortality,[10,36] NICU admissions,[37] length of hospitalization, ventilation requirement, intraventricular hemorrhage, transient tachypnea of the newborn, neonatal sepsis, pneumonia, respiratory distress syndrome, and convulsions.[36,38] There were no differences in outcomes between infants of Tdap-vaccinated and unvaccinated mothers in these studies. Our study included a longer follow-up period than these previous studies and still showed no increased risk of infant mortality or hospitalization after maternal Tdap vaccination.

Other long-term outcomes that have previously been studied after maternal Tdap vaccination include childhood development scores at 13 months of life,[13] infant growth up to 5 to 7 months of age,[24] and complex chronic conditions at 12 months of age.[17] The researchers for these studies did not find an increased risk of these infant outcomes after maternal Tdap vaccination during pregnancy. Our study managed a larger number of infants and had similar findings to these studies, further demonstrating long-term safety in infants of Tdap vaccine exposure in pregnancy.

We did find a protective association between maternal Tdap during pregnancy and infant respiratory hospitalizations, which is consistent with results of other published studies that have looked at infant pertussis as an outcome.[7,39–42] However, only 3% of infants hospitalized for respiratory causes had a pertussis ICD-9 code. This could indicate that infants with pertussis are not being appropriately diagnosed and tested.[43] It is also possible that other factors (eg, the healthy adherer effect[44] and other differences in people who choose vaccination and those who do not) are contributing to this finding.

This study does have some limitations. The VSD captures data on an insured population, which could translate to better health outcomes than the general population. Additionally, VSD has a high rate of women with adequate prenatal care on the basis of the Kotelchuck index, which can translate to better infant outcomes.[31] A recent study has revealed that despite being a fully insured population, the VSD is comparable to the total US population on many important demographic factors.[45] Moreover, the VSD population size is large, and even groups that typically comprise a smaller proportion of insured populations (ie, lower income populations) still have a substantial (>2 million individuals) presence in the VSD. There may have been bias related to requiring controls to have a DTaP vaccine record to be included in the study. We did this to ensure we had access to health care utilization data to avoid misclassifying case patients as controls. To look for bias, we repeated our analysis of hospitalizations requiring case patients to have a DTaP vaccine (98.0% of case patients) and found similar results to our main findings. We looked at broad safety outcomes (hospitalizations, respiratory hospitalizations, and deaths) and may not capture true increases in a specific outcome, if such an association was present. We relied on vaccination data from our VSD electronic data files and may not have captured vaccines in pregnancy occurring outside the health care system. However, previous internal work looking at influenza vaccination in pregnancy revealed that the VSD vaccine files are over 98% complete in capturing these data (J. Donahue, DVM, PhD, unpublished observations). We did not evaluate the risks of infant hospitalizations and mortality in multiple gestation infants, very preterm infants, and those with major birth defects because these infants are at a much higher risk of the outcomes we studied; therefore, our results are not generalizable to these

populations. Finally, we were sufficiently powered for our outcomes of hospitalizations and hospitalizations from respiratory causes but underpowered for the outcome of death.

This is the first study in which infant hospitalizations and mortality in the first 6 months of life after maternal influenza vaccine and Tdap vaccines are evaluated. In this large case-control study, we found no increased risk of infant hospitalization and death after vaccination in pregnancy.

Our findings support the safety of influenza and pertussis vaccinations during pregnancy for infants of vaccinated mothers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CI	confidence interval
DTaP	diphtheria-tetanus-acellular pertussis
ICD-9	*International Classification of Diseases, Ninth Revision*
ICD-10	*International Classification of Diseases, 10th Revision*
PCR	polymerase chain reaction
Tdap	tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis
VSD	Vaccine Safety Datalink

Biography

Dr Sukumaran conceptualized and designed the study, participated in data collection, conducted the analysis, drafted the initial manuscript, and reviewed and revised the manuscript; Ms McCarthy participated in the conceptualization and design of the study, designed the data collection instruments, collected data, participated in the analysis, and reviewed and revised the manuscript; Mr Weintraub participated in the conceptualization and design of the study, participated in the analysis, and critically reviewed the manuscript; Drs Kharbanda, Vazquez-Benitez, Lipkind, Jackson, Klein, Naleway, McClure, Hechter, Kawai, and Glanz contributed to the acquisition and interpretation of data and reviewed and revised the manuscript; all authors critically reviewed the final manuscript; and all authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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WHAT'S KNOWN ON THIS SUBJECT:

Influenza and tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccines are recommended in pregnancy. Although there is evidence that these vaccines are safe in pregnant women, there are limited long-term data on infants born to mothers vaccinated during pregnancy.

WHAT THIS STUDY ADDS:

Influenza and tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccines in pregnancy are not associated with an increased risk of hospitalization or death in infants. Our findings contribute to the knowledge of the long-term safety of vaccination during pregnancy.

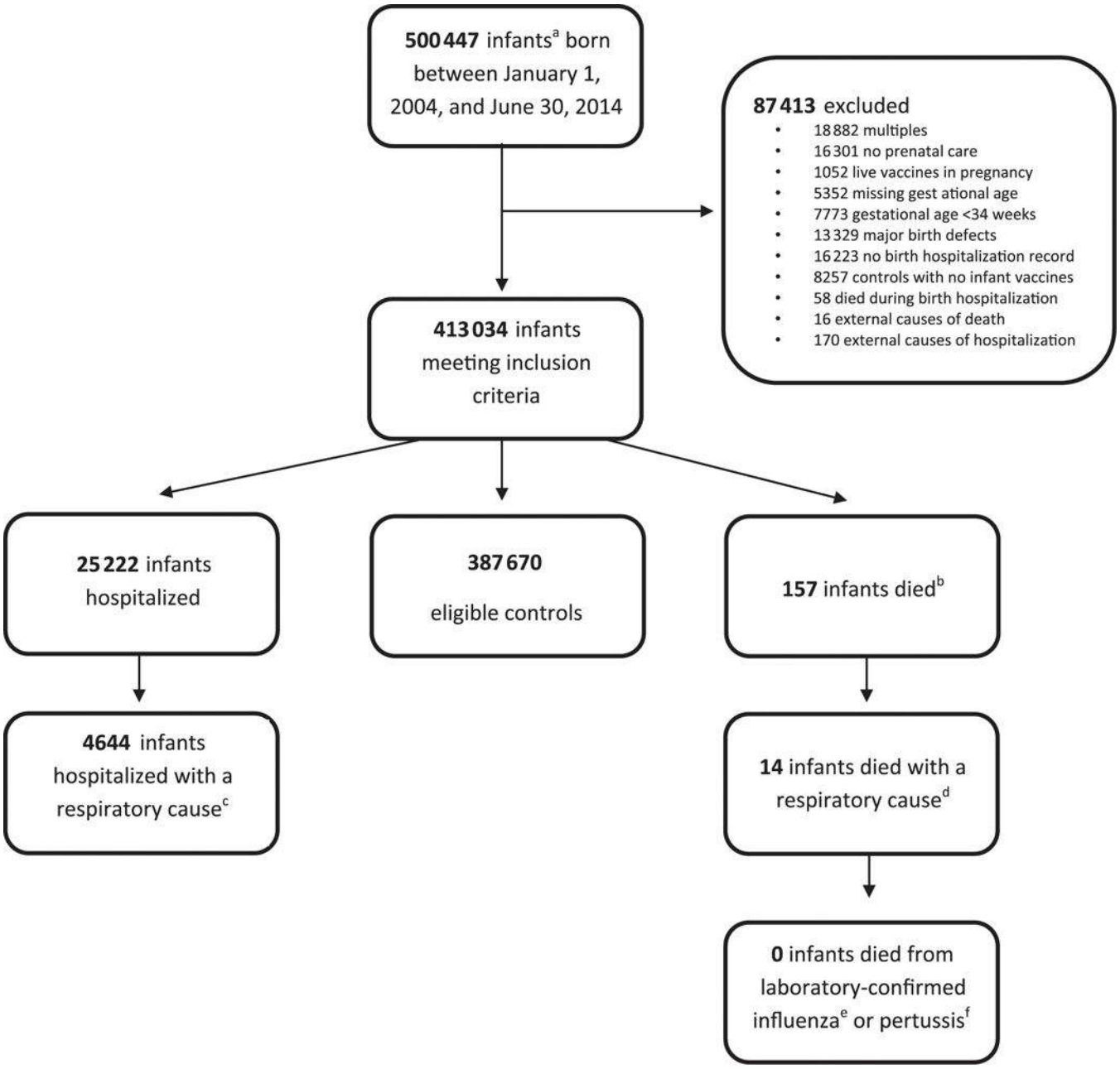

FIGURE 1.

Study population of infants with hospitalization or death in the first 6 months of life in the VSD, 2004–2014. [a] Infants with continuous enrollment in the VSD until 6 months of age or until the time of death whose mothers were enrolled for the duration of their pregnancy. [b] Fifteen infants were hospitalization and death case patients. [c] Defined as ICD-9 codes: 033, 460–488, 491–496, 510–519. [d] Defined as ICD-10 codes: A37, J00–J99. [e] Positive influenza A or B antigen, viral culture, PCR, or direct fluorescent antibody test results within 14 days of hospitalization. [f] Positive *B pertussis* PCR or culture test results within 14 days of hospitalization.

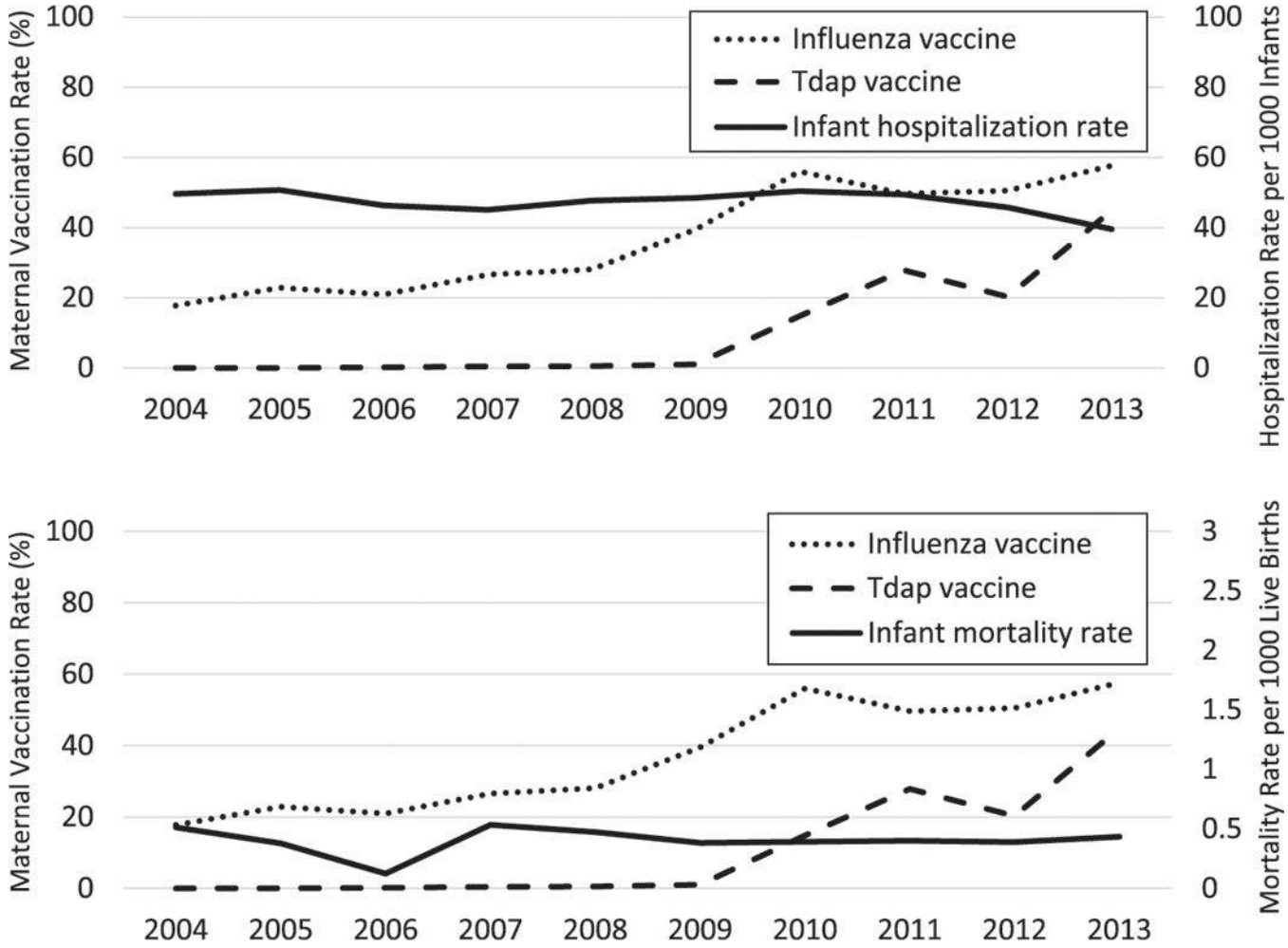

FIGURE 2.

Rates of maternal influenza and Tdap vaccination, infant hospitalization, and infant mortality in the VSD, 2004–2013.

TABLE 1

Characteristics of Matched Case Patients and Controls for Infant Hospitalizations and Mortality in the First 6 Months of Life in the VSD, 2004–2014

	Hospitalization Case Patients (*n* = 25 222)	Matched Controls (*n* = 25 222)	*P*[a]	Death Case Patients (*n* = 157)	Matched Controls (*n* = 157)	*P*
Mean age at event in d (range)	36 (1–183)	—	—	61 (1–180)	—	—
Mean gestational age at delivery in wk (range)	39 (34–43)	39 (34–43)	<.0001	39 (34–41)	39 (34–41)	.88
Mean maternal age in y (range)	31 (13–54)	31 (13–55)	.0005	30 (15–41)	30 (16–41)	.23
Mean length of delivery hospitalization in d (range)	2.2 (0–103)	2.2 (0–110)	<.0001	3.5 (0–41)	2.2 (0–18)	.43
Smoking during pregnancy, %	8.9	9.2	.39	15	10	.35
Pregnancy complications, %[b]	31.0	28.7	<.0001	34	27	.14
Cesarean delivery, %	23.6	27.9	<.0001	36	31	.52
Adequate prenatal care by Kotelchuck index, %[31]	94.2	93.9	.30	92	92	.92
African American non-Hispanic or American Indian race, %	5.9	7.2	<.0001	10	6	.14

—, not applicable.

[a] *P* values calculated by χ^2 tests for categorical variables and Wilcoxon median 2-sample tests for continuous variables.

[b] Pregnancy complications include hemorrhage, hypertensive disorders, renal disease, diabetes, thyroid disease, cardiovascular disease, and epilepsy.

TABLE 2

Matched Case-Control Analysis of Infant Hospitalizations and Death in the First 6 Months of Life in the VSD After Maternal Vaccination, 2004–2014

	1:1 Matched Analysis of Hospitalizations (*n* = 50 444)			1:1 Matched Analysis of Respiratory Hospitalizations (*n* = 9288)			1:1 Matched Analysis of Deaths (*n* = 314)		
Vaccine in pregnancy	Influenza[a]	Tdap[b]	Both[c]	Influenza	Tdap	Both	Influenza	Tdap	Both
Case patients exposed, %	38.7	12.8	8.6	38.4	9.9	7.1	32	6	3
Controls exposed, %	39.3	13.4	9.0	38.1	11.4	7.4	37	12	8
Crude OR (95% CI)	0.97 (0.93–1.01)	0.91 (0.85–0.97)	0.94 (0.87–1.01)	1.02 (0.93–1.12)	0.79 (0.67–0.93)	0.93 (0.78–1.13)	0.81 (0.49–1.31)	0.41 (0.17–0.99)	0.25 (0.07–0.89)
aOR[d] (95% CI)	1.00 (0.96–1.04)	0.94 (0.88–1.01)	0.97 (0.90–1.05)	1.08 (0.97–1.19)	0.79 (0.67–0.94)	0.97 (0.80–1.17)	0.96 (0.54–1.69)	0.44 (0.17–1.13)	0.32 (0.08–1.24)
P[e]	.93	.09	.44	.15	.007	.73	.87	.09	.10

aOR, adjusted odds ratio; OR odds ratio.

[a] Influenza vaccine in pregnancy given with or without Tdap vaccine.

[b] Tdap vaccine in pregnancy given with or without influenza vaccine.

[c] Both influenza and Tdap vaccines given in the same pregnancy.

[d] Adjusting for pregnancy complications, adequacy of prenatal care, smoking during pregnancy, race, maternal age, infant DTaP receipt before event, length of birth hospitalization in days, and gestational age at delivery in weeks.

[e] *P* values correspond to the aOR.

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Medical Education

Institute of Medicine (US) Committee on Conflict of Interest in Medical Research, Education, and Practice; Lo B, Field MJ, editors. Conflict of Interest in Medical Research, Education, and Practice. Washington (DC): National Academies Press (US); 2009.

5 Conflicts of Interest in Medical Education

Medical education prepares physicians for a lifetime of professional work. Education that is objective and that teaches students how to critically evaluate the evidence prepares physicians to keep current with scientific advances throughout their professional lives.

This chapter is organized around the concept of the learning environment, which shapes and reinforces the professional attitudes and behavior of physicians throughout the continuum of learning that begins in medical school and extends through residency training and to lifelong learning. Learning environments in medicine are diverse. They include conference rooms and lecture halls, patient care locales (such as inpatient service and outpatient practice locations), laboratories, and the Internet. Some continuing education programs take place at restaurants or resorts.

If the learning environment provides the stage for education, the curriculum provides the script. Reviews of undergraduate and graduate medical education often emphasize the "formal curriculum" (i.e., required courses and explicit educational objectives).[1] That formal curriculum aims to help students develop the core competencies that are defined by accreditation agencies. Each educational activity has learning objectives, and the totality of educational sessions must address all the core competencies.

The learning environment also includes two other elements: the informal curriculum (i.e., ad hoc interactions among teachers and students) and the hidden curriculum (i.e., institutional practices and culture) (see, e.g., Hafferty [1998], Ratanawongsa et al. [2005], Cottingham et al. [2008], and Haidet [2008]). Ideally, these two elements convey messages that are consistent with the formal curriculum, but in practice they may not. For example, the formal curriculum might include course work on medical ethics, research methodology, and appropriate relationships with industry. Concurrently, the informal and hidden curricula might be characterized by disparaging faculty comments on their institution's conflict of interest policies and the failure of institutions to adopt and implement sound policies.

Unfortunately, some aspects of each curriculum may contribute to undesirable attitudes or practices. The Association of American Medical Colleges (AAMC) observed in a 2008 report that the conflicts created by a range of common interactions with industry can "[f]or medicine generally, and for academic medicine in particular … have a corrosive effect on three core principles of medical professionalism: autonomy, objectivity, and altruism" (AAMC, 2008c. 4). Members of the U.S. Congress have also expressed concern about commercial relationships in medical education, primarily continuing medical education (see, e.g., Finance Committee, U.S. Senate [2007]). In contrast to the requirements for recipients of U.S. Public Health Service research awards, the federal government does not require the recipients of direct or indirect funds for medical education to establish and administer conflict of interest policies.

This chapter next provides a brief background on the current context of medical education. It then examines the literature on conflict of interest issues and responses in the learning environments of undergraduate, graduate, and continuing medical education. The discussion covers access to educational environments by sales representatives of medical product companies (e.g., drug detailing, which is a visit to a doctor by a sales representative for a pharmaceutical company), the provision of drug samples and other gifts to faculty and students, and industry-sponsored scholarships and fellowships. A separate section considers a concern that cuts across all phases of education: intellectual independence in presentations and publications and the risks associated with speakers bureaus and ghostwritten publications. (Chapter 4 discussed concerns about how researcher conflicts of interest might affect their advice or supervision involving the research of medical students, residents, fellows, and junior faculty.)

The committee concluded that, in general, industry financial relationships do not benefit the educational missions of medical institutions in ways that offset the risks created. The chapter thus ends with recommendations that are intended to protect the integrity and limit the potential for undue industry influence in medical education. As explained in Chapter 1, the committee focused on conflicts of interest involving physicians and biomedical researchers; but much of the core rationale for the recommendations may be relevant to nursing, pharmacy, dentistry, and other professions, even though

some of the specifics might differ. Chapter 6 considers many of the same issues in the context of physicians in practice outside academic settings.

BACKGROUND AND CONTEXT

Scale and Oversight of Medical Education

American medical education evolved during the 19th and early 20th centuries from pure apprenticeships to proprietary medical schools of variable quality to a reformed and formal educational system that stresses both science and professionalism. During the middle decades of the 20th century, an increasingly elaborate structure of graduate (post-M.D.) medical education emerged, characterized by multiyear residencies in medical specialties beyond the traditional internship year. The latter half of the century saw the growth of requirements by state licensing boards and specialty certification boards for demonstrated participation in accredited continuing education activities (Caplan, 1996).

Today, the scale of American medical education is impressive. The United States has

- 130 accredited medical schools (AAMC, 2008d),[2] approximately 400 major teaching hospitals (Salsberg, 2008), more than 100,000 faculty members (Salsberg, 2008), and approximately 75,000 medical students (AAMC, 2008e);
- 8,355 accredited residency programs for 126 specialties and subspecialties (2006–2007) and more than 107,000 active full-time and part-time residents (2005–2006) (ACGME, 2007b); and
- 740 national providers of accredited continuing medical education (and 1,600 accredited state providers)[3] that reported more than 7 million physician participants in their programs (ACCME, 2008a, 2009), a number that includes multiple registrations among the nation's more than 800,000 active physicians (a count that includes medical residents) (Salsberg, 2008).

The Liaison Commission on Medical Education (LCME) is the oversight agency that is responsible for the accreditation of the nation's medical schools. Its members are appointed by AAMC and the American Medical Association (AMA). The Accreditation Council for Graduate Medical Education (ACGME) accredits residency training programs in the United States. The sponsoring institution for a residency program may be a hospital, medical school, university, or group of hospitals (ACGME, 2008). Accreditation bodies define the core competencies for students, residents, and fellows and ensure that the formal curriculum covers all essential aspects of medical education. ACGME board members are appointed by AAMC, AMA, the American Board of Medical Specialties, the American Hospital Association (AHA), and the Council of Medical Specialty Societies (CMSS). Accredited continuing medical education providers are accredited by the Accreditation Council for Continuing Medical Education (ACCME). Its member organizations are AHA, AMA, AAMC, CMSS, the Association for Hospital Medical Education, and the Federation of State Medical Boards. State medical societies may also accredit providers within a state.[4] In addition, AMA, the American Academy of Family Physicians, and certain other groups set standards and certify credits for specific courses that physicians can take (from accredited providers) to meet state licensure board and other requirements for accredited continuing medical education (see, e.g., AMA [2006, 2008b]).[5] Accredited providers usually issue certificates to document that a physician has completed a certified course. Consistent with common usage, this report uses the phrase accredited continuing medical education to refer to education that is (1) presented by accredited providers and (2) certified for course credits.

Changing Environment and Fiscal Challenges

Academic medical centers dominate the provision of undergraduate and graduate medical education. The institutions consist of two related enterprises: a medical school that trains physicians and conducts research and a system that provides health care services. The latter system may include teaching hospitals, satellite clinics, and physician office practices. Academic health centers include other health professions schools, such as a school of dentistry, nursing, or pharmacy (Wartman, 2007).

In recent years, academic medical centers have struggled financially because of low levels of payment for poor and uninsured patients, reductions in the Medicare indirect medical education adjustment for hospital payment rates, and lower profit margins for the provision of hospital services to Medicare patients. (In the late 1990s, medical schools also

faced declining admissions, but admissions increased from 2003 to 2007 [AAMC, 2008a].) At the same time, teaching hospitals have faced rising costs because of the incorporation of new medical informatics systems and expensive medical technologies and restrictions on the numbers of hours that residents may work. The Medicare Policy Advisory Commission has characterized 53 percent of major teaching hospitals as being under high financial pressure—compared to 28 percent of hospitals overall (MedPAC, 2009). Given these circumstances, financial support from industry may seem attractive.

Physicians in training also face financial challenges. In 2006, the median levels of debt of medical students graduating from public and private medical schools were $120,000 and $160,000, respectively (Jolly, 2007). Medical school graduates can expect to pay approximately 9 to 12 percent of their after-tax income after graduation for educational debt service (Jolly, 2007). This level of indebtedness and the delayed gratification of a profession that requires years of training before independent practice is permitted can contribute to a sense of entitlement, which, in turn, may position medical students, residents, and fellows to be strongly influenced by gifts and attention from representatives of pharmaceutical and medical device companies (see, e.g., Levine [2008]). Sierles and colleagues (2005) found that 80 percent of the medical students that they surveyed believed that they were entitled to gifts. In addition, as discussed in Chapter 6, once they are in practice, limits on reimbursements for physician services make debt repayment more of a burden than in the past and may make gifts and other financial relationships with industry more appealing.

Industry Funding of Medical Education

During most of the 20th century, medical product companies were not major participants in medical education. The exception was sales representatives, who provided information to residents and faculty as well as to nonacademic physicians. In the latter decades of the century, however, medical product companies became increasingly involved in sponsoring continuing medical education, including grand rounds and other academic-based programs. In a 2008 report on industry funding of medical education, a task force of AAMC observed generally that

> Over recent decades, medical schools and teaching hospitals have become increasingly dependent on industry support of their core educational missions. This reliance raises concerns because such support, including gifts, can influence the objectivity and integrity of academic teaching, learning, and practice, thereby calling into question the commitment of academia and industry together to promote the public's interest by fostering the most cost-effective, evidence-based medical care possible. (AAMC, 2008c. iii)

The committee found no data on the amount or proportion of undergraduate or graduate medical education supported by industry. It also found little systematic information on specific categories of financial support, for example, grants for residencies or fellowships, direct or indirect financial support for grand rounds, or donations for buildings or other capital items. The most extensive information on academic institutions' ties with industry comes from a 2006 survey of department chairs at medical schools and the 15 largest independent teaching hospitals (67 percent response rate). The responses indicated that 65 percent of clinical departments received industry support for continuing medical education, 37 percent received industry support for residency or fellowship training, 17 percent received industry support for research equipment, and 19 percent received unrestricted funds from industry for department operations (Campbell et al., 2007b). The committee did not categorize industry payments for meals, gifts, and visits by sales representatives as support for medical education because these activities do not fit the learning objectives in the formal curriculum.

Information on industry funding for accredited continuing medical education comes from yearly surveys by ACCME. Figure 5-1 shows that commercial sources (excluding advertising and exhibits at programs organized by accredited providers) provide a substantially larger share of income for education providers today than they did in 1998. By 2003, about half of all funding for accredited continuing medical education programs came from commercial sources. The fees paid by program attendees once provided the majority of provider income, but today industry-supported programs are often provided free or at reduced cost to physicians (Steinbrook, 2008a).

LEARNING ENVIRONMENTS IN MEDICAL SCHOOLS AND RESIDENCY PROGRAMS

The ultimate mission of medical education is to prepare physicians to provide effective, safe, high-quality, efficient, timely, affordable, and patient-centered care to patients. In revising the standards that provide the framework for essential aspects of medical education, both LCME and ACGME have recently emphasized how the learning environment can affect the development of core professional values and core competencies, including how to critically review the evidence and to commit to lifelong learning about scientific advances.

Both LCME and ACGME recognize the power of the local learning environment to shape the knowledge, skills, behaviors, and attitudes of the next generation of physicians. To achieve accreditation, institutions providing undergraduate or graduate medical education must have curricula and resources that, among other requirements, (1) promote the development of appropriate professional attributes; (2) help learners at all levels think critically and appraise the evidence base for research reports, practice guidelines, and marketing materials; and (3) provide appropriate role models and mentoring. In addition, a standard on the creation of the appropriate learning environment must be implemented (LCME Standard MS-31-A). Recently, ACGME has required institutions to have a statement or institutional policy that addresses interactions between vendor representatives or corporations and residents and their programs (Requirement III. B.13 [ACGME, 2007a]).

The Learning Environment in Undergraduate and Graduate Medical Education as a Target of Industry Influence

Scope of Relationships Between Industry and Students, Medical Schools, and Teaching Hospitals

Interactions between medical students and industry are common. Table 5-1 summarizes the results from a survey of third-year medical students at eight major medical schools. Almost all students had received an industry-provided lunch or other gift. More than one-third had attended a social event hosted by a drug company.

Information from two surveys of residency directors similarly documents frequent interactions with pharmaceutical companies. For example, a 2002 survey of emergency medicine residency program directors found that approximately 40 percent allowed industry to fund social activities, and a similar percentage allowed pharmaceutical representatives to teach residents (Keim et al., 2004). Twenty-nine percent said that industry travel support could be made contingent on residents attending an industry event. Only 50 percent said that they always or very frequently followed ACGME recommendations for industry funding of core lectures, and 10 percent said that they always or very frequently allowed pharmaceutical representatives unrestricted access to residents. In a 2002 survey of psychiatric residency program directors, 88 percent reported that they allowed industry to provide lunches for their residents, and among this group, the mean was about five lunches per week (Varley et al., 2005). Approximately a third of the programs solicited travel funds from industry (31 percent) or allowed residents to seek such funding from industry on their own (34 percent).

Value of Relationships

Some interactions with industry can have educational value, for example, when an industry scientist participates in a seminar on drug development strategies or when a device company representative provides supervised training on a complex and innovative medical device that has recently been approved for marketing. Other examples may include unrestricted grants to academic medical centers that support student or resident research stipends or participation in scientific conferences. On a much larger scale, universities have benefited from company gifts for buildings, research programs, and auditoriums.

