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List of Abbreviations

Abbreviation	Definition
ACE-2	angiotensin-converting enzyme 2
Alum	aluminum hydroxide
ASC	antibody-secreting cell
BAL	bronchoalveolar lavage
cDC	conventional dendritic cell
CDS	conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein
cISH	chromogenic in situ hybridization
CoV	coronavirus
dLN	draining lymph nodes
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ELISA	enzyme-linked immunosorbent assay
EMBP	eosinophil major basic protein
ERD	enhanced respiratory disease
FACS	fluorescence-activated cell sorting
GLP	Good Laboratory Practice
H&E	hematoxylin and eosin
IC_{50}	half-maximal inhibitory concentration
ICS	intracellular cytokine staining
ID_{50}	50% inhibitory dilution
IFN	interferon
Ig	immunoglobulin
IHC	immunohistochemistry
IM	intramuscular, (-ly)
mAb	monoclonal antibody
MFI	mean fluorescence intensity
mRNA	messenger RNA
NHP	nonhuman primates
NS	nasal swabs
NT	nasal turbinates
NTFIX	noncoding mRNA formulated into the same lipid nanoparticle dispersion as mRNA-1273
NTD	N-terminal domain

Abbreviation	Definition
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
pDC	plasmacytoid dendritic cell
PEG2000-DMG	1,2-dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PFU	plaque-forming units
PRNT	plaque reduction neutralization tests
RBD	receptor binding domain
qRT-PCR	quantitative reverse transcription PCR
S	spike
S1_NTD	N-terminal domain of S1
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
SARS	severe acute respiratory syndrome
SARS-CoV	severe acute respiratory syndrome coronavirus
SARS-CoV-1	severe acute respiratory syndrome coronavirus-1
SARS-CoV-1 DIV	double-inactivated severe acute respiratory syndrome coronavirus-1
SARS-CoV-2	2019 novel coronavirus
(b) (4)	
SD	standard deviation
sgRNA	subgenomic RNA
SM-102	heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate)
Tfh	T follicular helper
Th	T helper
WT	wild type

2.6.2.1 BRIEF SUMMARY

ModernaTX, Inc. (Sponsor) has used its messenger RNA (mRNA)-based rapid-response proprietary vaccine platform to develop mRNA-1273, a novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine against the 2019 novel coronavirus (CoV; SARS-CoV-2). mRNA-1273 contains a single mRNA that encodes the full-length SARS-CoV-2 spike (S) glycoprotein modified with 2 proline substitutions within the heptad repeat 1 domain (S-2P) to stabilize the S protein into the prefusion conformation. The mRNA-1273 is combined in a mixture of 4 lipids common to the Sponsor's mRNA vaccine platform: SM-102, cholesterol, DSPC, and PEG2000-DMG.

As SARS-CoV-2 is a newly emerged CoV, there were no established animal models for the evaluation of prophylactic vaccines and therapeutics. Therefore, nonclinical studies were initiated in multiple animal species in order to gain a comprehensive understanding of the effects of mRNA-1273 immunization. As a proof of concept, the expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro in HEK293T cells and in vivo in BALB/c mice (Report MOD-4112.1273).

Wild-type (WT) mice are convenient and easy-to-use model to assess vaccine immunogenicity; however, the angiotensin-converting enzyme 2 (ACE-2) receptor, the primary route for SARS-CoV-2 binding and entry, differs significantly between mice and humans and, as a result, WT SARS-CoV-2 does not infect mice. Therefore, a mouse-adapted SARS-CoV-2 strain, which was developed by the laboratory of Dr Ralph Baric at the University of North Carolina at Chapel Hill, was used to assess protection of immunized mice from SARS-CoV-2 challenge. The mouse-adapted SARS-CoV-2 contains 2 targeted amino acid mutations (Q498T/P499Y) in the receptor binding domain (RBD) a region of the S protein that binds to the mouse ACE-2 receptor (Dinnon et al 2020). Although this mouse-adapted SARS-CoV-2 strain infects young mice and induces mild disease symptoms, more severe disease symptoms are evident in aged mice (> 12 months) (Dinnon et al 2020). Aged mice were therefore included in the nonclinical pharmacology program to further characterize the immune response and the level of protection from mouse-adapted SARS-CoV-2 challenge. In addition, this model was used to characterize the quality of the immune response to determine if the mRNA-1273-induced immunity would be predicted to promote vaccine-associated enhanced respiratory disease (ERD), observed previously with vaccines against respiratory syncytial virus (Kim et al 1969), measles (Polack 2007), and in animal models of SARS-CoV vaccination [Czub et al 2005; Deming et al 2007; Bolles et al 2011; Corbett et al 2020]). The immunogenicity and protection study in aged mice was designed to directly address this concern through the evaluation of dose levels predicted to drive optimal or suboptimal protection from viral challenge.

Golden Syrian hamsters were also selected as a relevant model for evaluation. Wild-type SARS-CoV-2 productively infects hamsters, causing weight loss and moderate to severe lung pathology. This model was selected because the hamster ACE-2 receptor is only 3 amino acids different versus the human ACE-2 receptor allowing for productive infection with wild-type SARS-CoV-2, and it is currently the only animal species in which severe respiratory disease is evident after virus challenge (Chan et al 2020).

Nonhuman primates (NHPs) are the species most closely related to humans and have previously recapitulated several important aspects of SARS-CoV infection, including pneumonia, lung inflammation, and upper and lower airway infection and viral replication (Lu et al 2020). Although SARS-CoV-2 infection in NHPs result only in mild clinical symptoms, infection does causes illness with evidence of pneumonia (Johansen et al 2020).

A summary of the pharmacology studies conducted with mRNA-1273 is presented in Table 1. Nonclinical pharmacology evaluations were conducted in vitro in HEK293T cells and in vivo in young and aged WT mice (BALB/c, C57BL/6J, and B6C3F1/J strains), golden Syrian hamsters, and rhesus macaques (NHPs) animal models were conducted to characterize the expression and the immunogenicity of mRNA-1273, as well as its effects on viral replication and disease progression after SARS-CoV-2 challenge, and to evaluate its safety profile and potential to promote vaccine-associated ERD after viral challenge. Additionally, the immunogenicity of mRNA-1273 was assessed as part of a non-Good Laboratory Practice (GLP) pharmacology study with safety endpoints in Sprague Dawley rats (Report 2308-123; Module 2.6.6).

The expression of mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo. HEK293T cells transfected with a dose range (0.003125 through 0.2 µg) of mRNA expressed the encoded SARS-CoV-2 S-2P antigen, as demonstrated by surface-protein staining with monoclonal antibodies (mAbs) specific to the RBD (CR3022) or the N-terminal domain (NTD; 4A8) epitopes of the SARS-CoV-2 S protein. The expression of these epitopes was similarly confirmed in spleen and draining lymph node (dLN) immune cells (conventional dendritic cells [cDCs] and plasmacytoid dendritic cells [pDCs]) in BALB/c mice administered a single intramuscular (IM) dose (2 or 10 µg) of mRNA-1273.

Immunogenicity was characterized in young and aged mice as well as rats, hamsters and NHPs through the evaluation of the humoral (immunoglobulin [Ig] G binding antibodies), cellular (T-cell cytokines and T helper [Th] 1-directed CD4+ and CD8+ responses), and/or neutralizing antibody responses elicited by prime-only or prime/boost immunization schedule with a range of mRNA-1273 dose levels.

To assess protection by mRNA-1273, young and aged mice as well as hamsters, and NHPs were immunized with a prime-only or prime/boost schedule, followed by viral challenge with high-dose SARS-CoV-2 (mouse-adapted or WT strain). mRNA-1273 dose levels and immunization schedules predicted to drive optimal and suboptimal protection were included in these studies to identify immune signatures for each regimen and to assess the level of protection mediated by different dose levels. Suboptimal dose levels that confer only partial protection were also included to evaluate the theoretical risk of disease enhancement. Viral load and replication in the upper (nasal turbinates) and lower (lungs) airways as well as lung pathology and inflammation, were evaluated after viral challenge.

The potential of mRNA-1273 to promote vaccine-associated ERD was assessed in viral challenge studies in immunized young and aged mice, hamsters, and NHPs through the evaluation of immunogenicity endpoints indicative of a protective or disease enhancement phenotype. In addition, mice immunized with SARS-CoV-1 DIV (double-inactivated severe acute respiratory syndrome coronavirus-1) aluminum hydroxide (alum) adjuvanted or CDS (conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein) alum adjuvanted were included as positive controls for immune signatures associated with ERD to compare against the immune signature of mice immunized with mRNA-1273. Binding and neutralizing antibody titers were measured in these studies, as high levels of binding antibodies but low levels of neutralizing antibodies have been associated with ERD mediated by immune complex deposition and complement activation. Additionally, a Th2-directed response has been associated with ERD. Therefore, T-cell cytokine levels were analyzed by intracellular cytokine staining (ICS) and antibody subclasses profiling (immunoglobulin [Ig]G2a/c and IgG1) was performed to identify Th1- and Th2- directed CD4+ T-cell responses. Lung and nasal virus measurements were performed post-challenge in these animals and measurements of total virus and actively replicating virus were performed to verify that virus loads did not increase at suboptimal dose levels. Lastly, lung histopathology assessments were performed to assess immune complex deposition and immune cell invasion in response to viral challenge in immunized animals.

These studies demonstrated that mRNA-1273 is immunogenic in all the species assessed, showing a dose-dependent response in IgG binding antibody titers and neutralizing antibody activities. Antigen-specific T-cell responses were observed in studies in mice and NHPs. Direct measurement of Th1-directed responses in mice and NHPs, indirect measurement of Th1-directed responses (IgG2a/c:IgG1 antibody subclasses) in mice, and the high levels of neutralizing antibody in all species lessen the concerns regarding the risk of ERD associated with mRNA-1273 immunization. Additionally, a robust and dose dependent CD8+ T-cell response in

mice and a low CD8+ T-cell response in NHPs were observed after boosting with a second dose of mRNA-1273.

In addition to measurements of the immune response, mice, hamsters, and NHPs were challenged with high doses of SARS-CoV-2 (Mice: mouse-adapted SARS-CoV-2; Hamsters and NHPs: WT SARS-CoV-2 strain, Washington state isolate). mRNA-1273 dose levels predicted to be optimal (fully protective) and suboptimal (subprotective) were included in these studies. At the high doses, mice, hamsters, and NHPs were fully protected from viral replication in both lungs and nasal passages. At suboptimal dose levels, animals were either fully protected in the lungs or had reduced viral burden post-challenge compared to control animals. There were no observations of increased viral load in animals immunized with suboptimal dose levels of mRNA-1273, which further supports that mRNA-1273 immunization does not promote ERD. Lung histopathology assessments were performed to verify reduction of inflammation, immune complex deposition, and immune cell invasion in response to viral challenge in animals immunized with mRNA-1273 compared to PBS-control (phosphate-buffer saline [PBS]) vaccinated animals. In animals immunized with either optimal or suboptimal mRNA-1273 dose levels, histopathological evaluation of the lungs of mice and NHPs confirmed the lack of evidence of ERD, as demonstrated by minimal inflammation and no noteworthy neutrophilic-associated alveolar disease or eosinophil-dominant inflammatory response, which have been historically associated with vaccine-associated ERD. In contrast, moderate to severe inflammation involving the small airways and the adjacent alveolar interstitia was elicited by SARS-CoV-2 infection in PBS control animals.

Overall, nonclinical animal studies demonstrate that mRNA-1273 is well-tolerated, is immunogenic, fully protects animals from viral challenge at optimal immunization dose levels, and does not promote vaccine-associated ERD at either optimal or suboptimal dose levels.

Table 1: Summary of Pharmacology Studies Supporting mRNA-1273 Development

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Primary Pharmacology					
Evaluation of in vitro expression of SARS-CoV-2 mRNA and in vivo expression of mRNA-1273	SARS-CoV-2 S-2P mRNA: 0.003125 through 0.2 µg mRNA-1273: 2 or 10 µg	HEK293T cells BALB/c mice	In vitro transfection In vivo IM; single injection	No	MOD-4112.1273

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Evaluation of immunogenicity, protective capacity, and safety in young mice	mRNA-1273: 0.01, 0.1, 1 or 10 µg SARS-CoV-2 S-2P: 0.01, 0.1 or 1 µg (b) (4)	Mouse (young), BALB/cJ, C57BL/6J and B6C3F1/J	IM; prime only or prime/boost (3-week interval) prime/boost (4-week interval)	No	VRC01
Immunization and protein restimulation in young BALB/c mice with enhanced respiratory disease endpoint monitoring	mRNA-1273: 1 or 10 µg SARS-CoV-2 S-2P: 10 µg (+ alum)	Mouse (young), BALB/c	IM; prime/boost (2-week interval)	No	MOD-3937
Immunogenicity and determination of titer dynamic range in young BALB/c mice	mRNA-1273: 0.0025 through 20 µg	Mouse (young), BALB/c	IM; prime/boost (3-week interval)	No	MOD-3938 / MOD-3940
Immunogenicity and characterization of cellular response in young BALB/cJ mice	mRNA-1273: 0.1, 1 or 10 µg SARS-CoV-1 DIV: 0.2 µg or 1 µg (+ alum) CDS: 0.2 µg or 1 µg (+ alum)	Mouse (young), BALB/cJ	IM; prime/boost (3-week interval)	No	VRC05
Efficacy and enhanced respiratory disease in aged BALB/c mice	mRNA-1273: 0.1 or 1 µg SARS-CoV-1 DIV: 0.1 µg (+ alum)	Mouse (aged), BALB/c	IM; prime/boost (3-week interval)	No	VRC02
Five-week (2 doses: prime/boost) repeat dose immunogenicity with safety endpoints	mRNA-1273: 30, 60, or 100 µg	Rat, Sprague Dawley	IM; prime/boost (3-week interval)	No	2308-123
Protection from WT SARS-CoV-2 in hamsters using optimal and suboptimal doses	mRNA-1273: 1, 5, or 25 µg	Hamster, golden Syrian	IM; prime only or prime/boost (3-week interval)	No	UTMB01
Immunogenicity and protective efficacy in NHPs	mRNA-1273: 10, or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime/boost (4-week interval)	No	VRC04

Study Type/Description	Test Article Dose (µg)	Species, Strain	Method of Administration; Immunization Schedule	GLP	Report Number
Evaluation of immunogenicity and efficacy from expanded dose range in NHPs	mRNA-1273: 2.5, 30, or 100 µg	NHP, rhesus macaque (Indian-origin)	IM; prime only or prime/boost (4-week interval)	No	VRC07

Abbreviations: alum = aluminum hydroxide; CDS = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein; GLP = Good Laboratory Practice; IM = intramuscular; mRNA = messenger RNA; NHP = nonhuman primate; SARS-CoV-1 DIV = double-inactivated severe acute respiratory syndrome coronavirus-1; SARS-CoV-2 = 2019 novel coronavirus; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; (b) (4) WT = wild-type.

2.6.2.2 PRIMARY PHARMACODYNAMICS

2.6.2.2.1 Expression of SARS-CoV-2 S-2P Encoded by mRNA (In Vitro) or mRNA-1273 (In Vivo)

The objectives were to evaluate the in vitro expression of SARS-CoV-2 S-2P-encoding mRNA and the in vivo expression of mRNA-1273 (Report MOD-4112.1273).

Methods:

Evaluation of In Vitro Expression of mRNA Encoding SARS-CoV-2 S-2P Antigen

In vitro expression of SARS-CoV-2 S-2P antigen was evaluated in HEK293T cells transiently transfected with 0.003125 to 0.2 µg of an mRNA construct that encodes the SARS-CoV-2 S-2P antigen. After 24, 48, and 72 hours of transfection, the cells were collected and assessed for expression of the encoded antigen, as measured by flow cytometry analysis of cell surface staining using mAbs specific to the RBD (clone CR3022) or the NTD (clone 4A8) epitope of the SARS-CoV-2 S protein.

