nature research

Peer Review File

Manuscript Title: SARS-CoV-2 mRNA Vaccine Design Enabled by Prototype Pathogen Preparedness

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

In their manuscript Corbett and colleagues describe the evaluation of an mRNA vaccine candidate for SARS-CoV-2 in the mouse model. It is the same vaccine candidate that currently is also assessed in clinical trials by Moderna. The manuscript is, of course, interesting. But compared to data for other vaccine candidates recently published it provides little data and some experiments lack rigor.

Major points

1) A large majority of experiments performed lack negative controls. Vaccination with PBS is never a proper negative control in in vivo vaccine experiments. An mRNA formulation coding for an irrelevant protein needs to be included. mRNA packaged in LNPs can induce many off-target effects e.g. interferon stimulation (as evidenced by grade 3 local side effects in many Moderna vaccine trials). These could of course have some effect on viral challenge. Proper controls need to be added to experiments shown in Figure 1, Figure 3, Figure 4, Extended Data Figure 4, Extended Figure 8 etc.

2) In addition, for all serological readouts, negative control sera need to be added. Those are missing throughout the paper as well.

3) No information is given on the mouse-adapted challenge virus. What is the LD50? What is the sequence? Which mutations are present? How was it generated? A detailed description needs to be included.

4) The authors investigate if vaccination with stabilized S protein is inducing a balanced Th1/Th2 response. To do that mice are vaccinated with the stabilized S protein plus a TLR4 agonist as control. The second control is S protein adjuvanted with alum. Without explanation the authors use a commercially available S protein of questionable quality for the S+alum group instead of their own stabilized protein. This is an apples and oranges comparison. Please either remove the alum group or replace it by stabilized protein plus alum.

5) The 'PP' version of the SARS-CoV-2 spike does not express better than the wild type versions and structural biologists complain that only a fraction of it is actually in the pre-fusion conformation. For this reason, the McLellan lab has recently developed a better version of the protein (hexapro). The authors need to discuss this since it seems that a suboptimal vaccine construct is moving forward.

6) The authors use pseudotyped virus particle entry assays throughout the manuscript. Please change neutralizing activity to entry inhibition activity in figures and the manuscript. The concordance testing in the supplementary figures would be nice but has no significance with an n of 3. Please add more samples or remove it.

7) The authors mention in line 131 that whole inactivated SARS-CoV-1 vaccines led to enhanced disease. This is also true for vectored vaccines and RNA replicon vaccines (which are similar to mRNA vaccines in some aspects). Please broaden this statement. Maybe it should also be mentioned that BPL whole inactivated vaccines protected nicely from challenge.

8) It is unclear if the mouse model is actually the right model to study VAERD. An NHP model would likely have more significance. This should be discussed.

9) In many experiments only the highest dose used really leads to significant reductions in virus titers in lungs and the nasal turbinates. The highest does tested by Moderna was discontinued. It would be helpful if the authors could discuss this.

10) Several manuscripts describing vaccine candidates in detail (NHP data, cross-neutralization of divergent strains etc.) have been published. The authors should discuss this and bring their data into perspective of other published candidate vaccines.

Minor points

1) Many abbreviations are not defined in the manuscript (including in the abstract).

2) Line 50: Please update the numbers.

3) Line 58: CFR? IFR? Please clarify what is meant.

4) Line 86 and following lines: 'MERS-CoV', not 'MERS'

5) Line 340: What is meant by 'HI'?