Pharmaceutical companies argue that their representatives provide information on new drugs. Yet, medical students, residents, and fellows have ready access to the latest scientific information through faculty members, information technologies that allow them to search the medical literature, and open-access sources of evidence-based literature reviews and summaries. The committee recognizes that some medical students and residents who have become accustomed to interactions with representatives may value the meals that they receive as a respite and may view the gifts that they bring as either inconsequential or as an appropriate reward for their demanding schedules and economic sacrifices.

The discussion below focuses on several different types of academic-industry relationships and the literature about their consequences. Each section includes a discussion of private- and public-sector responses to concerns about the extent and consequences of these relationships. In addition to consulting reports by AAMC and other groups, the committee examined the policies of a number of medical schools. It found many of these policies at or available through links from the websites of the American Medical Student Association (AMSA) and the Institute on Medicine as a Profession (IMAP). The AMSA website also includes the organization's scorecard, which presents school-by-school ratings of various policy elements (e.g., the policy on the acceptance of gifts) and which has received considerable attention from the media.[6]

The committee notes that the recommendations in the 2008 AAMC report on medical education apply off campus as well as on campus. The report calls for academic medical centers to "communicate to off-site training facilities their expectation that the off-site venues will adhere to the standards of the academic center regarding interactions with industry" (AAMC, 2008c. 10).

Site Access by Drug and Device Company Representatives

Issues and Evidence

Drug detailing, that is, a visit to a doctor by a sales representative for a pharmaceutical company, is a common way that companies promote their products and establish relationships with physicians in academic and community settings. In 2004, an estimated 36 percent of the $57.5 billion that pharmaceutical companies spent on product promotion went for detailing (Gagnon and Lexchin, 2008).

Medical device companies also employ sales representatives to promote their products to physicians and hospitals, although the responsibilities of some of these representatives may be more complex. They may provide training, equipment calibration, and additional services or advice related to implants and other sophisticated technologies used in the operating room and elsewhere (see, e.g., ECRI Institute [2007]). In one instance, the Food and Drug Administration (FDA) has required physicians to be trained by company representatives as a condition for the approval of a device (see, e.g., FDA [2004b] and Dawson [2006]).

The committee did not locate any information about how drug or device detailing activity differs between academic and nonacademic settings or how specific tactics of detailing and their effects may vary by setting or type of physician (e.g., resident versus faculty member versus community physician). Interactions with drug company representatives are common in academic settings. Medical students average about one interaction with drug company representatives a week, and 80 to 100 percent of students report interactions (see, e.g., Bellin et al. [2004], Sierles et al. [2005], and Fitz et al. [2007]). As described by one faculty member,

> [d]rug company representatives are a major presence. They sponsor Journal Club (where trainees learn to review new data and research), they pay for many of our weekly speakers and regularly offer free dinners for the residents and faculty. They enjoy free access to our mailboxes and regularly detail our trainees in their offices, hallways and in our little kitchen. (Shapiro, 2004, p. F5)

Medical students and residents reported that they received insufficient training in interacting with drug representatives. Studies also indicate that students and residents believe that their own prescribing behavior is not affected by drug company gifts, although they believe that the prescribing behavior of their colleagues is (Sierles et al., 2005; Zipkin and Steinman, 2005). Limited evidence suggests that educational interventions "show some promise" in affecting the attitudes and behaviors related to relationships with industry (Carroll et al., 2007).

Overall, research suggests that drug company representatives may influence prescribing patterns and requests for additions to hospital formularies. The effects appear to be modest but consistent across various kinds of research and disciplines. One review concluded that the "pharmaceutical industry has a significant presence during residency training, has gained the overall acceptance of trainees, and appears to influence prescribing behavior" (Zipkin and Steinman, 2005, p. 777). Another review (which was not limited to educational settings) concluded that detailing "affects physician

prescription behavior in a positive [i.e., the more detailing that there is, the more of an effect that it has] and significant manner" (Manchanda and Honka, 2005, p. 787).

Taken together with the information reviewed below on the role of drug samples and gifts (which typically accompany sales visits), the literature suggests that academic medicine and the public have reason to be concerned about the easy access of sales representatives to medical students, residents, and faculty. In addition, the committee could find no evidence that the exposure of students and residents to drug and device sales representatives—without additional training and supervision—contributes to the achievement of learning objectives or the development of core competencies, for example, increasing an individual's ability to critically evaluate presentations or promoting adherence to evidence-based clinical practice guidelines.

Responses

AAMC has recommended tight limits on site access by sales representatives from medical product companies, particularly uninvited and unscheduled visits and unsupervised access to individual students and residents (see Box 5-1) (see, e.g., AMSA [2008a] and AAMC [2008c]). The recommended rules for device representatives are somewhat less stringent than those for drug representatives and allow limited exceptions for training on the use of complex new devices and the other activities mentioned above. A number of medical schools and teaching hospitals have adopted policies consistent with the AAMC recommendations.

A quality assurance and risk management document prepared by the ECRI Institute (2007) recommends several additional safety and administrative provisions for device representatives who are allowed access to the operating room.[7] The recommendations include training requirements for device representatives as well as procedures to ensure patient safety, privacy, and informed consent and to prevent kickbacks (ECRI Institute, 2007). In addition, the ECRI Institute document suggests that medical schools have not provided adequate training in the use of devices. It emphasizes that hospitals and physicians are responsible for seeing that personnel have the appropriate training on the use of the devices that they regularly use, so that reliance on device representatives is limited and appropriately supervised.

Drug Samples

Issues

Physicians and patients often value drug samples provided as gifts because they allow physicians to send a patient home with a medication that can be evaluated for its short-term effects and side effects without requiring the patient to fill and pay for a full prescription. For low-income patients, many of whom are treated at academic medical centers and teaching hospitals, samples can provide access to needed medications (Daugherty, 2005). Some research has, however, suggested that poor or uninsured patients are somewhat less likely than higher-income or insured patients to receive a drug sample (Cutrona et al., 2008). Drug samples may also be used by physicians themselves or their families. In a 1997 survey of residents, 32 percent of all medications used by residents were obtained from drug sample cabinets or directly from drug representatives (Christie et al., 1998). As discussed in Chapter 6, some professional societies approve such use.

Other research points to risks associated with physician acceptance of drug samples. In academic medical centers, drug samples may be associated with the prescription of new brand name drugs in situations in which the sample drugs are different from the physician's preferred drug or are not recommended by evidence-based practice guidelines or in situations in which less expensive drugs or generic equivalents are available for the same indication. One study of a sample of university-based physicians' responses to several clinical scenarios found that from 17 to 82 percent of the physicians would dispense a drug sample, and, in two of three scenarios, a great majority would do so instead of using their usually preferred drug—largely on the grounds that use of the sample would avoid costs to the patient (Chew et al., 2000). Residents were more likely than attending physicians to report that they used drug samples. In a second study, which involved residents in an inner-city clinic, half were randomized to forgo the use of available free drug samples. They were more likely than the control group to choose unadvertised drugs and were more likely to use over-the-counter drugs. The authors concluded that access to drug samples influences residents' prescribing decisions (Adair and Holmgren, 2005). A third study found that physicians who prescribed angiotensin-converting enzyme inhibitors or calcium channel blockers (a departure from the recommendations of the Joint National Commission on High Blood

Pressure Treatment) were more likely than other physicians to report that they provided patients with samples of antihypertension medications (Ubel et al., 2003). This relationship persisted even after physician and practice variables were taken into account.

Responses

Concerns about the possible negative effects of drug samples have led some academic health centers to restrict or ban their provision. For example, some medical schools require drug samples to be received and distributed by a medical center pharmacy and prohibit their direct provision to individual physicians (see, e.g., University of Massachusetts [2008]). Other policies may allow donation of products only for purposes of evaluation or education and not to support "patient care purposes on an ongoing basis" (University of California, 2008, p. 4). When the University of Michigan Health System (2007) prohibited the distribution of drug samples in patient care and non-patient care areas, it provided committee-approved vouchers for starter medications for clinic patients and for limited exceptions if a clinic director believed that a sample of a specific drug was clinically necessary. The most common provision among the policies reviewed by the committee was a prohibition on the personal use of samples by physicians or their family members.

AAMC (2008c) recommends that samples—if their distribution is by the institutions—should be centrally managed, when feasible (e.g., when timely access to the medications is possible). It warns that the "acceptance and use of drug samples transmits the message to students and trainees that information about samples received from industry sales personnel is sufficient without independent critical evaluation" (p. 16). The recommendation does not mention the personal use of samples by physicians or their family members or staff.

In a March 2009 report, the Medicare Payment Advisory Commission recommends that the U.S. Congress require manufacturers and distributors of drugs to report their distribution of drug samples. It also recommends that the secretary of the U.S. Department of Health and Human Services make the information available for analysis through data use agreements.

Gifts from Medical Product Companies

Issues

As noted earlier in this chapter, surveys indicate that almost every medical student has received a meal and a small noneducational gift from a drug company and that other interactions are common as well (see, e.g., Sigworth et al. [2001], Bellin et al. [2004], Sierles et al. [2005], and Fitz et al. [2007]). In one study, residents were asked to empty their pockets of pens, penlights, calipers, and other items (Sigworth et al., 2001). Ninety-seven percent of the residents had at least one item marked by a pharmaceutical insignia, and about half of the items carried by residents were so branded. More than 90 percent of the residents said that they thought that interactions with drug company representatives influenced their prescribing.

The committee found no studies documenting an educational benefit of these kinds of gifts from industry. Although medical students or residents may find the gift of an expensive textbook welcome, nothing similar to the benefits of academic-industry collaboration in biomedical research has been argued for gifts from industry in medical education.

In contrast, studies of medical personnel combined with social science research provide reasons for concern about the risks of industry relationships and gifts, even small gifts. The paper by Jason Dana in Appendix D reviews this literature. It suggests that even small gifts can be influential. Furthermore, because influence may operate at an unconscious level, it can distort the choices of people who believe that they are objectively making decisions. Disclosure of interests and education about bias may be useful, but they cannot be relied upon to overcome the potential for undue influence and bias associated with conflicts of interest. A number of studies suggest that medical residents, faculty, and other physicians tend to think that they themselves are less likely than others to be influenced by gifts or other interactions (see, e.g., McKinney et al. [1990], Steinman et al. [2001], Halperin et al. [2004], Zipkin and Steinman [2005], and Morgan et al. [2006]).

Few studies have specifically investigated the effects of industry relationships on teaching. One study compared the attitudes of internal medicine residents and faculty about the impact of gifts or income from industry on teaching within and outside the institution (Watson et al., 2005). In general, students were more likely than faculty to perceive industry

influence in association with gifts or income. Both students and faculty perceived visiting attending faculty as more susceptible to such influence than regular faculty, and both perceived off-site teaching as more subject to influence than on-site activities. For example, residents were more likely than faculty to believe that gifts or income from industry influences how attending physicians teach on rounds (47 versus 34 percent), during in-hospital lectures and journal clubs (58 versus 30 percent), and during out-of-hospital dinner lectures and journal clubs (80 versus 57 percent). For responses about the effects on visiting attending physicians, the numbers were even higher, with 89 percent of residents and 72 percent of faculty reporting that they believed that gifts or income from industry affected teaching by this group during out-of-hospital dinner lectures and journal clubs. Moreover, 62 percent of residents and faculty believed that annual income or gifts of less than $10,000 could influence an attending physician's teaching. Sixty-five percent of residents and 74 percent of faculty preferred that speakers disclose all financial relationships with industry rather than just report relationships that speakers considered relevant to the educational topic. Although these findings are from a single study in a single institution, they do raise particular concerns about presentations given outside the medical school setting.

Responses

AAMC (2008c) recommends that schools ban the acceptance of industry-supplied food or meals, except in association with ACCME-accredited educational programs. This ban should apply both on and off campus. A few universities (e.g., the University of Michigan and Yale University by 2005) initiated restrictions some years before the AAMC statement. Schools that ban vendor-provided meals on campus (e.g., Stanford University) may not be explicit about the acceptance of meals at off-site locations, although several schools (e.g., Yale University) also discourage this.

As discussed in more detail in Chapter 6, AMA allows gifts of modest value that are viewed as having some benefit to patients (e.g., meals as part of an educational activity) or the physician's practice (e.g., notepads). The policies of several medical centers (e.g., Wake Forest University, Case Western Reserve University, and the University of Minnesota) are similar to this policy.

In addition to policy changes within the academic community, the Pharmaceutical Research and Manufacturers of America (PhRMA) recently revised its voluntary *Code on Interactions with Healthcare Professionals* (PhRMA2008, effective 2009). Except for the section on scholarships and education funds, the document does not refer specifically to interactions in academic settings. As discussed further in Chapter 6, the revised code more strongly discourages "noninformational" physician-company relationships, such as the provision of tickets to sporting events, token consulting arrangements, speaker training programs at resorts, and meals by sales representatives outside a physician's office or other medical setting.

Industry-Sponsored Scholarships and Training Positions

Issues

Little information on the extent of industry funding for undergraduate and graduate medical education is available, although AAMC has stated that medical schools have become increasingly dependent on such funding for such major activities. The committee is aware of industry-funded residencies or fellowships in a few areas, for example, dermatology residencies funded by companies making dermatologic products (Kuehn, 2005); industry-funded fellowships in rheumatology (Goldblum and Franzblau, 2006); and industry support for psychiatry resident fellowships, awards, and the Chief Resident Leadership Conference (APA, 2008).

The rationale for industry funding of residencies and fellowships seems to rest on physician or researcher shortages in certain specialties and the desire to attract more individuals to these areas through additional industry-supported training positions. For example, the American Academy of Dermatology (AAD) launched an initiative in 2004 to fund 10 dermatology residency positions (Kuehn, 2005). The AAD created a fund to accept donations from the academy, pharmaceutical companies, and other interested parties. Awards were assigned to 10 university programs ($60,000 per year for 3 years), and no recipient would be identified as having been funded by a particular company or companies.

Responses

AAMC (2008c) recommends that academic medical centers establish and implement policies requiring that industry funds for scholarships and similar purposes be given centrally to the administration of the medical center. In addition, industry should have no involvement in the selection of recipients, and no "quid pro quo [should] be involved in any way" (p. 21). The objective is to "prevent the establishment of one-on-one relationships between industry representatives and students and trainees" and minimize "the possibility that these funds will be perceived or used as direct gifts" (p. 21). The committee supports the AAMC recommendations. AMA and PhRMA both permit industry funding of scholarships for medical students, residents, or fellows to attend carefully selected educational conferences when the selection of recipients is made by the academic or training institution.

Changing the Environment or Creating Educational Interventions

To the extent that industry influence operates at an unconscious level, the most effective strategies for reducing the risk of undue influence may involve changing the environment in ways that eliminate or reduce the source, especially when the source offers little or no countervailing educational benefit. That is a major rationale for the policies cited above that eliminate gifts, meals, and other noneducational interactions from the learning environment. Some evidence suggests that the learning environment influences attitudes. Two studies have reported that residents who trained in environments that restricted interactions between industry representatives were less likely than residents who trained in environments without such restrictions to view promotional interactions as being beneficial (Brotzman and Mark, 1993; McCormick et al., 2001). One literature review found weak evidence that trainees who were exposed to educational interventions may be "less accepting of pharmaceutical industry marketing tactics" than those who are not (Carroll et al., 2007, p. e1533). The review noted that two studies that involved industry personnel in the design of the educational intervention found that the participants were more positive toward industry and industry representatives than they were before the intervention.

Some research—including research in academic medical centers as well as community settings (see, e.g., Solomon et al. [2001])—suggests the value of "academic detailing" or educational outreach programs provided by clinical pharmacists or other experts as an objective educational alternative to the activities of medical product companies. Because these programs are aimed at physicians outside academic institutions, this research is reviewed in Chapter 6.

THE LEARNING ENVIRONMENT IN ACCREDITED CONTINUING MEDICAL EDUCATION

Physicians commit to life-long learning to keep pace with new knowledge and skills and to maintain their current skills. Most state licensing boards, specialty boards, and hospitals require accredited continuing medical education for relicensure, recertification, or staff privileges. Thus, it is important to promote a constructive learning environment in this arena as well as in undergraduate and graduate education. This discussion focuses on accredited continuing medical education. (As noted earlier, this report uses the phrase accredited continuing medical education to refer to education that is presented by accredited providers and is certified for course credits.)

Providers of accredited continuing medical education are more numerous and diverse than providers of undergraduate and graduate medical education. The major ACCME-accredited providers are physician membership organizations (n = 270), publishing/education companies (n = 150), medical schools (n = 123), and hospitals and health care delivery systems (n = 93). In 2008, ACCME had 740 accredited providers of continuing medical education, and state medical societies accredited approximately 1,600 additional providers (ACCME, 2008a, 2009). What ACCME calls "publishing/education companies" are often described as "medical education and communication companies," or MECCs, and that term is used here. According to data reported by the Society for Academic Continuing Medical Education (SACME) for 2006, about 40 percent of medical schools held commercially sponsored "satellite" meetings in conjunction with national professional society meetings, and 70 percent of these meetings were managed by communications companies (SACME, 2007).

Table 5-2 shows the shares of total income, participants, hours of instruction, and activities (all providers) accounted for by several types of accredited continuing medical education providers. Medical schools accounted for a considerably larger share of total hours of instruction than might be expected from their share of the total income received by education providers. In contrast, MECCs (publishing/education companies) account for a considerably smaller share of all instructional hours than of total income.

Accredited continuing medical education programs embedded in medical schools are shaped in part by the missions, culture, and challenges of the larger institution. The programs' members are represented by SACME, which describes its mission as promoting "research, scholarship, evaluation and development" of educational and professional development programs "to enhance the performance of physicians … for purposes of improving individual and population health" (SACME, 2008anpaged). Professional society programs are also shaped by the missions, culture, and resources of the society. Most MECCs are for-profit organizations. They are represented by the North American Association of Medical Education and Communication Companies, which is "dedicated to providing representation, advocacy, and education for its members" (NAAMECC, 2009).

The curriculum for accredited continuing medical education is also diffuse. All states except Colorado, Indiana, Montana, New York, South Dakota, and Vermont have some requirements for accredited continuing medical education for physicians who want to maintain (reregister) their license (AMA, 2008a). The policies are generally not specific about the content of the accredited continuing medical education, although a number of states have certain content requirements, for example, palliative and end-of-life care or patient safety (AMA, 2008a). Medical specialty boards have more specific and coherent requirements. They have also recently adopted a "maintenance of certification" model for ensuring continuing physician competence, and this model has implications for the future content of accredited continuing medical education.[8] Approximately 85 percent of U.S. physicians are board certified, so recertification requirements affect the majority of physicians (ABMS, 2007).

In addition to accredited continuing medical education, physicians also have access to an array of nonaccredited education programs sponsored by a wide range of public and private organizations. Many conferences sponsored by the National Institutes of Health and other government agencies do not offer credit, although some do. Hospitals sponsor a range of medical staff education programs that do not offer credits. The committee heard testimony that a professional society may organize a scientific meeting of research presentations for which it controls the selection of topics and speakers (ASH, 2008; Kaushansky, 2008). The organization may then seek financial support from industry, often small grants from several companies. Because of limited budget and staff, a small society may not pursue the provision of continuing medical education credits even when it provides safeguards against commercial bias consistent with accreditation standards. When medical product companies organize nonaccredited continuing medical education, the offerings may range from dinner seminars to training on the use of a medical device and satellite symposia at professional society meetings (some satellite symposia offer credit). Some nonaccredited programs controlled by companies may be little more than marketing. Others, such as programs that provide training on the use of a complex new medical device, may meet legitimate education needs, although the presentations may still be more positive about the device than presentations by an independent educational source would be. The committee lacked the resources to investigate nonaccredited activities.

Some medical schools have policies that require their faculty to limit participation in industry-supported programs to programs that meet certain conditions. These conditions may be similar or identical to the standards for accredited continuing medical education (see, e.g., Boston University [2007] and the University of Pittsburgh [2007]).

As noted earlier, the committee commissioned a paper on conflict of interest concerns, policies, and practices in other professions. That paper, which is presented as Appendix C, examines conflicts of interest in law, accounting, engineering, and architecture. In general, other professions differ from medicine in that they have no authority similar to that of physicians to prescribe regulated products for client's personal use and, except to various degrees for law, do not have vulnerable clients.

In some respects, the current system of continuing legal education resembles the system of continuing medical education in decades past. Much continuing legal education is provided by law schools as part of their service mission, although law firms and commercial companies also offer programs. Programs may be offered at no charge or may be paid for by individual lawyers or their firms or employers. Programs sometimes have corporate sponsorship, but the sponsors' products tend to be resources for the lawyer (e.g., software and information resources) rather than for the lawyer's clients and thus do not present the same concerns about bias in presentations that occur in medicine. Although legal continuing education cannot be seen as an exact model for medicine, it does suggest that alternatives (e.g., higher fees and employer subsidies) to the major role of industry funding for continuing medical education may exist.

Industry Funding in Accredited Continuing Medical Education

Survey data from ACCME show that industry funding of accredited continuing medical education increased by more than 300 percent between 1998 and 2007 (ACCME, 2008a, Table 7).[9] Moreover, profit margins increased substantially, from 5.5 percent in 1998 to 31 percent in 2006 (Steinbrook, 2008b). For the many providers of accredited continuing medical education, this combination of increased reliance on industry funding and increased profitability provides strong incentives to resist efforts to curtail such funding.

The contribution of funding from industry (primarily from drug, medical device, and biotechnology companies) varies by the type of provider of accredited continuing medical education (Table 5-3). Funding from industry provides more than half of the total income for medical schools and almost three-quarters of the total income for MECCs. Professional societies (i.e., physician membership organizations) as well as MECCs show a significant margin of income over expenses.

Although professional societies are not as dependent on industry funding for their accredited educational programs as MECCs or medical schools, they receive nearly equal amounts of funding from commercial sources (24 percent) and advertising and exhibit income (25 percent). ACCME's survey does not count the latter as commercial support.

SACME surveys provide additional data on the significance of industry funding for medical school programs. In 2006, the typical (median) medical school received some commercial support for about 45 courses, which represented almost 70 percent of its educational activities (SACME, 2007). About 7 percent of schools reported that the majority of their courses were supported by a single commercial source, and the mean number of such courses across all respondents was two. Respondents also reported that "if commercial support were no longer provided, the typical school would no longer hold 11 courses, representing 23% of the school's courses" (p. 3).

Because they depend on industry for almost three-quarters of their income, MECCs could be severely challenged by an end to direct commercial funding, which some have proposed (Fletcher, 2008), or by a decision by medical product companies to shift their support to academic institutions, as one company recently did (Loftus, 2008). They could still have a role if academic medical centers continued to contract with them to manage or administer some of their continuing medical education programs.

Providers of accredited continuing medical education may solicit industry support for their programs. For example, a medical education company described opportunities to provide educational grants for a large meeting sponsored jointly with an academic medical center, as shown in Box 5-2. Other organizations sell sponsorship opportunities for everything from meeting coffee breaks to hand sanitizers and flash drives.

In addition to support for organizational programs, industry also provides support to individual physicians. On the basis of the findings from a 2004 survey, Campbell and colleagues (2007a) found that 26 percent of physicians reported that industry paid for their admission to continuing medical education meetings and 16 percent reported payments for serving as a speaker or on a speakers bureau.

Conceptually, industry support may be direct or indirect. Direct funding is from the company to the program provider. Indirect funding may occur in several ways. The company may set up a foundation that it substantially controls to provide the funding, or the provider may set up a foundation to receive the funds. Such arrangements may not provide any protection against the company influencing the content of the accredited continuing medical education. Alternatively, the company may provide funds to an intermediary, such as a central continuing medical education office in an academic health center. These arrangements are intended to separate the funding from decisions about the course content. The committee has heard criticisms that despite ACCME requirements that course directors review the course content for bias, the recipient of industry funds may have an implicit understanding that additional industry funds will not be offered in the future if the course does not present topics of interest to the company and use speakers who are favorable to the company's products.

Concerns About Industry Support for Accredited Continuing Medical Education

The substantial support that industry provides for accredited continuing medical education indirectly subsidizes physicians who pay less for many accredited continuing medical education programs than they otherwise would. As the preceding section indicates, industry support also contributes to the financial well-being of many educational providers that depend on it for the major part of their income for the provision of accredited continuing medical education.

The committee found little systematic research on other consequences of industry-supported continuing medical education, for example, whether it promotes bias in individual programs or in overall educational offerings. One study published before the adoption of the first ACCME standards for commercial support compared programs funded by rival pharmaceutical companies and found that the programs favored the products of their funders (Bowman, 1986). A study by Orlowski and Wateska (1992) focused on a kind of industry-sponsored activity that provoked considerable criticism and that now is not permitted for accredited education, that is, a program held at a resort with all expenses paid for attendees and with limited time actually devoted to the educational content. The authors found, using actual prescribing data obtained before and after the activity, that this "elaborate promotional technique … was associated with a significant increase in the prescribing of the promoted drugs at one institution" (p. 273). The investigators also found that the physicians involved did not believe that the activity would affect their practices.

Another study found that courses on primary care directed by academic faculty covered a broader range of topics than symposia sponsored directly by industry (Katz et al., 2002). Moreover, 91 percent of the industry-sponsored symposia were sponsored by a company that had recently obtained FDA approval for a drug related to the symposium topic. The industry-sponsored symposia did not cover prevention screening, dermatological diagnoses, child abuse, alcoholism, or the technology resources available for clinicians, which were considered important in the academic program. In that study, the university-based accredited continuing medical education courses received funding from multiple companies through a MECC to the university. University faculty determined the content of their courses, and the MECC handled marketing and meeting logistics. During meal breaks at these courses, symposia funded by industry were also offered.

Unfortunately, much information about accredited continuing medical education, particularly that offered by for-profit providers, is not based on good data but, rather, is based on personal experiences with covert relationships with providers or inferences made on the basis of the nearly total dependence of these providers on pharmaceutical, medical device, and biotechnology companies. One 2008 article, based on personal experience, describes how accredited continuing medical education providers can tailor programs to secure company grants (Gilbert, 2008, unpaged). A commer cial provider selected a program concept to "provide a platform for one of the sponsors," which was working on a drug covered by the program. The provider also organized informal workshops with experts who were hired on the basis of their support for the sponsor's message.

Using a checklist that they developed to assess bias in education programs, Takhar and colleagues (2007) concluded that 9 of the 17 continuing medical education programs that they assessed were biased (e.g., by limiting the discussion to the sponsor's product and ignoring alternatives). Work is needed to validate this and other instruments that are intended to be used to assess bias in presentations retrospectively or identify presentations at risk of bias during the planning stage (see, e.g., Barnes et al. [2007]).

The Senate Finance Committee staff report on the use of educational grants by pharmaceutical manufacturers noted that ACCME's reports documented numerous cases of undue influence by companies over "supposedly independent educational programs" (Finance Committee, U.S. Senate, 2007, p. 2). For example, during 2005 and 2006, 18 of 76 program providers were found to be out of compliance with at least one of the ACCME standards related to independence, and some were cited for being under the improper influence of industry.

More specific information on industry practices comes from litigation. Prompted in many instances by whistleblower complaints, the U.S. Department of Justice as well as state attorneys general have filed charges against a number of pharmaceutical and medical device companies for illegal practices related to purported educational activities as well as speaking and writing arrangements. In some cases, one focus of litigation has been the giving of educational grants as an inducement to use the company's products, which can be illegal under the Medicare law. In other cases, the focus has been on industry efforts to bias the content of educational programs and presentations, particularly as part of efforts to promote the off-label use of drugs (i.e., for purposes not approved by the FDA), which is also illegal.[10]

Box 5-3 lists some of the cases in which settlements have been reached. Internal company documents that were made public as a result of the first case described in the box provided insights into the use of speakers bureaus (which included chairs of neurology departments), "educational" teleconferences, and grants to medical education companies (with multiple ties to the company) to further marketing objectives for the drug Neurontin (gabapentin) (Steinman et al., 2006; see also Landefeld and Steinman [2009]). The conditions associated with the settlement in the case specified requirements for the company's reporting of its support for continuing medical education and its financial relationships with speakers and participants (OIG, 2004).[11]

Responses to Concerns About Bias in Industry-Funded Accredited Continuing Medical Education

Responses by Private Organizations

Expanded industry support for accredited continuing medical education and the involvement of commercial firms began to become a significant concern in the 1980s and led to ACCME-developed guidelines on commercial support in 1987 and then ACCME-developed standards in 1992. These standards have been criticized as doing little to curb industry influence over the content of accredited continuing medical education (see, e.g., Relman [2001, 2003]; see also Ross et al. [2000], Krimsky [2003], and Brody [2007]). In 2004, ACCME issued new, more restrictive standards.

The accreditation standards now require the disclosure of conflicts of interest by meeting planners as well as speakers. They also require the review of the educational content for bias and the resolution of conflicts of interest in some fashion (e.g., by finding an alternative speaker or identifying and eliminating biased content in a presentation). In addition to the standards, ACCME has developed tools (e.g., definitions, frequently asked questions, and slide presentations) to help educational providers with program implementation.

The SACME survey mentioned above reported that academic providers found the 2004 standards to be difficult to implement (SACME, 2007). Only 5 percent of the respondents considered the standard related to resolving conflicts of interest to be easy to implement. Slightly less than half of the respondents thought that the standards had reduced bias a little or somewhat.