The cell surface expression was calculated by multiplying the frequency of positive cells by the mean fluorescence intensity (MFI [(MFI × frequency]), which indicated the level of SARS-CoV-2 S-2P antigen expression.

Evaluation of In Vivo Expression of mRNA-1273

Mice were administered a single IM dose of 2 or 10 µg of mRNA-1273. Spleens and dLNs were harvested 24, 48, and 72 hours after injection and processed for evaluation by flow cytometry of antigen expression in pDCs and cDCs using the CR3022 and 4A8 mAbs together with a panel of

antibodies to cell surface markers. Cells isolated from mice injected with PBS were used as controls. The treatment regimen is summarized in Table 2.

Table 2: Study Design for the Evaluation of In Vivo Expression of mRNA-1273 – Report MOD-4112.1273

Test Article	Total Dose (µg)	Number of Animals/Treatment Group	Injection	Sample Collection (hours post Day 0)[a]	Endpoint Monitoring
PBS	NA	12	Day 0	24, 48, 72	Cell isolation and FACS
mRNA-1273	2	18			
mRNA-1273	10	18			

Abbreviations: NA = not applicable; FACS = fluorescence-activated cell sorting; PBS = phosphate-buffered saline
[a] At each time point, samples were collected from 4 mice/group (PBS) or 6 mice/group (mRNA-1273).
Source: Report MOD-4112.1273 (Table 2).

Results

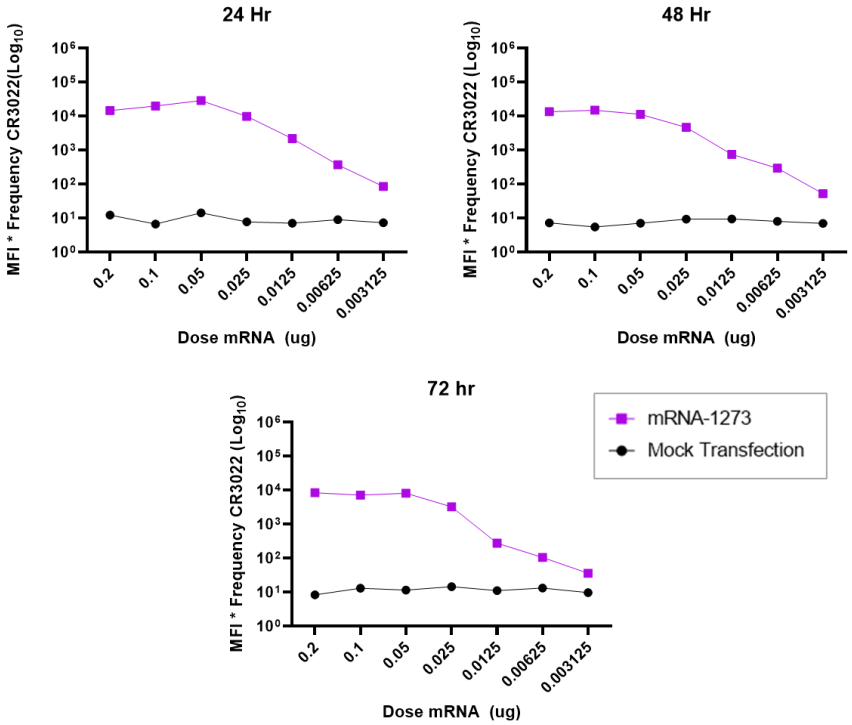
In Vitro Expression of mRNA Encoding SARS-CoV-2 S-2P Antigen

Surface staining of the SARS-CoV-2 S-2P antigen was observed through 72 hours post-transient transfection of HEK293T cells with 0.003125 through 0.2 µg of an mRNA construct that encodes the SARS-CoV-2 S-2P antigen (Figure 1).

In vitro evaluation of cell surface expression in cells transfected with 0.05 µg or 0.2 µg mRNA revealed an overall increase in the frequency of cells expressing the SARS-CoV-2 S-2P antigen, as demonstrated by cell staining with mAbs that bind the RBD (CR3022) or the NTD (4A8) epitopes of the SARS-CoV-2 S protein. A slightly higher frequency of cells expressing the SARS-CoV-2 S-2P antigen was observed at 48 hours when compared with that at 24 hours with both the CR3022 and 4A8 mAbs (Figure 2A and Figure 2C).

The MFI was similar in cells transfected with SARS-CoV-2 S-2P-encoding mRNA at 24 and 48 hours, evident after staining with CR3022 and 4A8, and a slight dose effect was observed (Figure 2B and Figure 2D). Mock-transfected cells showed no MFI signal.

Figure 1: SARS-CoV-2 S-2P Cell Surface Expression MFI*Frequency at 24, 48, and 72 hours With CR3022 Staining – Report MOD-4112.1273

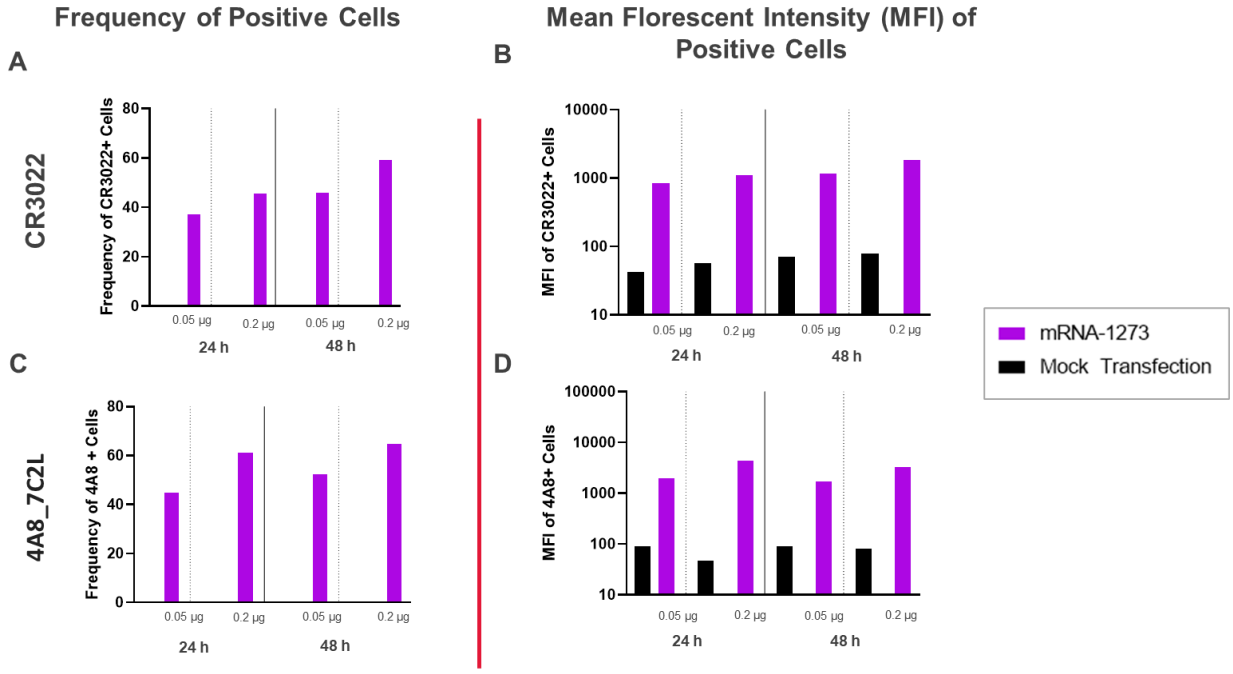


Abbreviations: MFI = mean fluorescence intensity; MFI*frequency = the frequency of positive cells multiplied by the MFI; mRNA = messenger RNA; SARS-CoV-2 S-2P = 2019 novel coronavirus spike protein with 2 proline substitutions within the heptad repeat 1 domain.

Notes: CR3022 is a monoclonal antibody for the receptor binding domain epitope of the SARS-CoV-2 spike protein; in this figure, mRNA-1273 refers to the mRNA construct that encodes the SARS-CoV-2 S-2P antigen.

Source: Report MOD-4112.1273 (Figure 1).

Figure 2: In Vitro Cell Surface Expression of the SARS-CoV-2 S-2P Antigen After Transfection of HEK293T Cells With mRNA Encoding SARS-CoV-2 S-2P, Measured by Flow Cytometry at 24 and 48 Hours – Report MOD-4112.1273



Abbreviations: MFI = mean fluorescence intensity; mRNA = messenger RNA; SARS-CoV-2 S-2P = 2019 novel coronavirus spike protein with 2 proline substitutions within the heptad repeat 1 domain.

Notes: 4A8 is a monoclonal antibody for the N-terminal domain epitope of the SARS-CoV-2 spike protein; CR3022 is a monoclonal antibody for the receptor binding domain epitope of the SARS-CoV-2 spike protein; in this figure, mRNA-1273 refers to the mRNA construct that encodes the SARS-CoV-2 S-2P antigen.

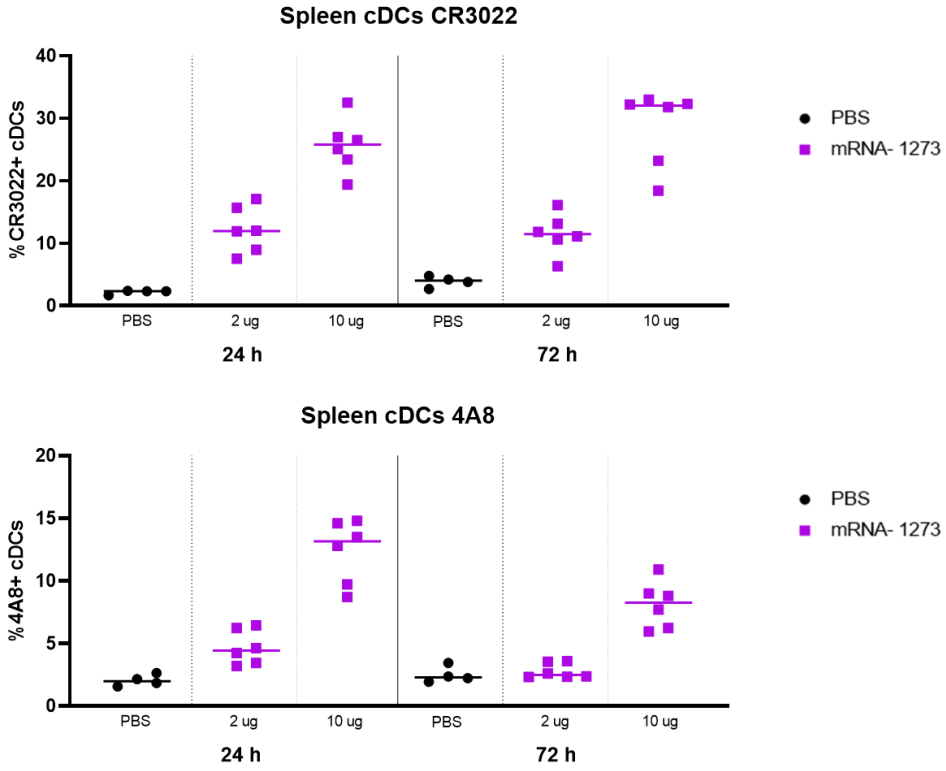
Source: Report MOD-4112.1273 (Figure 2).

In Vivo Expression of mRNA-1273

Evaluation of in vivo expression of mRNA-1273 showed very little difference in the level of antigen expression in splenic cDCs (Figure 3) or pDCs (Report MOD-4112.1273 [Figure 4]) between 24 hours and 72 hours, regardless of whether the staining was specific to the RBD (CR3022 mAb) or the NTD (4A8 mAb) epitopes of the SARS-CoV-2 S protein. The higher dose (10 µg mRNA-1273) induced higher levels of antigen expression in immune cells collected from spleens at each time point.

In the dLNs, SARS-CoV-2 S-2P antigen expression, as measured by cell surface expression of the RBD (CR3022 mAb) and NTD (4A8 mAb) epitopes, peaked at 48 hours after immunization with mRNA-1273 in cDCs (Figure 4) and pDCs (Report MOD-4112.1273[Figure 6]).

Figure 3: In Vivo Expression in Splenic Conventional Dendritic Cells – Report MOD-4112.1273

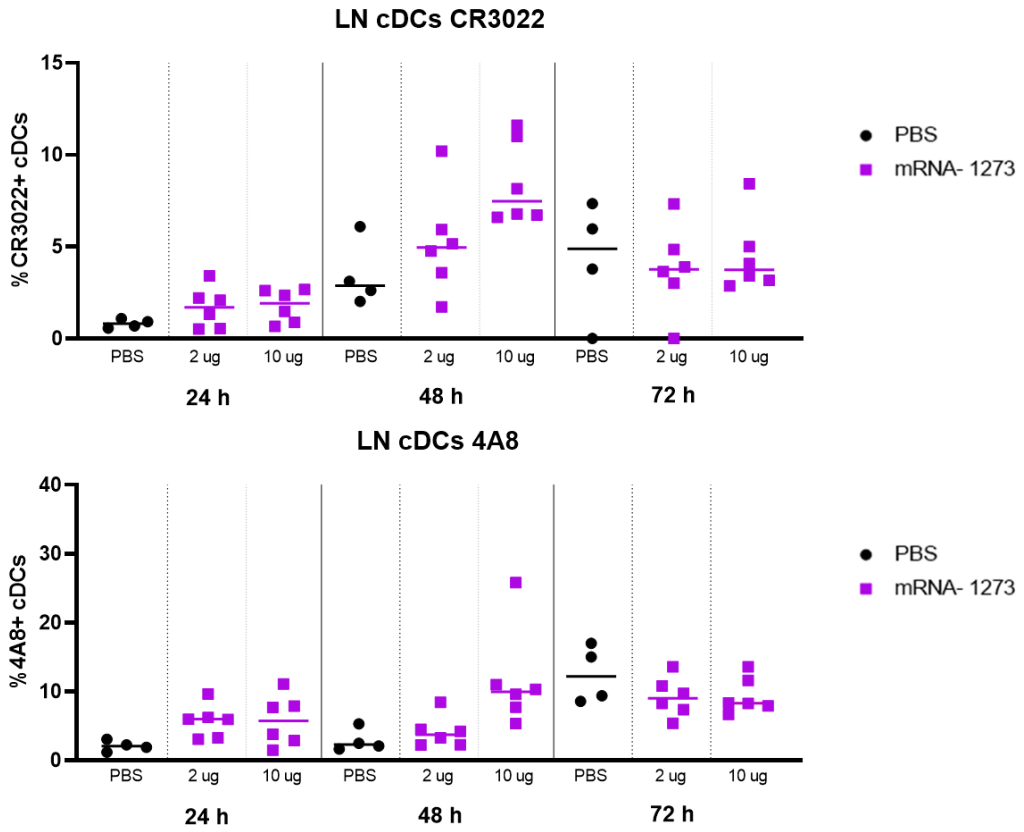


Abbreviations: cDC = conventional dendritic cell; PBS = phosphate-buffered saline.

Notes: 4A8 is a monoclonal antibody for the N-terminal domain epitope of the SARS-CoV-2 spike protein; CR3022 is a monoclonal antibody for the receptor binding domain epitope of the SARS-CoV-2 spike protein.

Source: Report MOD-4112.1273 (Figure 3).

Figure 4: In Vivo Expression in Lymph Node Conventional Dendritic Cells – Report MOD-4112.1273



Abbreviations: cDC = conventional dendritic cell; LN = lymph node; PBS = phosphate-buffered saline.
Notes: 4A8 is a monoclonal antibody for the N-terminal domain epitope of the SARS-CoV-2 spike protein; CR3022 is a monoclonal antibody for the receptor binding domain epitope of the SARS-CoV-2 spike protein.
Source: Report MOD-4112.1273 (Figure 5).