Referee #2 (Remarks to the Author):

In this report the investigators describe in vitro and pre-clinical data that was produced in the remarkably rapid development over only 2 months of the first mRNA-based vaccine to SARS-CoV-2 to enter clinical trials; the "Moderna vaccine." Clearly this work is of the utmost importance and urgency. Immunization of BALB/cJ mice demonstrated that the mRNA vaccine was highly immunogenic and induced high titers of SARS2 virus neutralizing antibodies. Further, there is concern that a sub-protective dosage or "take" of respiratory-based vaccines can drive a TH2-biased immune response leaving the recipients susceptible to VAERD. They report that the mRNA vaccine induces Ig isotype and T cell cytokine profiles balanced for TH1 and TH2 in comparison to immunization with SARS2 protein spike adjuvanted with alum. This at least provides evidence that the vaccine is not biased towards a strong TH2 response. Although not that convincing because of clear species differences in ACE2 receptor, some in vivo protection was noted against a mouse-adapted SARS-CoV-2 virus. The values of this in vivo assessment is questionable and ideally should be repeated using a more relevant in vivo model such as hamsters or at least mice expressing the human ACE2 receptor in relevant tissues. In the very least this section should be revised to acknowledge the major short comings of this in vivo system prior to publication.
The rapid development of this vaccine sets a new precedent for "emergency" vaccine development in the face of a pandemic outbreak using the mRNA vaccine strategy.

Referee #3 (Remarks to the Author):

The authors describe the incredible achievement that led to start phase I clinical trials with an RNA vaccine against SARS-Cov-2 66 days after the genomic sequence of the virus was available. This finding is a milestone in vaccine development which merits publication in a top journal.
The paper first reports the design and immunogenicity of an RNA vaccine against MERS, showing that the previously described substitution of prolines were necessary for the RNA to be immunogenic and that the construct containing the transmembrane domain induced better neutralizing antibodies. When the SARS-Cov-2 sequence became available on January 10th 2020, the authors were able to use the data derived from the structure-based design of the MERS antigen to design by analogy the RNA vaccine for Covid-19. The properties of the SARS-Cov-2 RNA vaccine described in the paper are as expected. They induce dose-dependent neutralizing antibodies, a balanced Th1/Th2 response and protect mice from infection with a mouse adapted SARS-Cov-2 virus. The quality of the data is good and the manuscript is well written.
In a normal condition, this would not be a manuscript for Nature because there is little scientific novelty in the preclinical studies reported in the paper. The stabilization of spike from MERS is not new, the stabilization of a related corona virus spike using the same structure-based design is not surprising, the POC that the structurally designed antigen can be deliver through a mRNA and is immunogenic in mice (neut, CD4 and CD8) is also not surprising. However, the speed by which all the available science has been used to develop a vaccine against an emerging pathogen is remarkable and unprecedented and this makes this paper a high priority for Nature.

Author Rebuttals to Initial Comments:

Referee #1 (Remarks to the Author):

In their manuscript, Corbett and colleagues, describe the evaluation of an mRNA vaccine candidate for SARS-CoV-2 in the mouse model. It is the same vaccine candidate that currently is also assessed in clinical trials by Moderna. The manuscript is, of course, interesting. But compared to data for other vaccine candidates recently published it provides little data and some experiments lack rigor.

> **RESPONSE:** Based on the currently available literature, the mRNA-1273 vaccine is the most immunogenic yet published. Also, the evaluation of T cell responses, pathology, cytokine production post-challenge, and the investigation of sub-protective doses to assess possible induction of VAERD, is more extensive than for other published vaccines. Importantly, as noted by the reviewer, mRNA-1273 is anticipated to be in Phase III efficacy evaluation within the next few weeks.

Major points

1) A large majority of experiments performed lack negative controls. Vaccination with PBS is never a proper negative control in in vivo vaccine experiments. An mRNA formulation coding for an irrelevant protein needs to be included. mRNA packaged in LNPs can induce many off-target effects e.g. interferon stimulation (as evidenced by grade 3 local side effects in many Moderna vaccine trials). These could of course have some effect on viral challenge. Proper controls need to be added to experiments shown in Figure 1, Figure 3, Figure 4, Extended Data Figure 4, Extended Figure 8 etc.