In 2008, the ACCME board of directors adopted a statement that indicated that accredited continuing medical education providers "cannot receive guidance, either nuanced or direct, on the content of the activity or on who should deliver that content" (ACCME, 2008b. 3). The organization also announced that it was devoting more resources to implementation and enforcement, which would eventually require an increase in member fees (ACCME, 2008b). In addition, ACCME issued a request for comments on a proposal related to commercial support, which included as options the elimination of commercial support, the continuation of the current situation, and the development of a new paradigm (ACCME, 2008d). The executive summary for the November 2008 board of directors meeting states that analysis of the comments is continuing and that action is not anticipated before the end of 2009 (ACCME, 2008c).

Notwithstanding the changes in ACCME standards, criticisms of industry funding and influence continue (see, e.g., Steinbrook [2005, 2008b] and Fletcher [2008]). ACCME's limited resources for monitoring adherence to its standards (as of early 2008, it had approximately a dozen staff members) are also a concern (Kopelow, 2008).

Other issues involve the monitoring of the content of presentations. Program-by-program and presentation-by-presentation assessments for bias are labor-intensive activities, and instruments for the systematic assessment for bias need further development and validation. The committee found no studies describing or evaluating the effectiveness, burdens, and adverse consequences of such monitoring for bias overall or by category of accredited continuing medical education provider. ACCME requirements for monitoring may stimulate research in this area.

Some critics raise broader questions about the value, goals, and structure of the current system of accredited continuing medical education (see, e.g., Fletcher [2008]). Some have also proposed ending direct industry support for continuing medical education (see, e.g., Brennan et al. [2006], Fugh-Berman and Batt [2006], CEJA [2008], and Fletcher [2008]). In 2008, the AMA House of Delegates referred back to its Committee on Ethical and Judicial Affairs a proposal that physicians and organizations not accept industry funding for professional medical education (AMA, 2008c; see also Relman [2008]). The summary of a 2008 consensus conference held at the Mayo Clinic describes a conclusion that continuing medical education requires a "strategic management process that focuses on the integrity of an enterprise" and

that deals "in a convincing, transparent and accountable manner issues such as commercial interest influence, conflicts of interest, bias, sources of evidence and the quality of product, process and delivery" (Kane, 2008, p. 8). It also stressed the need for research (and funding for research) to guide reforms.

In a 2008 report on industry funding of medical education, AAMC recommended that academic medical centers set up audit procedures to assess compliance with ACCME standards. The report observed that given "the heavy dependence by academic medical centers on industry funding" for continuing medical education, it was essential that they comply with "evolving" ACCME standards and take other steps to ensure the independence of their program offerings (AAMC, 2008c. 19). The report also recommended that academic medical centers establish a central office through which all requests for industry support and the receipt of funds for continuing medical education would be coordinated and overseen. It further proposed that institutions should prohibit faculty, students, residents, and fellows from participating in non-ACCME accredited industry events that are labeled as continuing medical education. Also, if medical centers allow faculty participation in industry-sponsored, FDA-regulated programs, they should set standards for appropriate faculty involvement.

In its revised code of conduct, PhRMA includes provisions on industry support for continuing educational programs. With an eye to federal kickback laws, it advises companies to separate decision making about educational grants from sales and marketing units and to "develop objective criteria for making CME grant decisions to ensure that … the financial support is not an inducement to prescribe or recommend a particular medicine or course of treatment" (PhRMA, 2008). For nonaccredited educational activities, the code provides that the organizers of the activity should control its content, faculty, materials, and similar details. As noted earlier, one pharmaceutical company announced that it would no longer fund educational programs offered by MECCs.

Most medical school policies reviewed by the committee already state that their programs should meet the standards for commercial support set forth by ACCME. Some have instituted further restrictions. In 2007, Memorial Sloan-Kettering Cancer Center announced a 6-month trial period during which it would no longer accept industry funding for its continuing medical education programs (industry provided about 25 percent of total funding for continuing medical education at that institution). To reduce costs, off-site programs were moved on-site, free lunches were eliminated, advertising was cut, and fewer external speakers were used. Although the fees for external participants were raised by 10 to 20 percent, program attendance stayed the same (Kovaleski, 2008). The ban on industry funding is now permanent. At least one other institution has also announced that it will no longer accept direct industry funding for specific accredited continuing medical education courses either on or off campus, nor will it accept payments from third parties that have received commercial support (Stanford University School of Medicine, 2008). Industry support is, however, permitted if it is not designated to a specific subject, course, or program but is for use in a broadly defined field and is provided through a central university office for continuing medical education.

Responses by Public Agencies

As described above, the U.S. Department of Justice and state attorneys general have charged a number of companies with illegal practices related to the funding of educational programs, including accredited programs in some instances. In addition, in its 2003 compliance guidelines for pharmaceutical manufacturers, the Office of the Inspector General (OIG) of the U.S. Department of Health and Human Services identified the provision of educational grants as an activity that place a company at high risk for violating federal antikickback rules and certain FDA regulations (OIG, 2003). These compliance guidelines advise manufacturers to separate their grantmaking activities from their sales and marketing activities to "help insure that grant funding is not inappropriately influenced by sales or marketing motivations and that the educational purposes of the grant are legitimate" (p. 21). Other activities identified as having a high potential for fraud and abuse include the provision of gifts, entertainment, and personal services compensation arrangements. The OIG guidelines also recommend (pp. 20–21) that manufacturers

1. separate grant-making functions from sales and marketing functions;
2. establish objective criteria for awarding grants that do not take into account the volume or value of the recipient's purchases;

3. establish objective criteria for awarding grants that ensure that the funded activities are bona fide; and

4. refrain from controlling speakers or content of educational activities funded by grants.

The 2007 Senate Finance Committee staff report cited above concluded that most large pharmaceutical companies had established written policies and procedures on educational grants, limited sales representatives from soliciting requests or promising funding, and established a centralized mechanism for administering grants.

GHOSTWRITING, SPEAKERS BUREAUS, AND INDEPENDENCE OF PUBLICATIONS AND PRESENTATIONS

Concerns about Ghostwritten Publications, Participation in Speakers Bureaus, and Other Industry-Controlled Work

Two hallmarks of academic integrity are intellectual independence and accountability for one's work. Certain practices by medical school faculty create a hidden curriculum that subverts the professional values endorsed by the formal curriculum. One example is taking credit as the author of a manuscript prepared by an unacknowledged or inadequately acknowledged industry-paid writer. (An adequate acknowledgment would specify the roles of these writers, for example, as the preparers of the first draft, as well as the roles of the listed authors.) Another example is participating in an industry speakers bureau or other long-term speaking arrangement with a company, regardless of how the relationship is labeled. One concern is that ongoing company payments for presentations (and travel to attractive locations) create a risk of undue influence. A second concern that is frequently tied to the speakers bureau label is that the company exerts substantial control over the content of a presentation. Industry influence in these arrangements may be direct (e.g., when a talk and slides are largely or entirely prepared by someone else or when speakers are instructed to provide the company-prepared responses to questions and avoid the favorable mention of competing products). Influence may also be less direct (e.g., when a company-trained and company-paid physician modifies talks to fit the objectives of the company) (see, e.g., Elliott [2006] and Carlat [2007]). The committee recognizes that companies have an interest in some oversight of presentations for a variety of reasons, including the need to comply with FDA prohibitions on promoting the use of drugs for the treatment of conditions not approved by the agency.

Serving on speakers bureaus appears to be common in clinical medicine. A 2006 survey of academic-industry relationships found that 21 percent of clinical department chairs reported being on a speakers bureau (whereas 2 percent of nonclinical department chairs reported being on a speakers bureau) (Campbell et al., 2007b). As reported earlier, another survey, which was not limited to academics and which asked less specific questions, found that 16 percent of physicians reported serving on a speakers bureau or as a speaker, which could have involved a single presentation (Campbell et al., 2007a). ACGME has expressed concern about "a new variation of a promotional activity in which residents and even medical students receive slides, lecture materials and honoraria and subsequently act as 'experts,' delivering the packaged information at continuing medical education events" (ACGME, 2002, p. 3).

Unacknowledged industry influence over publications is also common. In one study, 13 percent of research articles in major biomedical journals had "ghost" authors, that is, people who filled the criteria for authorship but who were not listed as authors (Flanagin et al., 1998). None of these ghost authors was even acknowledged in the paper. A review of documents obtained during litigation against a major pharmaceutical company concluded that review manuscripts were often prepared by writers for medical publishing companies but authorship was "subsequently attributed … to academically affiliated investigators who often did not disclose industry financial support" (Ross et al., 2008, p. 1800). One incident illustrates that such ghostwriting may be discovered only by accident. An academic physician reported that a MECC sent her a draft manuscript of a review article commissioned by a drug company and invited her to be its "author." She declined, but she was subsequently asked by a journal to review an article that was similar to that article and that now had another author (Fugh-Berman, 2005; see also Eaton [2005]). The analysis by Steinman and colleagues (2006) of documents obtained through litigation cited earlier found that those documents describe plans for recruiting academic authors of a series of ghostwritten articles to be prepared by a medical education company. Box 5-3 included examples of company settlements with the Department of Justice related to speaking and writing arrangements.

Another concern about industry relationships is that academic authors of research articles may not have full access to the data from an industry-sponsored study. This issue was discussed in Chapter 4.

In the setting of medical education, the question is not whether assistance by professional writers and others may improve publications and help busy researchers get important, objectively presented findings into print; it may do both. The questions are whether the assistance is hidden, whether it is intended to promote a company's interests rather than present unbiased information, and whether the author takes credit for work that he or she did not do and thus misrepresents the provenance of the article. Such arrangements (which are essentially gifts) send the wrong message about the values of intellectual independence, professional ethics, accountability, and evidence-based medicine. In the context of research, they raise questions about the objectivity of research reports that other researchers as well as practitioners and developers of practice guidelines rely on.

Responses to Concerns About Independence and Accountability in Writing and Speaking

Medical journal editors (including the International Committee of Medical Journal Editors and the World Association of Medical Editors) have taken steps to eliminate ghostwriting (see, e.g., Rennie et al. [1997], Davidoff et al. [2001], ICMJE [2008], and WAME [2008]). As stated by the International Committee of Medical Journal Editors, "[a]ll persons designated as authors should qualify for authorship, and all those who qualify should be listed" (ICMJE, 2008, p. 3; see also Ross et al. [2008]). The objective of authorship policies is to eliminate unethical practices and generally not to preclude legitimate and properly acknowledged writing assistance (see, e.g., Lagnado [2002] and Woolley et al. [2006]).

As described in Chapter 3, one journal has revised its conflict of interest disclosure form to include questions intended to detect commercial sponsorship and unacknowledged authors after concluding that such questions were necessary to detect ghostwritten or promotional submissions (AFMI, 2008). In its disclosure form for continuing medical education programs, the same professional society asks several questions about relationships with speakers bureaus (e.g., whether an individual is acting independently or as an agent) as well as questions about the receipt of assistance with manuscript preparation from commercial entities (AAFP, 2006b).

In its 2008 report on medical education, AAMC recommended, "[a]cademic medical centers should prohibit physicians, trainees, and students from allowing their professional presentations of any kind, oral or written, to be ghostwritten by any party, industry or otherwise" (AAMC, 2008c. 22). It noted that properly acknowledged collaborations with industry personnel or medical writers is not ghostwriting. The report also recommends that participation in industry-sponsored speakers bureaus be discouraged.

A few medical school policies reviewed by the committee mention speakers bureaus by name. For example, the University of Massachusetts views speakers bureaus as an "extension of the marketing process" and forbids faculty participation in them. The Mayo Clinic has long prohibited faculty from speaking on behalf of industry, and its current policy prohibits participation in the speakers bureaus of commercial firms because the linkage would imply endorsement by the Mayo Clinic (personal communication, Marianne Hockema, Administrator, Office of Conflict of Interest Review, Mayo Clinic, September 19, 2008). Faculty at the University of Louisville (2008) are "strongly discouraged" from serving as speakers hired by vendors (p. 4). A policy recently adopted by the Johns Hopkins University School of Medicine (2009) states that faculty may not participate on-site or off-site in "activities with any of the following characteristics … a company has the contractual right to dictate what the faculty member says; a company (not the faculty member) creates the slide set (or other presentation materials) and has the final approval of all content and edits; the faculty member receives compensation from the company and acts as the company's employee or spokesperson for the purposes of dissemination of company-generated presentation materials or promotion of company products; and/or a company controls the publicity related to the event" (p. 7). The policy notes that some of these activities occur in the context of speakers bureaus but it is the conditions of an activity that determine whether it is permissible.

In addition, a few medical schools (e.g., the University of California at San Francisco, the University of Louisville, and the University of Colorado) forbid ghostwriting (using that term). A few other medical schools (e.g., Stanford University, the University of Missouri, Emory University, and the University of Rochester) cover the practice of ghostwriting by forbidding medical school personnel from publishing, under their own name, articles that are written entirely or in significant part by an industry employee.

The ACCME standards for commercial support require that presenters disclose relevant financial relationships. They provide no explicit guidance or reference to the appropriateness of commercial assistance in the preparation of talks.

The 2008 PhRMA *Code on Interactions with Healthcare Professionals* notes that companies and speakers should understand the difference between (accredited) continuing medical education and company-sponsored speaker programs (PhRMA, 2008). For the latter, "[s]peaker training is an essential activity because the FDA holds companies accountable for the presentations of their speakers" (p. 9). This is a reference to FDA's ban on company promotion of the use of a medication for the treatment of conditions that have not been approved by the agency (FDA, 1997). The PhRMA code specifies that company policies should provide a cap on the total annual amount that it will pay a speaker and address the "appropriate number of engagements for any particular speaker over time" (p. 10).

RECOMMENDATIONS

Medical Schools and Residency Programs

Policies on Relationships with Industry

This chapter has documented the extensive relationships that exist between industry and medical institutions, faculty, students, and residents and the concerns that have been raised about the risks that these relationships pose to the basic educational missions of academic medical centers and the lack of benefits from such relationships, such as those that support academic-industry collaborations in medical research. It has cited research indicating that even small gifts can be influential and has reviewed the recommendations of organizations such as AAMC and PhRMA. The committee concluded that it is time for medical schools to end a number of long-accepted relationships and practices that create conflicts of interest, threaten the integrity of their missions and their reputations, and put public trust in jeopardy. The risks are substantial and are not offset by meaningful benefits.

RECOMMENDATION 5.1 For all faculty, students, residents, and fellows and for all associated training sites, academic medical centers and teaching hospitals should adopt and implement policies that prohibit

- **the acceptance of items of material value from pharmaceutical, medical device, and biotechnology companies, except in specified situations;**
- **educational presentations or scientific publications that are controlled by industry or that contain substantial portions written by someone who is not identified as an author or who is not properly acknowledged;**
- **consulting arrangements that are not based on written contracts for expert services to be paid for at fair market value;**
- **access by drug and medical device sales representatives, except by faculty invitation, in accordance with institutional policies, in certain specified situations for training, patient safety, or the evaluation of medical devices; and**
- **the use of drug samples, except in specified situations for patients who lack financial access to medications.**

Until their institutions adopt these recommendations, faculty and trainees at academic medical centers and teaching hospitals should voluntarily adopt them as standards for their own conduct.

This recommendation has several targets, most of which focus on promotional relationships. One target is the acceptance by faculty or trainees of items of material value (including small gifts and meals) from industry except in certain situations. These situations, which should be defined in institutional policies, include (1) appropriate payment for legitimate services (such as contracts, grants, and consulting arrangements); (2) charitable donations, which should be given to the institution; and (3) sharing of research materials or data. Under appropriate transfer agreements, the sharing of research materials or data is encouraged, as it promotes medical research. This recommendation covers not only

physical gifts, such as pens, notepads, and meals, but also preferences, such as paid speaking engagements that are intended as rewards or inducements. Consulting arrangements and drug samples are discussed further below.

The second target of this recommendation is the involvement of faculty or trainees in presentations or publications for which they cannot ethically claim credit or intellectual independence. Although no physician or researcher should accept authorship of a ghostwritten academic publication (see the discussion earlier in this chapter), failure to meet this standard is particularly troublesome when it involves faculty who have a special obligation to demonstrate intellectual independence and to act as role models. For similar reasons, faculty should not participate in speakers bureaus and similar promotional activities in which they either present content directly controlled by industry or formulate their remarks to win favor and continued speaking fees. If institutions fail to adopt these recommendations, then acceptance of authorship for ghostwritten publications or industry-controlled presentations would constitute a gift to be disclosed to the institution even if the institution's policies do not explicitly mention these arrangements as gifts.

The recommendation's third target is consulting arrangements. Faculty should engage only in bona fide consulting arrangements that require their expertise, that are based on written contracts with specific tasks and deliverables, and that are paid for at fair market value. As part of their administration of conflict of interest policies, university review of faculty consulting and other contracts is prudent and desirable.

The fourth target of this recommendation concerns access to educational environments by sales representatives of pharmaceutical, medical device, or biotechnology companies. Clinical teaching should be done by faculty, not by marketing agents. The recommended restrictions on site access should not discourage appropriate and productive research collaborations between industry and academic researchers. In addition to promoting scientific progress and the development of useful products, collaborations can provide educational benefits to medical students, graduate students, and postdoctoral fellows who might participate in legitimate collaborative research projects with industry under proper supervision.

As described earlier, the AAMC recommendations and some medical school policies set stringent restrictions on access by pharmaceutical sales representatives but establish slightly less restrictive conditions for access by representatives of medical device companies. The recommendations and policies reflect assessments that access by device representatives—if they are properly managed and appropriately limited—can contribute to patient safety. Nonetheless, the expectation is that faculty will quickly learn how to use complex new devices, including relevant surgical techniques, and will then instruct and supervise residents and fellows rather than rely on company representatives to do so. Access under these circumstances would occur after the institutional purchase of a complex device. For the purposes of device evaluation, access by the device representatives would occur before purchase of the device.

The fifth target of this recommendation, which covers drug samples, presents difficult issues. Caring for patients who cannot afford needed drugs is frustrating for physicians who are trying to meet their professional obligations to act in their patients' best interests. Despite the aid provided through Medicaid and Medicare, other public programs, and the patient access initiatives of pharmaceutical companies, many patients are not eligible for such aid and cannot afford to continue to take medications after they have used a sample. Moreover, although physicians and others may believe that drug samples allow low-income patients access to drugs that they could not readily obtain otherwise, this chapter has cited research that suggests that most samples are not, in fact, given to indigent patients and that access to samples may change trainee behavior such that they move away from practicing evidence-based and lower-cost care. Drug samples are not a satisfactory answer to the serious problem of the lack of affordability of medications for many patients, but the committee was reluctant to call on physicians to abandon them completely in the short term.

For academic medical centers, the use of drug samples may often be managed without a direct interaction between a physician and a company representative. Thus, AAMC recommends and this committee agrees that samples (if the institution permits them) should, whenever possible, be centrally managed in ways that allow timely and appropriate patient access.

In the absence of such centralized arrangements, institutions should limit the provision of free drug samples and provide them only to patients who lack financial access to medications in situations in which generic alternatives are not available and the sample medication can be continued at little or no cost to the patient for as long as it is needed. They should also

help physicians and patients use alternative public and private resources to obtain the needed medications. The proposal by the Medicare Payment Advisory Commission for company reporting and U.S. Department of Health and Human Services analysis of data about the distribution of drug samples cited earlier in this chapter could, if it is adopted, produce helpful information to guide future policies.

The elements of this recommendation apply both to campus settings and to off-site settings, for example, off-site locations for professional meetings and educational programs. They also apply to volunteer faculty who provide clinical education in their offices or in community hospitals. Chapter 6 presents a parallel recommendation (Recommendation 6.1) for physicians who are not affiliated with academic institutions. That chapter also presents a comprehensive recommendation (Recommendation 6.2) that calls for medical product companies to change their policies to be consistent with these recommendations. The committee recognizes that it takes time for academic medical centers to develop policies. It recognizes the value of policy development processes that involve the assessment of local conditions, the inclusion of those who will be affected, and investigation of the experiences of similar institutions.

Until institutions act, faculty, students, and trainees should still change their own behavior so that it is in line with the recommendations presented above. In addition, consistent with Recommendation 9.1, the committee encourages AAMC, AMSA, and similar membership organizations to continue or initiate survey, monitoring, and other activities to promote the reform of conflict of interest policies in medical education.

Education on Relationships with Industry

RECOMMENDATION 5.2 Academic medical centers and teaching hospitals should educate faculty, medical students, and residents on how to avoid or manage conflicts of interest and relationships with pharmaceutical and medical device industry representatives. Accrediting organizations should develop standards that require formal education on these topics.

Changing the environment within educational institutions is important, but medical schools also need to prepare trainees for practice in environments that may be characterized by more permissive standards of conduct regarding drug and device marketing. Faculty will continue to experience a range of situations in which they will interact with industry representatives and will also need to be prepared to act as educators and role models on industry relationships.

The committee recognizes that the evidence on the effectiveness of educational programs of this sort on physician attitudes and behaviors is not strong, but it believes that a basic level of education supports the development of core competencies and prepares students and trainees for future practice. The establishment of educational standards will help ensure that such education is of high quality and receives appropriate attention.

Accredited Continuing Medical Education

The members of the committee had extensive internal discussions about industry support for accredited continuing medical education. Overall, there was general agreement that continuing medical education has become far too reliant on industry funding and that such funding tends to promote a narrow focus on products and to neglect the provision of a broader education on alternative strategies for managing health conditions and other important issues, such as communication and prevention. Given the lack of validated and efficient tools for preventing or detecting bias, industry funding creates a substantial risk of bias, to the extent that industry-reliant providers want to attract industry support for future programs. Although the committee did not reach agreement on a specific path to reform, it concluded that the current system of funding is unacceptable and should not continue.

RECOMMENDATION 5.3 A new system of funding accredited continuing medical education should be developed that is free of industry influence, enhances public trust in the integrity of the system, and provides high-quality education. A consensus development process that includes representatives of the member organizations that created the accrediting body for continuing medical education, members of the public, and representatives of organizations such as certification boards that rely on continuing medical education should

be convened to propose within 24 months of the publication of this report a funding system that will meet these goals.

One option is for this broad-based consensus development process to be convened by the member organizations of ACCME. As described earlier in this chapter, they represent medical specialty boards (American Board of Medical Specialties), hospitals (AHA and the Association for Hospital Medical Education), organized medicine (AMA), medical schools (AAMC), medical specialty societies (CMSS), and state licensure boards (Federation of State Medical Boards). Although these organizations have interests in continuing medical education and in ensuring that continuing education is free of bias and supports core competencies, they do not all have a vested interest in the current system of funding that education.

The consensus development process convened by this or another group should be broad based and should also include representatives of other medical education accrediting bodies (LCME and ACGME), other interested state and federal agencies, public interest and patient advocacy groups, and organizations such as specialty certification boards that rely on continuing medical education. It should also include providers of accredited continuing medical education and industry funders. The deliberations should take into account the findings of other groups that have analyzed funding for continuing medical education or that have made recommendations about improving continuing medical educational methods.

Most committee members believed that a near-term end to industry funding would be unacceptably disruptive for the major providers of accredited continuing medical education, including medical schools and professional societies, which together provide 68 percent of the total number of hours of this type of education (see Table 5-2). A SACME survey found that 77 percent of respondents said that immediate elimination of commercial support would substantially reduce the number of courses at their academic centers and the scope of their programs and could potentially lead to the elimination of programs (SACME, 2008b). Eliminating all industry funding without having in place an alternative model could have other adverse consequences. For example, a surgical society may hold a premeeting accredited workshop involving hands-on teaching of surgical techniques, typically supported by indirect funds from industry. In the committee's experience, the costs of setup and materials for multiple simultaneous workshops can be several million dollars and would be hard to cover by payments from attendees. Furthermore, other innovative educational formats—for example, Internet-based training, simulation-based training, and performance improvement learning activities—also require funding for start-up and updating costs that could be prohibitive for providers to self-fund or fund entirely through nonindustry sources.

A majority of the committee supported the use of a consensus development process to develop a new funding system for accredited continuing medical education that would be free of industry influence but that would leave open the possibility of certain forms of indirect industry funding under conditions that minimized the risk of undue influence on program content. Some committee members supported the use of a consensus development process to develop an alternative funding model but believed that no form of direct or indirect industry funding was acceptable.

Among the options that the consensus development activity could consider are proposals for some kind of pooled funding mechanism. For example, companies could grant funds to some independent central or regional entity that would establish educational priorities and make decisions—perhaps within broad categories—about the distribution of funds on the basis of an independent review of applications from education providers.

Both direct company funding to institutions for specific continuing medical education programs and direct company provision of unrestricted grants to institutions offer clear opportunities for undue influence, particularly for continuing medical education providers that also receive the great majority of their funding overall from companies. A plan for a system free from industry influence would exclude such funding as well as funding from company-controlled foundations.

The committee recognizes that industry willingness to provide funds under a restructured system of funding accredited continuing medical education might be quite limited. Thus, the consensus development process would also need to consider alternative means of financing, steps to reduce program costs, and other strategies that would support high-quality continuing medical education. Options include increased fees for attendees; subsidies from academic medical centers as part of their educational missions; elimination of expensive program locales and amenities; reduced payments

to speakers; collaboration among education providers to share the costs of developing certain expensive programs; and rethinking the purpose and methods of continuing medical education, as is already being done in the development of programs for the maintenance of certification by specialty societies. Higher fees might be a particular burden for physicians with lower-than-average professional incomes, including rural physicians and physicians serving disadvantaged populations.

The committee members who opposed any industry funding of continuing medical education through any mechanism believed that physicians (or their employers) should bear the entire cost of accredited continuing medical education that is required for renewal of licensure and specialty certification. Even giving industry funding and program decision-making responsibility to a central office within a medical school, MECC, or other institution would unnecessarily retain conflicts of interest over the choice of course topics, directors, content and speakers, and the leadership of the continuing medical education office. In the view of these committee members, all industry support for accredited continuing medical education should be rejected, just as it is for most undergraduate and graduate medical education.

In the process of hearing testimony relevant to the issue of funding of continuing medical education, many committee members came to the conclusion that a number of other fundamental problems about the focus and the effectiveness of continuing medical education warranted attention. These issues were outside of the purview of the committee. Some will be considered by another committee of the Institute of Medicine, which is charged with making recommendations about the promotion of more effective methods of life-long education for health professionals (IOM, 2009). Analyses of the financing of continuing medical education are planned in conjunction with that project. Those analyses may provide a better understanding of the implications of different proposals about financing in the context of other changes in the system.

The committee focused on accredited continuing medical education. As noted earlier, some nonaccredited activities with industry support are educational rather than promotional and apply safeguards to prevent bias in the selection of topics, speakers, and materials presented. One example is the scientific symposium that is organized and controlled by a professional society and supported by unrestricted grants from companies. Such meetings may be particularly important for fields with many Ph.D. researchers and relatively restricted budgets. Another example is training in the use of complex medical devices provided by medical device companies under the conditions outlined elsewhere in this report (e.g., no gifts or inducements to use the product).

Other Recommendations in This Report

In addition to the recommendations in this chapter, other recommendations in this report would affect institutions that provide undergraduate, graduate, or continuing medical education. The standardization of institutional disclosure policies and formats (Recommendation 3.3) would require work to change policies and information systems, but in the long term, it should make institutional policies less burdensome across all educational institutions—as well as for individuals who must disclose potential conflicts of interest. Academic medical centers, which have repeatedly been embarrassed by revelations of incomplete and inaccurate faculty disclosures of payments from industry, would benefit from a national program of company reporting of payments to physicians and researchers that would allow the verification of certain disclosures (Recommendation 3.4). Because that reporting program would also cover payments to academic medical centers and other providers of medical education, it could provide an incentive for the adoption of institution-level conflict of interest policies, as recommended in this report (Recommendation 8.1). Accrediting organizations, membership groups such as AAMC and CMSS, and government agencies should also develop incentives for institutions to adopt and implement conflict of interest policies (Recommendation 9.2).

Adoption of the recommendation related to the conduct of research in which an investigator has a financial interest would encourage the development of management plans to protect trainees involved in such research if the institution concludes that the participation by the investigator with a conflict of interest in the research is essential (Recommendation 4.1). To the extent that physicians embrace Recommendation 6.1 to reject gifts and similar ties, it would reduce dissonance when students, trainees, and faculty interact with others in the medical community at professional society meetings and in other contexts. Further steps by companies to reform their policies and practices on gifts and payments to physicians (Recommendation 6.2) would allow medical centers to focus more attention on other issues, for example, consulting and

other contractual arrangements. Finally, academic institutions can play an important role in implementing a program of research on conflict of interest (Recommendation 9.2).

Footnotes

1 The committee follows the convention in medical education of referring to the years of medical school as "undergraduate medical education" and the post-M.D. years of residency and fellowship as "graduate medical education." Unless otherwise described (e.g., research fellows), fellows are physicians in subspecialty training programs. This report refers to "residents" and "fellows" rather than "trainees" (a description commonly used by medical educators).

2 The count includes four schools granted preliminary accreditation in 2008. It does not include accredited Canadian schools or the 20 accredited U.S. schools of osteopathic medicine.

3 These providers are accredited by state medical societies under the rules of the Accreditation Council on Continuing Medical Education.

4 As described by ACCME, "ACCME has two major functions: the accreditation of providers whose CME [continuing medical education] activities attract a national audience and the recognition of state or territorial medical societies to accredit providers whose audiences for its CME activities are primarily from that state/territory and contiguous states/territories" (ACCME, 2005).