Conclusions

The expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo.

Sustained levels of antigen expression were observed over 24, 48, and 72 hours in HEK293T cells transiently transfected with a dose range (0.003125 through 0.2 µg) of an mRNA construct that encodes the SARS-CoV-2 S-2P antigen, as demonstrated by an overall increase in the frequency of cells expressing the RBD (CR3022 mAb) or the NTD (4A8 mAb) epitopes of the SARS-CoV-2 S protein; a slight dose effect was observed.

Immunization of BALB/c mice with 2 or 10 µg mRNA-1273 induced in vivo expression of the SARS-CoV-2 S-2P antigen in spleen and dLN immune cells (cDCs and pDCs), as demonstrated by staining with mAbs specific to the RBD (CR3022) or the NTD (4A8) epitopes of the SARS-CoV-2 S protein. The level of antigen expression measured in cDCs was similar to that of pDCs. The higher dose (10 µg mRNA-1273) induced higher levels of antigen expression in these cells.

2.6.2.2.2 Evaluation of Immunogenicity, Protective Capacity, and Safety of mRNA-1273 in Young Mice

The objectives were to evaluate the immunogenicity, protective capacity, and safety of mRNA-1273 in BALB/cJ, C57BL/6J and B6C3F1/J mice (Report VRC01).

Methods:

Female young (approximately 6 to 8 weeks old) mice (BALB/cJ, C57BL/6J, and B6C3F1/J) were treated according to the study design presented in Table 3. Sera were collected at various time points post-immunization with mRNA-1273 or SARS-CoV-2 S-2P (b) (4) (b) (4) protein and assessed for SARS-CoV-2 S-2P binding antibodies, (including total IgG and IgG1 and IgG2a/c subclasses), as measured by enzyme-linked immunosorbent assay (ELISA), and IgG subclass ratios (IgG2a/c:IgG1) were calculated. Sera collected from mRNA-1273 immunized mice were also tested for neutralizing antibodies, as measured by pseudotyped lentivirus reporter neutralization assay and plaque reduction neutralization tests (PRNT). Spleens from mRNA-1273 and SARS-CoV-2 S-2P (b) (4) protein immunized mice were collected on Week 12 (9 weeks post-boost) for the evaluation of T-cell cytokine responses to in vitro restimulation with pools of overlapping peptides from S proteins (S1 and S2 peptides), as measured by flow cytometry analysis of cell surface and intracellular immunostaining. Lungs and nasal turbinates were collected from mRNA-1273-immunized mice on Day 2 and Day 4 post-challenge for the evaluation of viral load in the upper (nasal turbinates) and lower (lungs) airways, as measured by plaque assays, and for histopathology assessment of lung samples.

Table 3: Study Design – Report VRC01

Mouse Strain[a]	No. of Groups[b] (Mice/group)	Treatment Dose (µg)	Immunization Schedule[c]		Challenge[d]	Sample Collection; Experimental Assessments
			Prime	Boost		
BALB/cJ	6 (10)	0.01, 0.1 or 1 µg of mRNA-1273	Week 0 (Day 1)	Week 3 (Day 22)	NA	Week 5 (Day 36) (sera for SARS-CoV-2 S-2P bAbs [total IgG])
BALB/cJ C57BL/6J B6C3F1/J	3 (10)	0.01, 0.1 or 1 µg of mRNA-1273	Week 0 (Day 1)	Week 3 (Day 22)	NA	Week 2 (Day 15) and Week 5 (Day 36); sera for SARS-CoV-2 S-2P bAbs (total IgG)
BALB/cJ C57BL/6J B6C3F1/J	2 (10)	0.1 or 1 µg of mRNA-1273	Week 0 (Day 1)	Week 3 (Day 22)	NA	Week 2 (Day 15) and Week 5 (Day 36); sera for nAbs (pseudovirus)
BALB/cJ	3 (3)	1 µg of SARS-CoV-2 S-2P with (b) (4)	Week 0 (Day 1)	Week 3 (Day 22)	NA	Week 5 (Day 36); sera for nAbs (WT virus)
BALB/cJ C57/B/6 B6C3F1/J	6 (10)	0.01, 0.1 or 1 µg of mRNA-1273 or SARS-CoV-2 S-2P with (b) (4)	Week 0 (Day 1)	Week 3 (Day 22)	NA	Week 5 (Day 36); sera for bAbs (IgG1, IgG2a/c)
BALB/cJ	4 (10)	0.01, 0.1, 1 and 10 µg of mRNA-1273	Week 0 (Day 1)	NA	NA	Week 2 (Day 15) and Week 4 (Day 26); sera for SARS-CoV-2 S-2P bAbs (total IgG, IgG1, IgG2a) and nAbs (pseudovirus)
B6C3F1/J	6 (5)	0.01, 0.1 or 1 µg of mRNA-1273 or 0.1 or 1 µg SARS-CoV-2 S-2P with (b) (4)	Week 0 (Day 1)	Week 3 (Day 22)	NA	Week 12 (Day 81); spleens for cytokine analysis
BALB/cJ	3 (5)	0.1, 1, 10 µg of mRNA-1273	Week 0 (Day 1)	NA	Week 7 (Day 52)	Day 2 post-challenge; lungs for viral load
BALB/cJ	12 (5)	0.01, 0.1, 1 µg of mRNA-1273 or PBS[e]	Week 0 (Day 1)	Week 3 (Day 22)	Week 8 (Day 57) and Week 16 (Day 113)	Day 2 post-challenge; lungs and NT for viral load
BALB/cJ	12 (5)	0.01, 0.1, 1 µg of mRNA-1273 or PBS[e]	Week 0 (Day 1)	Week 4 (Day 29)	Week 7 (Day 52)	Day 2 post-challenge; lungs and NT for viral load
BALB/cJ	4 (5)	0.01, 0.1, 1 µg of mRNA-1273 or PBS[e]	Week 0 (Day 1)	Week 3 (Day 22)	Week 8 (Day 58)	Day 4 post-challenge; lungs and NT for viral load
BALB/cJ	3 (4)	0.01 and 0.1 µg of mRNA-1273 or PBS[f]	Week 0 (Day 1)	Week 3 (Day 22)	Week 8 (Day 58)	Day 2 and Day 4 post-challenge; lungs for histopathology
BALB/cJ	1 (4)	0.1 µg of mRNA-1273	Week 0 (Day 1)	NA	Week 7 (Day 52)	Day 2 and Day 4 post-challenge; lungs for histopathology

Abbreviations: bAbs = binding antibodies; Ig = immunoglobulin; NA = not applicable; nAbs = neutralizing antibodies; No. = number; NT = nasal turbinates; PBS = phosphate-buffered saline; PFU = plaque-forming units; (b) (4) S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; WT = wild-type.

[a] All mice were females and 6 to 8 weeks old.

[b] Number of groups per mouse strain.

[c] Treatment dose was administered via IM injection into the right hind leg with a volume of 50 µL.

[d] Mice were challenged intranasally with 10^5 PFU of mouse-adapted SARS-CoV-2 virus strain containing. 2 targeted amino acid mutations (Q498T/P499Y) in the receptor binding domain, the region of the S protein that binds to mouse ACE-2 receptor, which results in infection and replication in the upper and lower respiratory tract.

[e] PBS 1×.

[f] PBS 2×.

Source: Report VRC01 (Table 2).

Results:

mRNA-1273 elicited dose-dependent binding and neutralizing antibody responses in BALB/cJ, C57BL/6, and B6C3F1/J mice when administered as prime/boost immunizations at a 3 week interval with doses ranging from 0.01 to 1 µg (Figure 5). mRNA-1273 also induced binding antibodies and neutralizing activity after a prime-only immunization with 1 or 10 µg of mRNA-1273 (Report VRC01 [Figure 4]). Immunization with mRNA-1273 induced a robust CD8+ T-cell response and a Th1-directed CD4+ T-cell response, as demonstrated by i) the predominant production of interferon (IFN)-γ, TNF-α, and IL-2 by splenocytes from mRNA-1273-immunized mice upon in vitro restimulation with S1 and S2 peptide pools (Figure 6) and ii) the IgG antibody binding subclass ratio (Report VRC01 [Figure 4]).

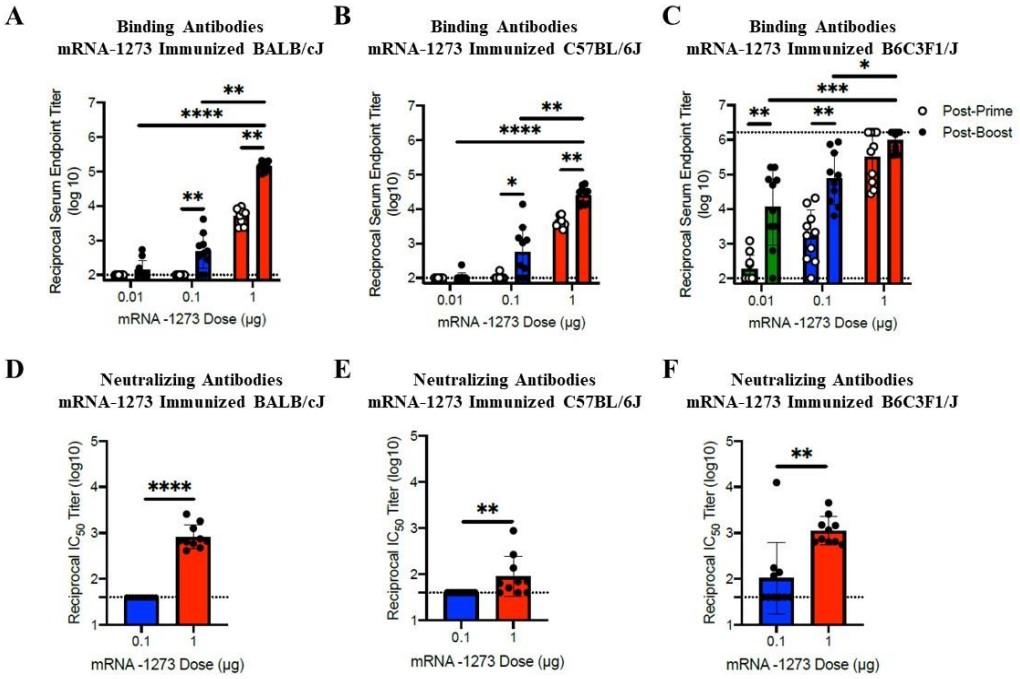
Mice immunized with 1 µg or greater doses of mRNA-1273 on a prime-only [Report VRC01 (Figure 6)], prime/boost schedule with a 3-week interval (Figure 7) and with a 4-week interval (Report VRC01 [Figure 8]) were fully protected from mouse-adapted SARS-CoV-2 viral challenge. In addition, animals immunized with less than 1 µg of mRNA-1273 were partially protected, with no indications of disease enhancement.

Post-challenge lung histopathology assessments showed no evidence of ERD in SARS-CoV-2 infected mice immunized (both optimal and suboptimal dose levels) as demonstrated by the presence of minimal inflammation and the lack of significant neutrophilic-associated alveolar disease or eosinophil-dominant inflammatory response (Figure 8). In contrast, moderate to severe inflammation was elicited by SARS-CoV-2 infection in PBS control animals, and this inflammation often involved the small airways and adjacent alveolar interstitia.

Conclusions:

Overall, the results from this study demonstrate that mRNA-1273 is well tolerated and immunogenic, fully protects mice from challenge at optimal dose levels, and does not drive ERD at optimal or suboptimal dose levels.

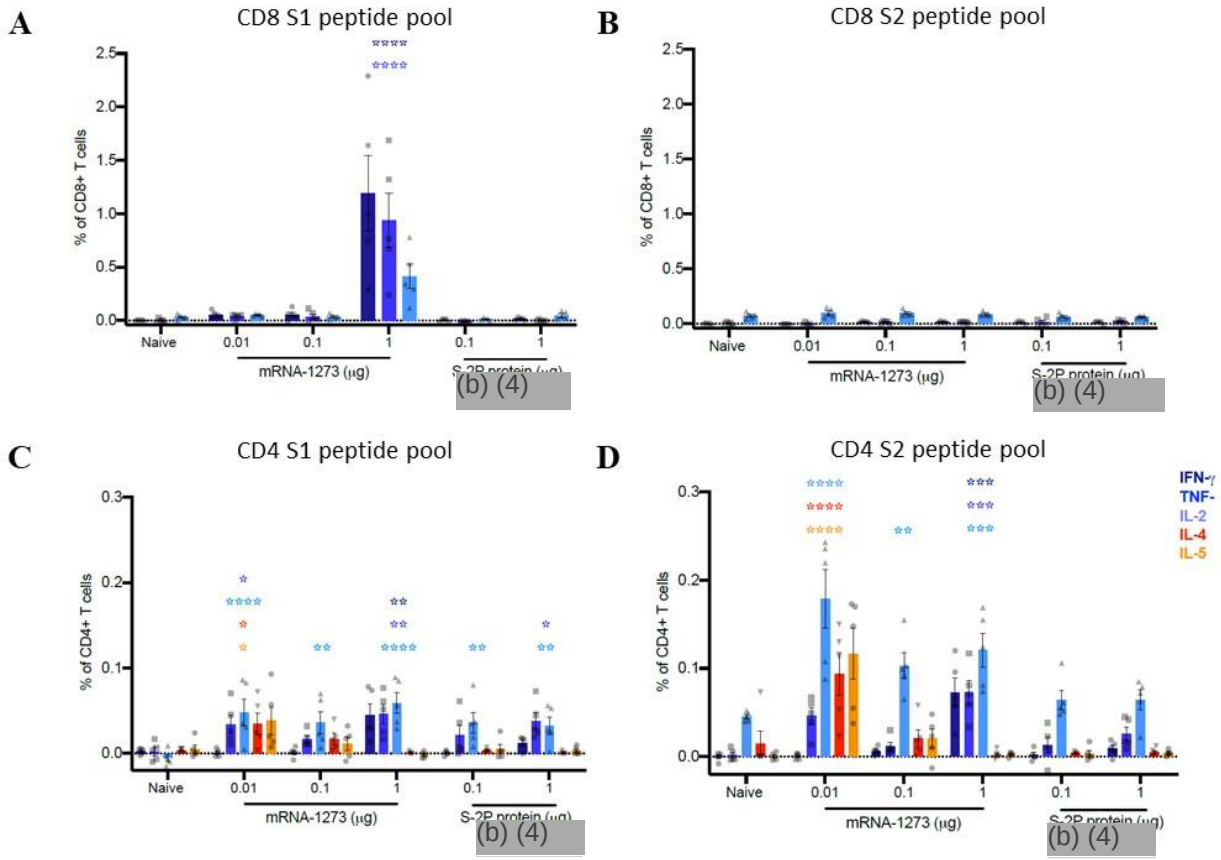
Figure 5: Binding and Neutralizing Antibody Titers in Young BALB/cJ, C57BL/6J, and B6C3F1/J Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC01



Note: Each dot represents an individual mouse, means are represented by the heights of bars, and error bars represent the corresponding SD. Dotted line indicates the assay limit of detection. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$.

Source: Report VRC01 (Figure 2).