> **RESPONSE**: We thank the reviewer for the observations on experimental controls as this is a critical point that we have now clarified. We have used empty LNPs or mRNA/LNP controls expressing irrelevant proteins in previously published *in vivo* studies for mRNA vaccines targeting other disease pathogens, including influenza, CMV, and RSV (John et al, Vaccine 2018; Bahl et al, Molecular Therapy 2017; Espeseth et al, Vaccines 2020). In each instance, the LNP formulated mRNA encoding for an irrelevant protein induced only transient innate signaling and

inflammation that quickly resolved (Liang et al, Molecular Therapy 2017). These innate responses did not translate into development of immunity against the pathogen under investigation, with no binding or neutralizing antibody measured (John et al, Vaccine 2018; Bahl et al, Molecular Therapy 2017; Espeseth et al, Vaccines 2020) and did not protect from viral challenge (Bahl et al, Molecular Therapy 2017; Espeseth et al, Vaccines 2020). Therefore, the transient and short-lived signaling driven by non-relevant mRNA/LNP and the lack of adaptive immunity would not impact viral challenge and would not be different from a PBS control (note that in the current study viral challenge occurred >1 month post-boost). Based on these foundational studies use of a non-relevant mRNA/LNP control was not considered to add additional benefit beyond the PBS-control mice.

1) CMV (John et al. Vaccines 2018) - (LNP2 in paper) does not induce Ab responses against CMV in NHP (Fig. 4 A/C)

2) Influenza (Bahl et al, Molecular Therapy 2017): - mRNA encoding for RSV F with reduced 5'cap/LNP does not protect mice from H7 challenge, either weight loss or survival (Fig 2).

3) RSV (Espeseth et al, Vaccines 2020) -

    a. Empty LNP does not induce RSV neutralizing activity in cotton rats (supplementary Fig. 5a)

    b. Empty LNP and luciferase-mRNA/LNP do not induce enhanced lung pathology after RSV challenge (Fig. 7a)

Relevant publications are provided for your review.

John S, Yuzhakov O, Woods A, et al. Multi-antigenic human cytomegalovirus mRNA vaccines that elicit potent humoral and cell-mediated immunity. *Vaccine*. 2018;36(12):1689-1699. doi:10.1016/j.vaccine.2018.01.029

Bahl K, Senn JJ, Yuzhakov O, et al. Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. *Mol Ther*. 2017;25(6):1316-1327. doi:10.1016/j.ymthe.2017.03.035

Espeseth AS, Cejas PJ, Citron MP, et al. Modified mRNA/lipid nanoparticle-based vaccines expressing respiratory syncytial virus F protein variants are immunogenic and protective in rodent models of RSV infection. *NPJ Vaccines*. 2020;5:16. Published 2020 Feb 14. doi:10.1038/s41541-020-0163-z

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

2) In addition, for all serological readouts, negative control sera need to be added. Those are missing throughout the paper as well.

RESPONSE: Due the pronounced dose-effect in mRNA-1273-immunized mice, mice immunized with 0.01 µg had little to no antibody responses, as shown in **Fig. 2**, **Extended Data Fig. 5**, and **Extended Data Fig. 6**. Thus, we initially opted to not include PBS-control sera on the graphs. To clarify that these assays were completed and further to highlight limited background antibody detection in negative control sera, sera from PBS-control BALB/c mice have been added to **Fig. 2** to show that

sera from mice administered PBS have no detectable S-specific IgG (A) or neutralizing activity (D). Additionally, in **Extended Data Fig. 6**, we have now included the sera from 0.01 µg mRNA-1273-immunized mice that did not have detectable S-specific IgG (A). IgG subclass ELISAs were completed using pooled negative control sera, as total IgG proved to be undetectable in sera from PBS-control mice and due to limited sera from PBS-control mice for all assays needed to fully assess humoral antibody responses as we have in this manuscript. We have summarized those data below:

Corresponding Figure	Experiment Details	PBS-control BALB/c Mouse #	Reciprocal Serum Endpoint Titer (Log_{10})				
			IgG	IgG1	IgG2a	IgG2c	IgG2a/c
Fig. 3	B6C3 - mRNA-1273 and S-2P protein + SAS - IgG1 and IgG2a/c	Pooled (9060-9070)	N/T[1]	2.0	N/T	N/T	2.0
		Pooled (9371-9380)	N/T	2.0	N/T	N/T	2.0
Extended Data Fig. 6	BALB/c - mRNA-1273 - IgG21 and IgG2	Pooled (9061-9070)	N/T	2.0	2.0	N/T	N/T
Extended Data Fig. 7	C57BL/6 - mRNA-1273 and S-2P protein + SAS - IgG1 and IgG2c	Pooled (9060-9070)	N/T	2.0	N/T	2.0	N/T
		Pooled (9371-9380)	N/T	2.5	N/T	2.0	N/T
Extended Data Fig. 7	BALB/c - mRNA-1273 and S-2P protein + SAS- IgG21 and IgG2a	Pooled (9060-9070)	N/T	2.0	2.0	N/T	N/T
		Pooled (9371-9380)	N/T	2.0	2.0	N/T	N/T
Extended Data Fig. 8	BALB/c - mRNA-1273 and S-2P protein + Alum - IgG, IgG1, and IgG2a	Pooled (9381-9382)	2.1	2.0	2.0	N/T	N/T
		Pooled (9383-9384)	2.6	2.0	2.0	N/T	N/T
		Pooled (9385-9386)	2.5	2.0	2.0	N/T	N/T

[1]N/T = Not Tested

3) No information is given on the mouse-adapted challenge virus. What is the LD50? What is the sequence? Which mutations are present? How was it generated? A detailed description needs to be included.

RESPONSE: We did not include an extensive description of the virus used for challenge, because it has been thoroughly described in another paper under review at *Nature* and is described in detail in a publicly available pre-print, which was aptly cited in the manuscript (Dinnon III KH et al. BioRxiv. 2020 May 7:2020.05.06.081497. doi: 10.1101/2020.05.06.081497). However, as requested, we have added the following sentences to line 172 summarizing the rationale and virus characteristics to make it easier for the reader to interpret the data in this paper. “SARS-CoV-2 MA contains RBD substitutions Q498Y/P499T, generated via site-directed mutagenesis in an infectious clone (Hu, et. al. Cell. 2020.) The substitutions effectively allow binding of the virus to the mouse ACE2 receptor and infection and replication in the upper and lower respiratory tract (Dinnon, et. al. BioRxiv. 2020).” Also, please see response to reviewer #2 for additional comments.

4) The authors investigate if vaccination with stabilized S protein is inducing a balanced Th1/Th2 response. To do that mice are vaccinated with the stabilized S protein plus a TLR4 agonist as control. The second control is S protein adjuvanted with alum. Without explanation the authors use a commercially available S protein of questionable quality for

the S+alum group instead of their own stabilized protein. This is an apples and oranges comparison. Please either remove the alum group or replace it by stabilized protein plus alum.

RESPONSE: Prefusion-stabilized S-2P protein was used in the experiment evaluating S-2P + alum. The method description "SARS-CoV-2 S protein (Sino Biological) + 250 µg alum hydrogel was delivered IM." was an error and has been changed to "For S-2P + alum immunizations, SARS-CoV-2 S-2P protein + 250 µg alum hydrogel was delivered intramuscularly (IM)" in line 345. **Extended Data Fig. 8** has also been updated from S to S-2P. We apologize for the mistake.

The point was not to control for specificity or conformational stability, but to have a positive control that induced a Th2-biased response, for which alum adjuvant is sufficient. Comparing a gene-based delivery with a protein-based vaccine is inherently "apples and oranges", but in this experiment, done in the same mice and analyzed with matching reagents, S-2P in alum induced a distinct IgG subclass ratio and cytokine pattern. The key finding shown by these data is that there is no evidence of a Th2-biased immune response that would pose a theoretical risk for vaccine-associated enhanced-respiratory disease (VAERD).