5 AMA also authorizes credits for other activities, such as publishing an article in a peer-reviewed journal or achieving and maintaining specialty board certification.

6 The AMSA ratings, the methodology, and other information can be found at http://amsascorecard.org/. The IMAP information can be found at http://www.imapny.org/coi_database/. Both groups use information and policies received in response to a survey conducted under the auspices of the Prescription Project with funding from the Pew Charitable Trust. Some schools did not respond initially, and others refused to supply their policies.

7 ECRI Institute is a technology assessment organization that has a long history of providing advice to health care institutions and government on medical device safety. It is one of the Evidence-Based Practice Centers designated by the Agency for Healthcare Research and Quality and is a Collaborating Center of the World Health Organization.

8 The American Board of Medical Specialties and its 24 member boards have been moving from a process of recertification based on an examination taken once every several years to a maintenance of certification program that emphasizes continuing self-evaluation of practice and knowledge and other activities to maintain competence. Boards may develop self-assessment programs that also offer continuing medical education credit that will meet state licensing board and other requirements.

9 One widely cited analysis estimated that every $1.00 of industry spending on physician meetings and events generated an average of $3.56 in increased revenue (cited in Walker [2001]; see also CEJA [2008] and NAAMECC and Coalition for Healthcare Communication [2008]). Descriptions of the reported analysis do not indicate the relative weight of accredited versus nonaccredited activities in the estimate or whether accredited continuing medical education was distinguished from other types of meetings, such as promotions. Nonetheless, it suggests a rationale for industry support of a range of educational activities.

10 In 1997, the FDA provided guidance on the characteristics of industry-supported educational activities that distinguish them from promotional activities, which are subject to the labeling and advertising provisions of the Federal Food, Drug, and Cosmetic Act (FDA, 1997). This guidance stresses the role of voluntary oversight, for example, through accreditation; it explicitly disavows an interest in regulating programs.

11 The corporate integrity agreement was signed by Pfizer, which had purchased Warner-Lambert, which, in turn, was the parent company of Parke-Davis, the company named in the case.

Figures

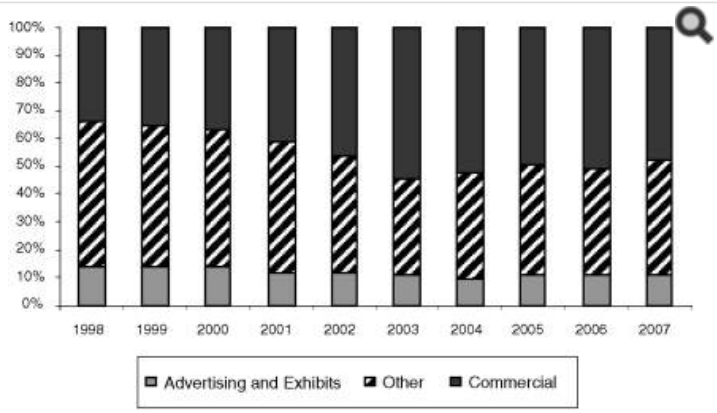


FIGURE 5-1

Sources of income reported by respondents (accredited providers of continuing medical education) to ACCME annual survey, 1998 to 2007. SOURCE: Compiled from ACCME, 2008a.

Tables

TABLE 5-1 Third-Year Medical Students' Interactions with Drug Companies

Type of Event	No. of Students. (*N* = 826)	No. (%) of Students Who Received a Gift or Participated in at Least One Event	Exposure Frequency per Month[a]	
			Mean (SD)	Range
A lunch provided by a drug company	793	768 (96.8)	1.08 (0.76)	0–4.2
A small, noneducational gift (e.g., pen or coffee mug)	801	754 (94.1)	0.87 (0.69)	0–3.5
A journal reprint or a glossy brochure from a pharmaceutical representative	800	716 (89.5)	0.53 (0.52)	0–3.5
A snack (e.g., donut, candy, coffee) provided by a pharmaceutical representative	800	713 (89.1)	0.75 (0.72)	0–8.5
A grand rounds sponsored by a drug company	798	690 (86.5)	0.54 (0.57)	0–2.4
A dinner provided by a drug company	801	405 (50.6)	0.13 (0.21)	0–2.4
A drug sample from a pharmaceutical representative	799	435 (54.4)	0.10 (0.20)	0–2.1
Another social event (e.g., party) sponsored by a drug company	799	272 (34.0)	0.06 (0.11)	0–0.8
A book donated by a drug company[b]	826	421 (51.0)		
Attendance at a workshop sponsored by a drug company[b]	826	214 (25.9)		
Registration fee for a conference paid for by a drug company[b]	826	37 (4.5)		
Participation in a market survey sponsored by a drug company[b]	826	29 (3.5)		
Participation in a research project sponsored by a drug company[b]	826	22 (2.7)		
Travel expenses for a conference paid for by a drug company[b]	826	15 (1.8)		
Nominated for an award sponsored by a drug company[b]	826	5 (0.6)		
Obtained a fellowship sponsored by a drug company[b]	826	4 (0.5)		

a For each student, an exposure index was calculated as the sum of the monthly frequencies for the first eight items.

b Monthly frequency data were not requested.

SOURCE: Sierles et al. Medical students' exposure to and attitudes about drug company interactions: a national survey. *Journal of the American Medical Association* 294(9):1034-1042 (September 7, 2005).

TABLE 5-2 Share of Total Accredited Continuing Medical Education Income, Instruction Hours, Participants, and Activities Accounted for by Major Types of ACCME-Accredited Providers

Provider Organization Type	Share (as %)			
	Total CME[a] Income	Total Hours of CME Instruction	Total CME Participants	All CME-Sponsored Activities
Medical school	17	45	31	30
Publishing/education company	33	9	30	30
Physician membership organization (nonprofit)	35	23	26	20
Other providers	15	23	13	20
TOTAL	100	100	100	100

a CME = continuing medical education.

SOURCE: ACCME, 2008a, Tables 2, 3, 4, 7.

TABLE 5-3 Income, Expenses, and Source of Support as Percentage of Income, by Type of Accredited Provider of Continuing Medical Education, 2007

Organization Type (No. of Organizations)	Total Income	Expenses as % of Total Income	Total Commercial Support (% of Total Income)	Advertising and Exhibits Income (% of Total Income)
Nonprofit (physician membership organization) (270)	$887,181	68	$215,388 (24)	$217,907 (25)
Publishing/Education Company [MECC](150)	830,811	74	594,420 (71)	10,831 (1)
School of medicine (123)	427,668	88	245,790 (57)	23,203 (5)
Hospital/health care delivery system (93)	105,014	95	47,498 (45)	7,407 (7)
Nonprofit (other) (38)	160,397	79	78,412 (49)	11,852 (7)
Not classified (33)	55,188	79	29,263 (53)	2,423 (4)
Government or military (15)	69,452	100	255 (0)	376 (0)
Insurance company/managed care company (14)	3,489	193	318 (9)	35 (1)

NOTE: Monetary data for 2007 are in 1,000s of dollars. Data for a third category of income (other) are not shown here. As categorized by ACCME, other income represents income other than commercial support and advertising and exhibit income. Data for providers accredited by state medical societies are not included, but ACCME survey data show that commercial sources accounted for about 25 percent of their income.
SOURCE: ACCME, 2008a (Table 7).

Boxes

BOX 5-1 AAMC Recommendations on Site Access by Sales Representatives

Site Access by Pharmaceutical Representatives

- To protect patients, patient care areas, and work schedules, access by pharmaceutical representatives to individual physicians should be restricted to non-patient care areas and nonpublic areas and should take place only by appointment or invitation of the physician.
- Involvement of students and trainees in such individual meetings should occur only for educational purposes and only under the supervision of a faculty member.
- Academic medical centers should develop mechanisms whereby industry representatives who wish to provide educational information on their products may do so by invitation in faculty-supervised structured group settings that provide the opportunity for interaction and critical evaluation. Highly trained industry representatives with M.D., Ph.D., or Pharm.D. degrees would be best suited for transmitting such scientific information in these settings.

Site Access by Device Manufacturer Representatives

- Access by device manufacturer representatives to patient care areas should be permitted by academic medical centers only when the representatives are appropriately credentialed by the center and should take place only by appointment or invitation of the physician.
- Representatives should not be allowed to be present during any patient care interaction unless there has been prior disclosure to and consent by the patient, and then only to provide in-service training or assistance on devices and equipment.
- Student interaction with representatives should occur only for educational purposes under faculty supervision.

SOURCE: AAMC, 2008c.

BOX 5-2 Example of a Solicitation of Industry Support (Educational Grants) for a Large Accredited Continuing Medical Education Program

Several support levels are listed below. Please note that educational support is appreciated at any dollar level. Please contact our office for further details. We appreciate that our supporters recognize the need for [the organization] to maintain authority and autonomy in decisions regarding program format, content, and faculty.

Cornerstone Supporter

Total: $195,000

Foundation Supporter

Total: $135,000

Leadership Supporter

Total: $80,000

Satellite Symposia

Open to Cornerstone and Foundation Supporters

1 Breakfast Symposium	Fee: $15,000
1 Lunch Symposium	Fee: $20,000
1 Breakfast Symposium	Fee: $15,000
1 Lunch Symposium	Fee: $20,000
1 Breakfast Symposium	Fee: $15,000

Symposium fee includes:

- Program listing on the [meeting] website, linking to the program provider's online registration site for the satellite symposium.
- Program listing and schedule in the meeting materials distributed to all meeting attendees.
- One complimentary email to the preregistration mailing list for use in promotion of the satellite symposium.
- One time complimentary use of the preregistration mailing list for use in promotion of the satellite symposium (restrictions apply).
- One insert into the delegate literature bag for use in promotion of the satellite symposium.

SOURCE: Excerpted from Oncology Congress, 2008, 2009.

BOX 5-3 Settlements Involving Educational Activities and Speaking and Writing Arrangements

In 2004, Warner-Lambert paid $430 million to settle U.S. Department of Justice charges that the company promoted off-label uses of the drug Neurontin in violation of the Food, Drug, and Cosmetic Act. "This illegal and fraudulent promotion scheme corrupted the information process relied upon by doctors in their medical decision making, thereby putting patients at risk." Tactics included "[paying] doctors to attend so-called 'consultants meetings' in which physicians received a fee for attending expensive dinners or conferences during which presentations about off-label uses of Neurontin were made; … [and sponsoring] purportedly 'independent medical education' events on off-label Neurontin uses with extensive input from Warner-Lambert regarding topics, speakers, content, and participants. … In at least one instance, when unfavorable remarks were proposed by a speaker, Warner-Lambert offset the negative impact by 'planting' people in the audience to ask questions highlighting the benefits of the drug" (DOJ, 2004, unpaged).

In 2007, Orphan Medical, Inc., agreed to pay $20 million and accept a corporate integrity agreement to settle charges that it had illegally promoted the drug Xyrem (sodium oxybate) for off-label uses. Among other charges, the company was accused of using unrestricted "educational grants" as an inducement for off-label use and paying tens of thousands of dollar in speaker fees to physicians for their promotion of these uses. One of these physicians has been charged criminally for his behavior (DOJ, 2007b). The associated corporate integrity agreement required, among other provisions, that the company create procedures to ensure that sponsored continuing medical education and educational activities be independent and nonpromotional (OIG, 2007).

In 2008, in a stipulated agreement filed in Oregon, Merck & Co, Inc., agreed to pay $58 million to 30 states and to end certain deceptive practices used to promote the drug Vioxx (rofecoxib). The stipulation prohibits, among other practices, company use of ghostwriting of published journal articles and the nondisclosure of promotional ties with speakers at independent continuing medical education programs (Oregon DOJ, 2008a).

Bookshelf ID: NBK22945

Financial Incentives

CHCS

Center for
Health Care Strategies, Inc.

Provider Incentive Programs: *An Opportunity for Medicaid to Improve Quality at the Point of Care*

Resource Paper

By:
Dianne Hasselman
Center for Health Care Strategies, Inc.

Made possible through support from The Commonwealth Fund.

March 2009

Contents

Introduction

Can there be such a thing as *too much* focus on quality? Imagine a primary care physician whose performance in diabetes care is assessed through incentive programs from multiple health plans. Each health plan uses slightly different performance indicators, requires different chronic care interventions, and provides different feedback reports for a subsection of the physician's patient panel — an overwhelming scenario, but unfortunately all too real.

Although the purpose of pay-for-performance (P4P) programs is to use financial incentives to "move the quality needle" in a deliberate manner and to increase value-based purchasing, the proliferation of incentive programs — particularly at the individual physician or practice level — is creating a patchwork of quality efforts with negative and unintended consequences. Many providers, frustrated with the numerous and fragmented performance reports they receive, discount or simply discard the data as confusing, inefficient, inaccurate, and unhelpful.

In recent years, there has been a groundswell among health policy experts, public and private purchasers, and payers toward greater standardization of quality improvement activities. Purchasers increasingly recognize the need for standardization around evidence-based guidelines. Significant movement has occurred in adopting nationally-recognized performance indicators to assess health outcomes. The National Quality Forum and its many partners are establishing national priorities and goals around performance measurement and reporting. National initiatives like *Aligning Forces for Quality*,[1] the *Regional Quality Improvement* initiative,[2] and *Bridges to Excellence*[3] are also helping to align public and private purchasers and payers around uniform quality improvement goals, common performance measures, and, in some instances, common payment.

By jointly developing incentive programs to improve quality at the point of care, purchasers and health plans can replace well-meaning but redundant and often conflicting pay-for-performance (P4P) programs. The resulting standardization of provider incentive programs could dramatically improve physician response to P4P efforts.

[1] For more information about Aligning Forces for Quality, visit **www.forces4quality.com**.
[2] For more information about the Regional Quality Improvement initiative, visit **www.chcs.org**.
[3] For more information about Bridges to Excellence, visit **www.bridgestoexcellence.org**.

Medicaid and Provider Incentive Programs

With 63 million beneficiaries — 66 percent of whom are in managed care — and more than $361 billion in annual expenditures, state Medicaid programs are in an excellent position to impact quality at the point of care and to foster greater alignment across health plans and delivery systems.[4] Indeed, P4P programs are not new to states. Currently more than 25 states have P4P programs with their health plans or primary care case management (PCCM) programs.[5] Yet, while many states use P4P programs to motivate improvements at the health plan level, few have designed effective programs at the provider level.

Historically, states delegate responsibility for provider incentive programs to their managed care partners, particularly in risk-based managed care delivery systems. States have been reluctant to micromanage managed care operations and have encouraged plans to innovate. States have also been challenged to work within the regulatory parameters established by the Centers for Medicare and Medicaid Services (CMS) including a cap on total incentive payments in risk-based systems.

In addition to acknowledging the benefits of standardization, states are increasingly aware that quality ultimately must occur at the point of care. Many states understand that in the highly competitive managed care environment, collaboration and alignment across Medicaid plans — even around quality — occurs most readily when the regulatory and purchasing authority of the state is used. As such, there is growing involvement of state Medicaid agencies in provider incentive programs.

In 2006, with funding from The Commonwealth Fund and additional support from the Robert Wood Johnson Foundation, the Center for Health Care Strategies (CHCS) launched the *Pay-for-Performance Purchasing Institute* to help state Medicaid agencies design provider incentive programs. Seven states — Arizona, Connecticut, Idaho, Ohio, Massachusetts, Missouri, and West Virginia — worked with CHCS to develop and test physician-level financial and non-financial incentives, choose performance measures, engage providers effectively, and increase alignment across incentive programs. This resource paper presents examples, including several from that initiative, of how states are becoming increasingly involved in P4P at the practice level, particularly around efforts to improve alignment and standardization.[6]

[4] HMA projections for total spending and enrollment for federal FY 2008, based on: CBO, *Budget and Economic Outlook*, January 2008; CBO, *Medicaid Baseline*, 2008; CMS, Office of the Actuary, National Health Statistics Group, 2008; and NASBO, *State Expenditure Report*, December 2007.

[5] K. Kuhmerker. *Pay-for-Performance in State Medicaid Programs: A Survey of State Medicaid Directors and Programs*, The Commonwealth Fund, April 2007. Available at: **http://www.commonwealthfund.org/usr_doc/Kuhmerker_P4PstateMedicaidprogs_1018.pdf?section=4039**

[6] For more about the *Pay-for-Performance Purchasing Institute* as well as information about structuring P4P programs, selecting measures, choosing financial and non-financial incentives, and engaging physicians, visit **www.chcs.org**.

Innovative State Models

The provider incentive program models described in this resource paper are based on the efforts of five state Medicaid programs:

- **Rhode Island** designed and implemented a provider incentive program that required Medicaid health plans to adopt standardized program goals, but allowed plans and providers to test different approaches to achieving those goals.

- **Arizona** is exploring the development of a provider incentive program that would require all Medicaid health plans to adopt common program goals, common performance measures, and to aggregate provider financial incentives across plans using a third-party broker.

- Since 2006, **Minnesota** has been participating in a provider incentive program where Medicaid and commercial purchasers and plans adopt uniform program goals and performance measures, and combine incentive dollars into "bucket" for provider payment.

- **Massachusetts** and **Missouri** are both focusing efforts around creating P4P program strategies and tools for use across different Medicaid delivery systems.

In considering the models above, a Medicaid program needs to recognize factors in its unique marketplace and circumstances, which may include:

- **Delivery system:** Will the incentive program operate in a risk-based managed care, primary care case management delivery system, or both?

- **Focus of P4P program:** Is the program targeting a chronic condition with a nationally-recognized measure set and a strong evidence base around impacting care (e.g., diabetes), or encouraging testing a new area with a less robust evidence base (e.g., reducing inappropriate emergency room utilization)?

- **Infrastructure:** Does the state have the infrastructure and staff to operate aspects of the program in house? The program design steps are consistent across models; however, "who does what" varies by state. In Rhode Island's model, the state is responsible for establishing and funding the provider incentive program, but the plans are responsible for the remaining steps. In Arizona's model, the state is more involved in all program design steps.

- **Political support:** Does the state have the political will and support to maximize alignment across plans? To join a multi-payer P4P program?

One size does not fit all in state-designed provider incentive programs, as illustrated by each of the following models, so states should consider their own unique circumstances when designing provider incentive programs.

Model 1: Alignment of Program Goals across Health Plans (Rhode Island)

Model 1 is based on Rhode Island's RIte Care P4P program. In 2005, the state required its health plans to implement a physician-level P4P component to compliment a health plan P4P program to reduce inappropriate emergency room (ER) use. The state had an incentive program at the plan level; however, with the prudent layperson laws and the Emergency Medicaid Treatment and Active Labor Act (EMTALA), the state and plans noted a gradual increase in ER utilization. The state decided to expand the incentive program to the point of care.

The state included a $0.95 per member per month (PMPM) increase to RIte Care's health plan capitation rate to be used for a primary care provider (PCP) incentive program. The state required that the additional PMPM payment be used to reward PCPs based on performance. Each plan was charged with designing a provider incentive program that promoted timely access to quality care, including preventive care, urgent care, and care during evening hours. Plans were not required to adopt the same provider-level measures or aggregate data into one rate per practice.

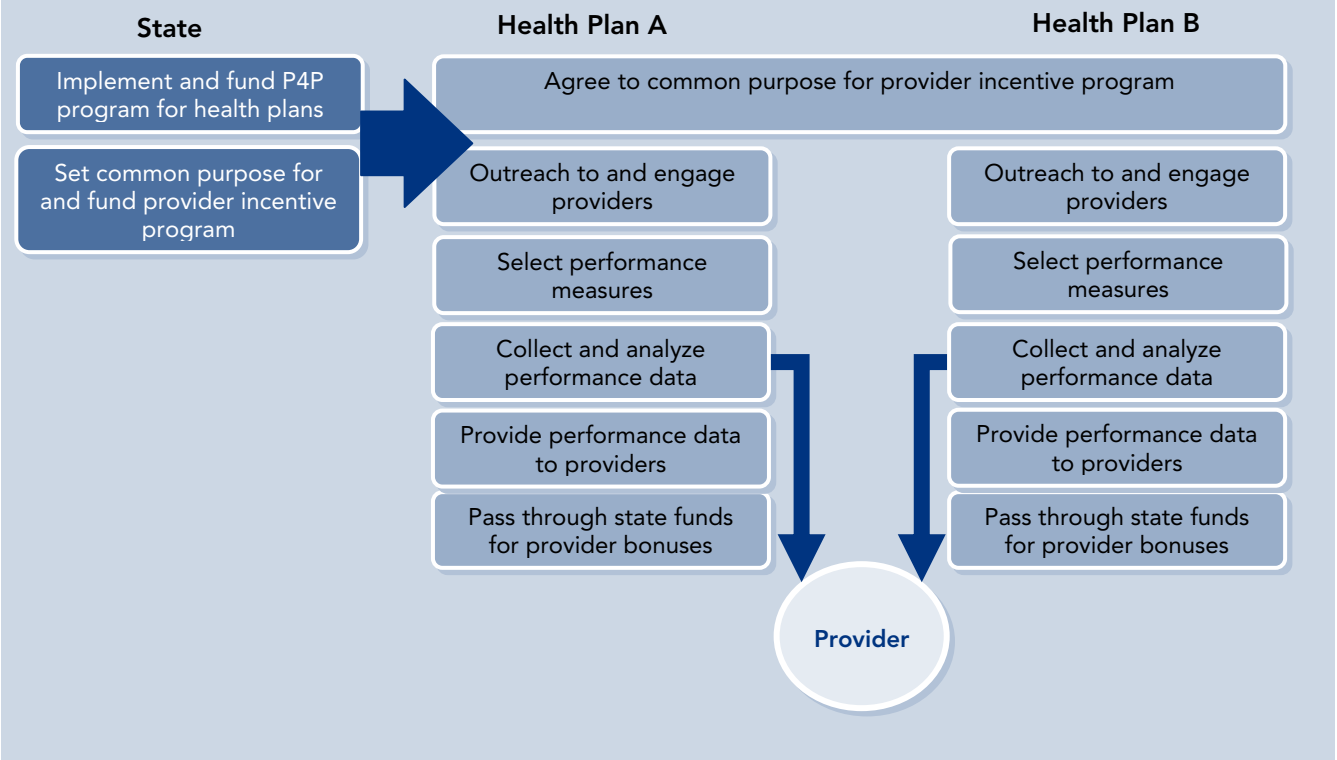
One of the plans, Neighborhood Health Plan of Rhode Island (NHPRI), targeted primary care practices with at least 200 NHPRI RIte Care members, creating a critical mass of potential new incentive dollars to "get the practice's attention." NHPRI divided the incentive payment into components. All eligible practices automatically received a payment of $0.30 PMPM during the first year. Practices that extended business hours or were open during the weekend received an additional PMPM amount. Practices that had multilingual practitioners on call after hours received an additional PMPM amount. Finally, practices that reduced ER utilization received an increase amount. Using this strategy, all eligible practices received some funding initially to work toward expanding access. Additional funding was not guaranteed or unlimited — progress had to be demonstrated over time. A subset of high-performing practices received the maximum amount for achieving specific outcomes.

Rhode Island's approach reflects its unique marketplace and delivery system. The state has a risk-based managed care program, so it operated the provider incentive program through its health plans. The program focus — reducing inappropriate ER utilization — did not have a strong evidence base of "what works." As such, the state wanted to leave ample room for the plans and providers to experiment. Adopting such an approach allowed the state and plans to test different provider incentive program designs and to compare and contrast what worked and what did not. The state is currently assessing outcomes data and convening its health plans to review, retool, and identify best practices.

States that are interested in testing provider incentive program options but are not yet ready to require greater alignment across plans or delivery systems might consider this strategy. Comparing and contrasting outcomes from different approaches would allow states to make more informed decisions in the future about how, where, and why to create greater standardization.

The figure below, based on Rhode Island's model, illustrates how a Medicaid program might design a provider incentive program across plans.

Model 1: Alignment of Purpose across Plans (Rhode Island)



Model 2: Alignment of Purpose, Measures, and Payment across Medicaid Plans Using a Third-Party Broker (Arizona)

Model 2 presents a provider incentive program that creates significant alignment across its Medicaid plans. It is based on a provider incentive approach that the Arizona Health Care Cost Containment System (AHCCCS) is exploring.

AHCCCS is considering a P4P program to improve care for 88,000 adult Medicaid beneficiaries with diabetes. In the proposed model, AHCCCS would aggregate claims data across Medicaid plans to calculate a provider's performance rate for all of his or her diabetic patients. In other words, the physician or practice would receive one consolidated diabetes performance report from the AHCCCS program, as opposed to a provider profile form each plan with whom the practice contracts. The proposed strategy aims to improve the validity and reliability of measurement and reduce the administrative burden on plans and providers alike.

Like the Rhode Island model, funds for provider bonuses would be included prospectively in the health plans' capitation rates. The health plans would be required to pass all state funds on to the providers. Incentive payments will be aggregated across plans into one payment per physician or practice. Thus a practice would receive one larger check, as opposed to multiple smaller checks. Because plans receive capitation rates prospectively, and provider performance would be measured and rewarded retrospectively, it

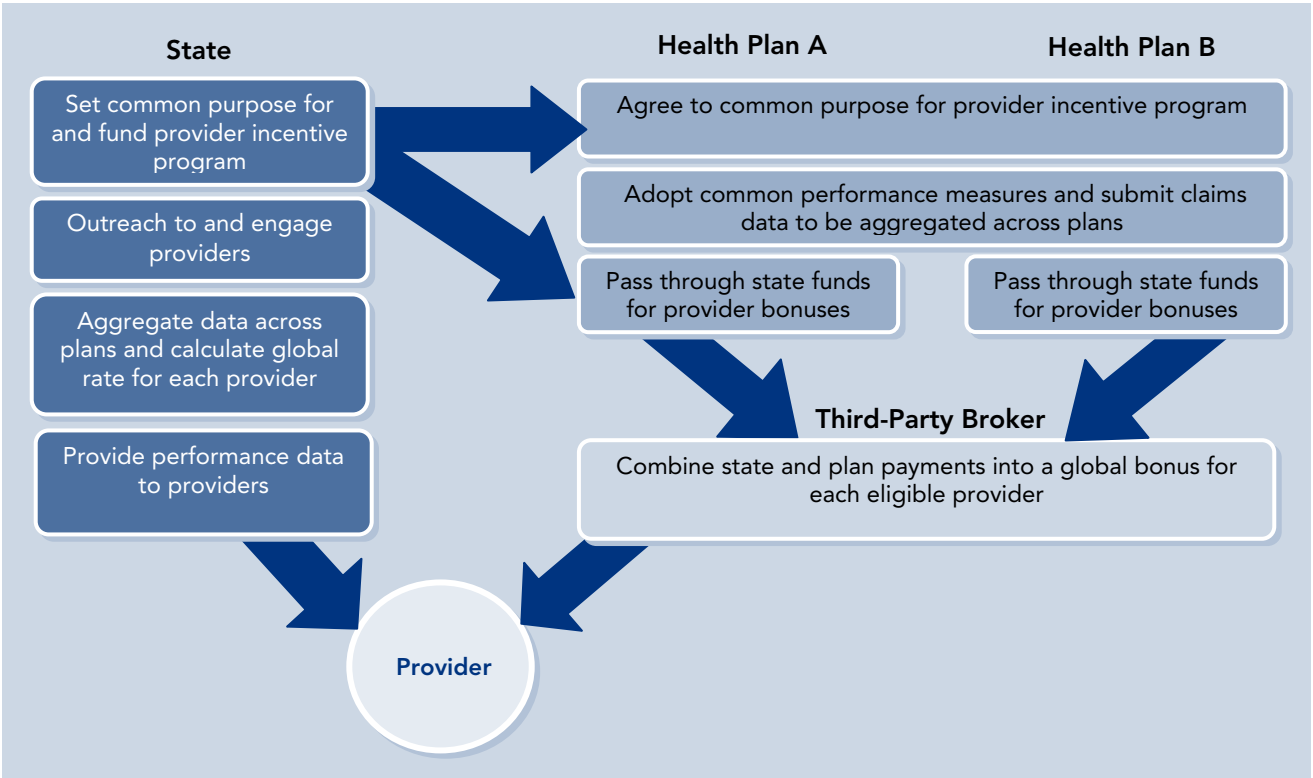
is possible that funds available for provider incentive payments might not be used in totality. Therefore, any remaining funds would accumulate and be used for provider incentive payments in the future.

The model that Arizona is exploring would use a third-party broker to receive and aggregate dollars from the plans, and to calculate and distribute bonus payments to practices. The state and CMS are currently exploring this broker model and whether and how it might be implemented.

Arizona's approach reflects its unique circumstances and delivery system. Like Rhode Island, Arizona operates a risk-based managed care delivery system, so any provider incentive program would need to operate through its plans. The potential program's focus — diabetes — has a nationally recognized set of measures and a strong evidence base around effective interventions. As such, the state could require plans to agree to a common set of measures. The state also has a rich source of high quality encounter data and a team of highly skilled data analysts who could calculate per practice or physician performance rates. Lastly, the large size of Arizona's program and the competitiveness of the managed care marketplace means that the state has significant purchasing leverage to direct quality improvement initiatives, if it so chooses.

The figure below, based on the model Arizona is exploring, illustrates how a Medicaid program might design a provider incentive program that is fully aligned across plans.

