World Health Organization

WHO/RNA/DRAFT/22 DECEMBER 2020

ENGLISH ONLY

Evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases: regulatory considerations

NOTE:

This document has been prepared for the purpose of inviting comments and suggestions on the proposals contained therein, which will then be considered by the Expert Committee on Biological Standardization (ECBS). Publication of this early draft is to provide information about the proposed document- *Evaluation of the quality, safety and efficacy of RNA-based prophylactic vaccines for infectious diseases: regulatory considerations,* to a broad audience and to improve transparency of the consultation process.

The text in its present form does not necessarily represent an agreed formulation of the Expert Committee. Written comments proposing modifications to this text MUST be received **by 31 January 2021** in the Comment Form available separately and should be addressed to the World Health Organization, 1211 Geneva 27, Switzerland, attention: Department of Health Products Policy and Standards. Comments may also be submitted electronically to the Responsible Officer: **Dr Tiequn Zhou at email: zhout@who.int**.

The outcome of the deliberations of the Expert Committee will be published in the WHO Technical Report Series. The final agreed formulation of the document will be edited to be in conformity with the "WHO style guide, second edition" (KMS/WHP/13.1).

Regulatory considerations documents published by WHO are intended to be scientific and advisory in nature. Each of the following sections constitutes regulatory considerations for national regulatory authorities (NRAs) and for manufacturers of biological products.

Contents

1. Introduction

2. Background

3. Purpose and scope

4. Terminology

5. General considerations

6. Quality issues

7. Nonclinical issues

8. Clinical issues

Authors and acknowledgements

References

1. Introduction

While the immunostimulatory effects of RNA have been known for nearly 60 years (1), the possibility of using direct in vivo administration of in vitro-transcribed (IVT) messenger RNA (mRNA) as a means to introduce genes expressing proteins (including antigens) was demonstrated in 1990 following direct injection of "naked" nucleic acids (2, 3). Subsequent improvements to stabilize mRNA, to increase the feasibility to manufacture RNA-based products and to decrease RNA-associated inflammatory responses have led to significant advances in the development of mRNA-based vaccines and therapeutics (4). Despite the lack of any licensed mRNA-based product, either therapeutic or prophylactic, as of November 2020, there are several reasons that have brought the mRNA-based platform technology to the forefront as a potential vaccine technology. Among these include: the rapid speed at which mRNA-based candidate vaccines can be constructed and manufactured and the necessity to rapidly develop vaccines against newly emergent pathogens, such as emerging influenza virus strains, Zika virus, and most recently SARS-CoV-2, the causative agent of COVID-19. It should be noted that in December 2020, several National Regulatory Authorities (NRA) have granted or are imminently considering granting either emergency use authorization or full marketing authorization to mRNA-based COVID-19 vaccines.

Although there are recent publications that have discussed some of the safety, production, and regulatory issues associated with this new technology (5-9), as of December 2020, there is no formal regulatory guidance specifically for mRNA-based vaccines. The rapidity with which clinical trials are progressing for COVID-19 candidate vaccines and the potential for global usage have created a pressing need for the World Health Organization (WHO) to address relevant characteristics for the evaluation of the quality, safety, and efficacy of mRNA-based products for prophylactic use against infectious diseases. Of note, the inherent immunological and structural properties of mRNA, the need for formulation for stability and efficient delivery, and the novel manufacturing process all need to be considered in evaluating the quality, safety, and efficacy of mRNA-based vaccines. Because detailed information on the methods for production and control for safe, efficacious mRNA-based vaccines is not yet standardized, and for candidate vaccines certain details remain proprietary and thus not publicly available, it is not feasible to set specific international guidelines at this time. The detailed production and control procedures as well as any significant changes in them that may affect the quality, safety and efficacy of mRNA vaccines should be discussed with and approved by the NRA. Nevertheless, the key principles described in this document are applicable for the class of mRNA vaccines in general. For mRNA-based vaccines that target diseases for which there are existing vaccines and corresponding guidelines, it may be appropriate to consider in tandem Part A from these guidelines and Parts B and C from the existing vaccine/disease-specific guidelines.

The WHO Expert Committee on Biological Standardization discussed these issues at its meeting in August 2020 and supported the development of a document on regulatory considerations for the evaluation of mRNA vaccines, which could be updated as more scientific and clinical data became available (10).

This document provides information and regulatory considerations on key aspects of the manufacture and quality control, nonclinical and clinical evaluation of prophylactic mRNA-based vaccines. In the light of the current COVID-19 pandemic and the corresponding mRNA vaccine development against COVID-19, it is expected that this document provides special considerations for this class of vaccines as rapidly as possible. However, it should be noted that there remain knowledge gaps in the scientific understanding of the pathogenesis of COVID-19 and what immunogenicity is needed for a successful COVID-19 vaccine. These knowledge gaps are being addressed by ongoing research and development. This document has been developed in light of the available knowledge to date and will need to be updated as new data become available in future. Given that this is a very dynamic field, both in terms of technologies and clinical-trial designs, this document should be read in conjunction with other relevant recent guidelines, including disease-specific guidelines, if available.

2. Background

For those mRNA-based vaccines most advanced in the clinic, the manufacturing of the RNA is enzymatic rather than cell-based, which is used for most other biologicals (1, 3). One either begins with DNA plasmids, which are grown in bacteria, similar to how biologicals like plasmid DNA vaccines are produced, or with a linear DNA molecule produced via the polymerase chain reaction (PCR). Whether the manufacture of the RNA starts from a plasmid DNA converted to a linear molecule or an otherwise already linear DNA sequence, the mRNA production occurs in vitro by means of a DNA-dependent RNA polymerase that transcribes the DNA template into an mRNA molecule. The mRNA sequence consists of the usual elements of cellular mRNA, such as 5’ and 3’ untranslated regions (UTRs) that regulate the mRNA translation, a 5’ cap, and a 3’ poly(A) tail. In addition, for the purposes of stabilizing the mRNA, optimizing for codons preferred for expression in humans and reducing the inflammatory nature of the RNA in vivo, some mRNA-based products have included modified nucleosides, e.g., pseudouridine (4, 11-14). Among the most clinically advanced mRNA vaccines are those using modified nucleosides that are naturally occurring (3, 16).

Of relevance to regulatory considerations of the safety and efficacy of mRNA-based vaccines is the structures that RNA takes. Unlike DNA, which is normally in the form of a double-stranded double-helix, most RNA is single-stranded; however, based on its sequence, an RNA forms a complex structure with portions of short double-stranded stretches with various single-stranded

loops in between. This is relevant because truly double-stranded RNA, a form taken by the genome of some RNA viruses, induces cells to produce interferon, triggering immune reactivity, which is an innate response to viral infection. However, cellular mRNA does not induce such an effect despite partial double-stranded segments. Whether mRNA candidate vaccines might behave more like viral RNA or like cellular RNA in vivo is a topic to be addressed in the vaccine design, nonclinical studies, and clinical trials.

RNA-based products can take different forms. The most advanced clinical candidate vaccines take the form of mRNA encoding the target antigen (3, 15, 16). However, mRNA (and RNA in general) is subject to degradation by nucleases. Therefore, the most advanced mRNA candidate vaccines are formulated in lipid nanoparticles (LNP), which aid stability and delivery (3, 15, 16). Other stabilizing and delivery systems will likely be developed in the future.

Some candidate products contain the components needed to permit the mRNA vaccine to be self-amplifying (self-amplifying mRNA or samRNA) (7, 17). These products include the gene sequences for replicase proteins in addition to the target antigen. Thus, the mRNA can be replicated in vivo, leading to higher expression of the target antigen. Current samRNAs are also formulated in LNPs (17).

samRNAs can be compared with and contrasted to viral replicons, which are packaged in viral structural proteins whereas samRNAs are packaged in LNPs or some other formulations. This means that the cells in vivo that take up samRNAs and viral replicons likely differ because samRNAs enter cells via the formulation for delivery whereas viral replicons enter the cells via the viral receptor (3). Finally, these RNA-based products can be contrasted with viral-vectored vaccines and live viral vaccines (of RNA viruses) by the lack of genes encoding the structural proteins of the virus being used as the vector or live vaccine. Thus, there is a spectrum of products that may seem similar and the distinctions may seem subtle, but when considering not only the structure and coding sequences of the RNA contained, and also the manufacturing methods and the delivery system into which the RNA is packaged or encapsulated, one can discern into which category the product fits. It is anticipated that future products may blur these distinctions further, so the scope of this document is limited at present.

3. Purpose and Scope

This document introduces quality, safety, and efficacy topics relevant for evaluating mRNA prophylactic vaccines for infectious diseases. Furthermore, because mRNA vaccines are novel and different from other types of vaccines, even other nucleic acid vaccines (e.g. plasmid DNA vaccines), a short introduction to mRNA-specific vaccine topics is provided where deemed useful. While not all of these topics would typically need to be included in a guidance document, because of the novelty of mRNA vaccines and their manufacturing process, a more

comprehensive approach has been taken in writing this document in order to ensure that all relevant aspects may be considered by regulators in evaluating and manufacturers in developing this type of product.

The scope of this document is limited to mRNA and samRNA, packaged in LNP, for in vivo delivery of the coding sequences of a target antigen relevant to an infectious disease intended to be prevented. It is acknowledged that mRNA or samRNA, in formulations other than LNP, are also in development, and portions of this document may be applicable to those products as well. Replicating agents, viral vectors, and RNA replicons (packaged in viral proteins or encoded by plasmid DNA) are outside the scope of this document. mRNA and samRNA for therapeutic purposes are also outside the scope of this document, although some aspects discussed in Section 6 (Quality) may apply, as the manufacturing of mRNA-based therapeutic products may be similar to those described. However, because the nonclinical and clinical issues will be based on the therapeutic indication of such products, it is not feasible to include regulatory considerations for all such products within this document; hence, the scope is confined to vaccines to prevent infectious diseases.