Figure 6: T-Cell and Cytokine Responses to In Vitro Restimulation With S1 or S2 Peptide Pool in Splenocytes From Young B6C3FI/J Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC01

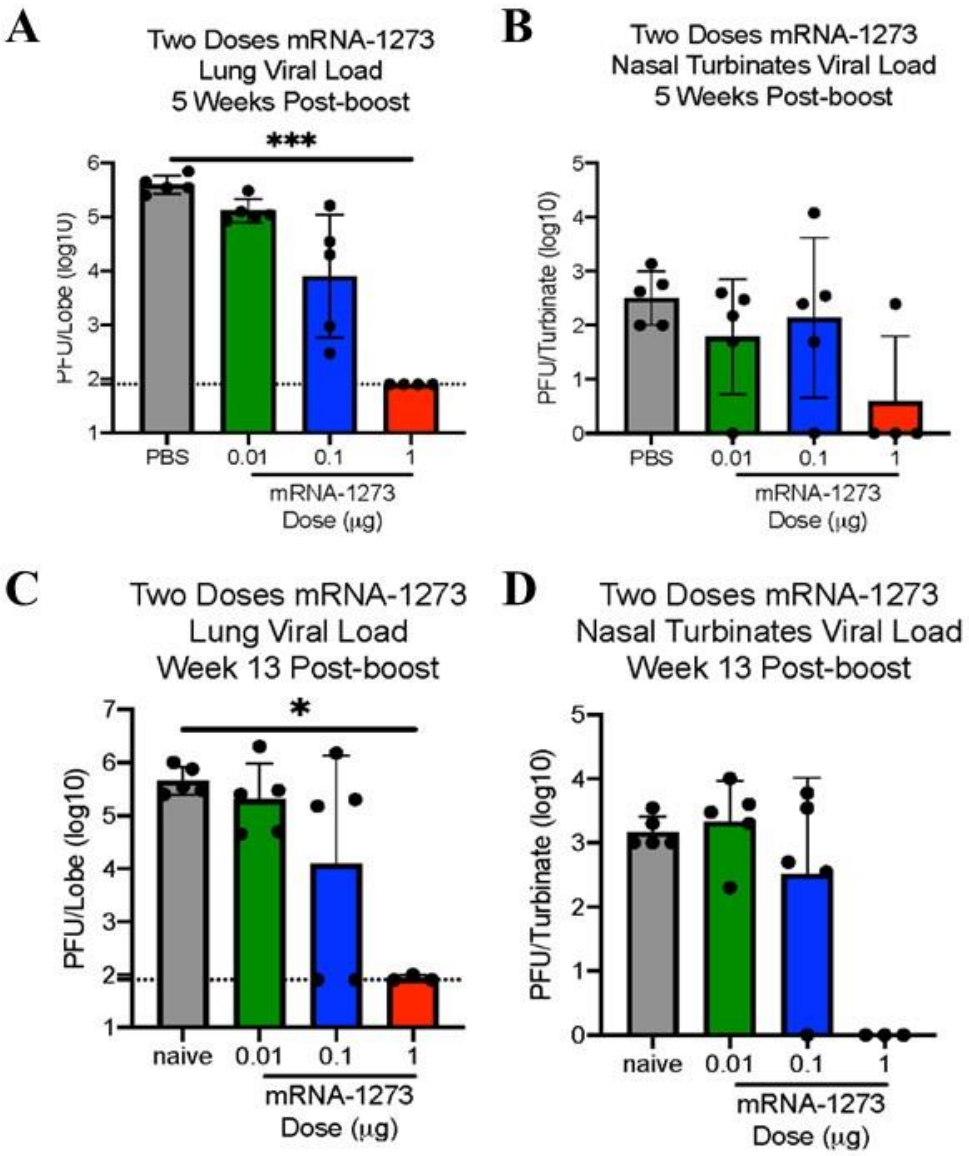


Abbreviations: IFN = interferon; IL = interleukin; S-2P (SARS-CoV-2 S-2P) = SARS-CoV-2 spike protein with 2 proline substitutions within the heptad repeat 1 domain; (b) (4) TNF = tumor necrosis factor.

Note: Each dot represents an individual mouse, means are represented by the heights of bars, and error bars represent the corresponding SD. Dotted line indicates the assay limit of detection. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$.

Source: Report VRC01 (Figure 5).

Figure 7: Lung and Nasal Turbinate Viral Loads 2 Days Post-Viral Challenge of Young BALB/cJ Mice Immunized With mRNA-1273 (Prime /Boost With 3-Week Interval) – Report VRC01

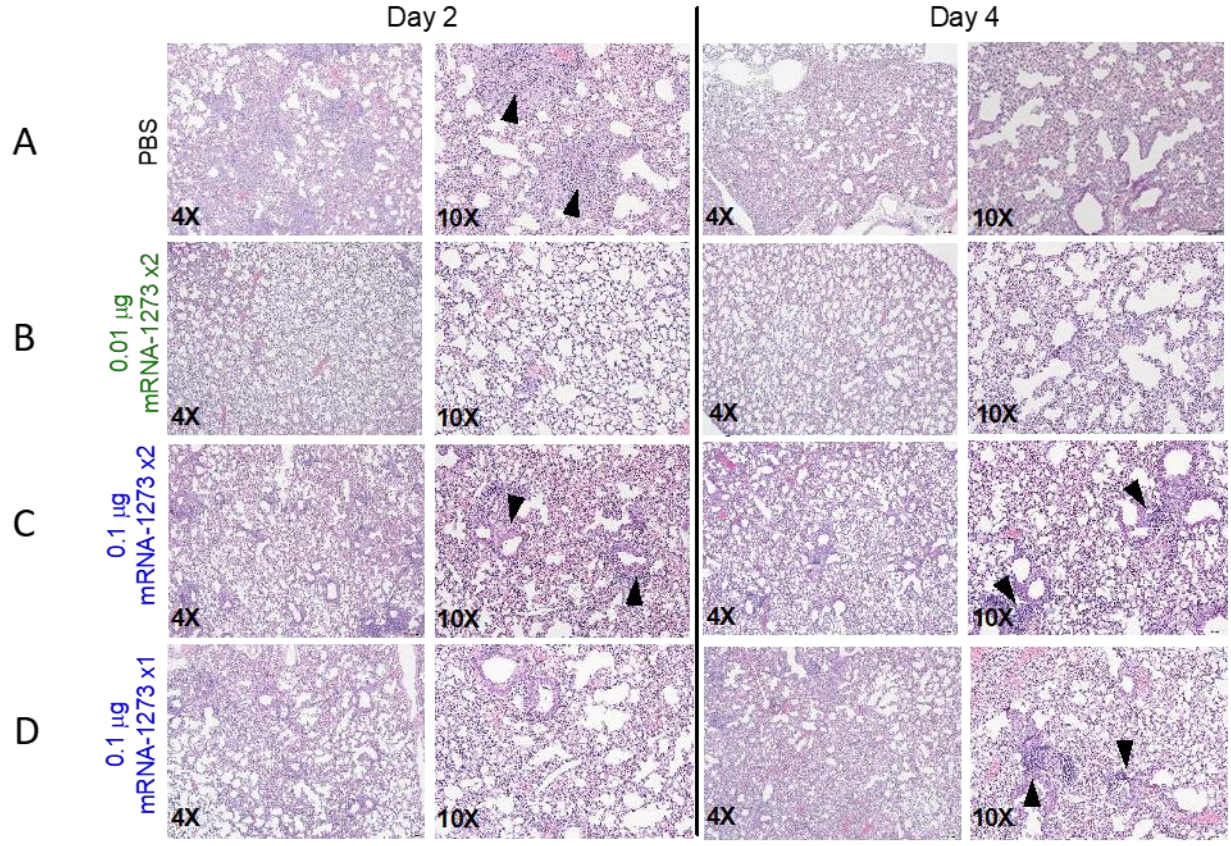


Abbreviations: PBS = phosphate-buffered saline; naïve = non-immunized; PFU = plaque-forming units; SARS-CoV-2 = 2019 novel coronavirus.

Note: Mice were immunized with mRNA-1273 at Week 0 and Week 3 and challenged with 10^5 PFU of mouse-adapted SARS-CoV-2 intranasally at 5 weeks post-boost (A and B) and 13 weeks post-boost (C and D). Each dot represents an individual mouse, means are represented by the heights of bars, and error bars represent the corresponding SD. Dotted line indicates the assay limit of detection. * $p < .05$, *** $p < .001$.

Source: Report VRC01 (Figure 7).

Figure 8: Lung Histopathology 2 Days and 4 Days Post-Viral Challenge of Young BALB/cJ Mice Immunized With mRNA-1273 (Prime-only and Prime/Boost With 3-Week Interval) –-Report VRC01



Abbreviations: PBS = phosphate-buffered saline; mRNA-1273 ×1 = prime-only mRNA-1273 schedule; mRNA-1273 ×2 = prime/boost mRNA-1273 schedule with 3-week interval;.
Note: Arrowheads indicate small bronchioles and their adjacent vasculature.
Source: Report VRC01 (Figure 10).

2.6.2.2.3 Immunization and Protein Restimulation in Young BALB/c Mice With Enhanced Respiratory Disease Endpoint Monitoring

The objectives were to evaluate the immunogenicity of mRNA-1273 and to address the theoretical concern of ERD in young female BALB/c mice relative to SARS-CoV-2 S-2P protein adjuvanted with alum (Report MOD-3937).

Methods:

Female young (approximately 8 to 9 weeks old) BALB/c mice were treated according to the study design presented in Table 4. Sera were collected at Week 4 (Day 28, 2 weeks post-boost) for the assessment of SARS-CoV-2 S-2P binding antibodies (including total IgG and IgG1 and

IgG2a subclasses), as measured by ELISA; IgG subclass ratios (IgG2a:IgG1) were also calculated. Sera were assessed for neutralizing antibodies as measured by pseudotyped lentivirus reporter neutralization assay. On Day 29, animals immunized with the alum (aluminum hydroxide)-adjuvanted SARS-COV-2 S-2P protein were restimulated in vivo with 10 µg of SARS-CoV-2 S-2P protein (without adjuvant). Spleens were collected on Day 33 (4 days after in vivo restimulation) for the evaluation of T-cell cytokine responses to in vitro restimulation with pools of overlapping peptides from S protein (S1 and S2 peptides), as measured by multiplex(b) (4) assay.

Table 4: Study Design - Report MOD-3937

Treatment Groups	Number of Mice[a]	Immunization Schedule[b]		In Vivo Restimulation Schedule	Sample Collection; Experimental Assessments
		Prime	Boost		
10 µg mRNA-1273	8	Week 0 (Day 1)	Week 2 (Day 15)	NA	Week 4 (Day 28); sera for SARS-CoV-2 S-2P bAbs (total IgG, IgG1, IgG2a) and nAbs (pseudovirus)
1 µg mRNA-1273	7				
10 µg SARS-CoV-2 S-2P + 250 µg alum[c]	8			Week 4 (Day 29)[d]	
PBS	3				Week 4 (Day 33); spleens for cytokine analysis

Abbreviations: alum = aluminum hydroxide; bAbs = binding antibodies; Ig = immunoglobulin; NA = not applicable; nAbs = neutralizing antibodies; PBS = phosphate-buffered saline; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus.

[a] All mice were female BALB/c mice approximately 8-9 weeks old.

[b] Treatment dose was administered via IM injection into the right hind leg with a volume of 50 µL.

[c] Aluminum hydroxide adjuvant used was (b) (4)

[d] On Day 29, mice immunized with SARS-CoV-2 S-2P were restimulated in vivo with 10 µg of SARS-CoV-2 S-2P (without adjuvant) administered intravenously; control animals were administered PBS intravenously.

Source: Report MOD-3937 (Table 2).

Results:

Immunization with 1 or 10 µg of mRNA-1273 administered on a prime/boost schedule with a 2-week interval induced serum SARS-CoV-2 S-specific binding antibody (total IgG) titers (Figure 9) and neutralizing activity (Figure 10) similar to those elicited 2 weeks post-boost in young female BALB/c mice immunized with alum-adjuvanted SARS-CoV-2 S-2P protein . A dose-dependent increase in neutralizing antibody titers was observed after immunization with mRNA-1273, with the 10-µg dose producing a significantly higher response than the 1-µg dose (Figure 10).

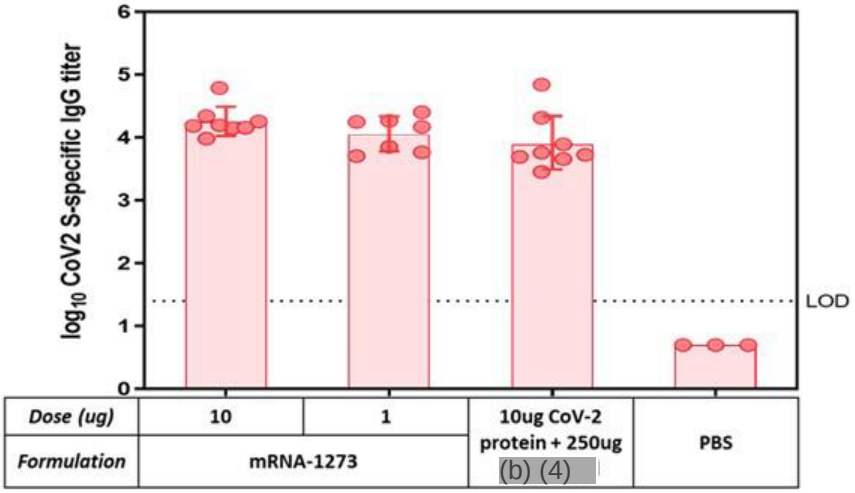
High titers of IgG1 and IgG2a were detected in mice immunized with 1 or 10 µg of mRNA-1273. In contrast, mice immunized with alum-adjuvanted SARS-CoV-2 S-2P protein had high titers of IgG1 and low titers of IgG2a (Figure 11). A comparison of the IgG2a/IgG1 ratios showed that mice immunized with 1 or 10 µg of mRNA-1273 had an antibody subclass profile that corresponds to a Th1-directed immune response (Report MOD-3937 [Figure 4]). This contrasted with the response to alum-adjuvanted SARS-CoV-2 S-2P protein, which was a predominant IgG1 antibody subclass response, a response profile that corresponds to a Th2-directed immune response.

After in vitro restimulation with the S1 and S2 peptide pools, splenocytes from mice immunized with mRNA-1273 secreted more IFN-γ than IL-4, IL-5, or IL-13, indicating a Th1-directed immune response; immunization with alum-adjuvanted SARS-CoV-2 S-2P protein induced a Th2-directed immune response (Figure 12).

Conclusions

Overall, these results demonstrate that, unlike alum-adjuvanted SARS-CoV-2 S-2P protein, which elicits a Th2-directed response, mRNA-1273 drives a Th1-directed immune response and induces robust binding and neutralization activity, an immune signature not predicted to drive vaccine-associated ERD.

Figure 9: Binding Antibody Titers 2 Weeks Post-boost in Young BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With 2-Week Interval) – Report MOD-3937

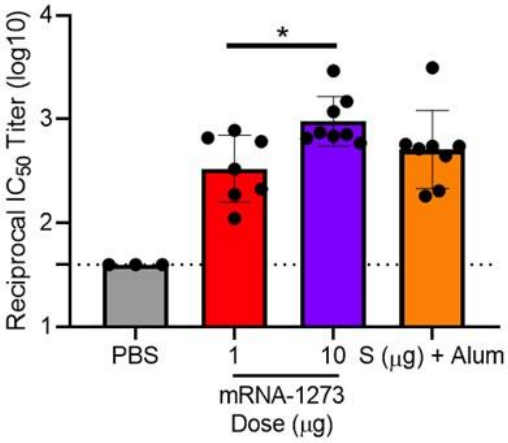


Abbreviations: CoV-2 = SARS-CoV-2; CoV-2 protein = SARS-CoV-2 S-2P; Ig = immunoglobulin; LOD = limit of detection; PBS = phosphate-buffered saline; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus.

Note: Dotted line indicates the assay limit of detection.

Source: Report MOD-3937 (Figure 1).