Model 2: Alignment of Purpose, Measures and Payment Across Plans Using a Third-Party Broker (Arizona)



Model 3: Alignment of Purpose, Measures, and Payment across Purchasers and Plans (Minnesota)

Model 3 is a cross-market collaboration that adopts a provider incentive program that is uniform across public *and* private purchasers (e.g., employers, Medicaid agencies, state employees, etc.) and plans (e.g., commercial and publicly funded). It is based on Minnesota's existing cross-payer provider incentive program. In this model, purchasers and plans use the same measures to assess provider performance and combine financial resources to reward high-performing physicians.

Such an approach has many benefits:

- Purchasers increase their buying power by banding together, focusing on value-based care, and demanding more efficient and effective health care.
- Plans must deliver value as purchasers make contracting decisions based on performance data. Plans benefit from creating economies of scale and reducing fragmentation of quality improvement activities.
- Practices are assessed by a common set of measures and a single report, which reduces administrative burden and confusion.
- When performance data is shared publicly, consumers have the opportunity to become more informed and active participants in their health care.

While public-private payer P4P programs are still in their infancy, the alignment and standardization created by this model holds great promise for Medicaid and commercial purchasers, providers, and consumers.

Minnesota's marketplace has unique characteristics that have helped accelerate innovations in quality. Its health care system is highly integrated. Hospitals and health systems own most primary care groups. As such, there is not only a strong business case to create alignment throughout a health care system, but the ability to do so.

Health care providers in Minnesota are required to serve the Medicaid population. This integration reinforces the importance of including Medicaid in cross-payer initiatives.

Lastly, Minnesota has created an infrastructure to support quality and innovation throughout its health care system. Three key building blocks have been particularly integral to Minnesota's achievements in value-based purchasing:

- **The Buyers Health Care Action Group** (BHCAG) is a coalition of private and public purchasers that seeks to promote purchasing strategies and develop tools that help purchasers buy and evaluate health care based on performance and value, not just price. BHCAG initiated Minnesota's diabetes provider incentive program in 2004.[7]
- **The Institute for Clinical Systems Improvement** (ICSI) develops evidence-based guidelines and measures for physician performance evaluation, and provides implementation support.[8]

[7] For more information about Minnesota's Buyers Health Care Action Group, visit **www.bhcag.com**.
[8] For more information about the Institute for Clinical Systems Improvement, visit **www.icsi.org**.

- **Minnesota Community Measurement** (MNCM) is a collaborative that receives and aggregates claims data from plans, collects clinical data from practices, and reports provider-level performance rates for conditions such as diabetes and cardiac disease. [9]

It is within this unique environment that BHCAG implemented a Bridges to Excellence (BTE) program to achieve optimal diabetes care in 2005. BTE is a national employer-led P4P program with a standard data exchange platform and performance measurements to foster cross-market collaborations in regions or states. Using evidence-based guidelines and measures developed by ICSI, MNCM collects, aggregates, and reports performance data for practices and clinics. BHCAG receives and aggregates financial incentives from purchasers, including Medicaid, and pays providers based on their performance.

In 2007, Minnesota's Department of Human Services (DHS), the state's Medicaid agency, joined BHCAG's effort and began to enroll Medicaid managed care recipients ages 18 to 75 with diabetes or cardiac disease into the initiative. DHS rewards practices based on their share of Medicaid patients, as opposed to clinical results. DHS includes dollars for provider incentives in health plan capitation rates. The plans, in turn, give those dollars to BHCAG.

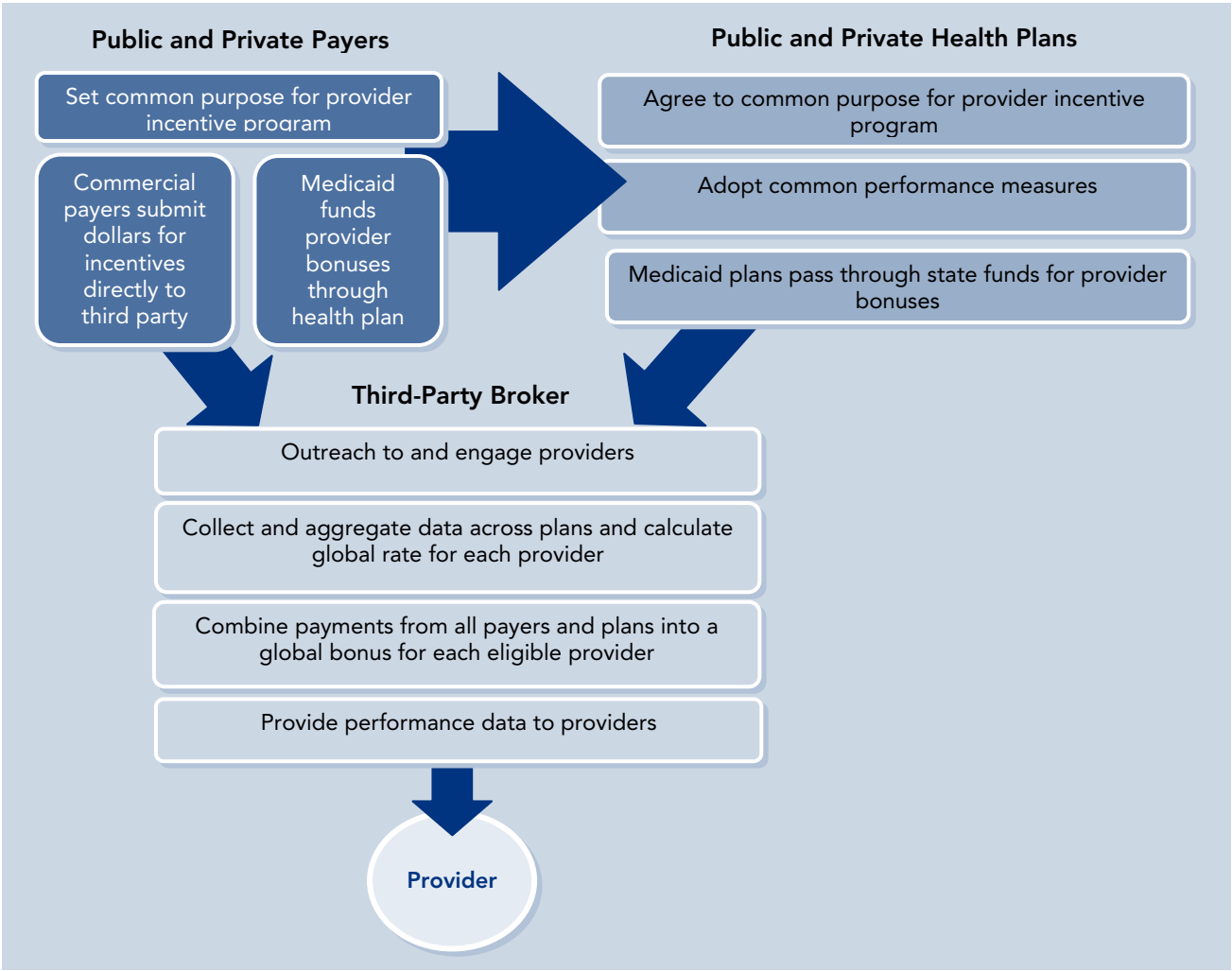
Through the BTE program, physicians providing optimal diabetes care to at least 10 percent of their patients with diabetes receive $100 per patient. In 2006, BHCAG paid physicians $97,000 in rewards, and rewarded $260,000 in 2007.[10] The percentage of Minnesotans receiving optimal diabetes care from providers participating in the BTE program has increased from 6 percent in 2004 to 22 percent in 2007.

DHS recently received approval from CMS for a new provider incentive program. This program will focus on Medicaid recipients remaining in fee-for-service. This population comprises 30% of Medicaid recipients, many of whom are disabled. Diabetes prevalence in this population is 10%, compared to 6% in the Medicaid managed care population. DHS will directly reward individual practices providing optimal care to Medicaid fee-for-service recipients with diabetes or cardiac disease. DHS will pay $125 per diabetic for the first year, and up to $500 for optimal performance in subsequent years. Practices will submit their data to DHS electronically.

The figure below, based on the model that Minnesota implemented, illustrates how to create full alignment across payers and plans.

[9] For more information about Minnesota Community Measurement, visit **www.mnhealthcare.org**.
[10] BHCAG March 2008 presentation at the CHCS Medicaid Purchasing Leadership Summit.

Model 3. Alignment of Purpose, Measures and Payment across Payers and Plans (Minnesota)



While several state Medicaid agencies have expressed interest in provider incentive programs that are aligned across purchasers and plans, Medicaid's engagement to date has been limited. States may face multiple challenges participating in a multi-payer P4P program. States have concerns about how data will be collected and used publicly. Public-private programs work best when there is a significant overlap of providers who serve both the commercial sector and Medicaid patients. States with comparatively low Medicaid reimbursement rates often have provider networks with a less integrated patient mix. Lastly, some Medicaid programs may struggle to obtain the funding necessary to adequately support and sustain provider incentive programs.

Model 4: Alignment of Purpose, Measures, and Interventions across Delivery Systems (Massachusetts and Missouri)

Model 4 depicts provider incentive programs being designed in Massachusetts and being implemented in Missouri. Both states developed P4P approaches within a non-risk-based context (PCCM or fee-for-service), with the intent to extend select program elements to the risk-based managed care delivery system in the future.

MassHealth, the Commonwealth's Medicaid program, is designing a provider incentive program for its Primary Care Clinician (PCC) Plan based on the following principles:

- Transparency and collaboration;
- Alignment with the existing data collection and reporting system;
- Minimization of provider reporting burden; and
- Consistency with established state and national P4P programs.

The program will measure performance of individual physician practices, group practices, community health centers, hospital-licensed health centers, and hospital outpatient departments. To participate in the clinical measures portion of the incentive program, providers must have a minimum number of Medicaid patients in the denominator of each clinical measure in the P4P program and must complete a practice survey.

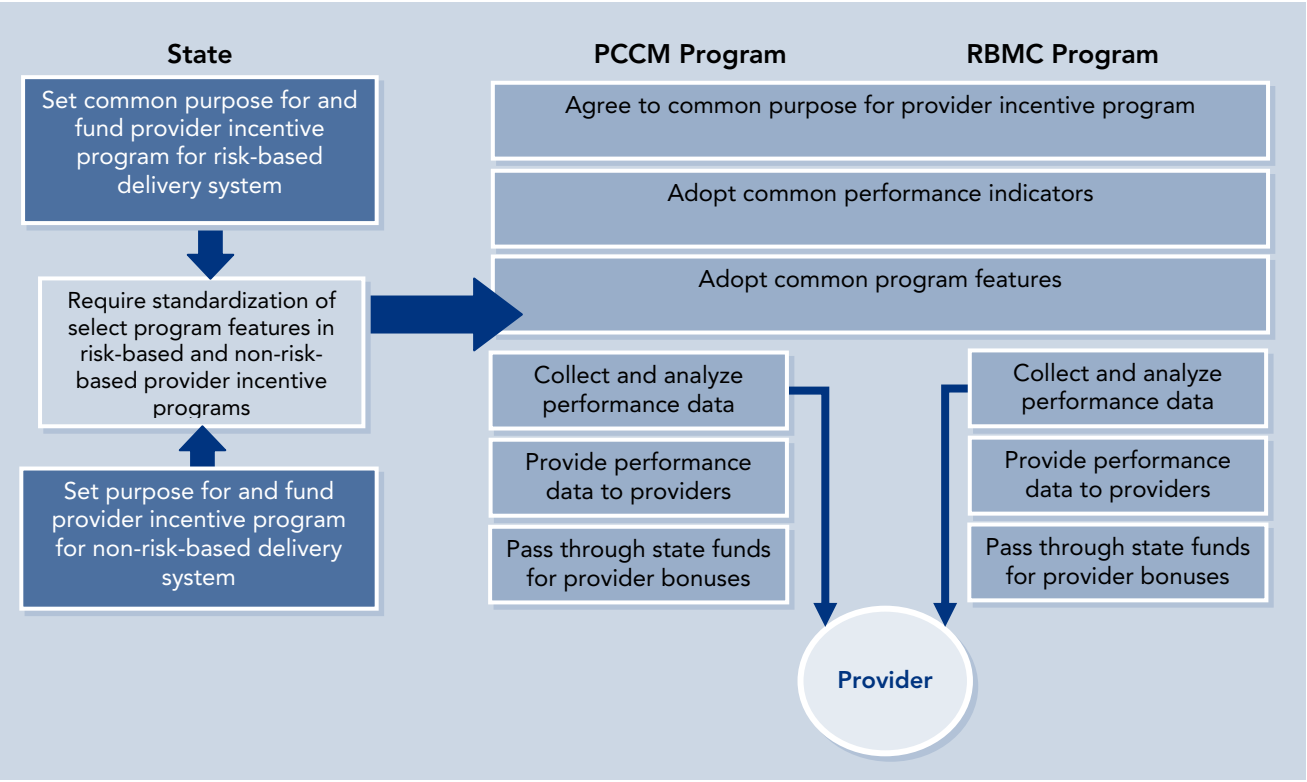
The practice infrastructure survey will be used to assess critical medical home components including: HIT capacity, follow-up from tests, referrals and acute events, guideline-based reminder systems, registries, access, and the process for gathering and tracking race and ethnicity information. During the first year of the program, practices will receive a "pay for reporting" amount if they fully complete and return the practice survey. Practices completing the survey will be assessed for eligibility for P4P funds based on their clinical indicator performance.

Provider performance around prevention and diabetes care will be assessed using HEDIS-based measures that are already collected and reported through the PCC Plan's profiling activities. MassHealth's new P4P program will allow providers to review their individual rates and submit additional information if they believe the rates are inaccurate. Payments will be based on achieving the performance benchmark for the clinical measures, or achieving improvement for clinical measures even though they do not meet the established benchmark rate. Incentives will be paid on a PMPM basis, based on the provider's total PCC Plan member enrollment. The state is still developing the specific PMPM amount.

The state is creating alignment across MassHealth's P4P programs. Specifically, all individual health plan provider incentive programs, including the new PCC Plan, will be required to use the same measures. They will also be required to use the same practice infrastructure survey tool.

The state of Missouri is also creating alignment with its fee-for-service (FFS) and risk-based managed care delivery system through a provider incentive program. Currently, physicians or mid-level practitioners in high-volume federally qualified health centers (FQHCs) in targeted geographic areas receive incentives for developing electronic care plans for patients with diabetes, asthma, gastroesophageal reflux disease, cardiovascular disease, or chronic obstructive pulmonary disease. The state pays providers $25 for developing the initial patient assessment online, and an additional $10 for updating web-based care plans. To assist providers with developing care plans, the state is placing care coordinators in the high-volume FQHCs to act as a liaison between the PCP and the patient. Missouri is currently revising its health plan contract to include the same provider incentive program.

Model 4. Alignment of Purpose, Measures, and Interventions Across Delivery Systems (Massachusetts and Missouri)



State Provider Incentive Program Profiles

Following are brief descriptions of additional state provider incentive programs:

Connecticut
Connecticut is working closely with its health plans to design and phase in a provider incentive program aimed at improving EPSDT rates. The first phase is to reward PCPs for completeness of EPSDT encounters. To do this, the state added payment incentives directly to the EPSDT reimbursement process so that medical practices can devote the time and resources necessary for care coordination. The state is now focusing on options for measuring and rewarding care coordination at the practice level. Specifically, the state will provide bonus payments through the health plan capitation rate to PCPs who coordinate care with specialists as appropriate based on EPSDT screening results.

Idaho
Idaho is piloting a provider incentive program within its PCCM delivery system and disease management program. Although the program will ultimately target five chronic diseases (diabetes, asthma, depression, hyperlipidemia and hypertension), the state made a strategic decision to "start small" by focusing first on diabetes. The state is targeting the pilot program to three high-volume FQHCs equipped with electronic medical records to facilitate data capture.

The diabetes program aligns with the state's existing disease management program and targets approximately 500 diabetics within the state. While Idaho is using nationally-recognized performance measures to assess changes in outcomes, it is initially rewarding practices based on process measures. Specifically, practices receive $50 per patient enrolled in the state's diabetes disease management program. They also receive $10 for each of the six diabetes measures reported. To date, the state has paid $20,000 to the three FQHCs. The state has $500,000 available for incentive payments as it continues to expand the P4P program. Next steps include establishing a secure web-based data submission and collection system.

Ohio
Ohio began exploring options for provider incentive programs by soliciting feedback from its health plans, provider community and other key stakeholders. A series of focus groups revealed physician frustration regarding the variety of measurement sets across different payers and plans. One medical director of a large primary care network described nine different measurement sets for which his organization is accountable. Physicians also voiced concerns with the accuracy of administrative data and were more likely to support P4P if their own data was used for measurement.

The state also surveyed health plans regarding the range of P4P methodologies, potential performance indicators, and estimated distribution of physician rewards over the various performance measurement domains. One key finding was that physician-level measurement, reporting and incentives could be complicated by the small numbers of encounters and measurable events at the physician-level. The state identified that only 12 percent of practices had a volume of 30 or more Medicaid patients — the number they estimate to sufficiently evaluate performance.

One option being considered is joining Cincinnati's Bridges to Excellence Diabetes Care Link program. Cincinnati, one of the original BTE pilot markets, has been active since 2003. Physicians in the program who demonstrate they are top performers in diabetes care can earn up to $100 for each patient covered by a participating employer. Employers (currently private only) fund incentives from documented savings achieved through lower health care costs and increased employee productivity that results from improved diabetes care.

Due to budgetary constraints, Ohio Medicaid has "tabled" short-term plans around developing a provider incentive program, but continues to consider its opportunities moving forward.

West Virginia
West Virginia is considering a provider incentive program that would support the state's Mountain Health Choices program. Mountain Health Choices offers a two-tiered benefit system — beneficiaries must sign a "personal responsibility" agreement to receive enhanced benefits. Through the agreement, the beneficiary acknowledges the role he/she plays in his/her health care delivery. The state is considering implementing a provider incentive program that rewards physicians and mid-level practitioners as they encourage and work with Medicaid recipients in completing the agreements. Due to budgetary constraints and limited resources, the state plans to revisit its P4P programs — both at the plan and provider level — in the near future.

Conclusion

While the number of provider-level incentive programs is low compared to plan-level programs, provider P4P initiatives are increasing with the greater awareness that health care is local and quality ultimately occurs at the point of care when the patient meets face-to-face with the provider.

The provider P4P models highlighted herein can help states identify options for creating greater standardization in their quality improvement activities, particularly in a risk-based managed care delivery system. Alignment of P4P programs can improve the validity and reliability of performance measurement, reduce administrative burdens on plans and providers, and create economies of scale for plans and breakthroughs in quality for patients — all of which lead to reductions in future cost growth. This resource paper deliberately highlights states that have chosen different levels of alignment, recognizing that states will vary in terms of their interest in and ability to create standardization.

This resource paper reflects the growing recognition of the need to standardize quality improvement initiatives to send a stronger message to providers. Fragmentation in quality improvement efforts creates duplication and confusion for providers. Provider incentive programs offer purchasers an opportunity to become more involved in improving quality at the point of care and in achieving a greater level of standardization across P4P programs. Although P4P is just one tool in the quality improvement "arsenal," Medicaid programs can play an important role in creating much needed alignment in P4P.

Appendix

Background on P4P

Pay-for-performance (P4P) programs are voluminous and growing throughout the public and private sectors as payers increasingly look for ways to link payment and quality. While many events have contributed to the proliferation of P4P programs, a few seminal events and initiatives are highlighted in this section.

The earliest P4P efforts were initiated by plans seeking to measure provider performance around cost and utilization, more so than quality. As employers saw their health care costs rising, they sought to link payment with health outcomes through health plans, which were responsible for a growing proportion of their employees. Plans were a logical starting place because they already collect standardized performance measures through the Healthcare Effectiveness and Data Information Set (HEDIS®) measures and customer satisfaction information through the Consumer Assessment of Healthcare Providers and Systems (CAHPS®) tool. Plans also have the capacity to collect and report data and are responsible for performance in many settings.

In 2001, the Institute of Medicine (IOM) released its groundbreaking report, *Crossing the Quality Chasm*, which revealed that up to 98,000 Americans die each year as a result of medical errors.[11] The IOM report stated that payers need to align payment policies to support quality improvement, as they often were paying more for poorer outcomes. This report was followed by one from RAND in 2003 that documented that patients receive the recommended care approximately half the time.[12] Both of these reports resonated deeply with health care purchasers, payers, providers, and consumers, and reinforced the need to link payment with performance.

As P4P grew in the private sector, public payers also began to link payment to performance. Medicare, for example, launched several P4P demonstration programs, targeting hospitals and physician practices. State Medicaid programs also began implementing P4P programs — some as early as the 1990s. Initial efforts focused on accountability, rather than quality or value, and targeted health plans. Because states began managed care enrollment with the Temporary Assistance for Needy Families (TANF) population, early P4P programs tended to focus on measures specific to services that mothers and children typically received, such as prenatal care visits, well-child checkups, and immunizations. Over time, as states have enrolled high-cost, high-need Medicaid beneficiaries into care management programs, P4P programs have expanded to focus on outcomes related to complex conditions and special needs.

As states have become more sophisticated purchasers of care and more proficient at collecting and using performance data and measures, P4P programs have become more advanced and targeted. As of July 2006, 28 state Medicaid agencies operated P4P programs, and half of those programs were operating for five or more years. Again, the majority of these programs were at the health plan level, followed by those targeting primary care case management (PCCM) programs, nursing homes, hospitals, behavioral health care providers, and lastly, individual physicians. In 2006, 19 states were planning to expand existing P4P programs in the next five years, and 15 Medicaid agencies were planning to start their first P4P programs.[13]

[11] Institute of Medicine. *Crossing the Quality Chasm: A New Health System for the 21st Century*. National Academy Press, 2001.
[12] RAND. *The Quality of Health Care Delivered to Adults in the United States*. McGlynn, June 2003.
[13] The Commonwealth Fund. *Pay-for-Performance in State Medicaid Programs: A Survey of State Medicaid Directors and Programs*. Kuhmerker and Hartman. April 2007.

The growing availability of performance data, the increasing demand for value-based purchasing, and a greater national focus on creating more alignment and standardization around quality have contributed to the proliferation of P4P initiatives, particularly to measure performance at the point of care. A few of the most notable ones are described below.

Rewarding Results: Aligning Incentives with High-Quality Health Care

Rewarding Results was a three-year effort funded by the Robert Wood Johnson Foundation, the California HealthCare Foundation, and The Commonwealth Fund. The three foundations selected seven demonstration projects that made providers eligible for financial and non-financial rewards based on the achievement of specific quality goals linked to clinical quality. The demonstration projects offered varied approaches, typically targeting primary care physicians or physician organizations, and represented several types of insurance arrangements, (e.g., health maintenance organizations (HMOs), preferred provider organizations (PPOs), and Medicaid.)

Through use of incentives, the *Rewarding Results* projects significantly increased patient visits to the doctor; pushed physicians to embrace health information technology (HIT) and electronic medical records (EMRs) at a faster pace; increased the number of patients receiving annual mammograms and other screenings; and motivated physicians to monitor patient care more aggressively, particularly for chronically ill patients. The initiative included seven experimental projects — three of which are described below — designed to test a variety of P4P models.

Integrated Healthcare Association (IHA)
Created in 1996, the Integrated Healthcare Association is a California-based, statewide coalition of health plans, physicians, health care systems, purchasers, and consumers working to create the business case for quality at the physician group level. In 2003, IHA initiated its P4P program with the goal of rewarding physician groups for performance in clinical care, patient experience, and HIT investment based on common metrics and public reporting. Key to the program's success has been the use of uniform measures to evaluate performance across multiple health plans, physician groups, and patient populations. To date, it is the largest P4P initiative in the country.

Bridges to Excellence
Bridges to Excellence (BTE) is the largest employer-sponsored effort rewarding and recognizing physicians for meeting specific quality benchmarks. For the *Rewarding Results* initiative, the BTE employer coalition focused on four locations across the country and financially rewarded physicians per patient per year for excellence in diabetes and/or cardiac care. The BTE model is now in several markets across the country and has found that physicians who are recognized for providing high-quality and more efficient care deliver it at 15 to 20 percent lower cost than physicians not participating in the program.

Local Initiative Rewarding Results
Local Initiative Rewarding Results was the largest collaborative P4P effort to improve the health of babies and teens in Medicaid. The California-based project involved seven health plans that collaborated to test the impact of financial and non-financial incentives on provider quality. The program, which ran from 2002 through 2004, ultimately paid $5 million in provider incentives and involved 3,300 physicians touching the lives of 350,000 babies, adolescents, and parents. Five of the seven plans improved the rate of well-baby visits, with increases from 4 to 35 percent. Visits to the doctor by teens increased from 7 to 14 percent at six of the seven plans. Of the seven *Rewarding Results* projects nationwide, the *Local Initiative Rewarding Results* project was the only activity focusing on the Medicaid population and the first known collaborative effort to establish financial incentives within Medicaid among multiple plans with the same objective.

The Leapfrog Group

The Leapfrog Group was established in 2002 to mobilize employers' purchaser power in relation to health care services and to influence the quality and affordability of care. The *Crossing the Quality Chasm* report focused Leapfrog initially on reducing preventable medical mistakes, recommending that large employers provide more market reinforcement for the quality and safety of health care. The Leapfrog Group launched its Hospital Rewards Program in 2005 and continues to measure hospital cost and quality performance. Hospitals that demonstrate excellence or show improvement along both dimensions receive rewards.

Medicare P4P Demonstration Programs

The Centers for Medicare and Medicaid Services (CMS) has been instrumental in establishing demonstration projects for P4P at the point of care. Two of its key P4P demonstration projects focused on physician practices are described below.

Physician Group Practice (PGP) Demonstration
In 2005, CMS launched its two-year Physician Group Practice (PGP) Demonstration focusing on improving the quality of care delivered to patients with congestive heart failure, coronary artery disease, and diabetes mellitus. Ten large, multi-specialty group practices participated in the demonstration project and received $16.7 million in incentive payments for improving health outcomes and coordinating the overall health care needs of Medicare patients assigned to their groups.

Physician Quality Reporting Initiative (PQRI)
A related CMS value-based purchasing initiative is the Physician Quality Reporting Initiative (PQRI), which uses a pay-for-reporting approach. Under PQRI, physicians and other health care professionals earn incentive payments for reporting measurement data about the quality of care they provide to Medicare patients. CMS is now developing a program that moves from the PQRI pay-for-reporting approach to a performance-based payment plan.

CMS Payment Polices and Federal Regulations

The Centers for Medicare and Medicaid Services provides policy guidance on provider incentive programs that states must consider as they develop P4P programs in risk-based managed care and PCCM programs.

Payments to Providers
Under risk-based managed care, because a contractual relationship exists between the state and its plans, states are prohibited from paying providers outside of the health plan contract. In other words, states are restricted from making direct incentives payments to providers. As an alternative, a state can include funds for a provider incentive program in the health plan's capitation rate, then contractually require the health plan to pass the full incentive payment on to eligible providers.

In a PCCM environment, a state can make incentive payments directly to eligible providers or have the PCCM administrator pass through the bonuses to eligible providers. The state must specify the incentives in its State Plan, and the incentives must be tied to payments for services, as specified in the State Plan.

Incentive Payment Amounts
Incentive payments from the state cannot exceed 105 percent of the payments attributable to services covered by the incentive arrangement.[14] In risk-based managed care, total payments (capitation payments

[14] 42 CFR 438.

plus any incentive amounts) cannot exceed 105 percent of the approved capitation rate attributable to services covered by the incentive arrangement. A state could not, for example, offer a 3 percent incentive payment to its health plans, and an additional 3 percent as a "pass through" to physicians.

In a PCCM delivery system, the 105 percent ceiling is based on services that impact the P4P program target, and may include inpatient hospital, emergency room services, and other services as well as those provided or authorized by the physician, practice, or other PCCM provider. A state should work with CMS to develop an incentive methodology that fits its PCCM program design. Research shows that incentive payments must be large enough to be meaningful in order to motivate a change in behavior. As such, limits on incentive payments may present an even greater reason to collaborate with other purchasers, particularly when Medicaid can benefit from pre-existing quality improvement programs.

The IEHP Pay For Performance Program

IEHP Pay for Performance Program (P4P)

- ⇨**Goals**
- ⇨**Methodology**
- ⇨**Evolution**
- ⇨**Outcomes**

Goals

- Motivate Physicians to Provide Services
  - Outreach to assigned Members
  - 'Capture' when Member in office
  - Report the event
- Increase Physician Reimbursement
  - Beyond Capitation payments
  - Pediatricians
  - OB/Gyns
- 'Bind' Physicians to IE
  - Direct Payments from IEHP
  - Significant Percent of Income

Methodology

- Direct to Physician Payments
- Fee For Service Model
- 'Easy' Billing
- Timely Submission
- Timely Payments

➢NOT CHDP or CPSP

The Beginning

The Evolution of P4P at IEHP

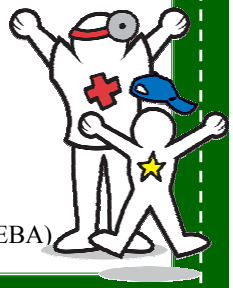
- The IEHP Immunization Program was the first attempt at a physician incentive program
- Launched September 1997
- Goal was to increase the immunization rate of IEHP Members 0-2 years of age.

The Beginning cont….