5) The 'PP' version of the SARS-CoV-2 spike does not express better than the wild type versions and structural biologists complain that only a fraction of it is actually in the pre-fusion conformation. For this reason, the McLellan lab has recently developed a better version of the protein (hexapro). The authors need to discuss this since it seems that a suboptimal vaccine construct is moving forward.

RESPONSE: The rapid development of mRNA-1273 was enabled by data generated with MERS-CoV, SARS-CoV, and hCoV-HKU1 showing that the 2P mutation largely stabilized S proteins in their prefusion conformation. Importantly, this prior work allowed for immediate SARS-CoV-2 antigen design and selection without additional experimentation. The 2P mutation has now been used by many labs to solve structures of more than a dozen coronavirus spike proteins that had previously resisted structural definition, in part due to instability. Included on that list are structures for SARS-CoV-2 S (Wrapp, et. al., Science, 2020. & Walls, et. al., Cell. 2020). In fact, the S-2P mutated protein was stable and uniform enough for cryo-EM determination of structure within about 20 days of acquiring the sequence, leading to publication 40 days after sequence release **(Extended Data Fig. 2)**. Our data show that protein expression from the 2P mRNA construct is superior to expression from the WT construct **(Extended Data Fig. 3)**. Notably, the S-2P sequence has been selected for the antigen in several leading SARS-CoV-2 vaccine candidates across multiple platforms. As noted in the response to the general comment, this mRNA-1273 vaccine is more immunogenic than other published candidates, so we have no evidence that it is suboptimal, rather that it is highly immunogenic, inducing potent neutralizing activity in mice (shown here), NHP, and humans. It has also been shown that as a reagent in ELISAs, the 2P version of the protein is more highly sensitive and specific for detecting S-specific antibodies in human sera, as referenced in line 111. Expression as a transmembrane-anchored protein provides additional stability to the native trimer structure. We collaborate closely with the McLellan lab and have been aware of the "hexapro" version of the SARS-CoV-2 S. It has 4 additional prolines capping various helices from the heptad repeat 1 region and is indeed more stable when stressed with extreme heat. So far, there is no evidence that it is more

immunogenic or that it presents different antigenic sites than the S-2P although those studies are continuing.

6) The authors use pseudotyped virus particle entry assays throughout the manuscript. Please change neutralizing activity to entry inhibition activity in figures and the manuscript. The concordance testing in the supplementary figures would be nice but has no significance with an n of 3. Please add more samples or remove it.

RESPONSE: The purpose of **Extended Data Table 1** is to show that the "Homotypic neutralizing activity induced by mRNA-1273 was also similar to that achieved by immunizing with 1 μg of SAS-adjuvanted S-2P protein", as written in the text on line 131, not to show concordance with the PRNT. Full concordance of this pseudovirus neutralization assay and the PRNT are being published with Phase I clinical trial results *(in press at NEJM)*. With that, we have changed the name of the table to "Extended Data Table 1. Pseudovirus neutralization and PRNT analysis of sera pooled from mRNA-1273- and S-2P protein-immunized mice". To address the concern of limited samples being tested by the PRNT, we have now amended **Extended Data Table 1** to include PRNT analysis of pooled sera from 9 mRNA-1273-immunized mice (N = 3 mice/pool). Notably, the pseudovirus neutralization assay used here has been adopted by several laboratories globally, vetted by the FDA for use for clinical trial analysis, and will be used for measuring neutralizing activity in phase II and III trials to come. We acknowledge that the pseudovirus NT assay, as the reviewer points out is a single-round infection assay that does not take into account viral replication or other post-entry antibody blocking mechanisms. However, the term "neutralization" is routinely used when reporting pseudovirus entry inhibition, because fundamentally blocking the initial steps of infection (viral attachment and entry) effectively neutralizes the virus.

7) The authors mention in line 131 that whole inactivated SARS-CoV-1 vaccines led to enhanced disease. This is also true for vectored vaccines and RNA replicon vaccines (which are similar to mRNA vaccines in some aspects). Please broaden this statement. Maybe it should also be mentioned that BPL whole inactivated vaccines protected nicely from challenge.