Figure 10: Neutralizing Antibody Titers 2 Weeks Post-boost in Young BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With 2-Week Interval) – Report MOD-3937

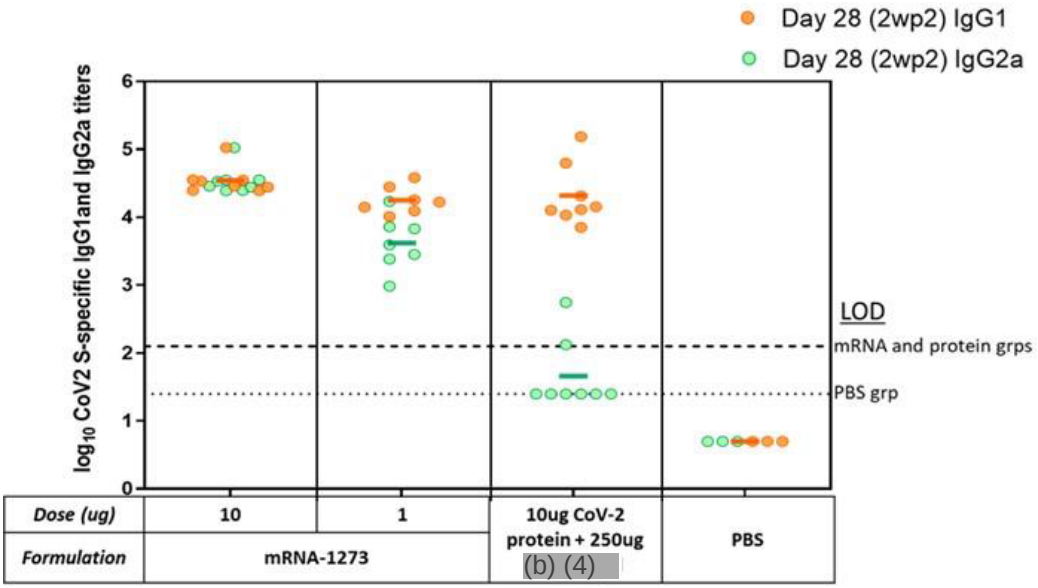


Abbreviations: alum = aluminum hydroxide; IC50 = half-maximal inhibitory concentration; PBS = phosphate-buffered saline; S = SARS-CoV-2 S-2P; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus.

Note: Dotted line indicates the assay limit of detection. * $p < .05$.

Source: Report MOD-3937 (Figure 2).

Figure 11: IgG1 and IgG2a Titers 2 Weeks Post-boost in Young BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With 2-Week Interval) – Report MOD-3937

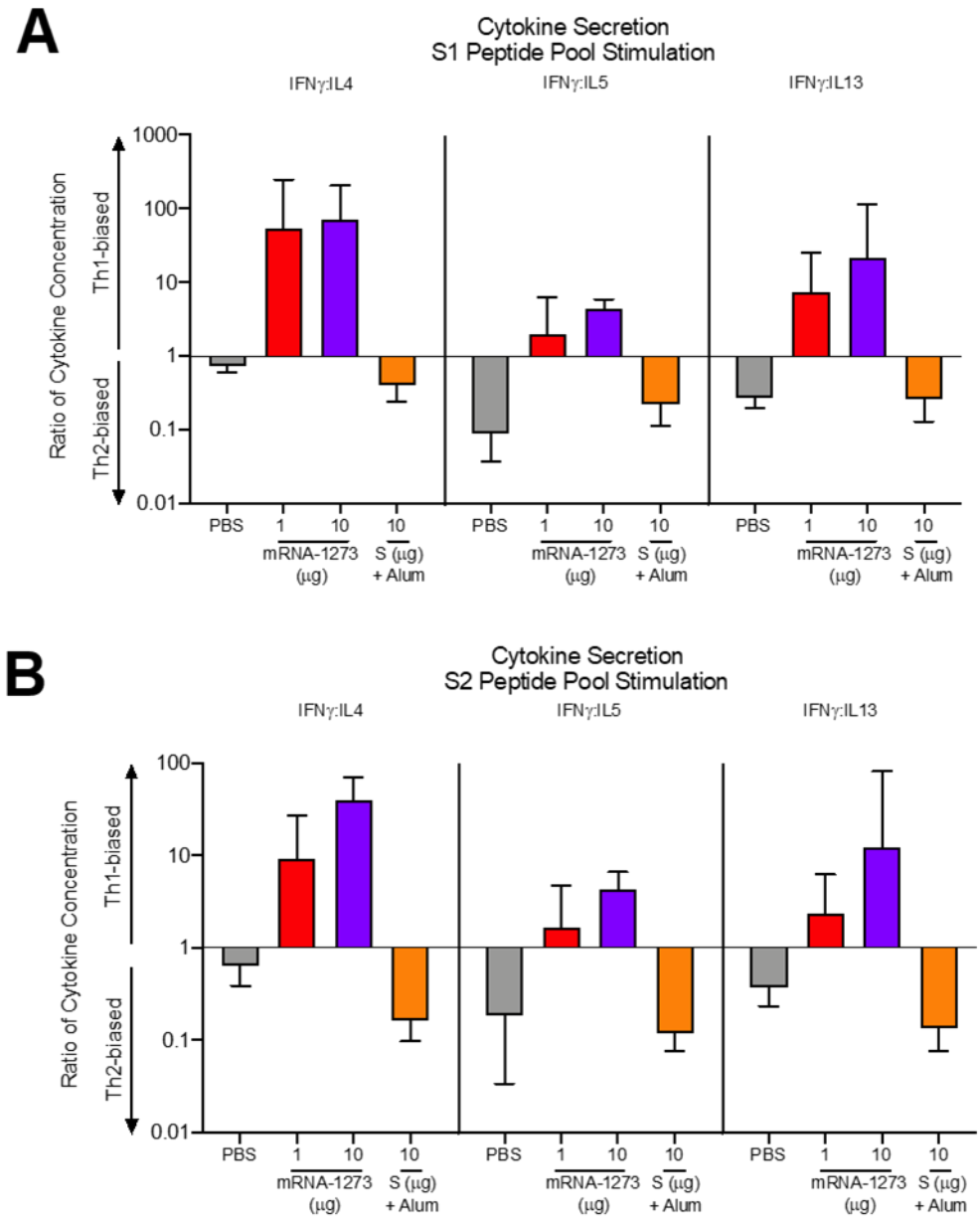


Abbreviations: (b) (4) = aluminum hydroxide; 2wp2 = 2 weeks post-boost; CoV2 = SARS-CoV-2; CoV-2 protein = SARS-CoV-2 S-2P; grp(s) = group(s); Ig = immunoglobulin; LOD = limit of detection; PBS = phosphate-buffered saline; S = spike; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus.

Note: Dotted line indicates the assay limit of detection.

Source: Report MOD-3937 (Figure 3).

Figure 12: Cytokine Levels in In Vitro Restimulated Splenocytes From Young BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With a 2-Week Interval) – Report MOD-3937



Abbreviations: alum = aluminum hydroxide; IFN = interferon; IL = interleukin; PBS = phosphate-buffered saline; S = SARS-CoV-2 S-2P protein; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; Th1 = T helper 1; Th2 = T helper 2.

Source: Report MOD-3937 (Figure 5).

2.6.2.2.4 Immunogenicity and Determination of Titer Dynamic Range of mRNA-1273 in Young BALB/c Mice

The objectives were to evaluate the immunogenicity and determine the titer dynamic range of the immune responses to mRNA-1273 (Study MOD-3938), repeated for confirmation of dose responses (Study MOD-3940), in young BALB/c mice (Report MOD-3938/MOD-3940).

Methods:

Female young (approximately 8 to 9 weeks old) BALB/c mice were treated according to the study design presented in Table 5. In both Study MOD-3938 and Study MOD-3940, sera were collected on Day 21 (1 day pre-boost) and Day 36 (2 weeks post-boost) for the assessment of SARS-CoV-2 S-2P binding antibodies, as measured by ELISA.

In Study MOD-3938, sera collected on Day 36 were also tested for RBD-, NTD-, and S1 subunit-specific binding antibodies (all groups), as measured by ELISA, and for neutralizing antibodies against a homotypic SARS-CoV-2 pseudovirus (0.02 through 20 µg groups). Spleens were collected on Day 36 (0.04 through 20 µg groups) for the evaluation of cytokine responses to in vitro restimulation with S peptide pools, as measured by flow cytometry analysis of cell surface and intracellular staining.

In Study MOD-3940, antigen-reactive IgG antibody-secreting cells (ASCs) collected from spleens and draining lymph nodes on Day 36 (doses from 10 and 1.25 µg) were analyzed, as measured by ELISpot.

Table 5: Study Design – Report MOD-3938/MOD-3940

mRNA-1273 Dose (µg)	Number of Mice[a]	Immunization Schedule[b]		Sample Collection; Experimental Assessments	
		Prime	Boost	MOD-3938	MOD-3940
20	8	Week 0 (Day 1)	Week 3 (Day 22)	Week 3 (Day 21) and Week 5 (Day 36); sera for SARS-CoV-2 bAbs (SARS-CoV-2 S-2P and subunits RBD-, NTD-, and S1-specific IgG), and nAbs (pseudovirus)[e] Week 5 (Day 36); spleens for cytokine analysis[f]	Week 3 (Day 21) and Week 5 (Day 36); sera for SARS-CoV-2 S-2P bAbs (total IgG) Week 5 (Day 36); LN and spleens for ASC analysis[h]
10	8				
5	8				
2.5	8				
1.25	8				
0.63	8				
0.32	8				
0.16	8				
0.08	4/8[c]				
0.04	8				
0.02	8				
0.01	8				
0.005	8				
0.0025	8				
0[d]	4/8[c]				

Abbreviations: ASC = antibody-secreting cells; bAbs =binding antibody; LN = lymph nodes; nAbs = neutralizing antibodies; NTD = N-terminal domain; PBS = phosphate-buffered saline; RBD = receptor binding domain; S1 = spike S1 subunit; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus.

[a] All mice were female BALB/c mice approximately 8- to 9-weeks old . Total number of mice was n =112 in Study MOD-3938 study and n = 120 in Study MOD-3940.

[b] Treatment dose in 50 µL dose volume was administered via IM injection into the right hind leg.

[c] n = 4 in Study MOD-3938 and n = 8 in Study MOD-3940.

[d] PBS 1x as negative-control.

[e] Serum neutralizing antibody titers were assessed in mice from the 0.02 through 20 µg groups.

[f] Spleens were collected from mice in the 10 highest mRNA-1273 dose levels (0.04 through 20 µg/dose) and from the control (PBS) mice.

[h] Lymph nodes and spleens were collected from mice immunized with 1.25 and 10 µg/dose of mRNA-1273.

Source: Report MOD-3938/MOD-3940; Table 2 (MOD-3938) and Table 3 (MOD-3940).

Results:

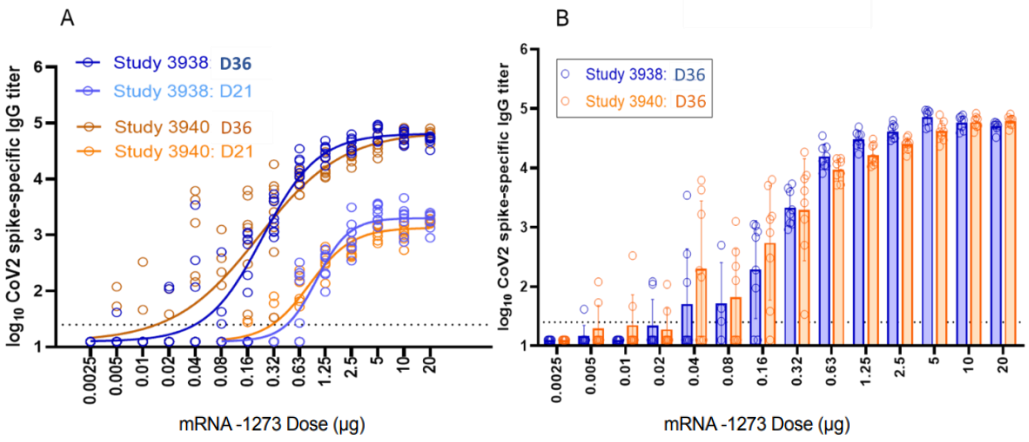
Immunization of young BALB/c mice with mRNA-1273 induced a dose-dependent response in SARS-CoV-2 whole S protein-specific IgG binding antibody titers that was similar across the 2 studies (Study MOD-3938 and Study MOD-3940) (Figure 13). In Study MOD-3938, neutralizing antibody titers showed similar dose-dependent responses with a strong correlation between binding and neutralizing antibody titers ($r > 0.9$) (Figure 14); in addition, RBD-, NTD-,

and S1 subunit-specific IgG antibody titers also showed similar dose-dependent responses (Report MOD-3938/MOD-3940 [Figure 3]; Study MOD-3938). mRNA-1273 induced IgG antigen-reactive ASCs in both the spleen and lymph nodes in a dose-dependent manner (Report MOD-3938/MOD-3940 [Figure 4]; Study MOD-3940). mRNA-1273 produced a Th1-directed CD4+ T-cell response and a CD8+ T-cell responses in splenocytes restimulated in vitro with S1 and S2 subunit overlapping peptide pools encompassing the entire SARS-CoV-2 S protein. Although a dose-dependent CD4+ Th1 cytokine response was not observed, the CD8+ T-cell response decreased with decreasing dose (Report MOD-3938/MOD-3940 [Figure 5 and Figure 6]; Study MOD-3938).

Conclusions:

Overall, these results demonstrate that mRNA-1273 is immunogenic and induces dose-dependent binding and neutralizing antibodies in young BALB/c mice at doses ranging from 0.02 to 20 µg. In addition, a strong correlation between binding and neutralizing antibody titers was demonstrated.

Figure 13: SARS-CoV-2 Spike-Specific Binding Antibody Titers 1 Day Pre-boost and 2 Weeks Post-boost in Young BALB/c Mice Immunized with mRNA-1273 (Prime/Boost With 3-Week Interval) — Report MOD-3938/MOD-3940

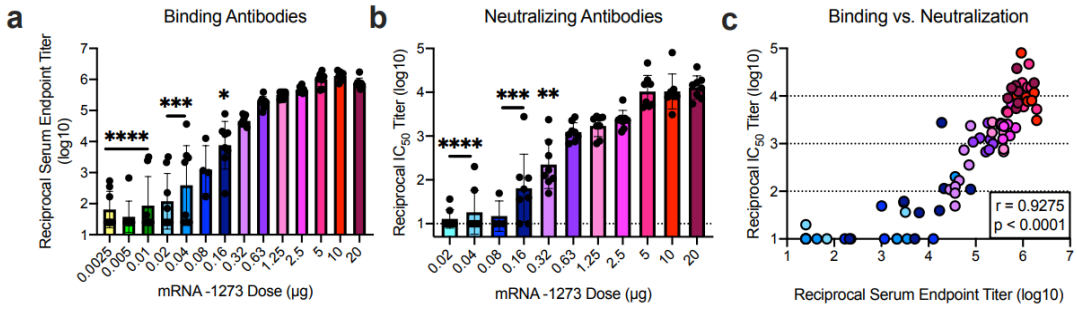


Abbreviations: CoV2 = SARS-Cov-2; D = day; Ig = immunoglobulin; SARS-Cov-2 = 2019 novel coronavirus.

Notes: Figure A shows 4-parameter curve of log10 titer vs log10 dose at both Day 21 (1 day pre-boost) and Day 36 (2 weeks post-boost). Figure B shows mean log10 titer and standard deviation for each dose level at Day 36 of both study MOD-3938 and Study MOD-3940. Geometric means or arithmetic means are represented by the heights of bars or symbols; error bars represent the corresponding SD.

Source: Report MOD-3938/MOD-3940 (Figure 1).

Figure 14: Pseudovirus Neutralizing Antibody Titers and Correlation to Binding Antibody Titers in Young BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report MOD-3938/MOD-3940 (Study MOD-3938)



Abbreviations: IC_{50} = half-maximal inhibitory concentration.