- Program provided direct reimbursement to physicians for immunizations
- Immunizations were submitted to IEHP via the PM160 form - only change was adding series #

PIP: The Physician Incentive Program

- In April 2000, the Physician Incentive Program was launched
- The new PIP program consisted of 5 components
  1. Immunizations
  2. Well Child Visits
  3. IHA/Adult Physical
  4. Perinatal Services
  5. Health Education Behavioral Assessment (HEBA)



PIP: The Physician Incentive Program cont…

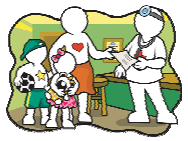
- Well Child Visit Component
  - Physicians were reimbursed $50 for each well child visit done in accordance with the IEHP Well Child Visit schedule for Members 0 to 18 years old
  - Exams done during the first 120 days of enrollment were paid an additional $50 bonus

PIP: The Physician Incentive Program cont…

- Perinatal Services Component
  - Designed to ensure that all IEHP Members receive timely prenatal and postpartum care
  - Reimbursement Schedule
    - $200 if date of service for initial visit in the 1st trime
    - $100 if the 2nd trimester
    - $50 if in the 3rd trimester
    - $50 for a postpartum exam within 8 weeks of delive

PIP: The Physician Incentive Program cont…

- In January 2001 the PIP program was redesigned
  - The IHA and HEBA components were removed
  - Reimbursement for Pap Tests was added - $25 reimbursement on CMS 1500
- In August 2001, Chlamydia Screening was added to the P4P program - $25 on CMS 1500



PIP: The Physician Incentive Program cont…

- In January 2003, the Diabetes component was added to PIP
- Providers were reimbursed $25 for each of the following:
  - HbA1c Tests
  - LDL Screening
  - Retinal Exams
  - Foot Exams

Pay For Performance

- With an increasing importance being placed on HEDIS results by DHS, MRMIB, and NCQA, IEHP decided to overhaul the PIP program
- In July 2004, the new Pay For Performance Program (P4P) was launched
- The P4P program is HEDIS-centered

Pay For Performance cont…

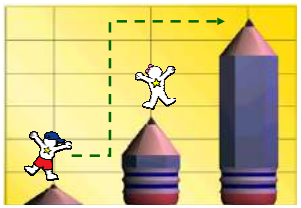
- Implemented a $100 bonus for
  - Completion of 6 well child visits by 15 months
  - Submission of a complete immunization record prior to age 2
- Significantly increased perinatal payments
- Implemented outcomes bonuses for Diabetes:
  - $50 For HgbA1c of 7.0 or less
  - $50 For LDL of 100 or less
- Added Asthma Component (9/1/05)
  - $25 for asthma progress note on-line
  - $20 for paper

Pay For Performance

Questions

The Payoff

- The Program is designed to increase the provision of preventive health services to our Members as well as to improve HEDIS results and we have achieved success in both areas
- Our P4P program has made a tremendous impact on our HEDIS results



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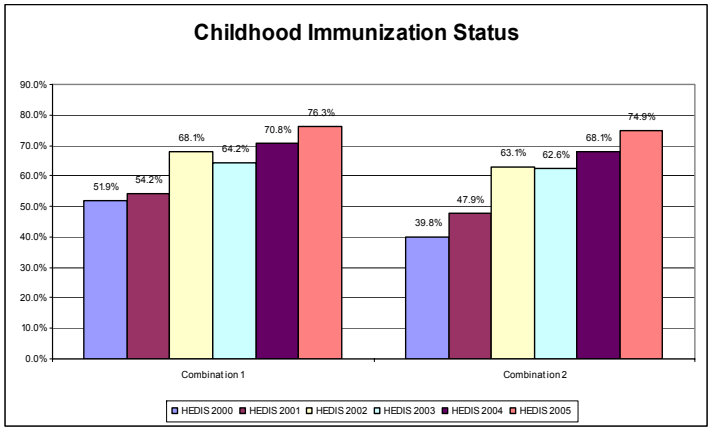

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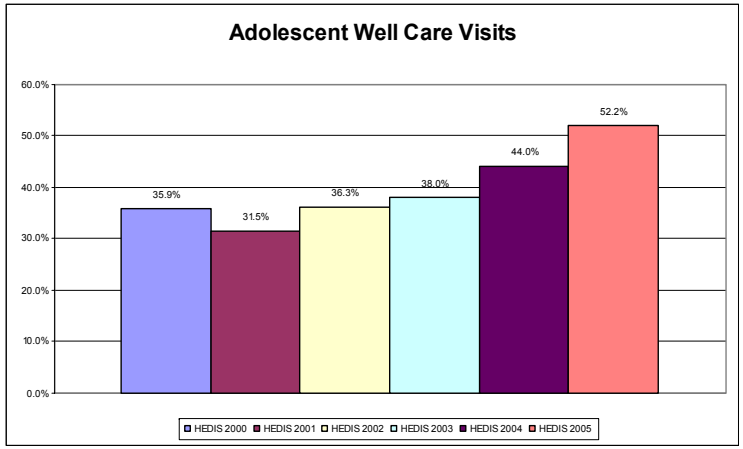

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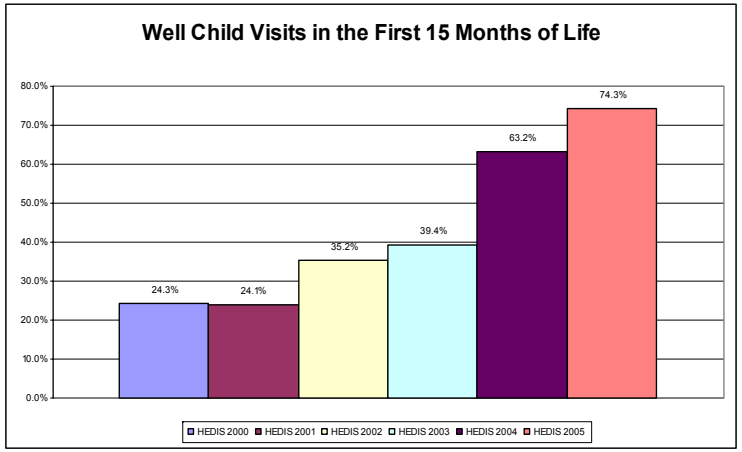

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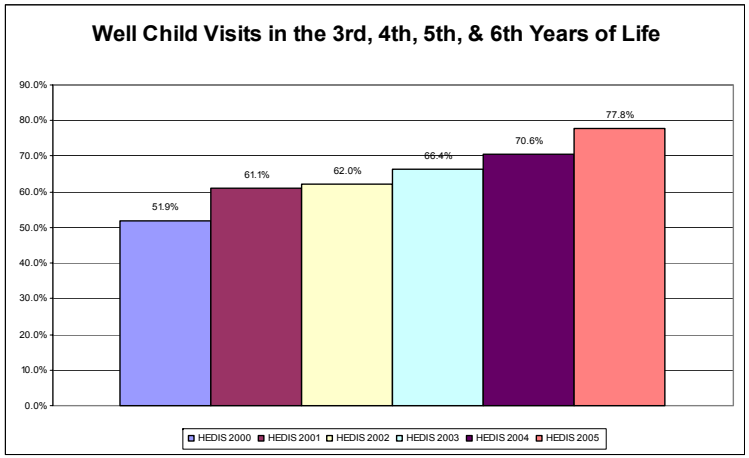

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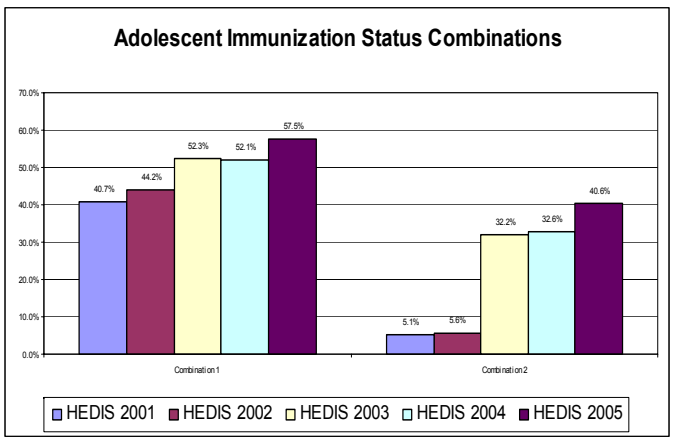

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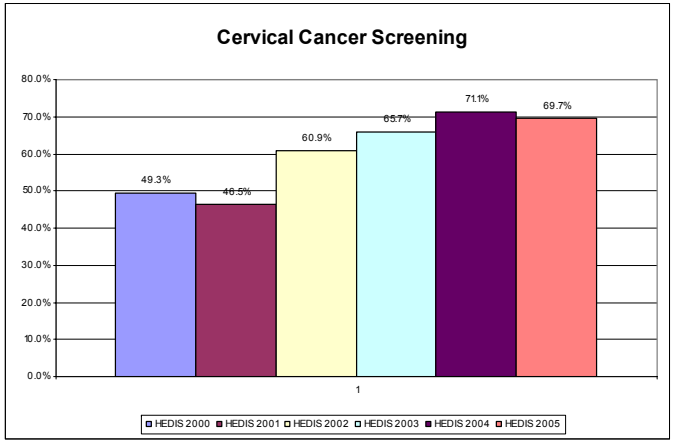

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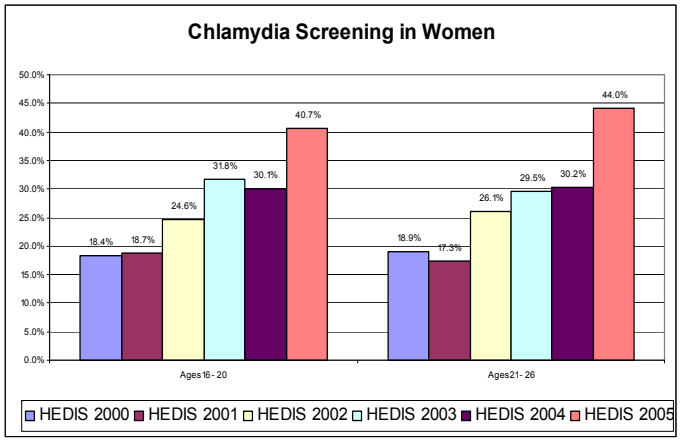

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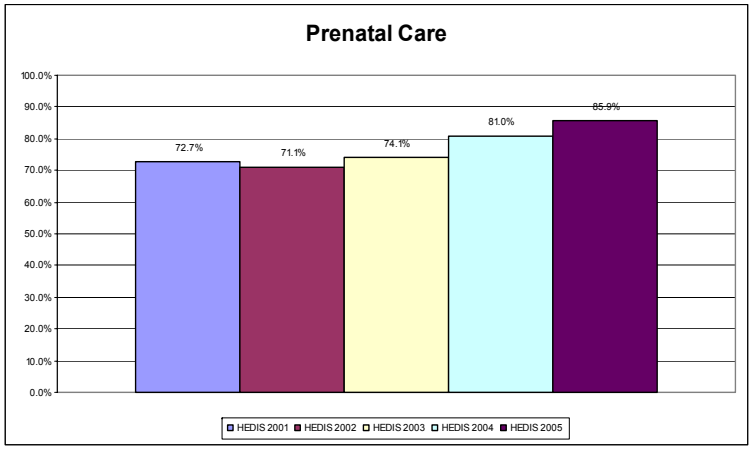

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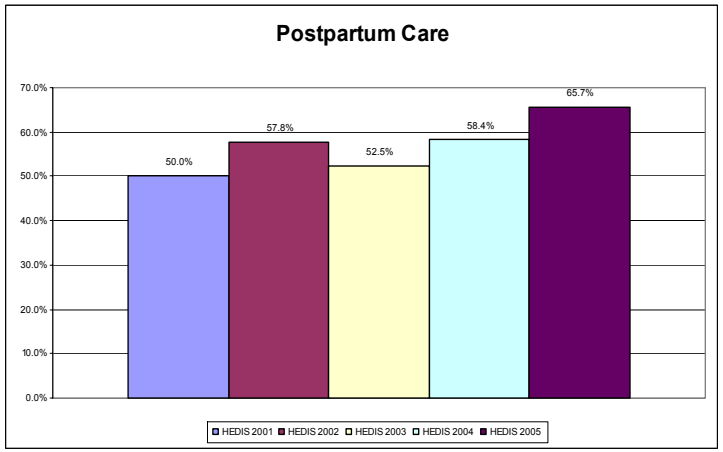

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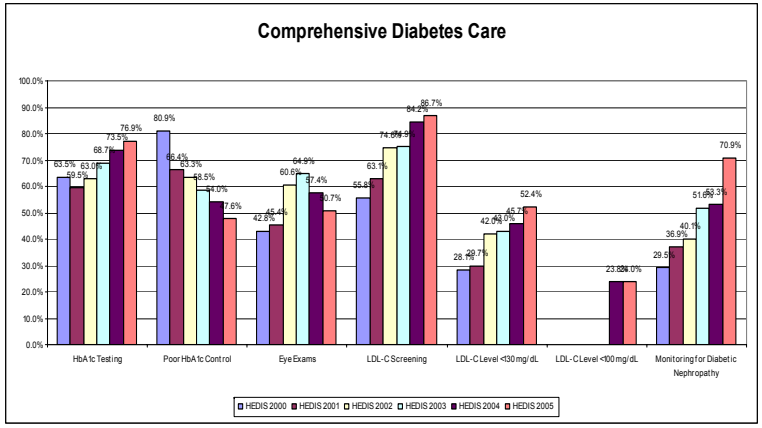
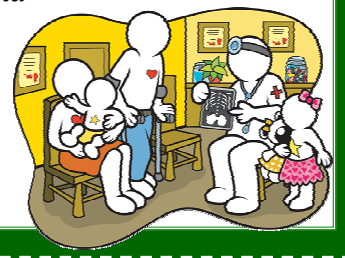


Update

- Program is now $12 million annually
- IPA P4P
  - HEDIS Measures
  - Physician Specific Web Data
  - Further Motivation



Pay For Performance

Questions

2016 Performance Recognition Program

PROVIDER INCENTIVE PROGRAM FOR:

- **BCN Commercial HMO**
- **BCN Advantage℠ HMO-POS**
- **BCBSM Medicare Plus Blue℠ PPO**

CONTENTS

2016 PERFORMANCE RECOGNITION PROGRAM

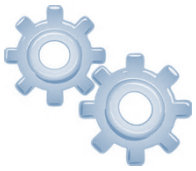
The Provider Performance Recognition Program rewards Blue Care Network Commercial providers and Medicare Advantage providers for both Blue Cross Blue Shield of Michigan and BCN for their role in helping Blue Cross and BCN achieve the objectives of the Healthcare Effectiveness Data and Information Set, or HEDIS®, and the Centers for Medicare & Medicaid Services' star ratings program. These objectives include:

- Better care
- Healthier people and communities
- Affordable care

Each program rewards providers who encourage their patients to get preventive screenings and procedures, such as eye exams and mammograms, and for achieving patient outcomes such as ensuring diabetic members have their blood sugar controlled.

Our philosophy is to use meaningful payments to encourage positive clinical results as well as increase HEDIS outcomes and CMS star ratings.

The components of the program, including the performance measures that are based on HEDIS benchmarks, are described in this booklet. Primary care physicians must have attributed or assigned members to participate in the program.



BLUE CROSS BLUE SHIELD OF MICHIGAN AND BLUE CARE NETWORK
2016 PHYSICIAN QUALITY INCENTIVE MEASURES

QUALITY INCENTIVE MEASURES	BCN COMMERCIAL HMO	BCN ADVANTAGE℠ HMO	BLUE CROSS MEDICARE ADVANTAGE PPO
Adult BMI assessment		●	●
Aspirin or antiplatelet therapy		■	
Breast cancer screening	●	●	●
Childhood immunizations — combo 10	●		
Colorectal cancer screening		●	●
Comprehensive diabetes care: HbA1c < 8%	●		
Comprehensive diabetes care: HbA1c ≤ 9%		●	●
Comprehensive diabetes care: monitoring for nephropathy	●	●	●
Controlling blood pressure		■	
Controlling high blood pressure for hypertension	●	●	●
Depression management — PHQ9 testing	●		
Disease modifying antirheumatic drug therapy for rheumatoid arthiritis		●	●
Medication adherence for diabetes medication		●	●
Medication adherence for hypertension medication		●	●
Medication adherence for cholesterol medications		●	●
Smoking/tobacco cessation counseling	●	■	
Weight assessment and counseling for children: BMI percentile, counseling for nutrition and physical activity	●		

Key

● = Performance Recognition Program
■ = CMS Million Hearts

BLUE CROSS BLUE SHIELD OF MICHIGAN AND BLUE CARE NETWORK
2016 PAYOUT SUMMARY — BCN COMMERCIAL

BCN Commercial HMO payment calculation

Payments for each eligible provider are calculated using the following methodology, regardless of membership level.

1. Quality score: A quality score for each program measure is computed for each provider using the following formula:

   a) Numerator = Eligible members meeting criteria

   b) Denominator = Total members eligible

   c) Numerator ÷ Denominator: The individual provider's quality score for each program measure

2. Compare the individual provider's quality score to the plan goal for quality. The payment for services will be calculated once the plan goal is met, based upon the Numerator.

   For measures with no specific plan goal, a flat fee will be paid for each service completed.

BCN Commercial HMO payment table

QUALITY INCENTIVE MEASURES	PLAN GOAL	PAYOUT
Breast cancer screening	80%	$100
Childhood immunizations — combo 10	63%	$400
Weight assessment and counseling for children: BMI percentile, counseling for nutrition and physical activity	63%	$150
Comprehensive diabetes care: HbA1c < 8%	68%	$250
Comprehensive diabetes care: monitoring for nephropathy	90%	$125
Controlling high blood pressure for hypertension	75%	$100
Depression management — PHQ9 testing	Flat Fee	$200
Smoking/tobacco cessation counseling	Flat Fee	$30

BLUE CROSS BLUE SHIELD OF MICHIGAN AND BLUE CARE NETWORK
2016 PAYOUT SUMMARY — MEDICARE

Medicare Advantage payment calculation

Program payments for each eligible provider are calculated using the following methodology.

1. Quality score: A quality score for each program measure is computed for each provider by determining:
   - Numerator = Eligible members meeting criteria
   - Denominator = Total members eligible
   - Numerator ÷ Denominator: The individual provider's quality score for each program measure
2. Compare the quality score for each measure to the **CMS star rating scale** for that measure to determine a star score for each measure.
3. Average the star scores for all measures to determine an overall star rating by provider.
4. Convert the overall star rating into a per-member-per-month payment using the **Medicare Advantage payment table**.

Note: Providers are scored separately for BCN Advantage and Medicare Advantage PPO products.
See next page for **CMS star rating scale** and **Medicare Advantage payment table**.

BLUE CROSS BLUE SHIELD OF MICHIGAN AND BLUE CARE NETWORK
2016 PAYOUT SUMMARY — MEDICARE

CMS star rating scale

QUALITY INCENTIVE MEASURES	1 STAR	2 STAR	3 STAR	4 STAR	5 STAR	WEIGHT
Adult BMI assessment	< 70%	70 - 80.9%	81 - 89.9%	90 - 95.9%	≥ 96%	1
Breast cancer screening	< 39%	39 - 62.9%	63 - 73.9%	74 - 79.9%	≥ 80%	1
Colorectal cancer screening	< 51%	51 - 62.9%	63 - 70.9%	71 - 77.9%	≥ 78%	1
Comprehensive diabetes care: HbA1c ≤ 9%	< 49%	49 - 59.9%	60 - 70.9%	71 - 83.9%	≥ 84%	3
Comprehensive diabetes care: monitoring for nephropathy	< 85%	85 - 88.9%	89 - 92.9%	93 - 96.9%	≥ 97%	1
Controlling high blood pressure for hypertension	< 47%	47 - 61.9%	62 - 74.9%	75 - 81.9%	≥ 82%	1
Disease modifying anti-rheumatic drug therapy for rheumatoid arthiritis	< 64%	64 - 74.9%	75 - 81.9%	82 - 85.9%	≥ 86%	1
Medication adherence for diabetes medication	< 60%	60 - 68.9%	69 - 74.9%	75 - 81.9%	≥ 82%	3
Medication adherence for hypertension medication	< 58%	58 - 72.9%	73 - 76.9%	77 - 80.9%	≥ 81%	3
Medication adherence for cholesterol medications	< 50%	50 - 60.9%	61 - 72.9%	73 - 78.9%	≥ 79%	3

Medicare Advantage payment table

AVERAGE STAR	PMPM PAYOUT
5	$8
4.5 – 4.99	$7
4 – 4.49	$4
3.5 – 3.99	$2.50
< 3.5	$1 for each half-star improvement from 2015

BLUE CROSS BLUE SHIELD OF MICHIGAN AND BLUE CARE NETWORK
2016 PAYOUT SUMMARY — MEDICARE

Medicare Advantage payment calculation
Example #1: "Dr. A"

DR. A QUALITY SCORES BY MEASURE:	NUMERATOR	DENOMINATOR	SCORE	STARS	WEIGHTED STARS
Adult BMI assessment	32	32	100%	5	5
Breast cancer screening	15	15	100%	5	5
Colorectal cancer screening	25	35	72%	4	4
Comprehensive diabetes care: HbA1c ≤ 9% (weighted x 3)	11	12	90%	5	5 5 5
Comprehensive diabetes care: monitoring for nephropathy	10	10	100%	5	5
Controlling high blood pressure for hypertension	0	0	n/a	n/a	n/a
Disease modifying anti-rheumatic drug therapy for rheumatoid arthritis	1	1	100%	5	5
Medication adherence for diabetes medications (weighted x 3)	5	6	83%	5	5 5 5
Medication adherence for hypertension medications (weighted x 3)	12	16	75%	3	3 3 3
Medication adherence for cholesterol medications (weighted x 3)	20	24	83%	5	5 5 5
Total stars					78
Number of measures with a star score for Dr. A					17
Average star rating					4.59
Per-member-per-month payment					$7.00
Dr. A's 2016 member months					1,000
Dr. A's total 2016 program dollars earned					**$7,000**

- Dr. A scored an average of 4.59 stars for 2016
- 4.59 stars places Dr. A in the 4.5 to 4.99 star range
- Dr. A will earn $7 per member per month for 2016

BLUE CROSS BLUE SHIELD OF MICHIGAN AND BLUE CARE NETWORK
2016 PAYOUT SUMMARY — MEDICARE

Medicare Advantage payment calculation
Example #2: "Dr. B"

	Scoring
Total stars	59
Number of measures with a star score for Dr. B	18
Average star rating 2016 for Dr. B	3.28
Average star rating 2015 for Dr. B	2.17
Dr. B star improvement 2015 – 2016	1.11
Per-member-per-month payment	$2.00
Dr. B's 2016 member months	500
Dr. B's total 2016 program dollars earned	**$1,000**

- Dr. B scored an average of 3.28 stars, below the 3.5 stars threshold for 2016
- Dr. B showed a 1.11 star improvement from 2015 to 2016
- The 1.11 star improvement is divided by 0.5 to determine how many half-star increments Dr. B improved
- 1.11/0.5 = 2.22, the 2.22 is rounded down to the nearest whole number which is 2
- Dr. B improved 2 half-star increments
- Dr. B will earn two times the improvement per member per month of $1
- Dr. B will earn $2 per member per month for 2016

Medicare Advantage payment calculation
Example #3: "Dr. C"

	Scoring
Total stars	31
Number of measures with a star score for Dr. C	12
Average star rating 2016 for Dr. C	2.58
Average star rating 2015 for Dr. C	3.08
Dr. C star improvement 2015 – 2016	None
Per-member-per-month payment (Dr. C showed no improvement)	$0
Dr. C's 2016 member months	750
Dr. C's total 2016 program dollars earned	**$0**

- Dr. C scored average of 2.58 stars, below the 3.5 stars threshold for 2016
- Dr. C showed no improvement from 2015 to 2016
- Dr. C does not qualify for a program payment for 2016

2016 PROGRAM SCHEDULE

JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG

Measurement period: January to December, 2016

HEB/Supplemental Date: January 2016 to Early/Mid-January 2017

Claim/EMR Submission: January 2016 to February 2017

Payment: May to August 2017

Note: See Page 24 for the schedule for the depression management quality measure.

PROGRAM QUALIFICATIONS

1. The primary care physician or physician organization must sign the BCN 2016 Medical Services Agreement to participate in the BCN Commercial and BCN Advantage Performance Recognition Programs and the Blue Cross Medicare Advantage PPO Provider Agreement to participate in the Blue Cross Medicare Plus Blue PPO Performance Recognition Program.

2. The primary care physician or physician organization must comply with all terms and conditions of those agreements, including:
   - Providing timely and accurate encounter, referral and claims data
   - Remitting any funds due for prior contract years

3. The primary care physician must be affiliated for the entire 2016 calendar year.

4. The primary care physician must be affiliated at the time of payment to be eligible for any program payments unless the PCP recently retired.

5. The primary care physician or PCP office must have a Health e-Blue℠ sign-on and actively use the program.

6. BCN and Blue Cross retain the right to modify the Performance Recognition Program for any reason and at any time. Modifications may include, but are not limited to:
   - Exclusion or removal of program measures
   - Changes to program calculation methodologies

7. Blue Care Network and Blue Cross conduct periodic random audits on provider data returns. If you are randomly selected to be audited for Health e-Blue data entry or electronic medical records, you must pass the audit in order to be eligible for payment.

PERFORMANCE MEASUREMENT GUIDELINES

- Each primary care physician will be credited for services completed through **Dec. 31, 2016,** to members who meet all measurement requirements, are continuously enrolled with the plan for the entire year and are assigned to a primary care physician whether or not the primary care physician was the member's primary care physician at the time services were provided.

- Credit will be granted to the primary care physician for each component measure only when the specific identified service is documented as provided to the member (by the primary care physician, the member's previous primary care physician or a specialist). Members may be excluded from measures under certain circumstances, such as bilateral mastectomy for breast cancer screening, which should be indicated to Blue Cross or BCN by the primary care physician offices via the Health e-Blue *Treatment Opportunities by Condition/Measure* screen.

- Blue Cross and BCN recognize that many primary care physician offices send reminder letters or may not see certain members in their offices who are identified by Blue Cross or BCN as needing certain services. Such occurrences will not count as credit toward the component measure.

- Each primary care physician's quality performance measurement data comes directly from Blue Cross or BCN's Health Management Program reporting database accessible through Health e-Blue. The Health e-Blue *Treatment Opportunities by Condition/Measure* for the Performance Recognition Program will include:
  - A list of the cohort member population for each component measure that needs a specific health promotion, disease prevention or health management service according to evidence-based medicine
  - **Intervention** opportunities for physicians to supplement Blue Cross or BCN's databases by providing service or exclusion data of which Blue Cross or BCN had no knowledge
  - **A Quality Summary Report or Performance Recognition Program composite score** that shows the monthly quality composite rates for the primary care physician and provider organizations

ADMINISTRATIVE DETAILS

Health e-Blue

Health e-Blue provides a valuable opportunity for provider offices to assess their current performance and return data to Blue Cross or BCN. We accept electronic submission of data through the Healthy e-Blue application, EMR, claims and HEDIS initiatives. Entering missing information will help reduce reporting errors. If your office needs assistance with or has a question about BCN Health e-Blue, please contact Health e-Blue technical support at healtheblue@bcbsm.com. For Blue Cross Health e-Blue questions please contact MAHealtheblue@bcbsm.com.

Please remember that all data entered into Health e-Blue must be for services you provide, not for services ordered, reminders sent or referrals provided.

Distribution of Blue Cross and BCN Performance Recognition Program Payment Reports and Payments

Blue Cross and BCN will make every effort to send the 2016 payment reports and payments by summer 2017.

BCN payments will be made according to BCN's incentive payment policy, subject to the requirements outlined in this document. The primary care physician's payment will be associated with the medical care group the primary care physician is affiliated with as of December 31, 2016.

Reconsideration

Blue Cross and BCN strongly encourage primary care physicians to focus on the ongoing review and data submission using Health e-Blue during each Performance Recognition Program year. In the event any future reconsideration process is provided based on extenuating circumstances, Blue Cross or BCN will notify the affected primary care physician of the terms, conditions and limitations of such a process.

QUESTIONS

If you have questions or concerns about the Performance Recognition Program, please contact your **provider consultant**. You can find contact information for your provider consultant by following these steps:

- Go to bcbsm.com/providers.
- Click on *Contact Us* in the upper right corner of the page.
- Under *Physicians and professionals*, click on *Blue Cross Blue Shield of Michigan* or *Blue Care Network provider contacts*.
- Click on *Provider consultants*.
- Find your provider consultant either on the *physician organization consultants* list or the applicable regional list.