RESPONSE: Statement in line 131 (now 147) was provided as an example to raise the issue of vaccine-enhanced disease suggested by studies of SARS-CoV-1 vaccines. Additional references and examples have been added. We have rephrased the sentence to avoid implicating a particular type of vaccine as a risk for VAERD.

8) It is unclear if the mouse model is actually the right model to study VAERD. An NHP model would likely have more significance. This should be discussed.

RESPONSE: The mouse model has been previously used to demonstrate respiratory disease enhancement (ERD) for a double inactivated SARS-CoV vaccine (Bolles et al, Journal of Virology, 2011). Since there is no established model for ERD for SARS-CoV-2, we believe that mice are a good animal model to assess VAERD risks for SARS-CoV-2 vaccines at the present time. We are actively evaluating the mRNA-1273 in NHPs and in hamsters, but we think the introduction of data from other models is beyond the scope of this manuscript. We also feel that a long discussion of other animal model options for ERD investigations is premature, and that subject will need to be covered elsewhere when more data are available. We will continue to generate data on this important topic and monitor for ERD symptoms in future clinical

studies. The reference for evaluating VAERD in mice is provided here for convenience.

Bolles M, Deming D, Long K, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. J Virol. 2011;85(23):12201-12215. doi:10.1128/JVI.06048-11

9) In many experiments only the highest dose used really leads to significant reductions in virus titers in lungs and the nasal turbinates. The highest does tested by Moderna was discontinued. It would be helpful if the authors could discuss this.

RESPONSE: In this case, because of our prior experience with mRNA-delivered glycoproteins, we deliberately selected a relatively low dose as the top dose evaluated. It would have been plausible to start at 10 or 20 µg in mice rather than 1µg, however, we specifically wanted to assess the dose-down effects of mRNA-1273 and potential for VAERD at sub-protective doses. **Extended Data Fig. 5** shows neutralizing activity can be increased about 10-fold higher by going up to 20 µg doses although the response plateau is reached in mice at a dose of 5 µg above which very little incremental benefit is seen in terms of binding or neutralizing Ab titers. There were no adverse events noted in the mice even after the second dose of 20 µg. This is not easily relatable to the 250 µg doses used in humans. Clinical doses for mRNA-1273 in phase 1 were selected based on previous experience with the Moderna mRNA/LNP technology and not based on the doses used in mice. The safety data from the Phase 1 trial has been accepted for publication and will attest to the safety of mRNA-1273.

10) Several manuscripts describing vaccine candidates in detail (NHP data, cross-neutralization of divergent strains etc.) have been published. The authors should discuss this and bring their data into perspective of other published candidate vaccines.

RESPONSE: We know from prior work that spike-induced antibody has very little cross-reactivity to other coronaviruses and limited cross-reactivity even within clades of betacoronaviruses. However, there is no evidence that SARS-CoV-2 has evolved or drifted far enough to evade polyclonal sera induced by any other SARS-CoV-2 variant. To emphasize this point, we have now added data using a pseudovirus constructed to express the D614G variant (Korber, et, al., Cell, 2020) against which vaccine-induced immune sera shows no difference in neutralizing potency **(Extended Data Fig. 4)**.

Minor points

1) Many abbreviations are not defined in the manuscript (including in the abstract).

RESPONSE: We thank the reviewer for this observation and believe we have now removed or defined all abbreviations.

2) Line 50: Please update the numbers.

RESPONSE: The numbers have been updated as requested.

3) Line 58: CFR? IFR? Please clarify what is meant.

RESPONSE: Instead of using the phrase “1% mortality rate” we have changed the phrase to “case fatality rate of 1%” to clarify the meaning.

4) Line 86 and following lines: ‘MERS-CoV’, not ‘MERS’

RESPONSE: This has been corrected as requested.

5) Line 340: What is meant by ‘HI’?