Note: Geometric means or arithmetic means are represented by the heights of bars or symbols; error bars represent the corresponding standard deviation (SD). *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$.

Source: Report MOD-3938/MOD-3940 (Figure 2).

2.6.2.2.5 Immunogenicity and Characterization of Cellular Response in Young BALB/cJ Mice

The objectives were to evaluate the immunogenicity of the mRNA-1273, to address the potential risk of ERD, and determine the level of protection from viral challenge in young female BALB/c mice as compared to alum-adjuvanted Th2 skewing vaccine regimens (Report VRC05).

Methods:

Female young (approximately 6 to 8 weeks old) BALB/cJ mice were treated according to the study design presented in Table 6. Sera were collected on Day 35 (2 weeks post-boost) and assessed for SARS-CoV-2 S-2P binding antibodies (including total IgG and IgG1 and IgG2a subclasses) as measured by ELISA; IgG subclass ratios (IgG2a:IgG1) were calculated. Sera were also tested for neutralizing antibodies, as measured by pseudotyped lentivirus reporter neutralization assay. Spleens were collected on Day 36 and 39 (2 weeks post-boost) from a subset of mice (n = 5/group) for the evaluation of cytokine responses to in vitro restimulation with S peptide and N peptide pools, as measured by flow cytometry analysis of cell surface and intracellular immunostaining.

Mice were challenged (10^4 plaque-forming units [PFU] of a SARS-CoV-2 MA10 mouse-adapted virus) 4 weeks post-boost. Body weight was measured through Day 7 post-challenge. Lung and nasal turbinate samples were collected on Day 2, Day 4, and Day 7 post-challenge for the evaluation of viral titers (Day 2 and Day 4 samples) as measured by plaque assay, lung histopathology (Day 4 lung samples) as assessed by severity of inflammation (primarily

mononuclear and polymorphonuclear cells), immunohistochemistry (IHC) (using anti-SARS-CoV-2, or an anti-eosinophil major basic protein [EMBP] staining), and gross hemorrhage evaluation (Day 2, Day 4 and Day 7 lung samples) (Score 0 [no hemorrhage] to 4 [severe hemorrhage]).

Table 6: Study Design – Report VRC05

Treatment Group[a]	Immunization Schedule[b]		Challenge[c]	Sample Collection; Experimental Assessments
	Prime	Boost		
PBS	Week 0 (Day 1)	Week 3 (Day 22)	Week 7 (Day 54)	Week 5 (Day 35); sera for SARS-CoV-2 S-2P bAbs (total IgG, IgG1, and IgG2a) and nAbs (pseudovirus)
0.2 µg of SARS-CoV-1 DIV + alum				
1 µg of SARS-CoV-1 DIV + alum				
0.2 µg of CDS + alum				
1 µg of CDS + alum				Week 5 (Day 36 and 39); spleens for cytokine analysis
0.1 µg of mRNA-1273				
1 µg of mRNA-1273				Day 1 to Day 7 post-challenge[d]; weight measurement Day 2, Day 4, and Day 7 pot-challenge[d]; lungs for lung hemorrhage Day 2 and Day 4 post-challenge[d]; lungs and NT for viral titers Day 4 post-challenge[d]; lungs for histopathology: IHC[e] and inflammation scoring[f]

Abbreviations: alum = aluminum hydroxide; bAbs = binding antibodies; CDS = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein; EMBP = eosinophil major basic protein; Ig = immunoglobulin; IHC = immunohistochemistry; IM = intramuscular; nAbs = neutralizing antibodies; NT = nasal turbinates; PBS = phosphate-buffered saline; PFU = plaque-forming units; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-1 DIV = double-inactivated severe acute respiratory syndrome coronavirus-1; SARS-CoV-2 = 2019 novel coronavirus.

[a] All mice were female 6- to 8-weeks old BALB/cJ; n = 30 mice/group.

[b] Treatment dose was administered via IM injection into the hind leg(s) with a volume of 50 to 100 µL.

[c] Mice (n = 20 mice/group) were intranasally challenged with 10^4 PFU with SARS-CoV-2 MA10. SARS-CoV-2 MA10 virus is a virus adapted in mice by 10 in vivo passages in addition to the 2 targeted amino acid mutations (Q498T/P499Y) in the receptor binding domain (RBD) that allow for mouse angiotensin-converting enzyme 2 [ACE-2] receptor binding (Leist et al 2020).

[d] Day 2 and Day 4 post-challenge: n = 5 mice/group; Day 7 post-challenge: n = 8 mice/group.

[e] IHC performed using anti-SARS-CoV-2 or EMBP staining in lung samples.

[f] Inflammation severity was graded based on the severity scoring of neutrophil infiltrate and the proportion of airspace areas area affected.

Source: Report VRC05 (Table 2).

Results:

Mice immunized with an optimal dose (1 µg) of mRNA-1273 showed significantly higher SARS-CoV-2 S-2P IgG binding antibody titers and neutralizing antibody titers than mice immunized with suboptimal dose (0.1 µg) of mRNA-1273 or the SARS-CoV-1 DIV and CDS (a conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein) alum-adjuvanted controls at suboptimal (0.2 µg) and optimal (1 µg) dosing (Figure 15).

Evaluation of the IgG subclass ratio and T-cell cytokine data demonstrate that immunization with mRNA-1273 results in a Th1-directed CD4+ T-cell response; a robust CD8+ T-cell response was also measured. Immunization with 0.1 and 1 µg of mRNA-1273 elicited an S-binding antibody response with a higher IgG2a/IgG1 ratio than immunization with the controls as well as higher expression of the Th1-associated cytokines (IFN-γ, TNF-α, and IL-2) than the Th2-associated cytokines (IL-4, IL-5, and IL-13) (Figure 16 and Report VRC05 [Figure 3]). In addition, mRNA-1273 immunization elicited SARS-CoV-2-specific polyfunctional CD4+ Th1 and CD8+ T-cell responses, which may play an important role for protection (Oh et al 2012; Boyd et al 2015). In contrast, SARS-CoV-1 DIV and CDS alum-adjuvanted Th2-skewing positive vaccine controls induced a T-cell cytokine profile and produced a IgG antibody subclass distinct from that of mRNA-1273, indicating that mRNA-1273 elicits an immune profile that is distinct from positive vaccine controls and unlikely to be associated with ERD.

Analysis of the pathological features in lung tissues after virus challenge not only determine the pathogenesis of SARS-CoV-2 disease but also may yield data indicating the potential for ERD. Mice immunized with mRNA-1273 or the SARS-CoV-1 DIV and CDS alum-adjuvanted controls showed little or no weight loss and no evidence of lung hemorrhage 7 days after SARS-CoV-2 MA10 challenge as compared to the PBS control group (Report VRC05 [Figure 4]).

At Day 4 post-challenge, mice immunized with a 0.1 µg of mRNA-1273 dose (suboptimal dose) showed partial protection from viral replication in the lungs and nasal turbinates. However, mice vaccinated with 1 µg of mRNA-1273 dose (optimal dose) were completely protected from SARS-CoV-2 MA10 viral challenge in the upper and lower airways; this protection was higher than that provided by SARS-CoV-1 DIV and CDS alum-adjuvanted controls at optimal dosing (Figure 17) (Bolles et al 2011, Tseng et al 2012). No lung hemorrhage and little or no weight loss was observed post-challenge in mice immunized with mRNA-1273 or the active vaccine controls (Report VRC05 [Figure 4] and [Figure 5]), indicating no evidence of disease enhancement.

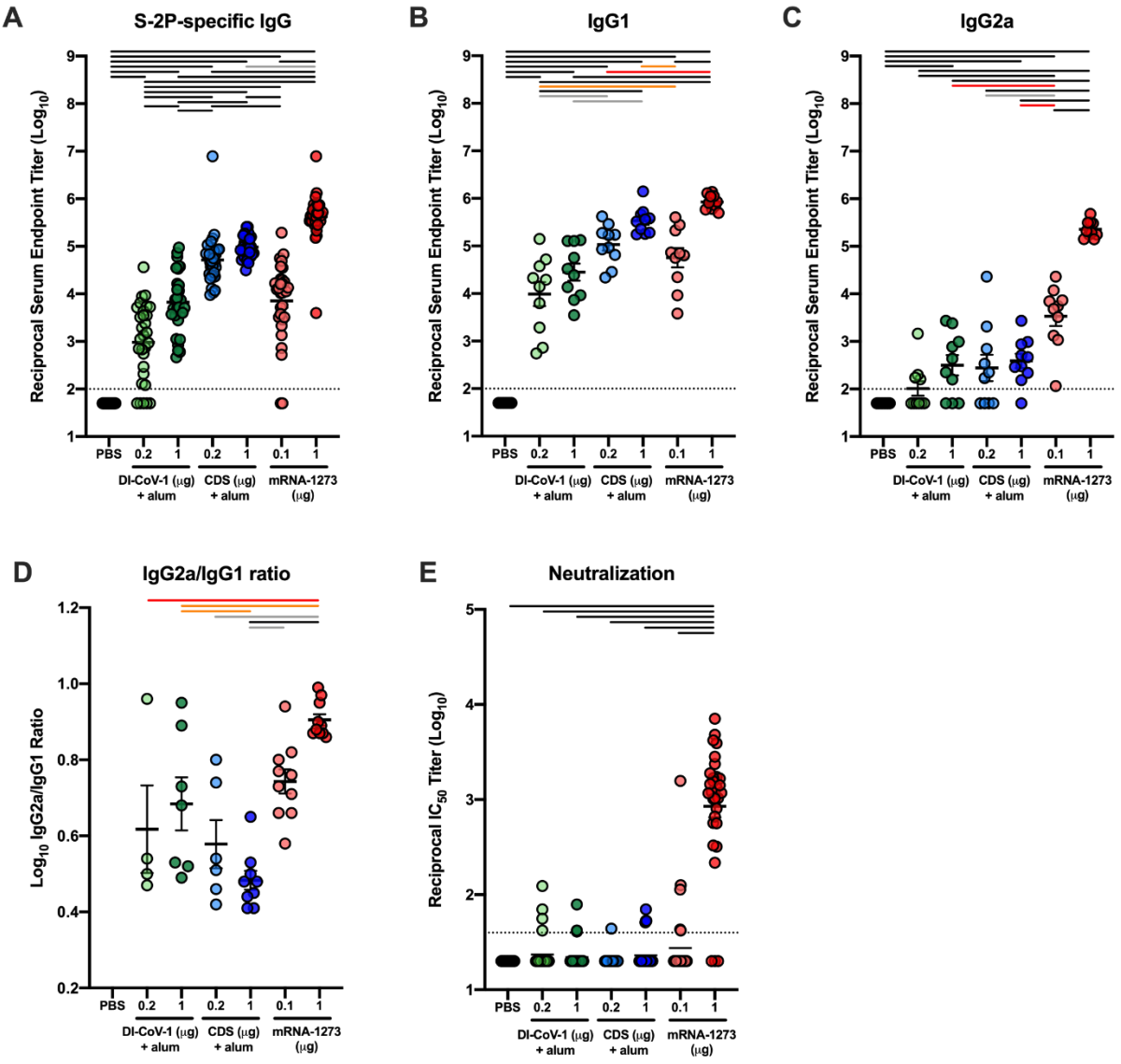
Furthermore, on Day 4 post-challenge, histopathological changes in the lungs of mRNA-1273-immunized animals were characterized by decreased inflammation, little to no viral

antigens, and sparse or minimally observed eosinophils, particularly after immunization with the optimal dose (1 μg). Animals immunized with SARS-CoV-1 DIV and CDS alum-adjuvanted controls had less abundant viral antigens but more severe histopathological alterations in the lungs than animals in the PBS control group. This increase in severity was associated with an abundance of eosinophils that were often closely associated with circumscribed airways and blood vessels (Figure 18).

Conclusions:

Overall, mRNA-1273 was immunogenic, efficacious and did not show evidence of promoting ERD even at subprotective dose in young BALB/cJ mice.

Figure 15: Spike-Specific Binding and Neutralizing Antibody Titers in Young BALB/cJ Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC05

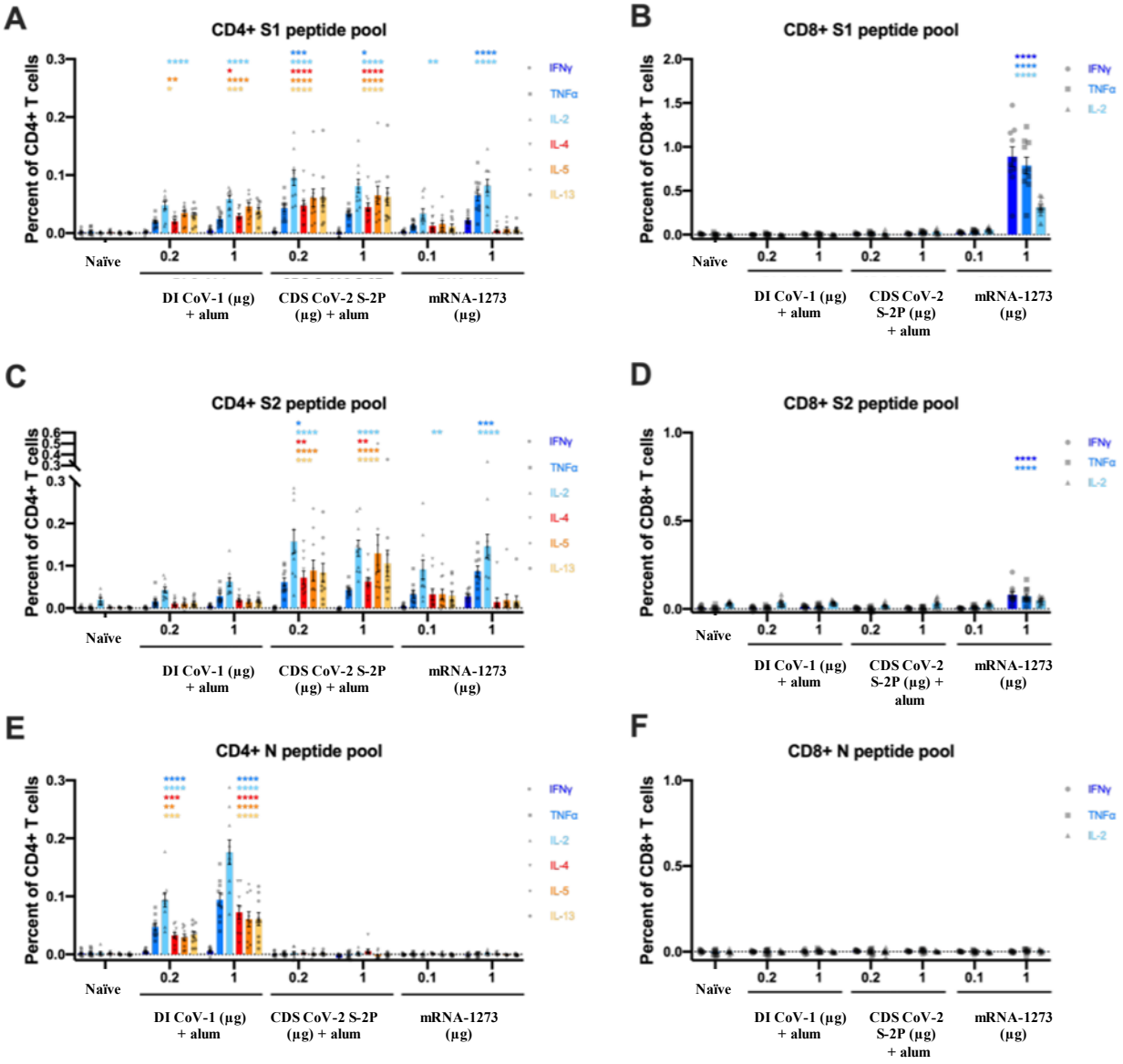


Abbreviations: alum = aluminum hydroxide; CDS + alum = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein alum adjuvanted; DI-CoV-1 + alum (SARS-CoV-1 DIV alum adjuvanted) = double-inactivated severe acute respiratory syndrome coronavirus-1 alum adjuvanted; IC_{50} = concentration of vaccine producing 50% inhibition; Ig = immunoglobulin; PBS = phosphate-buffered saline; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; S-2P = spike protein with 2 proline substitutions.