Additional Blue Cross and BCN contacts

Provider Outreach HEDIS/stars/Risk
Laurie Latvis, director
313-225-7778

Network Performance Improvement
Tracy Nelsen, Southeast and East Michigan
734-332-2181

Christine Wojtaszek, Mid and West Michigan
616-956-5769

Health e-Blue technical support

BCN Commercial and BCN Advantage
healtheblue@bcbsm.com

Blue Cross Medicare Plus Blue PPO
MAHealtheblue@bcbsm.com

HEALTH CARE OUTCOMES: PREVENTIVE HEALTH

ADULT BMI ASSESSMENT						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	HEDIS/CMS stars					
Description	Members 18-74 years of age who had an outpatient visit and whose weight and body mass index was documented during the measurement year or year prior to the measurement year					
Continuous enrollment	Must be continuously enrolled with the same Blue Cross or BCN plan for 2015-2016					
Age criteria	Members 18 years of age as of January 1, 2016 to 74 years as of December 31, 2016					
Numerator	Members as defined above					
Denominator	The eligible population					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	Weight
	< 70%	70 – 80.9%	81 – 89.9%	90 – 95.9%	≥ 96%	1
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

BREAST CANCER SCREENING						
Product lines	**BCN Commercial, BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	HEDIS/CMS stars					
Description	The percentage of women who had a mammogram to screen for breast cancer					
Continuous enrollment	Must be continuously enrolled with the same Blue Cross or BCN plan October 1, 2014 through December 31, 2016					
Age criteria	52 to 74 years of age as of December 31, 2016					
Exclusionary criteria	Women who have had a bilateral mastectomy The following criteria meets bilateral mastectomy: • Bilateral mastectomy • Unilateral mastectomy with bilateral modifier • Two unilateral mastectomies with services dates 14 days or more apart					
Numerator	A mammogram at any time on or between October 1, 2014, and December 31, 2016					
Denominator	The eligible population					
Level of measure	Provider level					
Target: COMM	80%					
Payout: COMM	$100 per service completed for each eligible member					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	Weight
	< 39%	39 – 62.9%	63 – 73.9%	74 – 79.9%	≥ 80%	1
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: PREVENTIVE HEALTH

CHILDHOOD IMMUNIZATIONS – COMBO 10	
Product lines	**BCN Commercial**
Source	HEDIS
Description	The percentage of children 2 years of age who meet the combination 10 criteria on or before their second birthday: • (4) DTaP* vaccinations • (3) IPV* vaccinations • (1) MMR vaccination • (1) VZV vaccination • (3) HiB* vaccinations • (3) Hepatitis B vaccinations • (4) PCV* vaccinations • (1) HepA vaccination • (2 or 3) RV* vaccinations • (2) Influenza** vaccinations *Vaccinations administered prior to 42 days after birth are not counted as a numerator hit. **Vaccinations administered prior to 180 days after birth are not counted as a numerator hit.
Continuous enrollment	Must be continuously enrolled 12 months prior to child's second birthday
Age criteria	Children who turn 2 years of age during 2016
Exclusionary criteria	Children who are documented with an anaphylactic reaction to the vaccine or its components
Numerator	The number of children who completed vaccinations as defined above
Denominator	The eligible population
Level of measure	Provider level
Target: COMM	63%
Payout: COMM	$400 per Combo 10 completed for each eligible member

HEALTH CARE OUTCOMES: PREVENTIVE HEALTH

WEIGHT ASSESSMENT AND COUNSELING FOR CHILDREN: BMI PERCENTILE, COUNSELING FOR NUTRITION AND COUNSELING FOR PHYSICAL ACTIVITY	
Product lines	**BCN Commercial**
Source	HEDIS
Description	Members 3 to 17 years of age who have an active BCN Commercial span through the end of 2016 and had an outpatient visit between January 1, 2016, and December 31, 2016, with a PCP or ObGyn, where BMI percentile, counseling for nutrition and counseling for physical activity were documented in the medical record. The member's outpatient visit was reflected on a claim and the BMI percentile, counseling for nutrition and counseling for physical activity was reflected on a claim, electronic data submission for an EMR or entered in Health e-Blue.
Continuous enrollment	Must be continuously enrolled with BCN for 2016
Age criteria	3 to 17 years of age as of December 31, 2016
Numerator	• BMI percentile documentation during the measurement period (January to December 2016). Documentation in the member's medical record must also include height and weight. • Counseling for nutrition during the measurement period (January to December 2016). • Counseling for physical activity during the measurement period (January to December, 2016).
Denominator	The eligible population
Level of measure	Provider level
Target: COMM	63%
Payout: COMM	$150 per eligible member for whom all services were complete

HEALTH CARE OUTCOMES: PREVENTIVE HEALTH

COLORECTAL CANCER SCREENINGS						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	HEDIS/CMS stars					
Description	The percentage of members who had appropriate screening for colorectal cancer					
Continuous enrollment	Must be continuously enrolled with the same Blue Cross/BCN plan for 2015-2016					
Age criteria	51 to 75 years as of December 31, 2016					
Exclusionary criteria	Either of the following any time during the member's history through December 31, 2016 • Colorectal cancer • Total colectomy					
Numerator	One or more screenings for colorectal cancer. Any of the following meet criteria: • Fecal occult blood test during 2016 (digital rectal exams do not count) • Flexible sigmoidoscopy 2012 through 2016 • Colonoscopy 2007 through 2016					
Denominator	The eligible population					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	Weight
	< 51%	51 – 62.9%	63 – 70.9%	71 – 77.9%	≥ 78%	1
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

COMPREHENSIVE DIABETES CARE: CONTROLLED HbA1c < 8%	
Product lines	**BCN Commercial**
Source	HEDIS
Description	The percentage of members with diabetes (type 1 or 2) and a documented HbA1c < 8% using the latest lab conducted in 2016
Continuous enrollment	Members must be continuously enrolled with the same BCN plan for 2016
Age criteria	18 to 75 years as of December 2016
Exclusionary criteria	• Diagnosis of gestational or steroid-induced diabetes, in any setting, during 2015 or 2016 and • Did not have a diagnosis of diabetes in 2015 or 2016
Numerator	The number of members with diabetes (type 1 or 2) with an HbA1c < 8.0%. This measure considers the most recent lab conducted in 2016. The member is not compliant if the most recent result is ≥ 8, if the member is missing a result or the test was not done during 2016.
Denominator	All members with diabetes as defined above
Level of measure	Provider level
Target: COMM	68%
Payout: COMM	$250 per service completed for each eligible member

COMPREHENSIVE DIABETES CARE: CONTROLLED HbA1c ≤ 9%						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	HEDIS/CMS stars					
Description	The percentage of members with diabetes (type 1 or 2) and a documented HbA1c ≤ 9% using the latest lab conducted in 2016					
Continuous enrollment	Must be continuously enrolled with the same Blue Cross or BCN plan for 2016					
Age criteria	18 to 75 years as of December 2016					
Exclusionary criteria	• Diagnosis of gestational or steroid-induced diabetes, in any setting, during 2015 or 2016 and • Did not have a diagnosis of diabetes in 2015 or 2016					
Numerator	The number of members with diabetes (type 1 or 2) with an HbA1c ≤9.0% This measure considers the most recent lab conducted in 2016. The member is not compliant if the most recent result is > 9, the member is missing a result or the test was not done during 2016.					
Denominator	All members with diabetes as defined above					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	**Weight**
	< 49%	49 – 59.9%	60 – 70.9%	71 – 83.9%	≥ 84%	**3**
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

COMPREHENSIVE DIABETES CARE: MONITORING FOR NEPHROPATHY						
Product lines	**BCN Commercial, BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	HEDIS/CMS stars					
Description	The percentage of members with diabetes (type 1 or 2) who have had one of the following: • A nephropathy screening or monitoring test (test for urine albumin or protein) in 2016 • Medical treatment for nephropathy in 2016 • Visit with a nephrologist in 2016 • At least one dispensing event of ACEI/ARB medication in 2016					
Continuous enrollment	Members must be continuously enrolled with the same Blue Cross or BCN plan for 2016					
Age criteria	18 to 75 years as of December 2016					
Exclusionary criteria	• Diagnosis of gestational or steroid-induced diabetes, in any setting, during 2015 or 2016 and • Did not have a diagnosis of diabetes in 2015 or 2016					
Numerator	Members with diabetes (type 1 or 2) who have had one of the following: • A nephropathy screening or monitoring test (test for urine albumin or protein) in 2016 • Medical treatment for nephropathy in 2016 • Visit with a nephrologist in 2016 • At least one dispensing event of ACEI/ARB medication in 2016					
Denominator	All members with diabetes as defined above					
Level of measure	Provider level					
Target: COMM	90%					
Payout: COMM	$125 per service completed for each eligible member					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	Weight
	< 85%	85 – 88.9%	89 – 92.9%	93 – 96.9%	≥ 97%	1
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

CONTROLLING HIGH BLOOD PRESSURE: HYPERTENSION						
Product lines	**BCN Commercial, BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	BCN and Blue Cross clinical guidelines					
Description	Members 18 to 85 years of age who were diagnosed with hypertension anytime on or before June 30, 2016 Control is demonstrated by: • Members 18 to 59 years of age with BP < 140/90 mm Hg • Members 60 to 85 years of age with diagnosis of diabetes with BP < 140/90 mm Hg • Members 60 to 85 years of age without a diagnosis of diabetes with BP < 150/90 mm Hg The last blood pressure reading between July 1, 2016 and December 31, 2016, will be counted.					
Continuous enrollment	Must be continuously enrolled with the same Blue Cross or BCN plan for 2016					
Age criteria	Members 18 to 85 years as of December 31, 2016					
Numerator	Members as defined above					
Denominator	The eligible population					
Level of measure	Provider level					
Target: COMM	75%					
Payout: COMM	$100 per service completed for each eligible member					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	Weight
	< 47%	47 – 61.9%	62 – 74.9%	75 – 81.9%	≥ 82%	1
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

DISEASE-MODIFYING ANTI-RHEUMATIC DRUG THERAPY FOR RHEUMATOID ARTHRITIS						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	HEDIS					
Description	The percentage of members ages 18 years of age or older diagnosed with rheumatoid arthritis who were dispensed at least one ambulatory prescription for a disease-modifying anti-rheumatic drug					
Continuous enrollment	Members must be continuously enrolled with the same Blue Cross or BCN plans for 2016					
Age criteria	18 to 85 years of age or older as of December 31, 2016					
Numerator	Members as defined above					
Denominator	The eligible population					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	Weight
	< 64%	64 – 74.9%	75 – 81.9%	82 – 85.9%	≥ 86%	1
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

MEDICATION ADHERENCE FOR DIABETES MEDICATIONS						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	CMS stars					
Description	The percentage of adult Medicare members who adhere to their prescribed drug therapy across the following classes of oral diabetes medications; biguanides, sulfonylureas, thiazolidinediones, DPP-IV inhibitors, incretin mimetics, meglitinides, and SGLT2 inhibitors					
Continuous enrollment	Members must be continuously enrolled with the same Blue Cross or BCN plan for 2016					
Age criteria	18 years of age by December 31, 2016					
Numerator	Number of adult members 18 years or older enrolled during 2016 with a proportion of days covered at 80 percent or more across the classes of oral diabetes medications Members are excluded if they have one or more fills for insulin during the measurement period.					
Denominator	Number of adult members 18 years or older enrolled during 2016 with at least two fills of medication across any of the drug classes					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	**Weight**
	< 60%	60 – 68.9%	69 – 74.9%	75 – 81.9%	≥ 82%	**3**
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

MEDICATION ADHERENCE FOR HYPERTENSION MEDICATIONS						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	CMS stars					
Description	The percentage of adult Medicare members who adhere to their prescribed drug therapy for ACEI or ARB medications					
Continuous enrollment	Members must be continuously enrolled with the same Blue Cross or BCN plan for 2016					
Age criteria	18 years of age by December 31, 2016					
Numerator	Number of adult members 18 years of age or older enrolled during 2016 with a proportion of days covered at 80 percent or more for ACEI or ARB medications					
Denominator	Number of adult members 18 years or older enrolled during 2016 with at least two fills of either the same medication or medications with the same active ingredient					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	**Weight**
	< 58%	58 – 72.9%	73 – 76.9%	77 – 80.9%	≥ 81%	**3**
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

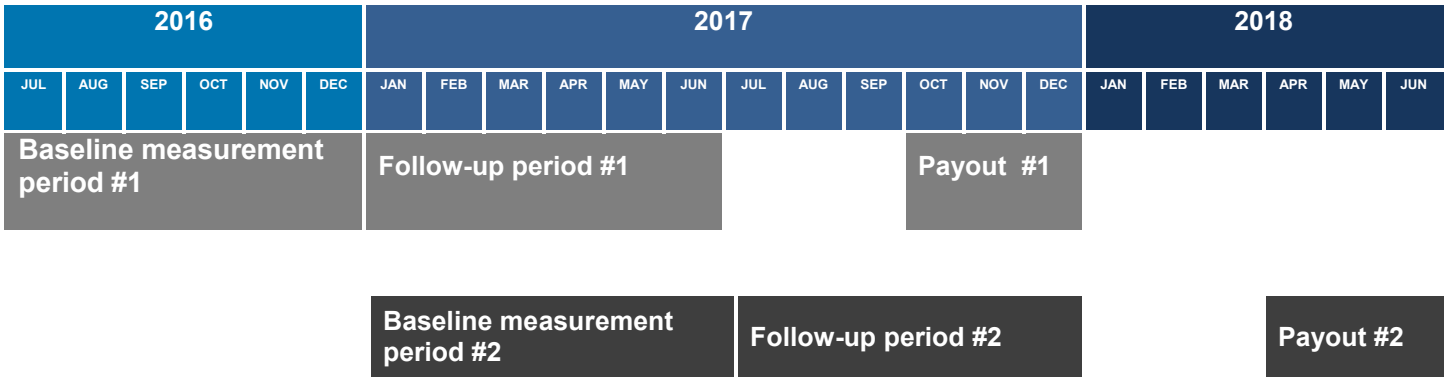
MEDICATION ADHERENCE FOR CHOLESTEROL MEDICATIONS						
Product lines	**BCN Advantage, Blue Cross Medicare Plus Blue PPO**					
Source	CMS stars					
Description	The percentage of adult Medicare members who adhere to their prescribed drug therapy for statin cholesterol medications					
Continuous enrollment	Members must be continuously enrolled with the same Blue Cross or BCN plan for 2016					
Age criteria	18 years of age by December 31, 2016					
Numerator	Number of adult members 18 years of age or older enrolled during the measurement period with a proportion of days covered at 80 percent or more for statin cholesterol medications					
Denominator	Number of adult members 18 years of age or older enrolled during 2016 with at least two fills of either the same statin medication or medications with the same active ingredient.					
Level of measure	Provider level					
Target: BCNA/MAPPO	1 star	2 stars	3 stars	4 stars	5 stars	**Weight**
	< 50%	50 – 60.9%	61 – 72.9%	73 – 78.9%	≥ 79%	**3**
Payout: BCNA/MAPPO	Per member, per month, based on overall average stars score for Medicare PRP measures					

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

SMOKING/TOBACCO CESSATION COUNSELING	
Product lines	**BCN Commercial**
Source	BCN Medical Administration
Description	Members who use tobacco and receive face-to-face cessation advice, information on medications and strategies to help them quit, and a follow-up letter from the physician to review the information discussed
Continuous enrollment	Not required
Age criteria	Members 18 years of age or older as of January 1, 2016
Numerator	Members as defined above who are smokers or tobacco users
Denominator	The eligible population
Level of measure	Provider level
Target: COMM	Flat fee per member who meets measure
Payout: COMM	$30 per service completed for each eligible member
Additional Details:	PCPs were provided with a sample member letter in the January-February 2016 *BCN Provider News* to send upon completion of an office visit that summarized the following that took place during the visit: • Face-to-face tobacco cessation advice • Information and medications that can assist the member in tobacco cessation • Tobacco cessation strategies to increase the member's chance of success These letters must be sent to the member upon completion of the visit and a copy must also be faxed to BCN at 1-**866-637-4972** to receive credit for this measure. The letter must be in the format provided by BCN in order to receive credit. A template for this letter can be found at **bcbsm.com**. 1. Login to Provider Secured Services. 2. Click on *BCN Provider Publications and Resources*. 3. Click on Forms and look under *Member materials*.

HEALTH CARE OUTCOMES: DISEASE MANAGEMENT

DEPRESSION MANAGEMENT: PHQ9 TESTING	
Product lines	**BCN Commercial**
Source	BCN Medical Administration
Description	Members who have any depressive condition and had a PHQ9 administered during the baseline period scoring greater than or equal to 10 and had a follow-up PHQ9 administered during the follow-up period, scoring below 5.
Continuous enrollment	Members must be continuously enrolled with the same BCN plan for the baseline and follow-up periods
Age criteria	12 years of age or older as of the first day of the baseline measurement period
Numerator	The last qualifying encounter (PHQ9 screening with a score < 5) in the follow-period determines the numerator events for the performance measure.
Denominator	The first qualifying encounter (PHQ9 Screening with a score ≥ 10) in the baseline determines the denominator events for the performance measure.
Level of measure	Provider level
Target: COMM	Flat fee per member who meets measure
Payout: COMM	$200 per service completed for each eligible member
Additional Details:	Measurement periods, follow-up periods and payouts will be on a rolling basis as outlined below:



CMS MILLION HEARTS INCENTIVE PROGRAM

Blue Care Network has implemented a program to prevent cardiovascular disease. The program is designed for BCN Advantage members, ages 40 and over, who have a history of cardiovascular disease or diabetes. The focus of the program is to reduce the morbidity and mortality related to cardiovascular disease in these members.

The program incorporates clinical practice guidelines for the management of ischemic heart disease and diabetes mellitus following the guiding principles behind the nation Million Hearts™ initiative. Million Hearts is a national initiative to prevent 1 million heart attacks and strokes over five years. It is led by the U.S. Department of Health and Human Services, the Centers for Disease Control and Prevention and the Centers for Medicare & Medicaid Services in partnership with other federal agencies.

CMS Million Hearts payment table

Quality incentive measures	Plan goal	Payout
Aspirin or antiplatelet therapy	Flat fee	$25
Blood pressure control	Flat fee	$25
Tobacco cessation counseling	Flat fee	$25

CMS Million Hearts payment calculation

CMS Million Hearts requires no specific plan goal. A flat fee is paid for each service completed.

CMS Million Hearts program qualifications

Providers must meet the Performance Recognition Program qualifications in order to be considered for a CMS Million Hearts incentive payment.

Providers can locate Million Hearts members in Health e-Blue under the Treatment Opportunity by Condition/Measures.

CMS Million Hearts data submission options

- Submit a claim with an appropriate CPT II code
- Health e-Blue entry
- Electronic medical record exchange

CMS MILLION HEARTS PROVIDER INCENTIVE
QUALITY INCENTIVE MEASURES

ASPIRIN OR ANTIPLATELET THERAPY	
Product lines	**BCN Advantage**
Source	CMS Million Hearts
Description	Members age 40 and over as of December 31, 2016, with a history of diabetes, cardiovascular disease or both who is prescribed or currently taking aspirin or antiplatelet therapy Report CPT II code 4086F for all patients meeting criteria
Level of measure	Provider level
Target: BCNA	Flat fee per member who meets measure
Payout: BCNA	$25 per service completed for each eligible member

BLOOD PRESSURE CONTROL	
Product lines	**BCN Advantage**
Source	CMS Million Hearts
Description	Members age 40 and over as of December 31, 2016 who meet both the systolic and diastolic blood pressure reading requirements: • Members 18-59 years of age as of December 31, 2016 whose BP was < 140/90 mm Hg • Members 60-85 years of age as of December 31, 2016 with a diagnosis of diabetes whose BP was < 140/90 mm Hg • Members 60-85 years of age as of December 31, 2016 without a diagnosis of diabetes whose BP was < 150/90 mm Hg • Systolic blood pressure value report one of the systolic codes – 3074F – SBP < 130 – 3075F – SBP 130-139 – SBP > 140 and < 150 (Needs to be documented in EMR or in HEB. No CPT Cat II codes are available) • Diastolic blood pressure value report one of the diastolic codes – 3078F – DBP < 80 – 3079F – DBP 80-89
Level of measure	Provider level
Target: BCNA	Flat fee per member who meets measure
Payout: BCNA	$25 per service completed for each eligible member

CMS MILLION HEARTS PROVIDER INCENTIVE
QUALITY INCENTIVE MEASURES

SMOKING/TOBACCO CESSATION COUNSELING	
Product lines	**BCN Advantage**
Source	CMS Million Hearts
Description	Members age 40 and over as of December 31, 2016 who are smokers and have been counseled on the importance of quitting smoking Providers can report 'Not a smoker' in Health e-Blue as an Exclusion Reason / Contra-Indication Report CPT II code 4000F or 4004F for each patient identified as a tobacco user and received tobacco cessation counseling
Level of measure	Provider level
Target: BCNA	Flat fee per member who meets measure
Payout: BCNA	$25 per service completed for each eligible member

Blue Cross
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Marketing

THE BLOG CDC CENTERS FOR DISEASE CONTROL FLU DEATHS

Don't Believe Everything You Read About Flu Deaths

The CDC's decision to play up flu deaths dates back a decade, when it realized the public wasn't following its advice on the flu vaccine. During the 2003 flu season "the manufacturers were telling us that they weren't receiving a lot of orders for vaccine," Dr. Glen Nowak, associate director for communications at CDC's National Immunization Program, told National Public Radio.

By Lawrence Solomon, Contributor

Columnist

Jan 24, 2014, 05:40 AM EST | **Updated** Mar 26, 2014

Flu results in "about 250,000 to 500,000 yearly deaths" worldwide, Wikipedia tells us. "The typical estimate is 36,000 [deaths] a year in the United States," reports NBC, citing the Centers for Disease Control. "Somewhere between 4,000 and 8,000 Canadians a year die of influenza and its related complications, according to the Public Health Agency of Canada," the *Globe and Mail* says, adding that "Those numbers are controversial because they are estimates."

"Controversial" is an understatement, and not just in Canada, and not just because the numbers are estimates. The numbers differ wildly from the sober tallies recorded on death certificates -- by law every certificate must show a cause -- and reported by the official agencies that collect and keep vital statistics.

According to the National Vital Statistics System in the U.S., for example, annual flu deaths in 2010 amounted to just 500 per year -- fewer than deaths from ulcers (2,977), hernias (1,832) and pregnancy and childbirth (825), and a far cry from the big killers such as heart disease (597,689) and cancers (574,743). The story is similar in Canada, where unlikely killers likewise dwarf Statistics Canada's count of flu deaths.

Even that 500 figure for the U.S. could be too high, according to analyses in authoritative journals such as the American Journal of Public Health and the British Medical Journal. Only about 15-20 per cent of people who come down with flu-like symptoms have the influenza virus -- the other 80-85 per cent actually caught rhinovirus or other germs that are indistinguishable from the true flu without laboratory tests, which are rarely done. In 2001, a year in which death certificates listed 257 Americans as having died of flu, only 18 were positively identified as true flus. The other 239 were simply assumed to be flus and most likely had few true flus among them.

"U.S. data on influenza deaths are a mess," states a 2005 article in the British Medical Journal entitled "Are U.S. flu death figures more PR than science? " This article takes issue with the 36,000 flu-death figure commonly claimed,

and with describing "influenza/pneumonia" as the seventh leading cause of death in the U.S.

"But why are flu and pneumonia bundled together?" the article asks. "Is the relationship so strong or unique to warrant characterizing them as a single cause of death?"

The article's answer is no. Most pneumonia deaths are unrelated to influenza. For example, "stomach acid suppressing drugs are associated with a higher risk of community-acquired pneumonia, but such drugs and pneumonia are not compiled as a single statistic," explained Dr. David Rosenthal, director of Harvard University

Health Services. "People don't necessarily die, per se, of the [flu] virus -- the viraemia. What they die of is a secondary pneumonia."

Pneumonia, according to the American Lung Association, has more than 30 different causes, influenza being but one of them. The CDC itself acknowledges the slim relationship, saying "only a small proportion of deaths... only 8.5 per cent of all pneumonia and influenza deaths [are] influenza-related."

Because death certificates belie claims of numerous flu deaths, CDC enlisted computer models to arrive at its 36,000 flu-death estimate. But even here it needed to bend conventional medical terminology to arrive at compelling death numbers.

"Cause-of-death statistics are based solely on the underlying cause of death [internationally defined] as 'the disease or injury which initiated the train of events leading directly to death,'" explains the National Center for Health Statistics. Because the flu was rarely an "underlying cause of death," the CDC created the sound-alike term, "influenza-associated death."

Using this new, loose definition, CDC's computer models could tally people who died of a heart ailment or other causes after having the flu. As William Thompson of the CDC's National Immunization Program admitted, influenza-associated mortality is "a statistical association ... I don't know that we would say that it's the underlying cause of death."

The CDC's decision to play up flu deaths dates back a decade, when it realized the public wasn't following its advice on the flu vaccine. During the 2003 flu season "the manufacturers were telling us that they weren't receiving a lot of orders for vaccine,"Dr. Glen Nowak, associate director for communications at CDC's National Immunization Program, told National Public Radio. "It really did look like we needed to do something to encourage people to get a flu shot."

The CDC's response was its "Seven-Step 'Recipe' for Generating Interest in, and Demand for, Flu (or any other) Vaccination," a slide show Nowak presented at the 2004 National Influenza Vaccine Summit.

Here is the "Recipe that fosters influenza vaccine interest and demand," in the truncated language that appears on his slides: "Medical experts and public health authorities [should] publicly (e.g. via media) state concern and alarm (and predict dire outcomes) - and urge influenza vaccination." This recipe, his slide show indicated, would result in "Significant media interest and attention ... in terms that motivate behavior (e.g. as 'very severe,' 'more severe than last or past years,' 'deadly')." Other emotive recommendations included fostering "the perception that many people are susceptible to a bad case of influenza" and "Visible/tangible examples of the seriousness of the illness (e.g., pictures of children, families of those affected coming forward) and people getting vaccinated (the first to motivate, the latter to reinforce)."

The CDC unabashedly decided to create a mass market for the flu vaccine by enlisting the media into panicking the public. An obedient and unquestioning media obliged by hyping the numbers, and 10 years later it is obliging still.

CDC | CENTERS FOR DISEASE CONTROL | FLU DEATHS | FLU SHOTS | FLU VACCINE

Lawrence Solomon, Contributor
Columnist

Increasing Awareness and Uptake of Influenza Immunization

Glen Nowak, Ph.D.
Acting Director of Media Relations, CDC
Associate Director for Communications, NIP/CDC

"Warning"

- Good (i.e., effective) communication is a necessary but usually only partially sufficient condition for achieving desired behaviors.
- Facts, figures, and statistics, in and of themselves, don't equate to good communication (nor does more information equal good communication).

Question

"It strikes 2 million Americans each year. And complications from this kill up to 200,000 people a year--more people than breast cancer, car crashes, and AIDS combined. The good news is, in most cases, this can be prevented."

What is it that causes this harm? (And does having this information change your behavior?)

IntelligenceReport

By Lyric Wallwork Winik

Stop A Deadly Killer

Skater Tara Lipinski suffered from DVT.

It strikes 2 million Americans each year and kills more people than breast cancer, car crashes and AIDS combined, yet most of us do not even know its name. The condition is called deep vein thrombosis, or DVT. It begins with a blood clot in the leg that can travel to the lungs, causing a pulmonary embolism and often death. Many of us are at risk—just sitting for a long time on a plane can produce DVT. But older people, pregnant women, smokers, the obese and others with a condition that limits mobility are at increased risk. Symptoms include leg tenderness, pain, swelling, discoloration or redness. If you suspect DVT, call a doctor immediately: There are quick, non-invasive tests to identify it, plus options ranging from blood thinners to simple exercises. National DVT Awareness Month begins tomorrow. Go to www.preventdvt.org to learn more.

A magnetic scan shows a leg blockage (white line), indicating deep vein thrombosis. Its clots can be fatal.

There are tests to identify DVT, and simple solutions.

"Recipe" for Fostering Public Interest and High Vaccine Demand (1)

1. Influenza's arrival coincides with immunization "season" (i.e., when people can take action)
2. Dominant strain and/or initial cases of disease are:
   - Associated with severe illness and/or outcomes
   - Occur among people for whom influenza is not generally perceived to cause serious complications (e.g., children, healthy adults, healthy seniors)
   - In cities and communities with significant media outlets (e.g., daily newspapers, major TV stations)

"Recipe" for Fostering Public Interest and High Vaccine Demand (2)

3. Medical experts and public health authorities publicly (e.g., via media) state concern and alarm (and predict dire outcomes)– and urge influenza vaccination.
4. The combination of '2' and '3' result in:
   A. Significant media interest and attention
   B. Framing of the flu season in terms that motivate behavior (e.g., as "very severe," "more severe than last or past years," "deadly")

"Recipe" for Fostering Public Interest and High Vaccine Demand (3)

5. Continued reports (e.g., from health officials and media) that influenza is causing severe illness and/or affecting lots of people– helping foster the perception that many people are susceptible to a bad case of influenza.
6. Visible/tangible examples of the seriousness of the illness (e.g., pictures of children, families of those affected coming forward) and people getting vaccinated (the first to motivate, the latter to reinforce)
7. References to, and discussions, of pandemic influenza– along with continued reference to the importance of vaccination.

Implications of the "Recipe"

- A large component of consumer demand for flu vaccination is contingent upon things we can't control (e.g., timing, severity, extent, duration of the disease and resulting illness).
- Fostering demand, particularly among people who don't routinely receive an annual influenza vaccination, requires creating concern, anxiety, and worry. For example:
  - A perception or sense that many people are falling ill;
  - A perception or sense that many people are experiencing bad illness;
  - A perception or sense of vulnerability to contracting and experiencing bad illness.