It should be noted that mRNA products are in development that express the gene product for a monoclonal antibody rather than a target antigen. While these may also be in development for prophylaxis against infectious diseases, they are considered to be outside the scope of this document. Some aspects discussed in Section 6 (Quality) may apply to the manufacturing of these products, as it may be similar to those described.

This document should be read in conjunction with other relevant WHO guidelines such as those on the following topics:

- Guidelines on nonclinical evaluation of vaccines (18)
- Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines (19)
- Guidelines on clinical evaluation of vaccines: regulatory expectations (20)
- WHO good manufacturing practices for pharmaceutical products: main principles (21)
- WHO good manufacturing practices for biological products (22)
- Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (23)
- Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products (24)
- Guideline for Stability Evaluation of Vaccines (25)
- Model guidance for the storage and transport of time-and temperature-sensitive pharmaceutical products (26)
- Guideline on the stability evaluation of vaccines for use under extended controlled temperature conditions (27)

- Guideline on procedures and data requirements for changes to approved vaccines (28)
- WHO policy statement: multi-dose vial policy (MDVP). Handling of multi-dose vaccine vials after opening (29)

4. Terminology

The definitions given below apply to the terms as used in this document. These terms may have different meaning in other contexts.

Adjuvants: substances that are intended to enhance relevant immune response and subsequent clinical efficacy of the vaccine.

Bulk substance: the purified mRNA before final formulation. It is prepared as a single homogeneous production batch, is kept in one or more containers designated as such, and is used in the preparation of the final dosage form (final formulated vaccine). In some cases, the bulk substance may be formulated (e.g., encapsulated in LNP) but stored concentrated in comparison to the final formulated vaccine.

Candidate vaccine: a vaccine that is regarded in national regulations as separate and distinct from other candidate and licensed vaccines.

dsRNA (double-stranded RNA): dsRNA that is fully double-stranded along its entire length rather than in distinct segments (such as the secondary structure of mRNA) is generated as a by-product during the *in vitro* transcription manufacturing process of making mRNA vaccines. Some viruses have genomes that are made of dsRNA and thus, dsRNA has the ability to activate immune/inflammatory responses. Consequently, it is an impurity that needs to be purified from the mRNA during the manufacturing process or at least its amount in the product determined.

Emergency Use Authorization: some NRAs have a mechanism for permitting shipping and use of a vaccine prior to its formal marketing authorization, which can be used in emergency situation, e.g., with COVID-19 vaccines. The decision to allow emergency use of a vaccine is based on the benefit/risk considerations in the face of an on-going epidemic/pandemic instead of routine use as would be considered for full marketing authorization.

Final formulated bulk: an intermediate in the manufacturing process of the final-formulated vaccine, consisting of a homogeneous preparation of the final formulation of bulk substance and excipients at the concentration to be filled into primary containers.

Final-formulated vaccine: a finished dosage form (e.g., frozen or liquid suspension or lyophilized cake) that contains an active ingredient, generally but not necessarily in association

with inactive ingredients (excipients) or adjuvants. Also referred to as "finished product" or "drug product" in other documents.

Final lot: a collection of sealed final containers that is homogeneous with respect to the composition of the product and the avoidance of contamination during filling. A final lot must therefore have been filled from a formulated bulk in one continuous working session.

Good Manufacturing Practice (GMP): a system that ensures that products are consistently produced and controlled to the quality standards appropriate to their intended use and as required by the marketing authorization.

Immunogenicity: the capacity of a vaccine to elicit a measurable immune response.

IVT mRNA (in vitro transcribed mRNA): the manufacturing process whereby mRNA is generated from *in vitro* from a linear DNA template using a T7, a T3 or an Sp6 phage RNA polymerase and nucleotide triphosphates.

Lipid nanoparticle (LNP): a delivery formulation consisting of various components to ensure that the mRNA is stabilized and encapsulated to facilitate its uptake into cells.

Marketing authorization: a formal authorization for a medicine (including vaccines) to be marketed. Once an NRA approves a marketing authorization application for a new medicine, the medicine may be marketed and may be available for physicians to prescribe and/or for public health use (also referred to as product licensing, product authorization, or product registration).

Modified nucleotides: naturally occurring modified nucleotides, such as pseudouridine, that can be substituted for the usual nucleotide, in this case, uridine, when making mRNA vaccines, with a resultant potential decrease in inflammatory activity and/or an increase in stability.

mRNA: a single-stranded RNA molecule that is translated in the cytoplasm of a cell into the protein that it encodes. It contains an Open Reading Frame (ORF) that encodes the target antigen, flanking untranslated regions, a 5′ cap, and a 3' poly(A) tail.

Self-amplifying mRNA (samRNA, sometimes referred to as self-amplifying molecule, SAM): an mRNA vaccine that in addition to encoding the desired antigen(s), also encodes nonstructural proteins of certain alpha viruses. When expressed *in vivo* following injection, these ORFs produce proteins of an alpha virus's replication machinery, which enables the cell to produce multiple copies of the mRNA that encodes the antigen protein. The goal of samRNA is to increase the *in vivo* potency of the mRNA vaccine.

tRNA (transfer RNA): an RNA molecule used by ribosomes, that translates the codons of the mRNA into a protein.

5. General considerations

As with all vaccines, the intended clinical target of the vaccine should be described, including the pathogen targeted and disease prevented, the anticipated clinical use and the target population(s). Given the novel structure and manufacturing of mRNA-based candidate vaccines in contrast to other vaccines already licensed with which regulators are familiar, this regulatory-considerations document raises the following potentially novel topics for evaluating their quality, safety, and efficacy:

- In particular, relevant aspects of the mRNA technology used, as further described below, such as, the inflammatory nature of the given mRNA, the quality, quantity and bias of the immune responses (e.g., T helper (Th1/2) cell phenotype), biostability, etc., should be described. To justify the vaccine design, whatever information is known or available about the type of immunity considered relevant to the specific pathogen and disease should be described.
- An overview should be provided on the development of the mRNA(s) vaccine, and this should include the rationale for the selection of the target antigen(s) or parts thereof and of any proteins (e.g. cytokines) that are encoded to contribute to the proposed mode of action of the vaccine. Likewise, the rationale for the selection of any coding sequences added to the target antigen, such as those used to fold the target antigen in a particular conformation, should be provided. Justifications for use of specific noncoding sequence and structural elements such as the chosen 5` cap structure should be provided. As regards samRNA viral replicon genes encoded in the vaccine construct to allow amplification of the mRNA in human cells after delivery should be described in detail. The anticipated function and role of each gene sequence encoded in the mRNA as well as of specific noncoding and structural elements should be explained in view of its contribution to the mode of action.
- The formulation of the final vaccine product and all excipients, including all components employed for the generation of lipid nanoparticles should be described. Information on the method of production of the final vaccine product including information on its composition and rationale for inclusion should be provided.
- For each novel excipient (i.e. not used before in any medicine licensed for human use or if licensed in any medicine for human use but not using the same route of administration), detailed information on its rationale for inclusion, the method of production and control, and data from non-clinical studies on the safety, need to be provided.

- The intended dosing, the route of administration, and a description and justification of any administration device should be provided.

6. Manufacture and control of mRNA vaccines

It is considered that mRNA vaccines are to be regulated as biologicals, and like other biologicals, adequate control of the starting raw materials and manufacturing process is as important as that of the final product. Regulatory considerations therefore place considerable emphasis on the control strategy of the manufacturing process of the vaccine as well as on comprehensive characterization and release testing of the bulk substance and the vaccine itself.
The general manufacturing requirements contained in good manufacturing practices (GMP) for pharmaceutical products: main principles (21) and biological products (22) should apply to the design, establishment, operation, control and maintenance of manufacturing facilities for mRNA vaccines. The requirements should also apply to the vaccine filled in the final form, records, retained samples for future studies and needs, labelling, distribution and transport, as well as storage and expiry dating for mRNA vaccines (25-28). Quality control during the manufacturing process relies on the implementation of quality systems, such as GMP, to ensure the production of consistent commercial vaccine lots with product characteristics similar to those of lots shown to be safe and efficacious in clinical trials. Throughout the process, a number of in-process control tests (with acceptable limits) should be established to allow quality to be monitored for each batch or lot from the beginning to the end of production. Release specifications should be agreed with the NRA(s) as part of the clinical trial or marketing authorization.

mRNA vaccines for use in clinical trials should also be prepared under GMP conditions suitable for the stage of clinical development (i.e. full compliance may not be possible in initial or early development when manufacturing and control procedures remain in development and may not be validated yet). Appropriate attention needs to be given to the quality of all reagents used in production and control. Particular attention to the sourcing of components of animal (including human) derivation is required. Many of the general requirements for the quality control of biological products, such as tests for endotoxin, stability and sterility, also apply to mRNA vaccines.

It is recognized that the level of detail required by a regulatory authority increases as product development proceeds. During the initial phases of clinical development, the information contained in a clinical trial application should be adequate to allow an assessment of the safety risks derived from the manufacturing process. This would include, for example, identification and specifications for all materials used in the process, assessment of risks from biologically-sourced materials, certification or phase-appropriate GMP compliance of the manufacturing facility, a brief description of the process and tests, results of testing of the clinical trial material

and preliminary stability of the final vaccine. As with all vaccines, for late-stage clinical trials, the level of detail on the quality (manufacturing and controls) expected would increase.