Note: — $p \leq .05$, — $p \leq .01$, — $p \leq .001$, — $p \leq .0001$.

Source: Report VRC05 (Figure 1).

Figure 16: In Vitro Cytokine Response in Splenocytes From Young BALB/cJ Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC05

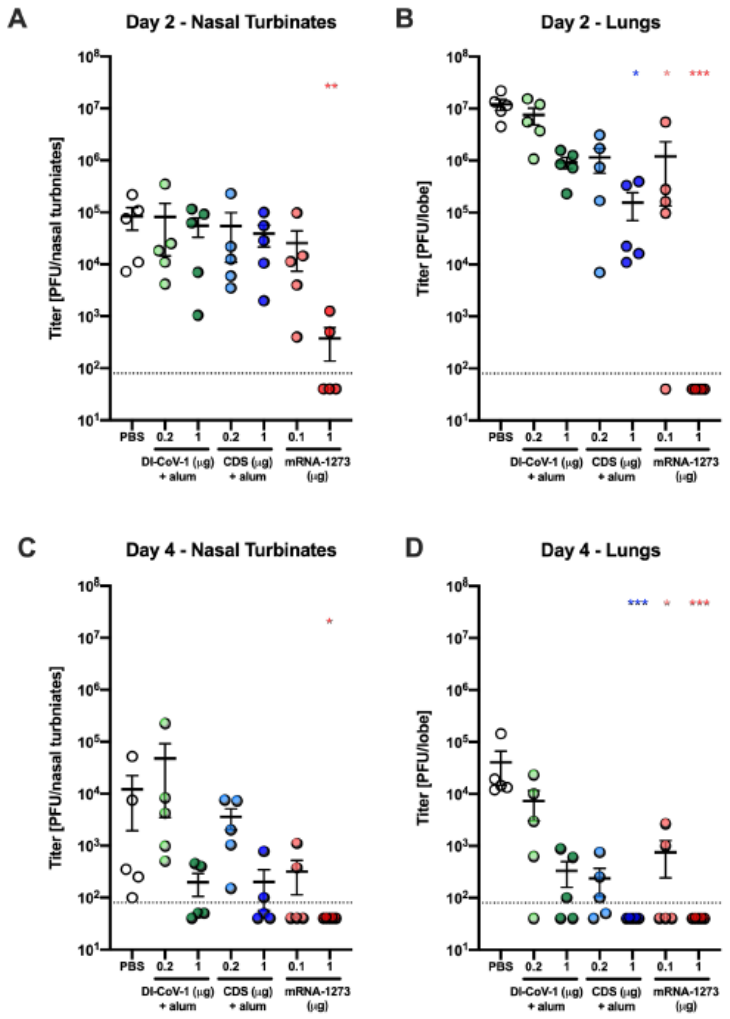


Abbreviations: CDS CoV-2 S-2P (CDS) + alum = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein alum adjuvanted; DI-CoV-1 + alum (SARS-CoV-1 DIV alum adjuvanted) = double-inactivated severe acute respiratory syndrome coronavirus-1 alum adjuvanted; IFN = interferon; IL = interleukin; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; TNF= tumor necrosis factor.

Note: Each symbol represents an individual mouse, bars represent geometric mean titers, and error bars indicate SD. *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$.

Source: Report VRC05 (Figure 2).

Figure 17: Viral Loads 2 Days and 4 Days Post-Viral Challenge in Lungs and Nasal Turbinates of Young BALB/cJ Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC05

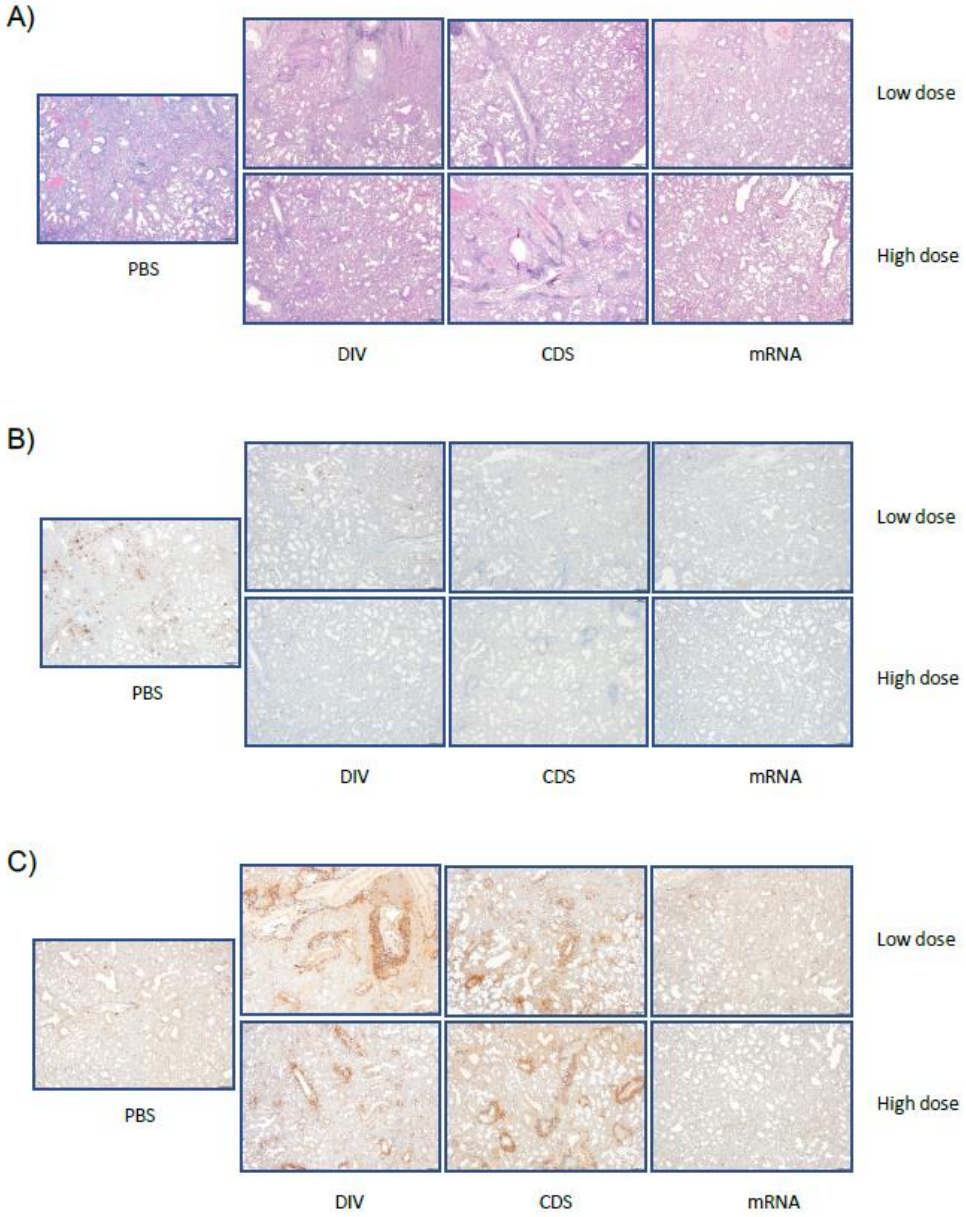


Abbreviations: CDS (CDS alum adjuvanted) = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein alum adjuvanted; DI-CoV-1 (SARS-CoV-1 DIV alum adjuvanted) = double-inactivated severe acute respiratory syndrome coronavirus-1 alum adjuvanted; PBS = phosphate-buffered saline; PFU = plaque-forming units.

Note: Suboptimal dose: 0.2 µg for DI-CoV-1 (SARS-CoV-1 DIV) and CDS alum adjuvanted groups and 0.1 µg for mRNA-1273 group. Optimal dose: 1 µg for all groups. Each dot represents an individual mouse, bars represent geometric mean titers, and error bars indicate SD. Dotted line indicates the assay limit of detection. *$p < .05$, **$p < .01$, ***$p < .001$.

Source: Report VRC05 (Figure 6).

Figure 18: Lung Histopathology 4 Days Post-Viral Challenge in Lungs of Young BALB/cJ Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC05



Abbreviations: CDS (CDS alum adjuvanted) = conformationally disrupted severe acute respiratory syndrome coronavirus-2 S protein alum adjuvanted; DIV (SARS-CoV-1 DIV alum adjuvanted) = double-inactivated severe acute respiratory syndrome coronavirus-1 alum adjuvanted; PBS = phosphate-buffered saline.

Note: A) Hematoxylin and eosin staining of lung section to show severity of inflammation. B) Immunohistochemistry for SARS-CoV-2 antigen-positive cells. C) Immunohistochemistry for eosinophil major basic protein. Suboptimal dose: 0.2 µg for DI-CoV-1 (SARS-CoV-1 DIV) and CDS alum adjuvanted groups and 0.1 µg for mRNA-1273 group. Optimal dose: 1 µg for all groups.

Source: Report VRC05 (Figure 7).

2.6.2.2.6 Efficacy of mRNA-1273 and Enhanced Respiratory Disease in Aged BALB/c Mice

The objective was to evaluate the immunogenicity of mRNA-1273 and protection from SARS-CoV-2 challenge and to determine if a subprotective level of immunity leads to increased viral loads or disease, compared with double-inactivated severe acute respiratory syndrome coronavirus-1 (SARS-CoV-1 DIV) in an aged mouse model (Report VRC02).

Methods:

Female BALB/c mice, approximately 1-year-old were treated according to the study design presented in Table 7. Sera were collected at Week 2 (2 weeks post-prime) and Week 5 (2 weeks post-boost) and assessed for SARS-CoV-2 S-2P binding antibodies (including total IgG and IgG1 and IgG2a/c subclasses) as measured by ELISA; IgG subclass ratios (IgG2a:IgG1) were calculated (only Week 5). Sera were also tested for neutralizing antibodies, as measured by pseudotyped lentivirus reporter neutralization assay.

Immunized mice were challenged with 10^3 PFU of SARS-CoV-2 MA10 at 4 weeks post-boost. Body weight was measured through Day 4 post-challenge. Lungs and nasal turbinates were collected on Day 2 and Day 4 post-challenge for the evaluation of viral titers as measured by plaque assays, lung cytokines as measured by relative cytokine RNA levels by quantitative reverse transcription (qRT) - polymerase chain reaction (PCR), and histopathology assessment of lung samples including IHC (using anti-SARS-CoV-2, or anti-EMBP staining). Evaluation of gross hemorrhage in the lung lobes was performed in collected lungs (Score 0 [no hemorrhage] to 4 [severe hemorrhage]).

Table 7: Study Design - Report VRC02

Treatment Group[a]	Immunization Schedule[b]		Challenge[c]	Sample Collection; Experimental Assessments
	Prime	Boost		
PBS	Week 0 (Day 1)	Week 3 (Day 22)	Week 7 (Day 50)[a]	Week 2 (Day 15) and Week 5 (Day 36); sera for SARS-CoV-2 S-2P bAbs (total IgG, IgG1, IgG2a) and nAbs (pseudovirus)
0.1 µg of mRNA-1273				
1 µg of mRNA-1273				Day 1 to Day 4 post- challenge; weight measurement.
0.1 µg of stock SARS-CoV-1 DIV + alum				Day 2 and Day 4 post-challenge lungs for lung hemorrhage; lungs and NT for histopathology[d] (including IHC)[e] and cytokines analysis; and NT for viral titers

Abbreviations: bAbs = binding antibodies; EMBP= eosinophil major basic protein; H&E = hematoxylin and eosin; IHC = immunohistochemistry; nAbs = neutralizing antibodies; NT = nasal turbinates; PBS = phosphate-buffered saline; PFU = plaque-forming unit; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-1 DIV = double-inactivated severe acute respiratory syndrome coronavirus-1; SARS-CoV-2 = 2019 novel coronavirus.

[a] Groups composed of aged (approximately 1 year old) female BALB/c mice.

[b] Treatment dose in 50 µL was administered via IM injection into the right hind leg.

[c] Mice were intranasally challenged with 10^3 PFU of SARS-CoV-2 MA10. SARS-CoV-2 MA10 virus is a virus adapted in mice by 10 in vivo passages in addition to the 2 targeted amino acid mutations (Q498T/P499Y) in the receptor binding domain (RBD) that allow for mouse angiotensin-converting enzyme 2 [ACE-2] receptor binding (Leist et al 2020).

[d] Inflammation assessed by H&E staining in lung samples.

[e] IHC performed using anti-SARS-CoV-2 or anti-EMBP staining in lung samples.

Source: Report VRC02 (Table 2).

Results:

mRNA-1273 elicited a robust binding and neutralizing antibody response in aged BALB/c mice in a dose-dependent manner after a prime/boost immunization schedule. mRNA-1273 groups showed significantly higher IgG binding antibody titers and neutralizing antibody response post-boost compared with the SARS-CoV-1 DIV alum adjuvanted group (Figure 19). Immunization with suboptimal (0.1 µg) and optimal (1 µg) doses of mRNA-1273 elicited a balanced IgG2a/IgG1 response. In contrast, SARS-CoV-1 DIV alum adjuvanted elicited SARS-CoV-2 S-2P IgG1 binding antibodies post-boost, but IgG2a subclass binding antibodies were not detected and therefore the IgG2a/IgG1 ratio could not be calculated. These results indicate that SARS-CoV-1 DIV alum adjuvanted induces a Th2-directed response (Bolles et al 2011) while mRNA-1273 is associated with a Th1-directed response that does not promote ERD (Report VRC02 [Figure 2]).

mRNA-1273 protected aged mice from SARS-CoV-2 infection in a dose-dependent manner. Mice immunized with optimal (1 µg) dose of mRNA-1273 were completely protected from SARS-CoV-2 MA10 viral challenge. Weight loss was not observed through Day 4 post-challenge (Figure 20A), viral titers in the lungs and nasal turbinates were below the limit of detection (LOD) at Day 4 post-challenge (Figure 20B-E), and no lung hemorrhage was observed at Day 2 or Day 4 post-challenge (Figure 20F and Figure 20G). Immunization with suboptimal (0.1 µg) dose of mRNA-1273 provided partial protection, characterized by weight loss, viral replication, and lung hemorrhage that was less than that observed in the SARS-CoV-1 DIV alum adjuvanted and PBS control groups (Figure 20).