RESPONSE: HI is an abbreviation for heat-inactivation that has now been clarified.

Referee #2 (Remarks to the Author):

In this report the investigators describe in vitro and pre-clinical data that was produced in the remarkably rapid development over only 2 months of the first mRNA-based vaccine to SARS-CoV-2 to enter clinical trials; the “Moderna vaccine.” Clearly this work is of the utmost importance and urgency. Immunization of BALB/cJ mice demonstrated that the mRNA vaccine was highly immunogenic and induced high titers of SARS2 virus neutralizing antibodies. Further, there is concern that a sub-protective dosage or “take” of respiratory-based vaccines can drive a TH2-biased immune response leaving the recipients susceptible to VAERD. They report that the mRNA vaccine induces Ig isotype and T cell cytokine profiles balanced for TH1 and TH2 in comparison to immunization with SARS2 protein spike adjuvanted with alum. This at least provides evidence that the vaccine is not biased towards a strong TH2 response. Although not that convincing because of clear species differences in ACE2 receptor, some in vivo protection was noted against a mouse-adapted SARS-CoV-2 virus. The values of this in vivo assessment is questionable and ideally should be repeated using a more relevant in vivo model such as hamsters or at least mice expressing the human ACE2 receptor in relevant tissues. In the very least this section should be revised to acknowledge the major short comings of this in vivo system prior to publication.

The rapid development of this vaccine sets a new precedent for “emergency” vaccine development in the face of a pandemic outbreak using the mRNA vaccine strategy.

RESPONSE: We thank the reviewer for acknowledging the rapid development timeline. The primary concern appears to be the virus used for challenge experiments. As noted in the response to Reviewer #1, comment #3, the virus used in this paper has been extensively characterized in a paper under review at *Nature*. We believe the relatively minor changes introduced into the RBD if anything put the vaccine candidate being evaluated at more of a disadvantage because of the mismatch. This virus can infect wild-type BALB/c mice. We believe it is a more physiologic challenge system than the human ACE2 transgenic mice that develop some illness when infected with the original SARS-CoV-2 strain, but not more than from the modified virus used unless it is serially passaged in mice to accumulate additional adaptive mutations. The pathogenesis of infection in hACE2 transgenic mice is also unlike infection restricted to the respiratory tract in humans, and mortality is usually associated with encephalitis more than lung disease. For those reasons, we elected to use the modified SARS-CoV-2 for mouse challenge. This also allows the use of mice from multiple backgrounds including KO and transgenic lines that may be helpful in evaluating mechanisms of immunity in future experiments. Studies in NHP and aged mice will further support the findings reported in the current manuscript.

Referee #3 (Remarks to the Author):

The authors describe the incredible achievement that led to start phase I clinical trials with an RNA vaccine against SARS-Cov-2 66 days after the genomic sequence of the virus was available. This finding is a milestone in vaccine development which merits publication in a top journal.

The paper first reports the design and immunogenicity of an RNA vaccine against MERS, showing that the previously described substitution of prolines were necessary for the RNA to be immunogenic and that the construct containing the transmembrane domain induced better neutralizing antibodies. When the SARS-Cov-2 sequence became available on January 10th 2020, the authors were able to use the data derived from the structure-based design of the MERS antigen to design by analogy the RNA vaccine for Covid-19. The properties of the SARS-Cov-2 RNA vaccine described in the paper are as expected. They induce dose-dependent neutralizing antibodies, a balanced Th1/Th2 response and protect mice from infection with a mouse adapted SARS-Cov-2 virus. The quality of the data is good and the manuscript is well written.

In a normal condition, this would not be a manuscript for Nature because there is little scientific novelty in the preclinical studies reported in the paper. The stabilization of spike from MERS is not new, the stabilization of a related corona virus spike using the same structure-based design is not surprising, the POC that the structurally designed antigen can be deliver through a mRNA and is immunogenic in mice (neut, CD4 and CD8) is also not surprising. However, the speed by which all the available science has been used to develop a vaccine against an emerging pathogen is remarkable and unprecedented and this makes this paper a high priority for Nature.