Changes made to the product composition (e.g. change in the content of mRNA, excipients or addition of preservatives) or manufacture (e.g. process, site or scale) during the development of clinical lots should be adequately described. Depending on how the final product composition is changed, e.g. addition of novel excipients, new preclinical studies might be warranted. For changes to the manufacturing process such as scale-up or change to the purification process, comparability of the -vaccine with one from previous processes should be evaluated. The comparability studies might include immunogenicity data from animal models, results from physico-chemical analyses, process and product-related impurity studies, and stability data. The WHO Guidelines on procedures and data requirements for changes to approved vaccines (28) should be consulted in this regard.

6.1 General Manufacturing overview

mRNA-based products represent a new class of vaccines. They are manufactured differently from traditional biologicals, most of which are propagated or produced in a cell-based (or organism-based) system. The mRNA component is manufactured via *in vitro*-transcription by an enzymatic process. The production process uses a linear DNA template to direct DNA-dependent RNA transcription with recombinantly-produced enzymes and nucleoside triphosphates. The nucleosides used may be native, naturally occurring but modified from the natural sequence, or non-naturally modified. In addition, optimized codon use may impact stability and enhance in vivo translation of the mRNA in humans (e.g., for translation by tRNAs more frequently used in human cells). Alternatively, codons may be selected for more infrequent tRNAs in order to slow translation permitting desired protein folding. Some changes to the mRNAs are designed both to increase the stability of the mRNA and to moderate activation of the innate immune system (14). Depending on the indication, it may be desirable to decrease innate-immune responses that might lead to inflammatory reactogenicity in vivo. For some prophylactic vaccines, some of the innate immunity may be considered useful for generating the desired immune response, and the mRNAs may be designed accordingly. The gene sequence encoding the antigen should contain start and stop codons and be flanked by 5’ and 3’ untranslated regions (UTRs), have a 5’ cap and a 3’ poly(A) tail. Of note, additional modifications such as adding a methylated cap may be made to increase the translation of the mRNA (2). Justification should be provided for the choice of the sequence or structure of not only the ORF but also the UTRs, the cap, and the poly(A) tail length.

After the mRNA has been transcribed, the template DNA is then digested by deoxyribonuclease (DNase) prior to purification of the mRNA. If the cap and poly(A) element are not added during the IVT process, or if a longer poly(A) tail is required, these can be added enzymatically after

synthesis and prior to purification (11-13). In addition to removing the DNA template, the unattached caps and unincorporated nucleosides, and the enzymes that have been used in production, all process-related and product-related (e.g., dsRNA) impurities should be removed to the extent feasible. The methods of purification and their purposes should be described and justified. Validation of the purification processes should be described in terms of the method of validation and the extent of purification from various impurities that has been demonstrated to be consistently achieved.

In most cases, the purified mRNA would be considered to be consistent with what is termed for other vaccines - the purified bulk antigen, although the mRNA is not the actual antigen but corresponds to the sequence of the gene encoding the antigen. This could also be thought of as the bulk biological substance and is referred to in this document as the bulk substance. In some cases, the purified mRNA is immediately formulated but stored concentrated in comparison to the final bulk vaccine. Whichever the case, the process step of manufacturing where the purified substance (mRNA) is stored and undergoes release testing should be identified by the manufacturer. For this reason and as would be expected for any vaccine, a flowchart of production should be provided that indicates each process step, the samples taken at that process step, and for which in-process control tests they are taken. This process flow diagram should also clarify what is considered by the manufacturer to be the bulk substance, the final formulated vaccine, and the filled vaccine and at which steps in the flow the samples are taken for in-process control and release testing.

In addition, the mRNA is not stable for clinical use unless it is enveloped with a given formulation. The formulation chosen for the most advanced clinical candidate vaccines so far is based on LNPs. There are other approaches to encapsulating mRNA-based products; however, this document will not detail methods other than use of an LNP. The formulation both stabilizes the mRNA and facilitates its entry into cells. In order to protect the mRNA from degradation by nucleases, the LNPs must be made inaccessible to such nucleases, but must also be able to release the mRNA once inside the cell. The LNPs must also be of a size suitable for uptake by cells. Hence, the process of encapsulation into the LNPs must be carefully controlled and the production methods and control measures described.

samRNA products are manufactured by essentially the same process. Although the RNA contains the coding sequences for additional proteins (viral nonstructural genes) that permit in vivo amplification of the RNA, but not packaging (viral structural genes), the method of manufacture by IVT, followed by purification and formulation in LNPs is essentially the same. Control measures would also be similar or the same. This explains why samRNAs are included in the scope of this document.

The key control points should include:

a. Raw materials: including, but not limited to, a linear DNA template, which could be a PCR-generated product or a plasmid DNA that has been linearized (generally by restriction endonuclease), nucleotides, enzymes (e.g., DNA-dependent RNA polymerases, capping enzyme, poly(A) polymerase, and DNase), buffers, solvents, column resins (if column chromatography is used in purification);
    i. In particular, any animal (including human)-derived raw materials or raw materials that were themselves produced using animal (including human)-derived raw materials should be subject to control by appropriate sourcing, by control testing, and by risk assessment.
b. The manufacturing processes: including the processes to manufacture the bulk mRNA substance (bulk substance) as well as the formulation and filling of the final filled vaccine; and
c. The mRNA vaccine bulk substance, formulated bulk vaccine, and final filled vaccine following manufacture.

For clinical trial usage, mRNA candidate vaccines should be manufactured under GMP conditions for pharmaceutical or biological (if any biological components are included) products appropriate for the stage of clinical development. It is still expected that clinical-trial material should be released on the basis of meeting appropriate quality-control standards.

Any manufacturing changes made during clinical development, particularly if made following completion of pivotal safety and efficacy trials but prior to seeking licensure, need to be described and justified. For post-approval changes, compliance would be expected with the WHO Guideline on procedures and data requirements for changes to approved vaccines (28).

6.2. General information and description of vaccine construct and composition

Information that describes the mRNA vaccine in terms of how it was constructed, what is its composition, and what is its structure and sequence should be detailed.

6.2.1 mRNA sequence and structure

a. The annotated sequence of the DNA template should be provided. The sequence of the mRNA including start and stop codons, flanking untranslated regions (UTRs), regulatory elements (e.g., promoter for the RNA polymerase), and 5’ cap and 3’ poly(A) tails should be provided, as well as the ORF for the target antigen. If any additional proteins are encoded, such as those for a self-amplifying construct or a cytokine, their sequence should be provided (see points d and e in 6.2.1).
b. Vaccine mRNA can be manufactured containing nucleosides that are native or modified. Presence of modified nucleosides in the mRNA potentially increases the

stability of the mRNA and decreases the inflammatory innate immune responses. The sequence information should thus include the specific nucleosides used, including modified nucleosides. The particular nucleosides used and modifications of structures may affect the clinical safety, potency, and ultimately efficacy of the product.

c. Additionally, optimized codons (e.g. codons that either better match the frequency of the appropriate tRNAs in mammalian cells or that are used to attain a specific secondary or tertiary structure), may be used rather than the native codons in the pathogen's genome; for example, to increase the stability of the mRNA and the yield and proper folding of the translated protein in vivo.

d. Certain mRNA constructs, known as samRNA, in addition to coding for the target antigen(s), also code for a viral RNA-dependent RNA polymerase complex. Such a construct constitutes a replicon, such that multiple copies of the mRNA coding for the antigen are then made in vivo upon delivery to and uptake by the vaccinee's cells, with the aim of increasing the efficacy of the vaccine. The sequences for any such replicon should be provided and their function explained.

e. If an mRNA vaccine includes sequences that code for any other immunomodulator, such as a cytokine, or non-coding sequences intended as an immunomodulator, the information for its purpose and its sequence should be provided.

6.2.2. Formulations and components

a. Chemical nature and formulation: The mRNA is formulated both for increased stability and for aiding in cellular uptake. While several potential types of delivery agents exist (such as protamine complexes, cationic liposomes, nanoparticles, lipoplexes), the candidate vaccines currently in the most advanced clinical trials rely upon LNPs. The characterization of these formulations both chemically and in terms of the physical parameters of the structural formulation (such as nanoparticles) with the mRNA need to be described and should address characteristics such as consistency and stability of the formulation and final product. Characteristics such as the capacity of the formulation to affect toxicity, adverse events, immune responses and efficacy, i.e., whether the effects are positive or negative, should be described.

b. Additional adjuvants: mRNA could also encode specific immunomodulatory molecules, such as cytokines. As a general principle regarding vaccines formulated with adjuvants, demonstration of the contribution to the vaccine immunogenicity of such an addition should be provided (19).

c. Additional sequences: If additional sequences are included to target the mRNA to antigen-presenting cells or to increase release of the mRNA from the endosome, the sequence and function of these additions need to be described and evidence provided for their function.

6.3 Control of raw materials

As with any vaccine, appropriate attention needs to be given to the sourcing and quality of all reagents used in production.

6.3.1 Quality of raw materials

The raw materials, including those used for the DNA template, as well as those used for the mRNA, such as nucleotides (which may include modified nucleosides), enzymes, buffer, solvents, any columns for purification, etc., should be described. Information should be provided as to their provenance, quality, control, stability, and role, including when each is used in the manufacturing process. The process for the derivation of any reagent (such as the linear template DNA) should also be given. Particularly, the quality of the lipids used to manufacture the LNPs, especially novel lipids present in LNPs that have not been previously studied nonclinically and clinically, should be detailed sufficiently to permit a meaningful assessment of safety and quality of these raw materials.

6.3.2 Release of raw materials

As with any vaccine, certificates of compliance (if applicable) and certificates of analysis should be provided for all raw materials, including a description of which testing is performed by the mRNA manufacturer or whether the material is accepted on the basis of the certificate of analysis provided by the manufacturer of the raw material.

Reference to internationally accepted pharmacopoeias or details on the specifications should be provided.