Additional (Pandemic) Influenza Communication Challenges

- Recommendations and perceptions regarding influenza vaccination are not "universal" (and achieving consensus by "fiat" is difficult)
- "Mass media" doesn't effectively reach "the mass"
- Mixed messages and advice are hard to avoid

Influenza Immunization Recommendations and Perceptions

- Until recently, influenza vaccination recommended primarily for 65 and older and people with certain chronic medical conditions– fostering perception that vaccination was for "elderly" and "frail"
- Now recommended for 50-64 year olds and 6-23 month olds– to many, implying a) its helpful primarily for older people and b) we have data that supports such precision
- Experts "nuance" recommendations, but the public (as well as many healthcare providers) don't similarly nuance their perceptions (e.g., "recommend" vs. "encourage," 6-23 month olds vs. 2 year olds)

Three Likely Population Segments

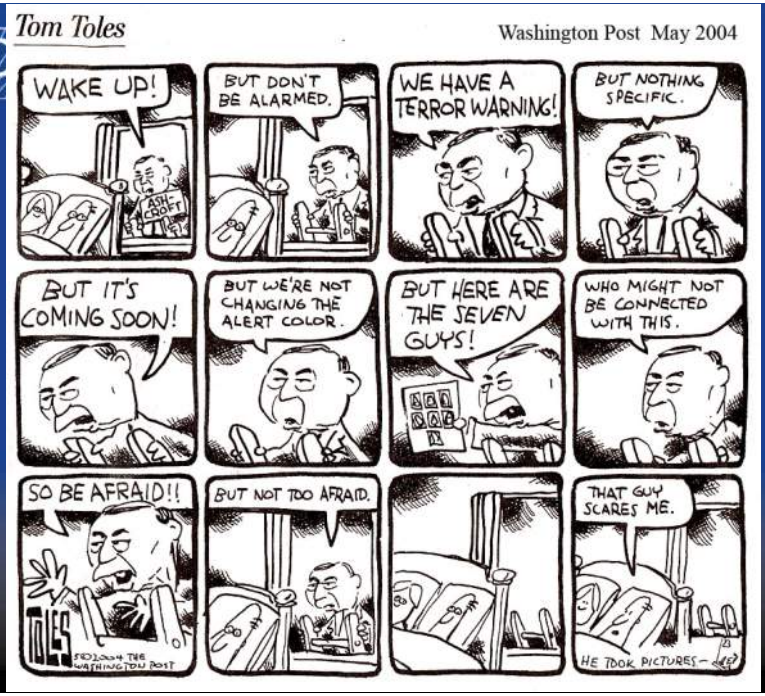
- **People who routinely receive an annual influenza vaccination, including those we recommend do so**
  - Primarily 65 years old and older
  - Primarily get vaccinated in Sept-November
- **People who sometimes receive an annual influenza vaccination, including those we recommend do so**
  - Interest is often contingent on perceptions of severity of the strain, likelihood they or someone they know will contract it, their belief they will experience or transmit a severe case
  - Appear to get vaccinated later (November, early December)
- **People who choose not to get an influenza vaccination, including those we recommend do so:**
  - Inversely related to age (e.g., most likely 18-49)
  - Among older people, often based on a firmly held belief/conviction

"Mass Media" Less Helpful

- Most people have 10 or so options when it comes to television viewing– many have 50-100 or more
- Hundreds of websites offer medical and health information
- Daily newspaper readership has been declining, particularly among 18-49 year olds
- Cultural and ethnic diversity is greater than ever
- Health literacy is a growing problem
- Belief that today you need to expose people to your message 10-12 times to achieve attention

The Challenge of Avoiding "Mixed Messages and Advice"

- Often arise when expert actions and behaviors don't seem to match or be consistent with policies and recommendations (e.g., healthcare providers not getting annual influenza vaccinations)
- Often fostered by a desire to improve our ability to provide services should large numbers of people act upon our advice
- Often recognized primarily in hindsight– and in contexts outside our own area of expertise



Some Recommendations

- Adopt more sophisticated approach to influenza-related communication:
  - Greater investment in communication research
  - Greater appreciation of need for a) less nuanced messages/advice and b) development/use of a portfolio of messages and materials
  - Plans that extend beyond news media reliance
- Recognition that the kind of communication activities envisioned (e.g., broad scope, high visibility, message frequency) require significant investment
- Greater understanding and use of risk communication principles (e.g., dilemma sharing, acknowledging uncertainty, providing coping strategies and advice)

Global Vaccine Market

- Differences between vaccines and medicines
- Main features of the vaccine market
- New trends since 2000?
- Implications for GAVI graduating and middle income countries?

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VACCINES VERSUS DRUGS

Vaccines	Medicines
Healthy People	Generally Sick People
High Risk Aversion	Moderate Risk Aversion
Induced Demand	Individual and Mixed Demand
Importance of UN, NITAG & Govt. Recommendations	Importance of Prescribers & Medical Societies
Public Funding (Govt, UN Donors)	High share of out of pocket and Health Insurance
Low mark up and taxes	Possible high mark up and taxes
No generics	Generics
Public good, positive externalities	Variable
Politically and Media sensitive	Variable

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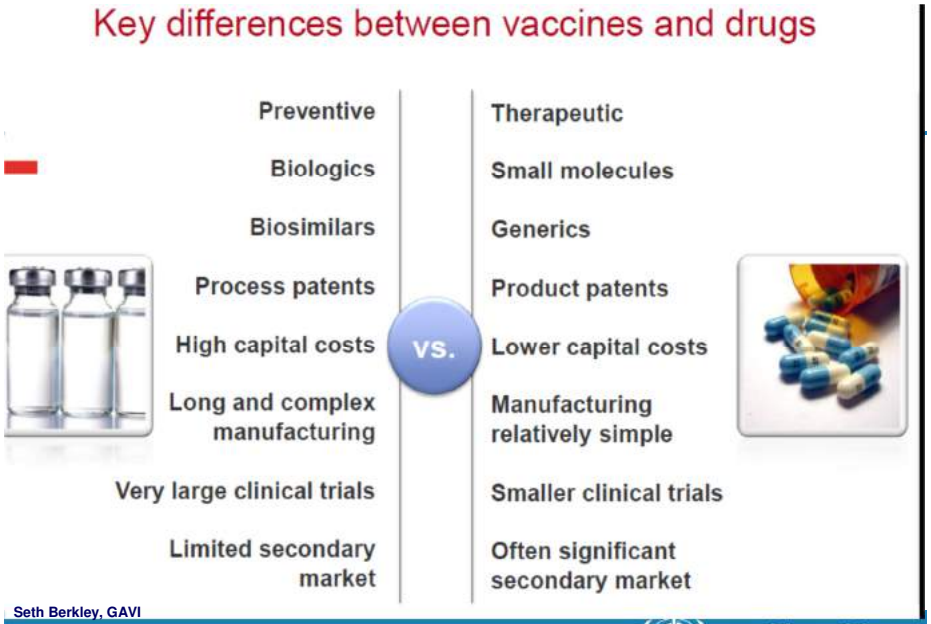
Key differences between vaccines and drugs



Seth Berkley, GAVI

World Health Organization

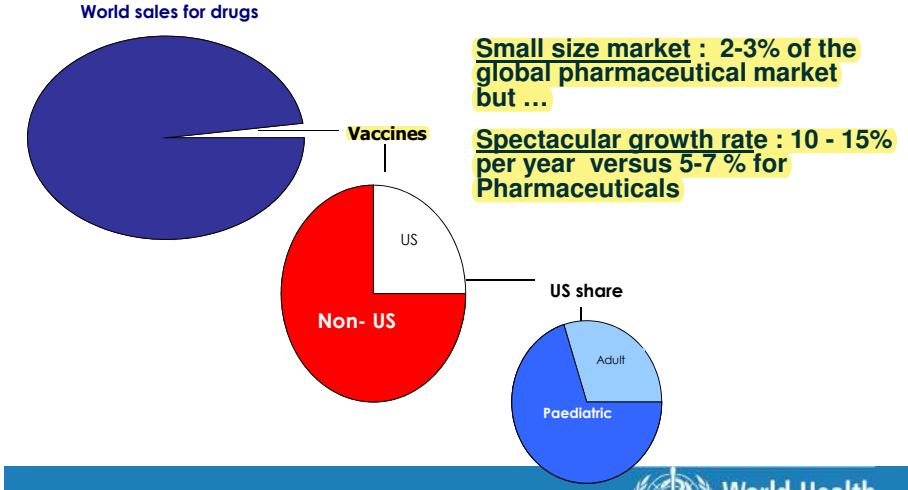
Vaccines are not commodity products and should not be treated as such (UNICEF SD)

Vaccine Product Profile

High Product Sensitivity	Limited Supply	Vaccine production and Market Competition
• Biological products	• 1- 5 suppliers per product	• Production of a dose: 6 -24 Months
• Significant risks of production failures	• High entry cost to manufacturers	• Capacity Increase: 2-3 years
• Quality is the OVER-RIDING criterion	• Diverse dynamics in individual vaccine markets	• New Plant: 5-7 years
• Highly regulated production environment	• New vaccines often result in monopoly supply	• New regulatory requirements can cause interruptions
• Dependency on well functioning NRA		• New vaccines being introduced globally, global competition
• Requiring constant temperature control ⇔ Cold Chain from Manufacturer to End user		• Initial selection has long term impact
• Limited shelf life		

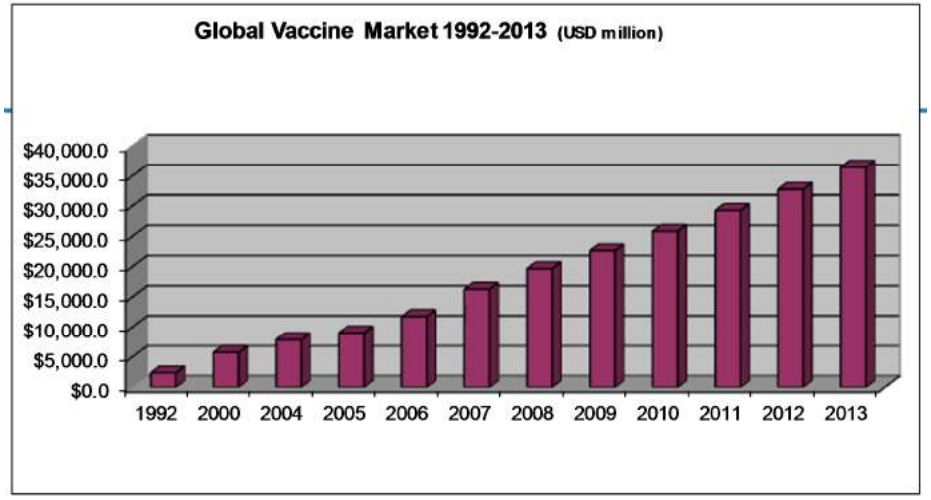
Requiring a specific approach to be developed for vaccine procurement

VACCINE MARKET STRUCTURE 2010



Small size market : 2-3% of the global pharmaceutical market but …

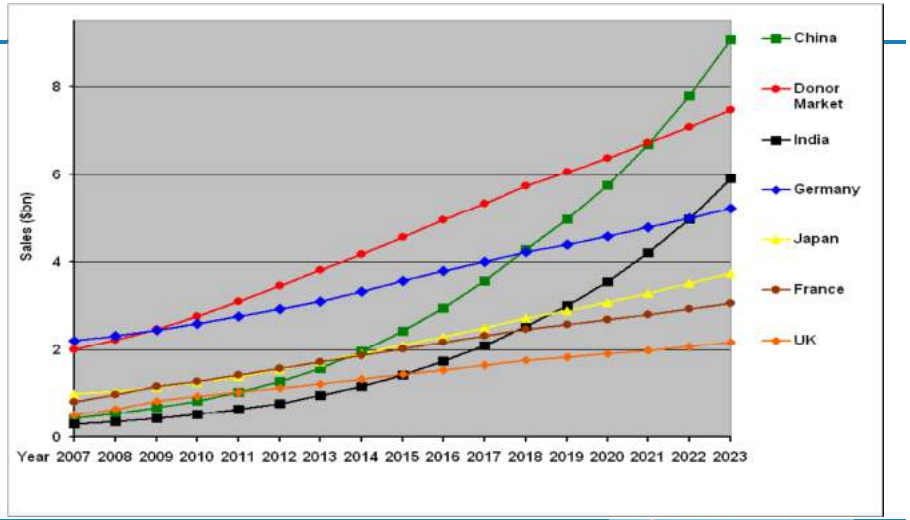
Spectacular growth rate : 10 - 15% per year versus 5-7 % for Pharmaceuticals



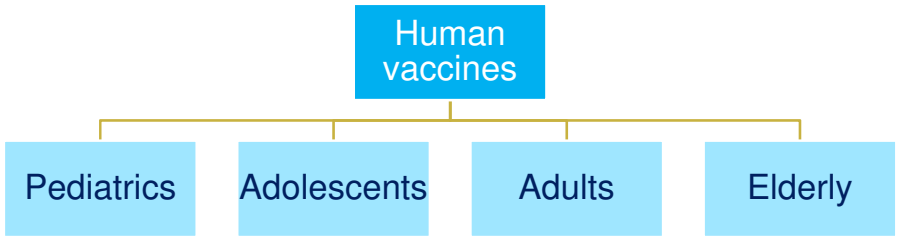
Sources: different estimates and projections (WHO, Industry, Frost and Sullivan, Biomarket group, Bionest, Kalorama,.)

2009-2012: projections

Market Growth Predictions



Vaccine segments



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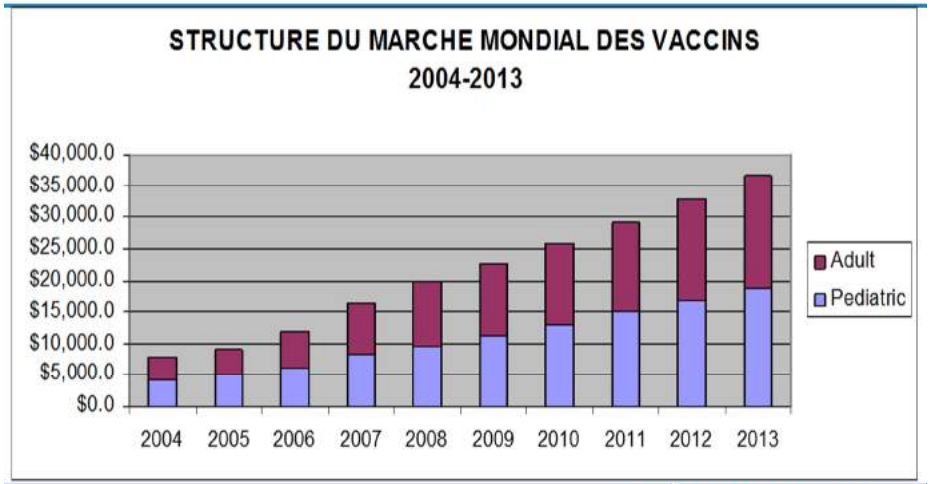
Value of Adult and Pediatric Vaccines



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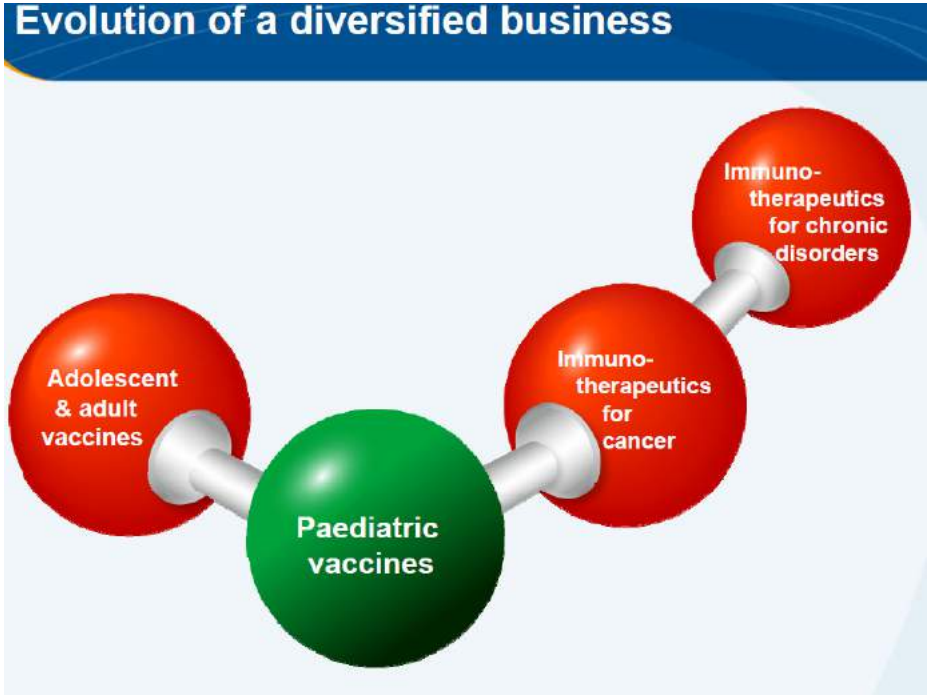
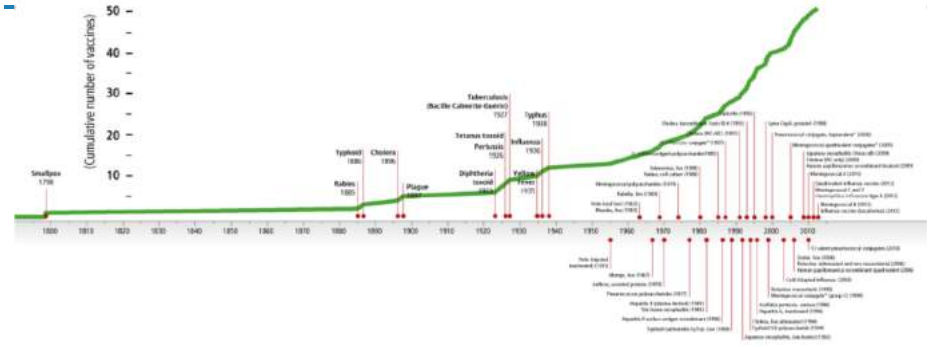


Cumulative number of vaccines developed



With Thanks to Stan Plotkin

Seth Berkley

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Vaccine Market: Growth Factors?

Combination of :

- Importance of communicable diseases and new threats
- New vaccines, new presentations and indications
- Cost effectiveness of immunizations
- New funding opportunities (Gov, PPP, donors, Foundations,...)
- New research techniques and manufacturing technologies
- Increasing demand, new target population, larger emerging markets
- Higher prices, improved profitability for the industry
- "Blockbuster" sales for some innovative vaccines

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Global Vaccine Market:
Rapid Growth and Changing Status

- Value from USD 5 bn in 2000 to almost USD 24 bn in 2013
- Global market projected to rise to USD 100 bn by 2025
- More than 120 new products in the development pipeline
- 60 are of importance for developing countries

➢ Vaccines: becoming an engine for the pharmaceutical industry

➢ Changing status of the vaccines within the pharmaceutical industry

➢ New business model for vaccines is emerging?

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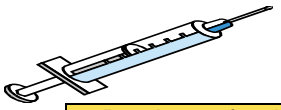

Top product sales in 2010

Brand name (producer)	Type/composition	2010 sales (US$)
Prevnar-13 (Pfizer)	13-valent pnenumococcal conjugate vaccine	$2.4 billion
Proquad (Merck/Sanofi-Aventis)	Measles-mumps-rubella and varicella combination vaccine (MMR-V)	$1.4 billion
Gardasil (Merck)	HPV	$1.35 billion
Prevnar (Pfizer)	7-valent pnenumococcal conjugate vaccine	$1.2 billion
Fluzone (Sanofi Pasteur)	Influenza (seasonal and H1N1 strains)	$1.2 billion
Infanrix and Pediarix) (GSK)	Infanrix = DTaP Pediarix = DTap-HepB-IPV (combination DPT-based vaccines with acellular pertussis)	$1.2 billion

Source: Krishan Maggon knoll (http://knol.google.com/k/krishan-maggon/global-vaccine-market-2010/3fy5eowy8sug3/152....)

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Total sales 2012 (1)

	Vaccine	Company	H1 Sales	Use
1	Prevnar 13	Pfizer	$3.718 billion	Pneumonia, otitis
2	Gardasil	Merck & Co. and Sanofi Pasteur MSD	$1.900 billion	Vulvar, vaginal, cervical cancer
3	PENTAct-HIB	Sanofi and Sanofi Pasteur MSD	$1.522 billion	Diphtheria, Pertussis/whooping cough; Tetanus; Polio; Haemophilus influenza type b
4	Infanrix/Pediarix	GlaxoSmithKline	$1.183 billion	Diphtheria; Tetanus; Pertussis; Hepatitis B; Poliomyelitis
5	Fluzone	Sanofi and Sanofi Pasteur MSD	$1.152 billion	Influenza
6	Hepatitis franchise	GlaxoSmithKline	$986 million	Hepatitis A, B
7	Varivax	Merck & Co. and Sanofi Pasteur MSD	$846 million	Varicella

Sources: Genetic Engineering and Biotechnology News; 8 July 2013

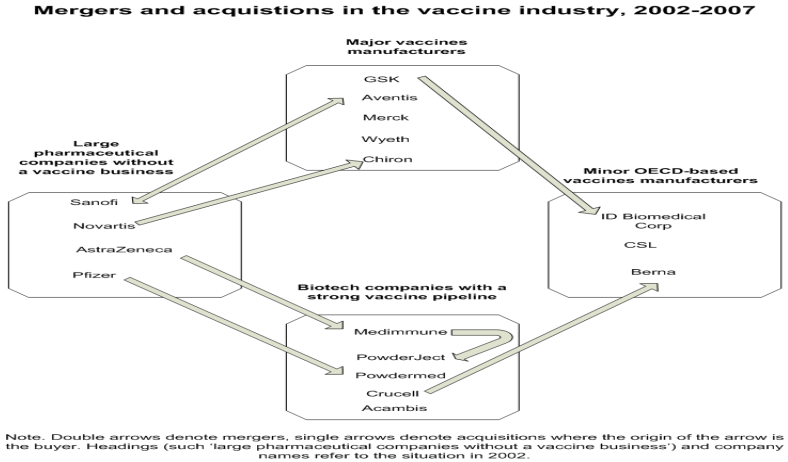
Total sales 2012 (2)

	Vaccine	Company	H1 Sales	Use
8	Menactra	Sanofi and Sanofi Pasteur MSD	$735 million	Meningococcal
9	Zostavax	Merck & Co. and Sanofi Pasteur MSD	$651 million	Shingles
10	RotaTeq	Merck & Co. and Sanofi Pasteur MSD	$648 million	Rotavirus gastroenteritis
11	Synflorix	GlaxoSmithKline	$587 million	Pneumococcal disease; Otitis
12	Pneumovax 23	Merck & Co. and Sanofi Pasteur MSD	$580 million	Pneumococcal disease
13	Rotarix	GlaxoSmithKline	$549 million	Rotavirus gastroenteritis
14	Adacel	Sanofi and Sanofi Pasteur MSD	$469 million	Diphtheria; Pertussis/Whooping Cough; Tetanus
15	Prevnar/Prevenar (7-valent)	Pfizer	$399 million	Pneumococcal disease

Sources: Genetic Engineering and Biotechnology News; 8 July 2013

New trends and features?

MERGERS AND ACQUISITIONS 2002-2007: Illustration

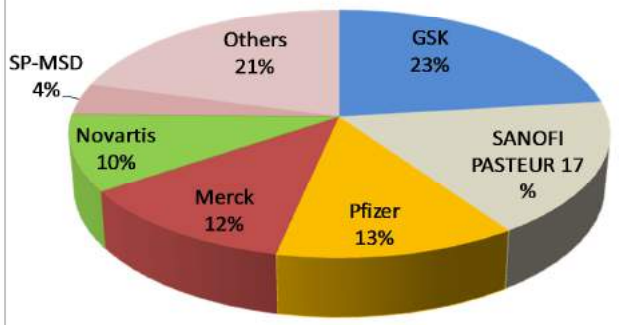


Overview of major vaccine related acquisitions (2005-2012)

Target Company	Acquiring Company	Investment Made	Date Announced
1. Bilthoven Bio of Netherlands	Serum Institute of India	Euros 80 mn	July 2012
2. Zhejiang Tianyuan Bio	Novartis	$125 mn?	March 2011
3. Wyeth	Pfizer	$68 bn	Jan 2009
4. MedImmune	AstraZenecea	$15.6 bn	April 2007
5. Chiron	Novartis	$5.1 bn	Oct 2005
6. Crucell	Johnson & Johnson	$2.6 bn	Sep 2009
7. ID Biomedical	GSK	$1.4 bn	Sep 2005
8. Shantha Bio	Sanofi Aventis	$781 mn	July 2009
9. Acambis	Sanofi Aventis	$549 mn	July 2008
10. Intercell	Novartis	$363 mn	July 2007
11. Corixa	GSK	$300 mn	May 2005
12. PowderMed	Pfizer	$230 mn	Oct 2006
13. Coley	Pfizer	$214 mn	Nov 2007

Source:VacZine Analytics + Fierce vaccine

Global vaccine leaders



- 5 large multi-national corporations make up 80% of the global market
- Major focus on new vaccine development for industrialised country markets

Newer and more expensive vaccines are coming into the market faster than ever before

- Hib containing vaccines: mono, tetra, pentavalent, hexavalent,…
- New products: PCV, RV, HPV
- New presentations and formulations: liquid/lyophilized, number of doses,…
- Vaccine pipeline: HPV *9-valent* (“nonavalent”), Malaria, TB, .. Hundreds of vaccines under development..
- Higher cost and prices

New business MNC model is emerging?

- More mapping, market segmentation and price differentiation
- Outsourcing selected part of R&D, production and commercialization to access promising markets and local capacities, lower production costs
- Aggressive marketing, “Pharma like" model
- Risk sharing with countries and donors

UN Market: UNICEF SD and PAHO RF

- Spectacular increase in the last 10 years
- Both UNICEF SD and PAHO
- Polio, measles, new vaccines,..
- National, regional and global priority
- MDGs, GIVS, GAVI, AMC, IFFim, GPEI, Measles partnership, BMGF, DOV/GVAP

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EMERGING MANUFACTURERS ARE PLAYING AN ACTIVE ROLE

- Brazil: Bio-Manguinhos, Butantan Institute
- China: Chengdu, Shanghai (SIBP), Sinovac, Shenzhen AVP, Shenzhen Kangtai
- Cuba: CIGB, Instituto Finlay
- India: Panacea Biotec, Shantha Biotechnics, Bharat Biotech, Biological E (BE), Serum Institute of India
- Indonesia: Biofarma
- Mexico: Birmex
- Republic of Korea: Berna Green Cross (Berna), LG Life Sciences (LG)

→ **Volume, EPI vaccines, prices,...**

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Emerging Market Suppliers

- Critical to competition and increased supply capacity and lowering prices
- Quality standards and requirements exactly the same as for MNC
- Emerging producers are playing a critical role in developing countries: EPI and combo vaccines
- Now supplying about 50% procurement through UNICEF by volume (less than 30% by value)
- Top country by value for UNICEF vaccine procurement 2012 - India

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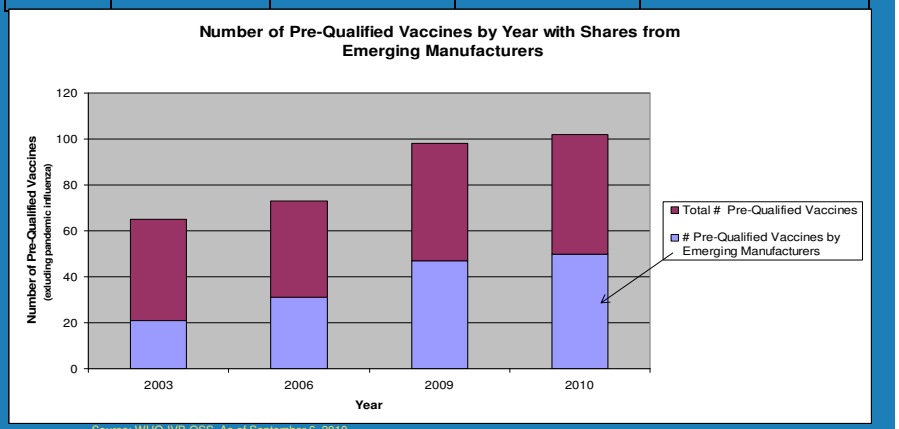
Year	Total # Pre-Qualified Vaccines (excluding pandemic influenza)	# Pre-Qualified Vaccines by Emerging Manufacturers (excluding pandemic influenza)	% of Pre-Qualified Vaccines by Emerging Manufacturers	# Emerging Manufacturer Countries with Functional NRA's
2003	66	21	32.3%	6
2006	73	31	42.5%	6
2009	98	47	48.0%	6
2010	102	50	49.0%	7



Source: WHO-IVB-QSS. As of September 6, 2010

GAVI's vaccine portfolio

Manufacturers with pre-qualified vaccines in 2013	Total
Pentavalent (DTP-HepB-Hib)	5
Rotavirus	2
Pneumococcal conjugate	2
Yellow fever	4
Meningococcal A	1
Measles-Rubella	1
HPV	2

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New global trends?

Demand side
- Vaccines and vaccinations: on the top of Govt. and UN agenda, unmet needs
- Accelerate uptake and increasing demand in Low income countries
- Middle Income countries promising but challenging

Supply
- Increasing capacity
- New products, presentations, indications, requirements,..
- New supply strategies
- Remaining supply tensions on almost all the products

Funding
- Government resources
- Donors
- Private foundations
- Increasing Co-financing
- Others..

More players on demand, supply and financing

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Potential implications for countries

1. Increased vaccine market knowledge (reducing knowledge asymmetry).
2. Information sharing on vaccine pipeline, supplier performance, quality, prices,…
3. Solid forecasting of demand.
4. Improved tendering and contracting approaches.
5. Strong quality control performing all necessary functions.
6. Flexible legislative environment.
7. Removing barriers to competition and market entry.
8. Optimised financing, payment and procurement terms to meet vaccine market dynamics.
9. Good coordination, communication and allocation of roles and responsibilities between stakeholders.

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