> **RESPONSE:** We appreciate the reviewer's positive comments and acknowledgement of the importance of this work that is supporting the rapid advancement of the mRNA-1273 vaccine into Phase III efficacy trials. This represents a new paradigm in vaccine development that can be applied to future threats. We also acknowledge that these are not normal times and in time additional studies will be done with serially passaged more virulent virus to provide additional insight into vaccine potency and viral pathogenesis. Apprised of mRNA-1273 data in nonhuman primates and clinical trials, we are confident that the data and preliminary findings described in this paper will hold up and be reinforced and would appreciate this consideration in the final editorial decision.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

The authors did a good job addressing the reviewers' comments but several concerns remain.

1) Original major point 1: I maintain that the correct negative controls are missing. This is not only important for this study but also for all next generation scientists who will read the paper. Setting bad examples of poor scientific rigor is problematic in my opinion.

2) Original major point 5: The authors response should be added to the discussion.

3) Original major point 6: Please refrain from using the term neutralization if an pseudotyped particle entry inhibition assay was used.

4) Original major point 10 was not addressed correctly.

Referee #2 (Remarks to the Author):

All concerns have been adequately addressed. Best of luck in phase 3.

Author Rebuttals to First Revision:

Referee #1:

The authors did a good job addressing the reviewers' comments but several concerns remain.

1) Original major point 1: I maintain that the correct negative controls are missing. This is not only important for this study but also for all next generation scientists who will read the paper. Setting bad examples of poor scientific rigor is problematic in my opinion.

RESPONSE: We appreciate the reviewer's perspective on this, but we stand by our initial response. We will not be able to go back and add irrelevant mRNA controls to these experiments.

2) Original major point 5: The authors response should be added to the discussion.

RESPONSE: The reviewer is asking that we defend the choice of the S-2P antigen design and add this to the discussion. Since part of the paper already describes the discovery of the coronavirus spike structure and stabilizing mutations, and how this provided the basis for advancing the current product into vaccine development, we think this discussion would be redundant. In addition, the fact that this product has outstanding immunogenicity and is protective in animal models, and that phase 1 clinical trial data also demonstrate outstanding immunogenicity with this product, justifying the choice of antigen is not necessary in our opinion. In future studies, we will compare the S-2P antigen design to next generation products, but those data will not be available for several more weeks.

3) Original major point 6: Please refrain from using the term neutralization if an pseudotyped particle entry inhibition assay was used.

RESPONSE: Having made this same point myself in prior discussions, I appreciate the reviewer perspective on terminology. We are indeed measuring the neutralization of viral entry, and this does not include some of the elements of the classical PRNT assay like multiple rounds of replication, cell-to-cell spread, and plaque formation. We have been explicit about using the term "pseudovirus" when referring to neutralization in the paper and have now added three sentences to the methods that state, "The pseudovirus neutralization assay measures the inhibition of pseudovirus attachment and entry including fusion-inhibiting activity. It is a single-round virus and does not replicate and does not express the spike protein in transduced cells. Therefore, pseudovirus infection will not cause cell-to-cell fusion or plaque formation that can be measured in a classical neutralization assay using live virus. This pseudovirus neutralization assay has been shown to correlate with live virus plaque reduction neutralization (Jackson, et. al. NEJM. 2020. DOI: 10.1056/NEJMoa2022483), and because it does not require BL3 containment, was chosen as the preferred assay for measuring neutralizing activity in these studies."

4) Original major point 10 was not addressed correctly.

RESPONSE: Perhaps the reviewer is asking for a more discussion of how these data compare to other data published from NHP models or phase 1 clinical trials. Comparing our results to other types of products evaluated by different assays would require a much longer discussion and would be speculative. There will eventually (within months) be an international serum standard that will help normalize values across assay formats that will be helpful in future publications.