6.4 Manufacture and control of bulk purified mRNA (bulk substance)

As stated above in Section 6.1, an overview of the development and manufacture of the mRNA should include a justification for the selection of the target antigen gene, other gene(s) contained in the mRNA sequence, UTRs, cap, poly(A) tail, and regulatory elements used. Any gene expression or other optimization modifications should be described. Annotated sequences of the complete DNA template and mRNA should be provided. Both an illustrative and annotated flowchart and a narrative description of the manufacture, in-process controls, and release tests should be provided. The detailed production and control procedures as well as any significant changes in them that may affect the quality, safety and efficacy of mRNA vaccines should be discussed with and approved by the NRA.

6.4.1 Process development and in-process controls

The development history of the manufacturing process should be provided. Tests and acceptance criteria for critical steps of the manufacturing process should be developed to ensure, and provide feedback on, the control of the process.

Validation of the manufacturing process should be shown to yield a product consistently meeting the predefined quality attributes, including demonstration of reproducible and consistent clearance of process and product-related impurities to levels acceptable for the intended use in humans.

Process validation is not generally required for a product used in early-stage clinical trials although critical steps such as aseptic processing and sterility of final product should be validated or carefully and convincingly controlled prior to initiation of manufacture of clinical materials.

6.4.2 Product characterization

A summary of the characterization of the mRNA and the formulated vaccine should be provided in addition to in-process and lot-release testing. Rigorous characterization by a range of orthogonal chemical, physical and biological methods will be essential. Characterization refers to studies and analyses that are not performed on every lot, but it permits the manufacturer to gain important knowledge about the structure, performance, and safety of their product in order to guide process and test development and improvements.

During development, the sequence of the population of manufactured mRNA should be determined and the degree of consistency of the proper sequence defined, as discussed further below in Section 6.4.3. The degree of consistency of the processes of capping and polyadenylation should also be characterized. Demonstration of expression of the full-length protein(s) without truncated or alternative forms should be provided. Particularly if expression of truncated or alternative forms of the target antigen are demonstrated during characterization studies and these alternative forms may result in neo-antigens or unwanted immune responses, this aspect may require quality control in-process or in release of the mRNA (bulk substance). The degree of consistency of encapsulation of the mRNA in the LNP should be addressed in characterization. During characterization, it should be determined whether any of these characteristics should be controlled as critical quality attributes.

The immunogenicity elicited by the mRNA should be characterized. Whenever other immunomodulatory elements or genes are included, their contribution to the mode-of-action (e.g., immunogenicity) of the mRNA should also be determined in order to justify their inclusion.

Potential impurities in the purified mRNA should be described and investigated. These impurities may include residual bacterial host-cell proteins (if used to manufacture the DNA template), endotoxins, residual bacterial host-cell RNA and chromosomal DNA (if used to

manufacture the DNA template), enzymes, unincorporated nucleotides, mis-folded or dsRNA, and other materials used in the manufacturing process. Data should be provided on the impurities present in the purified mRNA, with specifications set for their maximum acceptable or lowest achievable levels. For impurities and residuals with known or potential toxic effects, a toxicological risk assessment is expected. Degraded mRNA may be assessed as part of analytical procedures such as polyacrylamide or agarose gel electrophoresis, high performance liquid chromatography (HPLC) and/or capillary gel electrophoresis. An important characteristic to determine for the purified bulk substance is the degree to which the mRNA that has been produced is consistent in sequence and structure and that it produces a consistent protein when transfected into cells in vitro.

6.4.3 Consistency of manufacture

Like other biologicals, prior to seeking marketing authorization, a number of consecutive batches should be characterized and analyzed by employing validated methods to determine consistency of manufacture. Any differences between one batch and another outside the accepted range for the parameters tested should be noted and investigated. The data obtained from such studies, as well as clinical-trial outcomes with various lots, should be used as the basis for justification of the chosen specifications.

During early-stage development, few lots will have been made, and demonstration of production consistency may be limited. Demonstration of consistency will occur as manufacturing experience is gained during product development. Characterization of consistency of lots is generally done during advanced development, when the manufacturing process has been scaled up for commercial manufacture but prior to submission of application(s) for marketing authorization. However, in some cases, scale-up for commercial manufacture may be performed while marketing authorization is being sought for clinical trial-scale material. Whenever changes to the manufacturing process are implemented, comparability of lots, especially to those used in pivotal studies and made by the intended commercial process, should be demonstrated. Comparability protocols and strategies for demonstrating comparability are discussed in the WHO Guidelines on procedures and data requirements for changes to approved vaccines (28).

6.4.4 Control of bulk purified mRNA (bulk substance)

Specifications for critical quality attributes for the identity, purity, quality, quantity and physical state, and safety of the bulk purified mRNA should be established and justified. Descriptions of analytical methods used and the acceptance limits should be defined, including assay validation information. A summary of the results of testing of all batches produced at commercial scale should be provided.

Early in development, to support clinical trial authorization, results from testing batches made in accordance with GMP (21, 22) and, if available, engineering runs performed to establish manufacturing procedures should be summarized and provided.

It is recommended that the specifications for the bulk purified mRNA include, at a minimum, an assessment of the identity, purity, physical state and quantity of the mRNA, endotoxin content and sterility or bioburden. A justification of the specifications should be provided. Specifications should also be established for stability under storage conditions.

Early in development, the specifications may be limited and have somewhat wide acceptance criteria. Not all the tests conducted during product characterization need to be carried out on each batch of vaccine. Some tests are required only to establish the validity or acceptability of a procedure, whereas others might be performed on a limited series of batches to establish consistency of production. Thus, a comprehensive analysis of the initial commercial production batches should be undertaken to establish consistency with regard to identity, purity, quality, safety, and stability; thereafter, a limited series of tests may be appropriate.

6.4.4.1 Identity

mRNA batches should be tested to confirm their identity. Identity could include determination of the mRNA sequence by direct RNA sequencing, sequencing of an RT-PCR product, or next generation sequencing. If identity is based on a RT-PCR amplicon that represents only a portion of the complete mRNA sequence, then the sequence chosen should be unique to that mRNA product and not any others that might be manufactured in the same facility or using the same equipment. However, it might be more appropriate to select a method that serves both to address identity and purity, which would be to determine the sequence of the entire mRNA.

6.4.4.2 Purity and impurities

Each batch of bulk purified mRNA should be tested for purity, and the result should be within the limits established. Tests for impurities include bacterial endotoxins, as well as materials introduced during manufacture, such as the DNA template, unincorporated nucleotides, enzymes, mRNA fragments, and double-stranded RNA. The analyses should include sensitive and reliable assays for process- and product-related contaminants, and strict upper limits should be specified for their content in the bulk purified mRNA. A maximum allowable limit should be established and justified. It is important that the techniques used to demonstrate purity be based on as wide a range of physicochemical properties as possible.

Tests for residual levels of process- or product-related impurities as part of quality control may be reduced or discontinued after production processes have been adequately validated for their suitable removal and production consistency has been demonstrated, if agreed by the NRA. Plans and specifications for periodic re-validation of processes should be described. Until

validated, impurities should continue to be measured for a number of lots as agreed by the NRA. In the case of major changes to manufacturing, re-validation or continued measurement would be expected for the number of lots as agreed by the NRA. Container-closure system compatibility, leachables and extractables should be assessed and discussed in the application.

6.4.4.3 Quantification and physical state

It remains to be determined whether the integrity of the structure of the mRNA will be determined to be a critical quality attribute for release of the mRNA or whether this can remain a characterization, requiring re-characterization only when the sequence is altered. However, quantification of the mRNA is the basis for dosing. Thus, the method(s) used for quantifying the mRNA should be described.

6.4.4.4. Safety

Relevant safety tests should be described. These may include: tests for endotoxins, bacterial and fungal sterility (including demonstration of lack of bactericidal or fungicidal activity of the test article) or bioburden (including quantity, identification, and freedom from objectionable organisms). If required by an NRA, a test for pyrogenicity may be performed; however, animal testing should be avoided whenever alternative satisfactory testing is allowed. For ethical reasons, it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals in testing and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. Particularly, the test known as the innocuity, abnormal toxicity, or general safety test should not be required nor requested.

6.4.4.5 Quality

Additional important quality parameters should be established and controlled, such as appearance, pH, and if relevant, viscosity. In addition, there are critical quality attributes relevant to mRNA vaccines, such as poly(A) tail length and capping efficacy, which need to be determined and quantified. It should be determined whether the degree of integrity of the mRNA is a critical quality attribute that requires controlling. Whether this can be controlled by process validation (achieving a consistent level) or whether these measures need to be controlled on a batch-by-batch basis remains to be determined. Agreement with the NRA on the approach to be taken should be gained.

6.4.5 Reference materials

An in-house reference preparation should be established for use in assay standardization. Information on the reference standards or reference materials used for testing of the bulk purified mRNA should be provided by the time of application for marketing authorization.

A suitable batch, i.e. one that has been clinically evaluated, should be fully characterized in terms of its chemical composition, purity, biological activity, and complete sequence, and

retained for use as a chemical and biological reference material. A plan for replacing the initial reference material upon exhaustion should be agreed with the NRA.

In early development, an engineering run batch or a batch used to produce the lot of mRNA vaccine used in the pivotal nonclinical studies may be used until a suitable clinical trial batch has been identified and characterized for use in advanced development and commercial manufacture.

In the future, international standards (IS), expressed in International Units (IU), may be available from WHO. When such IS become available, it will be important to calibrate the internal reference material against the IS, so that IU may be assigned and in order to fully validate quality control tests or assays. In this manner, comparisons can be made in a more reliable and less variable way whenever new reference materials need to be prepared. Expression of results in IU, when appropriate, will also enable comparison of test results across laboratories and different products.

6.4.6 Stability

The stability assessment should, in principle, be in compliance with the WHO Guidelines for Stability Evaluation of Vaccines (25). The types of studies conducted, the protocols used, and the results of the studies should be summarized in an appropriate format such as tables, graphs or a narrative document. The summary should include results as well as conclusions with respect to appropriate storage conditions or shelf-life. Data on stability to support the shelf-life of the bulk and any future extension of it should be based on long-term, real-time stability studies under actual conditions.

Limited stability information would be expected during initial clinical development. For example, some regulators accept three months of real-time stability at the time of application for clinical trial authorization, but this should be agreed with the NRA.

6.5 Manufacture and control of final formulated vaccine

As stated above in Section 6.1, an overview of the development and manufacture of the vaccine should include both an illustrative and annotated flowchart and a narrative description of the manufacture, in-process controls, and release tests. The methods used to assure proper formation of LNPs should be detailed. If the formulation into LNPs occurs as part of the bulk substance, then these methods should be described in the appropriate earlier section of the marketing authorization application. The methods used for final formulation, fill and finish should be detailed.

6.5.1 Composition

The final composition of the vaccine, including the active substance and all excipients, should be described including quantity of the components in each posology, if marketing authorization is being sought for more than one dosage or dosage form.

6.5.2 Manufacture, filling and containers

An annotated flow chart should be provided that illustrates the manufacturing steps from the bulk purified mRNA to the final formulated vaccine. The chart should include all steps (i.e. unit operations), identification of materials and in-process and quality-control tests. In some cases, this may involve simple dilution of the purified bulk that has already been formulated in the LNPs.

A narrative description of each process step depicted in the flow chart should be provided. Information should be included on, for example, scale, buffers and other additives, major equipment, and process controls, including in-process tests and critical process operational parameters with acceptance criteria. In the case of simple dilution or no further formulation of the bulk purified mRNA other than filling into final containers for the final formulated vaccine, some quality control tests performed on the bulk purified mRNA may suffice as control for the final formulated vaccine.

The general requirements concerning filling and containers given in WHO good manufacturing practices for biological products (22) should apply to vaccine filled in the final form. Care should be taken to ensure that the materials of which the containers and closures (and, if applicable, the transfer devices) are made do not adversely affect the quality of the vaccine. To this end, a container closure integrity test and assessment of extractables and/or leachables for the final container-closure system are generally required for the qualification of containers and may be needed as part of stability assessments.

If multi-dose vaccine vials are used and these vaccines do not contain preservative then their use should be time-restricted, as is the case for reconstituted vaccines such as bacillus Calmette–Guérin (BCG) and measles-containing vaccines (29). In addition, the multi-dose container should prevent microbial contamination of the contents after opening. The extractable volume of multi-dose vials should be validated. If multi-dose vaccine vials are supplied as concentrate, an additional compatibility study should be conducted using the proposed reconstitution solutions and an appropriate hold period post-dilution should be established. The manufacturers should provide the NRA with adequate data to prove the stability of the product under appropriate conditions of storage and shipping.

6.5.3 Control of final formulated vaccine

Samples should be taken from each final formulated vaccine lot. These samples must fulfil the requirements of this section. All tests and specifications should be approved by the NRA. Specifications for the final formulated vaccine should be established and justified. As a principle,

the final specifications should be defined on the basis of the results of tests on lots that have been shown to have acceptable performance in clinical studies. Descriptions of analytical methods and acceptance limits for the vaccine, including information on assay validation should be provided. It is recommended that the specifications include an assessment of the identity, purity, physical state and quantity of the mRNA, any other relevant quality parameters, potency, endotoxin content and sterility. A justification of the specifications should be provided.

Early in development, the specifications may be limited with wide acceptance criteria.

A summary of the results of the testing on all lots produced at commercial scale should be provided. Early in development, results from testing lots made in accordance with GMP (21, 22) and if available, engineering runs performed to establish manufacturing procedures should be summarized and provided.

The appropriateness of performing tests on the bulk purified mRNA versus the final formulated vaccine should be considered on a case-by-case basis and justified.

Several consecutive lots of vaccine, in final dosage form, should be characterized and analysed by employing validated methods to determine manufacturing consistency. Any differences between one lot and another should be noted and investigated. The data obtained from such studies, as well as clinical trial outcomes with various lots, should be used as the basis for defining the vaccine specifications and acceptance criteria to be used for routine lot release.

Not all the tests conducted during product development need to be carried out on every lot of vaccine produced at commercial scale. Some tests are required only to establish the validity or acceptability of a procedure, whereas others might be performed on a limited series of lots to establish consistency of production. Thus, a comprehensive analysis of the initial commercial production lots should be undertaken to establish consistency with regard to identity, purity, quality, potency, strength/content/quantity, safety, and stability but thereafter a more limited series of tests may be appropriate.

6.5.3.1 Identity

Each lot of vaccine should be subjected to an appropriate test used to confirm the identity of the final product and distinguish it from other products made in the same facility or using the same equipment. Confirmation of the identity by sequence analysis should be considered. See section 6.4.4.1.

6.5.3.2 Purity and impurities

The purity of each lot of vaccine should be determined and be shown to be within specified limits. Tests including particle size and the proportion of/efficiency of mRNA encapsulated in

the LNPs should be determined. Container-closure system compatibility, leachables and extractables should be assessed and discussed. See also section 6.4.4.2.

6.5.3.3 Content, Strength, or Quantity

mRNA vaccines are dosed based on quantity of the mRNA by weight. Therefore, in addition to assessing potency, a quantification method should be described. See Section 6.4.3.3.

6.5.3.4 Safety, including sterility and endotoxin testing

Each lot of vaccine should be tested for sterility. If the vaccine is to be administered by a non-parenteral route, omission of the sterility test and inclusion of an appropriate alternative bioburden test needs to be appropriately justified. Further, a test for endotoxin should be conducted on each lot, and appropriate specifications should be defined. This may be determined by use of the monocyte activation test. If required by an NRA, a test for pyrogenicity may be performed; however, animal testing should be avoided whenever alternative satisfactory testing is available. For ethical reasons, it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals in testing, and consideration should be given to the use of appropriate in vitro alternative methods for safety evaluation. Particularly, the test known as the innocuity, abnormal toxicity, or general safety test should not be required nor requested.

6.5.3.5 Quality

Other important quality parameters should also be established and controlled, including appearance and pH. For the final formulated product additional parameters would include: lipid/polymer identification and content, lipid/polymer impurities, nanoparticle size, and polydispersity index of the liposomal finished product. The degree of encapsulation of the mRNA in the LNP may be a critical quality attribute. Depending on the product characteristics, control of other parameters such as osmolality or viscosity may be important. Further, quality may be assessed by methods used to evaluate purity or identity, such as gel or capillary electrophoresis and/or HPLC. Other tests, such as the test for residual moisture if the vaccine is lyophilized, may be required to confirm the physical characteristics of the product as well as the formulation.

6.5.3.6 Potency

The potency of each lot of the vaccine should be determined using a suitably quantitative and validated assay. Potency in the vaccinee is a complex function of the final vaccine including its formulation as well as the mRNA itself (which may include a self-amplifying replicon component). Thus, possible in vitro potency assays may include cell-based transfection systems or cell-free assays. However, because potency will be analyzed on the basis of not only the product type (in this case mRNA-based vaccines) but also the clinical indication of the disease to be prevented, it is not possible at this time to indicate one specific assay that should be used to

measure potency. Therefore, the assay used for vaccine potency should be discussed with and approved by the NRA.

The potency specifications for mRNA vaccines should be set based on the minimum dose used to demonstrate efficacy in clinical trials plus human immunogenicity data. An upper limit should also be defined based on available human safety data. It is recommended to avoid animal-based assays, as it is desirable to apply the 3Rs concept of "Replace Reduce Refine" to minimize the use of animals in testing. In addition, such bioassays tend to be highly variable and difficult to validate. Therefore, consideration should be given to the use of appropriate in vitro alternative methods for potency evaluation. However, it may be important to characterize the potency of the candidate vaccine in an animal model and bridge those findings to an alternative method that will be used for release. It is envisioned that in future, like plasmid DNA vaccines, a combination of biochemical measures, like quantity, sequence, and structure might be used to establish and monitor potency of mRNA-based vaccines. However, these measures only account for the mRNA and not the impact of any formulation, adjuvant, immunomodulators, etc. So, at present, discussion and agreement with the NRA is advised.

6.5.4 Reference materials

A suitable lot of the final formulated vaccine that has been clinically evaluated should be fully characterized in terms of its chemical composition, purity, biological activity, and full sequence, and retained for use as a chemical and biological reference material. This material should be used as the basis for evaluation of product quality for commercial production lots. See also Section 6.4.5.

IS may be useful for interpretation of nonclinical and clinical assays of immune responses or other biomarkers of relevance to an mRNA vaccine under development or being evaluated for marketing authorization (also see the WHO guidelines listed in Parts B and C for further guidance on this issue).

6.6 Records

The recommendations given in the WHO Good manufacturing practices for pharmaceutical products: main principles (21) should apply, as appropriate to the level of development of the candidate vaccine.

6.7 Retained samples

A sufficient number of samples should be retained for future studies and needs. A vaccine lot used in a pivotal clinical trial may serve as a reference material and a sufficient number of vials

should be reserved and stored appropriately for that purpose. Advance planning is required to enable the retention of an appropriate number of containers of the pivotal clinical trial lot.

6.8 Labelling

The labelling recommendations provided in the WHO Good manufacturing practices for biological products (22) should be followed as appropriate. The label of the carton enclosing one or more final containers, or the leaflet accompanying the container, should include, at a minimum, as agreed with the NRA:

- the name of the vaccine;
- the names and addresses of the manufacturer and distributer;
- a statement that specifies the nature contained in one human dose, if any;
- the immunization schedule and the recommended route(s) of administration;
- the number of doses, if the product is issued in a multi-dose container;
- the name and concentration of any preservative added;
- a statement on the nature and quantity, or upper limit, of any antibiotics present in the vaccine;
- the temperature recommended during storage and transport;
- the expiry/retest date;
- any special dosing schedules;
- contraindications, warnings and precautions, and information on concomitant vaccine use and on adverse events.

6.9 Distribution and transport

The recommendations provided in the WHO Good manufacturing practices for biological products (22) appropriate for the vaccine should apply. Further guidance is provided in the WHO Model guidance for the storage and transport of time- and temperature-sensitive pharmaceutical products (26). Shipments should be maintained within specified temperature ranges, as applicable, and packages should contain cold-chain monitors (27).

6.10 Stability testing, storage and expiry date

The recommendations given in Good Manufacturing Practices for biological products (22) and in the Guidelines on stability evaluation of vaccines (25) appropriate for the respective mRNA vaccine should apply. Furthermore, the Guideline on the stability evaluation of vaccines for use under extended controlled temperature conditions might apply (26). The statements concerning storage temperature and expiry date that appear on the primary and secondary packaging should be based on experimental evidence and should be submitted to the NRA for approval.

6.10.1 Stability

Adequate stability studies form an essential part of vaccine development. The stability of the final product in the container proposed for use should, therefore, be determined and the results used to set a shelf-life under appropriate storage conditions. Parameters that might be stability-indicating should be measured. These may include parameters such as appearance, quantity, and potency. The parameters to be measured should be described and specifications defined. Real-time stability studies should be undertaken for this purpose; but accelerated stability studies at elevated temperatures may provide complementary supporting evidence for the stability of the product and confirm the stability-indicating nature of the assays used to determine stability. If deep-freeze condition is recommended for long-term storage, alternate short-term storage conditions such as frozen and/or refrigerated should be explored to support distribution and dispensing. Container-closure system compatibility for storage stability, including leachables and extractables should be assessed and discussed. The stability assessment should comply with WHO Guideline for Stability Evaluation of Vaccines (25).

6.10.2 Storage conditions

Storage conditions should be fully validated. The vaccine should not be stored for a length of time and at a temperature greater than that shown by the manufacturer to be compatible with a minimal loss of potency before being distributed by the manufacturing establishment or before being issued from a storage site. The maximum duration of storage should be fixed with the approval of the NRA, based on results of stability studies, and should be such as to ensure that all quality specifications for the final product, including the minimum potency specified on the container or package, are maintained until the end of shelf-life. During clinical trials, this period should ideally be at least equal to the expected duration of vaccine administration in the clinical trial.

6.10.3 Expiry date

The expiry date should be defined on the basis of shelf-life of the final container supported by stability studies and should be approved by the NRA. The expiry date should be based on the date of blending of the final bulk, the date of filling, or the date of the first valid potency test on the final lot, as appropriate, and agreed with the NRA.

7. Nonclinical evaluation of mRNA vaccines

The nonclinical evaluation of candidate mRNA vaccines should be considered on a product-specific basis taking into account the intended clinical use of the product. The design, conduct and analysis of nonclinical studies including selection of appropriate studies relating to the

toxicology and pharmacology (immunogenicity and proof-of-concept) of the product should be based on either or both of the following WHO guidelines:

- WHO *Guidelines on the Nonclinical Evaluation of Vaccines (18)*
- WHO *Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines (19)*

There are several potential concerns that might be specific to mRNA vaccines because of the candidate vaccine design or because they have been seen clinically with other RNA-based products (e.g., therapeutic candidates). Because of the novelty of this product class and for sake of inclusiveness, many issues are listed in this Section. Not all will necessarily be relevant to mRNA vaccines, depending on their design. However, it is incumbent upon the vaccine developer/manufacturer to provide evidence demonstrating the safety and proof-of-concept (e.g., immunogenicity, challenge-protection) for their candidate vaccine. The types, design, and number of studies expected should be agreed with the NRA.

7.1 Safety/Toxicity in animal models

In addition to the expectations outlined in the WHO guidelines listed above (18, 19), study designs should consider how best to address the following:

a. Biodistribution and persistence: Developing a database of evidence about this potential concern will permit more rapid development of future clinical candidate vaccines (4,30-33). This issue may also depend on whether the vaccine targets specific cells or tissues. Nonclinical studies that address whether the mRNA vaccine distributes away from the tissue into which it was administered, into which tissues it distributes, and how long it persists there may be expected by the NRA. Agreement on these studies should be sought from the NRA.

b. Novel lipids: Because formulation may include lipids, when LNPs made with novel lipids are used that have not been previously tested nonclinically and clinically in mRNA products encapsulated in LNPs, then the toxicity of the novel lipids (or any novel excipients) should be assessed.

c. Novel formulations: Likewise, for novel formulations (other than LNPs), evidence on the toxicity of the formulation will be expected.

d. Inflammation: RNA is inflammatory via a number of pathways, particularly via the innate immune system with numerous sensors for RNA. While some of the activity may be beneficial for the immune response to the vaccine, it will be important to monitor for both systemic and local toxicity and inflammatory responses. In two independent clinical trials with different COVID-19 mRNA candidate vaccines, a dose-dependent systemic reactivity was observed. Therefore, lowering of clinical doses has been necessary due to systemic toxicity seen at the highest doses for the two different vaccines (15, 16). Nonclinical study design, including dosing levels that permit analysis of dose-

responsiveness data, needs to take into account this potential toxicity and look for any immune responses, reactogenicity, or toxicities that might predict indictors for serious adverse events (SAEs) or adverse events of special interest (AESI) in humans.

e. Unexpected and serious toxicities from modified nucleosides: Some antivirals and anti-cancer drugs that contain unnatural nucleoside analogues have caused mitochondrial toxicities, resulting in myopathy, polyneuropathy, lactic acidosis, liver steatosis, pancreatitis, lipodystrophy, and even fatality. Some of these toxicities had not been observed in nonclinical animal models. Thus, particularly for mRNA vaccines that include non-natural nucleoside modifications, careful safety evaluation needs to be considered for how these toxicities might be observed in appropriate animal models and nonclinical studies (34).

f. Anti-RNA antibody generation, anti-phospholipid antibody generation: Induction of anti-RNA or anti-phospholipid antibodies is a potential concern with this vaccine approach and should be monitored in the nonclinical studies for any new specific mRNA formulation, just as anti-DNA antibodies were evaluated in early plasmid DNA vaccine nonclinical and clinical trials. Building a database of evidence about this potential concern will permit more rapid development of future clinical candidate vaccines. This is particularly pertinent in light of concerns over AESI that are autoimmune or allergic in nature.

g. Potential immune tolerance: Because induction of tolerance rather than immunity has been observed when an antigen is expressed in cells other than professional APCs, the potential induction of immune tolerance is a theoretical risk. Particularly, dependent on the target antigen encoded and the immunological pathways it might induce, whether this theoretical risk should be evaluated with any given mRNA product should be agreed with the NRA. The formulation or delivery system and the cells it targets may guide decisions about the necessity for such a study.

It should be noted that while there was a theoretical concern for integration with regards to plasmid DNA vaccines, this concern is not shared for mRNA-based vaccines for the following reasons:

- The only known mechanism by which RNA can integrate into the host genome is in the presence of a retrovirus particle containing reverse transcriptase.
- Further, the design of the current candidate vaccines does not include specific RNA-binding domains required for the reverse transcriptase to bind and transcribe.
- Finally, the mRNA degrades within a relative short span of time once taken up in the body's cells, as does the cell's own mRNA. The mRNA vaccine is expected to remain during that time in the cytoplasm where it will be translated.

Therefore, it is not recommended for nonclinical studies to be performed to specifically address integration, as it considered an extremely remote and theoretical issue for mRNA-based vaccines to prevent infectious diseases (34).

As with any vaccine that is anticipated to be used widely in pregnant women or women of child-bearing potential, the guidance in the WHO Guidelines on Nonclinical Evaluation of Vaccines (18, see section 4.2.2) and the WHO Guidelines on Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines (19, see section D.2.3) should apply. The necessity for such studies is based on the target population for the given clinical indication of the vaccine. Often, if required, these studies are performed during advanced development of the candidate vaccine, e.g., based on commercial manufacturing methods and scale.

7.2 Pharmacology/Immunology/Proof-of-concept

In addition to the types of studies discussed in the guidelines above (18, 19), additional issues that might be expected by an NRA to be explored nonclinically may include:

a. Durability of immune responses, particularly those that are proposed to be related to the vaccine candidate's induction of protection.
b. Induction of RNA-specific immune responses (such as induction of type I interferon) that have been reported to decrease translation of the target antigen or that could affect the need or timing for boosts or subsequent doses.

In the situation of rapid development of a vaccine against priority pathogens for public health emergencies, consideration may be given to an abbreviated nonclinical program as follows:

- In the case where only minor changes are made to the sequence of the mRNA vaccine that has already been tested clinically for a related antigen with the same LNP (e.g. in the case of a pandemic influenza strain when a seasonal or other potential pandemic strain antigen has been tested, in the case of evolution in a SARS-CoV-2 spike protein), then the nonclinical program might be limited to an immunogenicity study(ies). However, that study should collect as many safety data as feasible given that many nonclinical immunogenicity studies are performed without full compliance to Good Laboratory Practices. Depending on the species used, if it is feasible to collect blood not only for immunogenicity analyses, but also for hematology and chemistry assessments, these analyses should be performed. In addition, depending on the species used, if the animals are sacrificed at the end of the immunogenicity study, then gross pathology and targeted histopathology should be performed. In addition, information about physical exams or clinical findings should be captured and reported to the NRA. If the species used are too small (e.g. mice) to permit individual clinical pathology or if a species used (e.g. non-human primates) that is not sacrificed because the animals will be used in other research

after the immunogenicity study is performed, then whatever safety data can be collected should be reported to the NRA. If there is safety information about veterinary vaccines expressing related antigens, this information might be useful to provide to the NRA.

- In the case where the LNP has been tested clinically with an unrelated mRNA such that the target antigen is novel (not related to another antigen that has been tested clinically), then this approach might not be sufficient. The decision about what type of nonclinical safety/toxicology information should be required might be guided by what and how much is known about the natural disease in terms of the pathology, particularly immunotoxicity. If the natural disease is associated with immunopathology due to cross-reactivity, autoimmunity, allergenic, or immunity-associated disease enhancement, then toxicology studies would likely be needed to assure that the novel target antigen is not associated with these effects. In the cases where natural disease is not associated with immunopathology or where little is known about the natural disease, discussion with the NRA should be undertaken on how the nonclinical program might be abbreviated.

- Finally, in the cases where the LNP and the encoded target antigen (and hence the mRNA structure and sequence) are both novel, nonclinical evaluation may be more complex and more extensive studies may be required; hence, discussion with the NRA also should be undertaken and it may not be possible to abbreviate the nonclinical program.

Decisions about abbreviating the nonclinical program should always take into account what is known about related products, if any have been previously tested. If clinical data from a related product(s) are available, these data are likely more meaningful to the safety of the candidate vaccine in humans than data from any given animal model.

8. Clinical evaluation of mRNA vaccines

The clinical evaluation expectations for clinical-trial authorization or marketing authorization will be driven by the disease against which the mRNA vaccine is being or has been developed and the vaccine mode-of-action (or mechanism-of-action), i.e., whether or not an immune correlate of protection has been identified. Clinical studies should adhere to the principles described in the WHO Guidelines for good clinical practice (GCP) for trials on pharmaceutical products (23) and the WHO Guidelines on clinical evaluation of vaccines: regulatory expectations (20). Likewise, post-marketing pharmacovigilance is discussed in this latter guideline.

Clinical trials should capture safety, immunogenicity, and efficacy data, as expected for any other type of vaccine, with particular consideration to the following potential concerns that may

be more related to mRNA vaccines than to other types of vaccines that might already be licensed.

8.1 Safety and immunogenicity evaluation

There should be sufficient data to permit evaluation of the following types of particular safety and immunological aspects that may be relevant to mRNA-based vaccines:

a. Adverse immune effects

Transient decreases in lymphocytes (Grades 1-3) a few days after vaccination were reported in the interim human clinical trial results of an mRNA COVID-19 vaccine, with lymphocytes returning to baseline within 6-8 days in all participants, and with no associated clinical observations (35). Because RNA induces interferon type 1, which have been associated with transient migration of lymphocytes into tissues, the phenomenon of any effect on lymphocyte counts in blood, may need specific attention in clinical trials (32, 36-38). Because this phenomenon may be important for the immune response to the vaccine candidate, it will be important to observe whether changes in leukocyte counts and subsets are associated with any adverse clinical signs or symptoms.

b. Types and scope of immune responses

In addition to the type and scope of immunogenicity described in the WHO *Guidelines on clinical evaluation of vaccines: regulatory expectations (20),* additional facets of safety and immunogenicity of mRNA vaccines may relate to*:*

i. Whether the mRNA vaccine candidate biases towards certain types of immune responses. Two clinical studies for COVID-19 mRNA vaccines reported to date have noted a Th1-type bias (15, 16 28).

ii. Whether immune interference may be observed with relevant co-administered vaccines or vaccines likely to be administered in a near-time-framework (e.g., COVID-19 and influenza vaccines). Which co-administered vaccines are considered relevant depends on the clinical indication and target population and should be agreed with the NRA.

iii. Whether there are any observed differences in the immune responses for different populations, such as people of different racial/genetic backgrounds, children, the elderly or patients with autoimmune disorders or with concomitant underlying diseases, such as hypertension (particularly relevant for clinical indications such as COVID-19). Observed differences may suggest the need for considering development of alternative posologies for these populations.

iv. Whether immune responses against vaccine components such as RNA or phospholipids are generated or, if pre-existing in the vaccinee, are increased or exacerbated, may be of concern. As with any new vaccine, events or evidence of adverse events of special interest, as defined in the WHO *Guideline for Clinical*

Evaluation of Vaccines: Regulatory Considerations (20), should be captured in clinical trials and in post-marketing evaluation.

It should be noted that in the conduct of clinical trials or in emergency use situations with mRNA-based COVID-19 vaccines, two types of immunologically relevant adverse events of particular note have been observed uncommonly. One is cases of Bell's palsy and the other is anaphylaxis or anaphylactoid cases. It should be further noted that the US FDA considered the rate of Bell's palsy cases to "be similar to the background rate in the general population and there is no clear basis for concluding causality at this time" (39). These types of adverse events will be assessed further in pharmacovigilance activities. Further, these events should be considered when designing clinical trials of future candidate mRNA-based vaccines against other diseases, as well as against COVID-19. It is not yet known what aspect of the formulation is associated with immunological adverse events and it is advised, as with other vaccines, that individuals with known allergies to vaccine components should not be vaccinated (39, 40). It will also be worth noting the Scientific Opinion of the European Medicines Agency, which is anticipated to be published on their website in due course. As stated before, this is a rapidly evolving arena and significant data are emerging in an on-going basis.

8.2 Efficacy evaluation

Efficacy evaluation depends on the disease against which the candidate vaccine is sought to protect, and the clinical indication determined in clinical trials. Factors that should be considered in evaluation of vaccine efficacy are described in the WHO *Guidelines on clinical evaluation of vaccines: regulatory expectations* (20).

It should be acknowledged that in countries where mRNA-based COVID-19 vaccines are receiving emergency use authorization, that the candidate vaccines remain investigational, i.e., under development. The ethical considerations regarding conduct of on-going COVID-19 trials with placebo controls has been discussed in open public meetings held in December 2020 (41, 42). Trial design issues including appropriate selection of comparators is discussed in the guideline above (20). In addition, of note, is the Expert Consultation on the use of placebos in vaccine trials (43). As with all candidate vaccines, both the scientific merits and the ethical considerations inform the trial design and decisions must be made on the current context of benefit and risk in the country in which regulatory authorization is being sought.

Finally, the WHO has more than sixty guidelines and recommendations for vaccines against specific diseases that may be relevant for any given mRNA vaccine (44).

Authors and Acknowledgements

A pre-draft was prepared by Dr M. Liu, Karolinska Institute, Stockholm, Sweden; and Dr H-N. Kang, World Health Organization, Switzerland. The pre-draft has been shared among a working group of experts for inviting comments during 1- 15 October 2020.

The first draft was then prepared by a WHO Drafting Group comprising by Dr R. Sheets, consultant, USA; Dr M. Liu, Karolinska Institute, Stockholm, Sweden; Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr K. Peden, FDA, USA; Dr S. Sankarankutty, Health Sciences Authority, Singapore, and Dr T.Q. Zhou, World Health Organization, Switzerland, taking into consideration the comments received from: Dr. E. Grabski and Dr H. Meyer, Paul-Ehrlich-Institut, Germany; Dr D. Kaslow, PATH - Vaccine Development Global Program, USA; Prof. S. Lu, University of Massachusetts Medical School, USA; Prof. E.E. Ooi, Duke-NUS Medical School, Singapore; Dr K. Peden, FDA, USA; Prof. P. Roy, London School of Hygiene & Tropical Medicine, United Kingdom; Dr S. Sankarankutty, Health Sciences Authority, Singapore; Dr J. Ulmer, GSK (former); Dr F. Verdier, Sanofi Pasteur, France; Drs. K. Karikó, A. Kuhn, U. Blaschke, C. Blume, J. Diekmann, BioNTech, Germany; Dr M. Watson, Dr C. Vinals, Moderna, USA; and Dr T.Q. Zhou, World Health Organization, Switzerland.

References

1. Sahin U, Karikó K, Türeci Ö; mRNA-based therapeutics--developing a new class of drugs. Nat Rev Drug Discov. 2014, 13:759-80.
2. Wolff JA et al. Direct gene transfer into mouse muscle in vivo. Science.1990, 247:1465-80.
3. Krammer F. SARS-CoV-2 vaccines in development. Nature 2020,586(7830):516-527. doi: 10.1038/s41586-020-2798-3.
4. Pardi N, Hogan MJ, Porter FW, and Weissman D; mRNA vaccines – a new era in vaccinology. Nat Rev Drug Discov, 2018, 17:261-279.
5. Hinz T, Kallen K, Britten CM, Flamion B, Granzer U, Hoos A, et al. The European regulatory environment of RNA-based vaccines. Methods Mol Biol vol 1499. Humana Press, New York, NY. 2017, 1499:203-222.
6. Jackson NAC, Kester KE, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. NPJ Vaccines 2020, doi: 10.1038/s41541-020-0159-8.
7. Niak R, Peden K. Regulatory Considerations on the Development of mRNA Vaccines. Current Topics in Microbiology and Immunology 2020, doi:10.1007/82_2020_220.

8. Schmid A. Considerations for producing mRNA vaccines for clinical trials. Current Topics in Microbiology and Immunology vol 1499. Humana Press, New York, NY. 2017, doi:10.1007/82_2020_220. doi: 10.1007/978-1-4939-6481-9_15.

9. Hager G. (2017) Nonclinical Safety Testing of RNA Vaccines. In: Kramps T., Elbers K. (eds) RNA Vaccines. Methods in Molecular Biology, vol 1499. Humana Press, New York, NY. doi: 10.1007/978-1-4939-6481-9_16.

10. WHO ECBS Executive Summary from August 2020: https://www.who.int/publications/m/item/WHO-ECBS-aug-2020-executive_summary

11. Zhong Z, McCafferty S, Combes F, Huysmans H, de Temmerman J, Gitsels A, et al. mRNA therapeutics deliver a hopeful message. Nano Today (2018), https://doi.org/10.1016/j.nantod.2018.10.005

12. Arbuthnot P, Ely A, Bloom K. A convenient method to generate and maintain poly(A)-encoding DNA sequences required for in vitro transcription of mRNA. BioTechniques 2019, 66: 37-39. doi: 10.2144/btn-2018-0120.

13. Trepotec Z, Geiger J, Plank C, Aneja M, and Rudolph C. Segmented poly(A) tails significantly reduce recombination of plasmid DNA without affecting mRNA translation efficiency or half-life. RNA 2020, 25:507–518. http://www.rnajournal.org/cgi/doi/10.1261/rna.069286.118.

14. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S; Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther 2008, 16:1833-40.

15. Jackson LA, et al., An mRNA Vaccine against SARS-CoV-2 —Preliminary Report. NEJM 2020, pre-publication; DOI: 10.1056/NEJMoa2022483

16. Sahin, U., Muik, A., Derhovanessian, E. et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature 586, 594–599 (2020). https://doi.org/10.1038/s41586-020-2814-7.

17. Lundstrom K. Nanoparticle-based delivery of self-amplifying RNA. Gene Therapy, 2020, 27(5):183-185. DOI: 10.1038/s41434-020-0132-1.

18. Guidelines on nonclinical evaluation of vaccines. In: WHO Expert Committee on Biological Standardization. Fifty-fourth Report. Geneva, World Health Organization, 2005, Annex 1 (WHO Technical Report Series, No. 927; http://www.who.int/biologicals/publications/trs/areas/vaccines/nonclinical_evaluation/ANNEX%201Nonclinical.P31-63.pdf?ua=1, accessed 1 Dec. 2020).

19. Guidelines on the Nonclinical Evaluation of Vaccine Adjuvants and Adjuvanted Vaccines, In: WHO Expert Committee on Biological Standardization. Sixty-Fourth Report. Geneva, World Health Organization, 2014, Annex 2 (WHO Technical Report

Series 987; http://www.who.int/biologicals/areas/vaccines/TRS_987_Annex2.pdf?ua=1, accessed 1 Dec. 2020)

20. Guidelines on clinical evaluation of vaccines: regulatory expectations. In: WHO Expert Committee on Biological Standardization. Sixty-eighth report. Geneva, World Health Organization, 2016, Annex 9 (WHO Technical Report Series, No. 1004; http://www.who.int/entity/biologicals/expert_committee/WHO_TRS_1004_web_Annex_9.pdf?ua=1, accessed 1 Dec. 2020).

21. WHO good manufacturing practices for pharmaceutical products: main principles. Replacement of Annex 3 of WHO Technical Report Series, No. 961. In: WHO Expert Committee on Specifications for Pharmaceutical Preparations: forty-eighth report. Geneva: World Health Organization; 2014: Annex 2 (WHO Technical Report Series, No. 986; http://www.who.int/medicines/areas/quality_safety/quality_assurance/TRS986annex2.pdf?ua=1 accessed 1 Dec. 2020).

22. WHO good manufacturing practices for biological products. Replacement of Annex 1 of WHO Technical Report Series, No. 822. In: WHO Expert Committee on Biological Standardization: sixty-sixth report. Geneva: World Health Organization; 2016: Annex 2 (WHO Technical Report Series, No. 999; http://www.who.int/biologicals/areas/vaccines/Annex_2_WHO_Good_manufacturing_practices_for_biological_products.pdf?ua=1 accessed 1 Dec. 2020).

23. Guidelines for good clinical practice (GCP) for trials on pharmaceutical products. In: WHO Expert Committee on the Use of Essential Drugs: sixth report. Geneva: World Health Organization; 1995: Annex 3 (WHO Technical Report Series, No. 850; https://www.who.int/medicines/areas/quality_safety/safety_efficacy/gcp1.pdf, accessed 1 Dec. 2020).

24. Guidelines on Transmissible Spongiform Encephalopathies in relation to Biological and Pharmaceutical Products. World Health Organization. https://apps.who.int/iris/handle/10665/68932, accessed 1 Dec. 2020.

25. Guideline for Stability Evaluation of Vaccines, In: WHO Expert Committee on Biological Standardization. Fifty-seventh Report. Geneva, World Health Organization, 2006, Annex 3 (WHO Technical Report Series, No. 962; http://www.who.int/biologicals/vaccines/Annex_3_WHO_TRS_962-3.pdf?ua=1, accessed 30 April 2020).

26. Model guidance for the storage and transport of time-and temperature-sensitive pharmaceutical products. In: Organization WH, editor. WHO Expert Committee on Specifications for Pharmaceutical Preparations Forty-fifth Report. Geneva: World Health Organization; 2011: Annex 9 (WHO Technical Report Series, No. 961;

http://www.who.int/medicines/areas/quality_safety/quality_assurance/ModelGuidanceForStorageTransportTRS961Annex9.pdf, accessed 2 Dec 2020).

27. Guideline on the stability evaluation of vaccines for use under extended controlled temperature conditions. In: WHO World Health Organization, editor. WHO Expert Committee on Biological Standardization, Sixty-sixth Report. Geneva: World Health Organization; 2016: Annex 5 (WHO Technical Report Series, No. 999; http://www.who.int/biologicals/areas/vaccines/Annex_5_Guidelines_on_Stability_evaluation_vaccines_ECTC.pdf?ua=1, accessed 1 Dec 2020).

28. Guideline on procedures and data requirements for changes to approved vaccines, In: WHO Expert Committee on Biological Standardization. Sixty-fifth Report. Geneva, World Health Organization, 2015, Annex 4 (WHO Technical Report Series, No. 993; https://www.who.int/biologicals/vaccines/Annex4_Guidelines_changes_to_approved_vaccines_eng.pdf?ua=1).

29. WHO policy statement: multi-dose vial policy (MDVP). Handling of multi-dose vaccine vials after opening. Revision 2014. Geneva: World Health Organization; 2014 (WHO/IVB/14.07; http://apps.who.int/iris/bitstream/10665/135972/1/WHO_IVB_14.07_eng.pdf)

30. Stokes A, Pion J, Binazon O, Laffont B, Bigras M, Dubois G, et al. Nonclinical safety assessment of repeated administration and biodistribution of a novel rabies self-amplifying mRNA vaccine in rats. Regul Toxicol Pharmacol 2020 Jun;113:104648. doi: 10.1016/j.yrtph.2020.104648.

31. Lou G, Anderluzzi G, Schmidt ST, Woods S, Gallorini S, Brazzoli M, et al. Delivery of self-amplifying mRNA vaccines by cationic lipid nanoparticles: The impact of cationic lipid selection. J Control Release. 2020 Sep 10;325:370-379. doi: 10.1016/j.jconrel.2020.06.027.

32. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, et al. Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses, Mol Ther. 2017 Jun 7; 25(6): 1316–1327.

33. Liang F, Lindgren G, Lin A, Thompson EA, Ols S, Rohss J, et al. Efficient Targeting and Activation of Antigen-Presenting Cells In Vivo after Modified mRNA Vaccine Administration in Rhesus Macaques. Mol Ther 2017 Dec 6;25(12):2635-2647. doi: 10.1016/j.ymthe.2017.08.006.

34. Moderna presentation to US FDA Vaccines and Related Biological Products Advisory Committee on December 17, 2020. https://www.fda.gov/media/144583/download, accessed 18 Dec 2020.

35. Walsh EE, Frenck RW, Falsey AR, Kitchen N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020; 383:2439-2450. DOI: 10.1056/NEJMoa2027906.

36. Heidenreich R, Jasny E, Kowalczyk A, Lutz J, Probst J, Baumhof P, et al.; A novel RNA-based adjuvant combines strong immunostimulatory capacities with a favorable safety profile. In J Cancer 2015, 137:372-384.

37. Alberer M, Gnad-Vogt U, Hong HS, Mehr KT, Backert L, Finak G, et al.; Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet 2017, 390:1511-1520.

38. Richner JM, Jagger BW, Shan C, Fontes CR, Dowd KA, Cao B, et al.; Vaccine mediated protection against Zika virus-induced congenital disease. Cell 2017, 170:273-283.

39. FDA presentation to their Vaccines and Related Biological Products Advisory Committee on December 17, 2020 regarding Moderna COVID-19 candidate vaccine. https://www.fda.gov/media/144585/download, accessed 18 Dec 2020.

40. UK Medicines and Health products Authority Public Assessment Report of COVID-19 mRNA vaccine BNT162b2. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/944544/COVID-19_mRNA_Vaccine_BNT162b2__UKPAR___PFIZER_BIONTECH__15Dec2020.pdf. Accessed 21 Dec 2020.

41. Goodman S. Considerations for placebo-controlled trial design if an unlicensed vaccine becomes available. Presentation to US FDA Vaccines and Related Biological Products Advisory Committee on December 10, 2020. https://www.fda.gov/media/144354/download, accessed 18 Dec 2020.

42. Goodman S. Considerations for placebo-controlled trial design if an unlicensed vaccine becomes available. Presentation to US FDA Vaccines and Related Biological Products Advisory Committee on December 17, 2020. https://www.fda.gov/media/144582/download, accessed 18 Dec 2020.

43. Expert consultation on the use of placebos in vaccine trials. Meeting report. Geneva: World Health Organization; 2013. http://apps.who.int/iris/bitstream/10665/94056/1/9789241506250_eng.pdf, accessed 18 Dec 2020.

44. WHO biological standardization website: https://www.who.int/biologicals/vaccines/en/, accessed 21 Dec 2020.