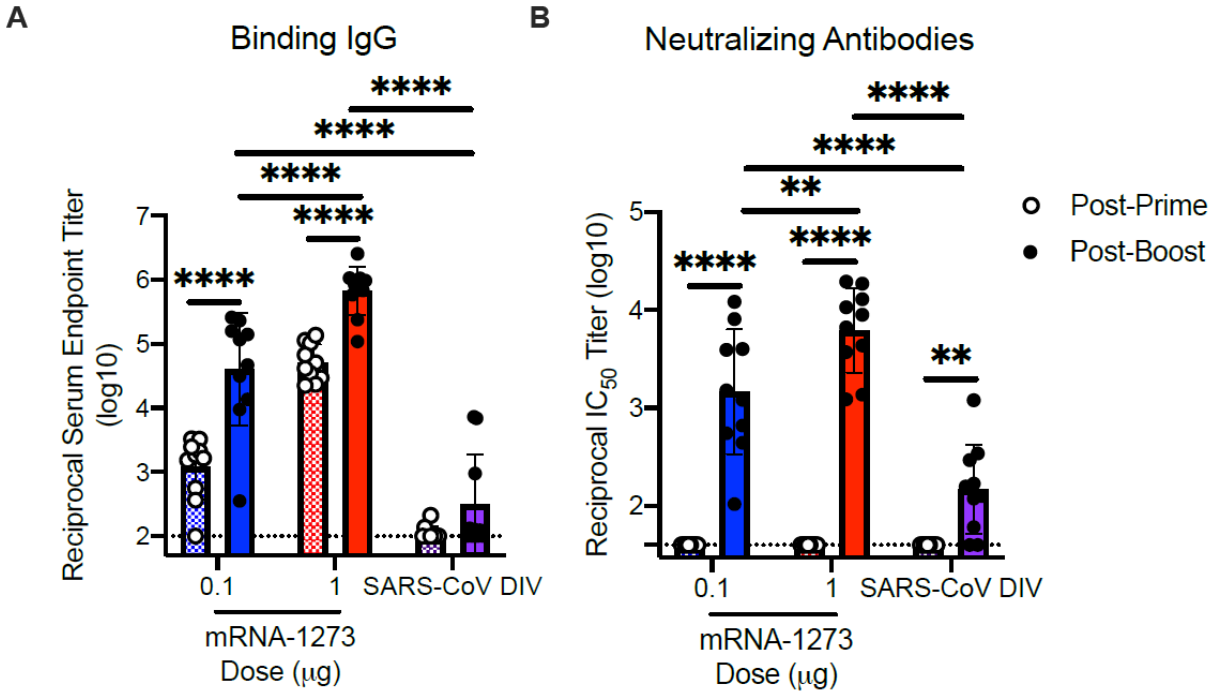
Th2-associated cytokine and chemokine expression and inflammatory cytokine expression in the SARS-CoV-1 DIV alum adjuvanted group were higher than in the PBS control group, indicating an exacerbated cytokine response suggestive of ERD. In contrast, mRNA-1273 induced a balanced Th1-directed cytokine response with lower expression of inflammatory cytokines than SARS-CoV-1 DIV alum-adjuvanted and PBS control groups, indicating a noninflammatory immune response (Report VRC02 [Figure 4]).

Lung histopathology and immunochemistry suggested no alterations related to ERD or inflammation and with no virus antigen-positive cells in immunized mice with optimal mRNA-1273 dose on Day 2 and Day 4 post-challenge (Report VRC02 [Figure 5]). Lung sections from immunized mice with suboptimal mRNA-1273 dose (0.1 µg) showed inflammation and SARS-CoV-2 virus antigen-positive cells on Day 2 post-challenge; however, the number of viral antigen-positive cells were substantially reduced by Day 4 post-challenge. The SARS-CoV-1 DIV alum-adjuvanted and PBS control groups showed marked alterations related to enhanced disease and inflammation with abundant SARS-CoV-2 virus antigen-positive cells (Report VRC02 [Figure 5]).

Conclusions:

Overall, mRNA-1273 was demonstrated to be immunogenic in a dose-dependent manner, efficacious, and did not induce vaccine-associated ERD in aged mice.

Figure 19: Binding and Neutralizing Antibody Titers in Aged BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC02

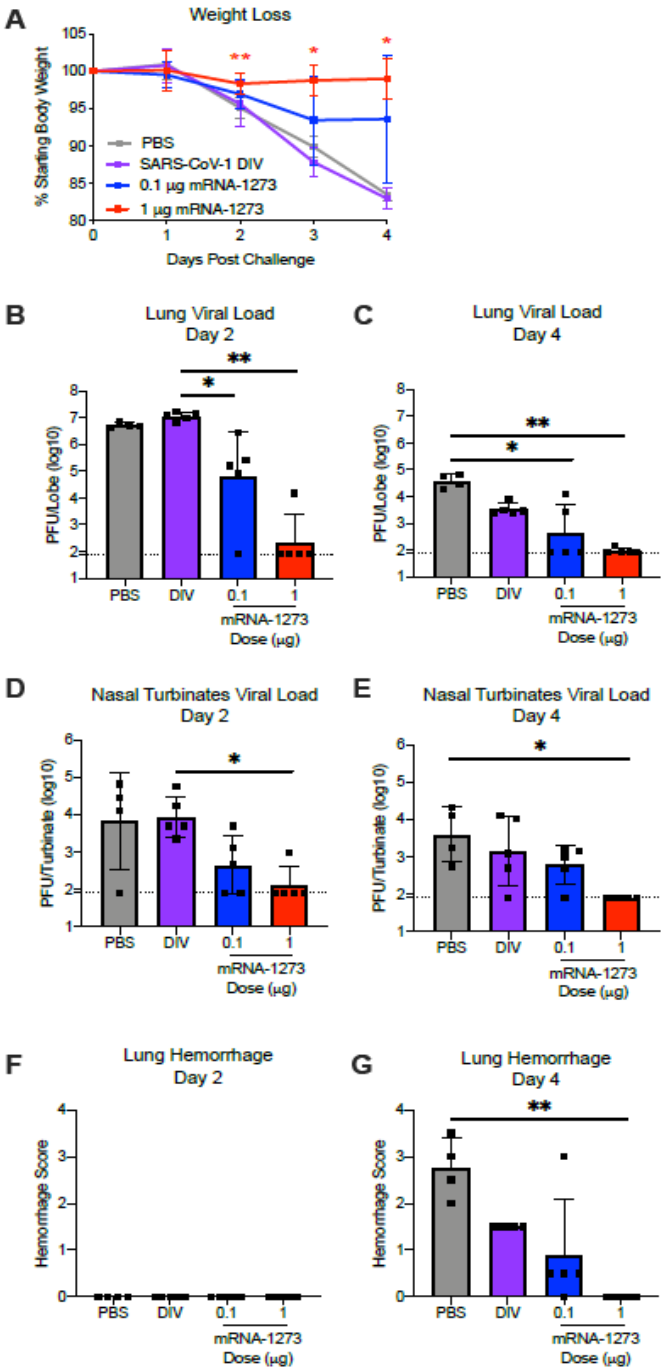


Abbreviations: IC_{50} = concentration of vaccine producing 50% inhibition; Ig = immunoglobulin; SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2; SARS-CoV DIV (SARS-CoV-1 DIV alum adjuvanted) = double-inactivated severe acute respiratory syndrome coronavirus-1 alum adjuvanted.

Note: ** $p < .01$, **** $p < .0001$.

Source: Report VRC02 (Figure 1).

Figure 20: Weight Change, Viral Load, and Lung Hemorrhage Scores 2 Days and 4 Days Post-Challenge in Aged BALB/c Mice Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report VRC02



Abbreviations: DIV (SARS-CoV-1 DIV alum-adjuvanted) = double-inactivated severe acute respiratory syndrome coronavirus-1 alum adjuvanted; PBS = phosphate buffered saline; PFU = plaque-forming unit

Note: Lung hemorrhage was measured on a score ranging from 0 (no hemorrhage in any lobe) to 4 (extreme and complete hemorrhage in all lung lobes). * $p < .05$, ** $p < .01$.

Source: Report VRC02 (Figure 3).

2.6.2.2.7 A Non-GLP Repeat-Dose Immunogenicity and Toxicity Study of mRNA-1273 by Intramuscular Injection in Sprague Dawley Rats

The immunogenicity of mRNA-1273 as measured by SARS-CoV-2 S-2P-specific antibody titers was evaluated in a non-GLP compliant pharmacology study with safety endpoints in Sprague Dawley rats (Report 2308-123; Section 2.6.6.11).

Methods:

Sprague Dawley rats were immunized IM (200 µL) with 0, 30, 60, or 100 µg of mRNA-1273 or control article (nCoV Formulation Buffer) on a prime (Day 1)/boost (Day 22) schedule (females n = 5 and males n = 5 per dosing group) (Table 8). Sera were collected before dosing on Day 1 and on Day 35 for analysis of SARS-CoV-2 S-2P binding antibodies as measured by ELISA.

Results:

Immunization with 30, 60, and 100 µg doses of mRNA-1273 elicited substantial antibody titers in Sprague Dawley rats 13 days after the second (boost) dose in a dose-independent manner.

Table 8: Binding Antibody Titers in Sprague Dawley Rats Immunized With mRNA-1273 (Prime/Boost With 3-Week Interval) – Report 2308-123

	mRNA-1273 Dose (µg)			
Sex	**0[a]**	**30**	**60**	**100**
M	LOQ[b]	2,486,970.54	3,571,545.26	2,361,125.79
F	LOQ	4,492,100.43	3,221,503.68	4,949,493.90

Abbreviations: F = female; LOQ = lower limit of quantitation; M = male.

[a] Control article is an nCoV Formulation Buffer composed of tris(hydroxymethyl)aminomethane and Sucrose Buffer.

[b] LOQ is ≤ 100.00

Note: Pretest antibody titer levels were not detectable and therefore are not presented.

Source: Report 2308-123 (Text Table 6 and Text Table 7).

Conclusions:

mRNA-1273 was immunogenic in Sprague Dawley rats, with high levels of binding antibody measured at all 3 dose levels assessed.

2.6.2.2.8 Protection From WT SARS-CoV-2 in Golden Syrian Hamsters Using Optimal and Suboptimal Doses of mRNA-1273

The objectives were to evaluate the immunogenicity of mRNA-1273 and protection it provides from SARS-CoV-2 challenge in golden Syrian hamsters (Report UTMB01).

Methods:

Female golden Syrian hamsters (6 to 7 weeks old) were treated according to the study design presented in Table 9. Sera were collected pre-boost (Day 21) and pre-challenge (Day 42) and assessed for SARS-CoV-2 binding antibodies (S-specific and RBD-specific IgG), as measured by ELISA. Sera collected were also tested for neutralizing antibodies, as measured by live virus plaque reduction assay.

At Week 6 (Day 43), hamsters were challenged intranasally with 10^5 PFU of WT SARS-CoV-2; a group of non-immunized animals were mock challenged with media to mimic virus inoculum. Body weight was measured through Day 14 post-challenge and sera were collected at Day 2, Day 4, and Day 14 post-challenge to assess SARS-CoV-2 binding antibodies (including S-specific IgG and nucleoprotein [NP]-specific IgG) as measured by ELISA. Sera were also tested for neutralizing antibodies, as measured by live virus plaque reduction assay. Lungs and nasal turbinates were collected on Day 2, Day 4, and Day 14 post-challenge for the evaluation of viral load (live but not actively replicating virus) as measured by viral plaque assays, and viral replicating virus (subgenomic RNA [sgRNA]) as measured by qRT-PCR. Histopathology assessment by IHC (using anti-SARS-CoV-2 staining) and inflammation (using hematoxylin and eosin [H&E] staining) were evaluated in lung samples collected on Day 2, Day 4, and Day 14 post-challenge.

Table 9: Study Design - Report UTMB01

Treatment Dose (µg)[a]	Schedule[b]			Sample Collection; Experimental Assessments
	Prime	Boost	Challenge	
PBS[c]	Week 0 (Day 1)	N/A	Week 6[d] (Day 43)	Week 3 (Day 21) and Week 6 (Day 42); Sera for bAbs (S-specific IgG and RBD-specific IgG) and nAbs (live virus) Day 1 to Day 14 post-challenge; weight measurement.
25 µg mRNA-1273				
25 µg mRNA-1273	Week 0 (Day 1)	Week 3 (Day 22)		Day 2, Day 4, and Day 14 post-challenge; Sera for bAbs (S-specific IgG and NP-specific IgG) and nAbs (live virus) Day 2, Day 4, and Day 14 post-challenge[e]; lungs and NT for viral load, viral replication and lungs for histopathology analysis[f]:; IHC[g]
5 µg mRNA-1273				
1 µg mRNA-1273				

Abbreviations: ELISA = enzyme-linked immunosorbent assay; H&E = hematoxylin and eosin; Ig = immunoglobulin; IM = intramuscular; N/A = not applicable; NP = nucleoprotein; PBS = phosphate-buffered saline; S = spike protein

[a] Groups (n = 15) composed of female 6 to 7 week old golden Syrian hamsters (Total n = 15/group).

[b] Treatment dose was administered via IM injection in each hind leg with a volume of 50 µL.

[c] PBS 1x as negative-control administered on a prime-only level (n =15).

[d] Hamsters were challenged intranasally with 10^5 PFU of WT SARS-CoV-2 (USA-WA1/2020) virus; a group of non-immunized (n = 5) animals were mock challenged with media to mimic virus inoculum.

[e] Day 2, Day 4 and Day 14: n = 5 hamsters/group.

[f] Inflammation assessed by H&E staining in lung samples.

[g] IHC performed using anti-SARS-CoV-2 staining in lung samples.

Source: Report UTMB01 (Table 2).

Results:

mRNA-1273 elicited robust S- and RBD-specific IgG antibody titers and neutralizing antibody responses in all animals in the prime/boost groups and a subset of animals in the prime-only group (Figure 21 and Report UTMB01 [Figure 2]). In general, the post-boost S- and RBD-specific IgG binding antibody titers and neutralizing antibody titers were higher than post-prime titers in animals immunized with 1, 5, and 25 µg of mRNA-1273. Complete neutralizing seroconversion in all animals was only achieved after administration of the boost dose. Post-boost (Day 42) RBD-specific IgG binding antibody titers and neutralizing antibody

titers were also higher in animals in the prime/boost groups than titers at Day 42 in animals immunized with a prime-only dose of 25 µg of mRNA-1273. The post-boost neutralizing antibody titers in animals immunized with 1, 5, and 25 µg of mRNA-1273 on a prime/boost schedule met or exceeded the highest titers observed in convalescent sera (Figure 21 and Report UTMB01 [Figure 2]).

Minimal weight loss (mean maximum weight loss of 2.25%) or little to no weight loss (6.2%) was observed post-challenge in immunized animals with prime-only or prime/boost mRNA-1273 schedule. In contrast, the PBS group showed a marked weight loss by Day 6 post-challenge with a lower mean weight compared to all other groups (12.0%) (Figure 22).

In the PBS group, S- and NP-specific IgG antibody titers and neutralizing antibody titers were detected at Day 14 post-challenge. In animals vaccinated with mRNA-1273 (all groups), S-specific IgG titers and neutralizing antibody titers were detected prior to the challenge; the levels increased significantly in response to the challenge virus by Day 14 post-challenge, except for the neutralizing antibody titers in the animals administered 1 µg of mRNA-1273 on a prime/boost schedule, in which the difference was not significant. In addition, NP-specific antibody titers were detected in all groups at Day 14 post-challenge, although the levels were lower in the animals vaccinated with mRNA-1273 than in the PBS group. These results indicate that despite protection from WT SARS-CoV-2 challenge, golden Syrian hamsters mounted an anamnestic immune response against the challenge virus after immunization with mRNA-1273 (Report UTMB01 [Figure 4]).

Measurement of viral load showed that animals immunized with 1, 5, and 25 µg of mRNA-1273 on a prime/boost schedule were protected from virus challenge, with mean viral titers that were below the LOD in the lungs and nasal turbinates at Day 4 post-challenge (Figure 23). In addition, all animals administered a prime/boost dose of 5 and 25 µg of mRNA-1273 and all but one animal administered a prime/boost dose of 1 µg of mRNA-1273 had no detectable viral replication (sgRNA) in the lungs by Day 4 post-challenge (Figure 23). In the nasal turbinates, all animals in the prime/boost groups were partially protected, as viral replication (sgRNA) observed on Day 2 and Day 4 post-challenge was significantly lower compared with the PBS control group. A prime-only dose of 25 µg of mRNA-1273 partially protected animals as live virus and viral replication were observed on Day 2 post-challenge in lungs and nasal turbinates; however, the mean viral titers were lower than those in the PBS group and by Day 4 post challenge, the mean viral titers were below the LOD (Figure 23).

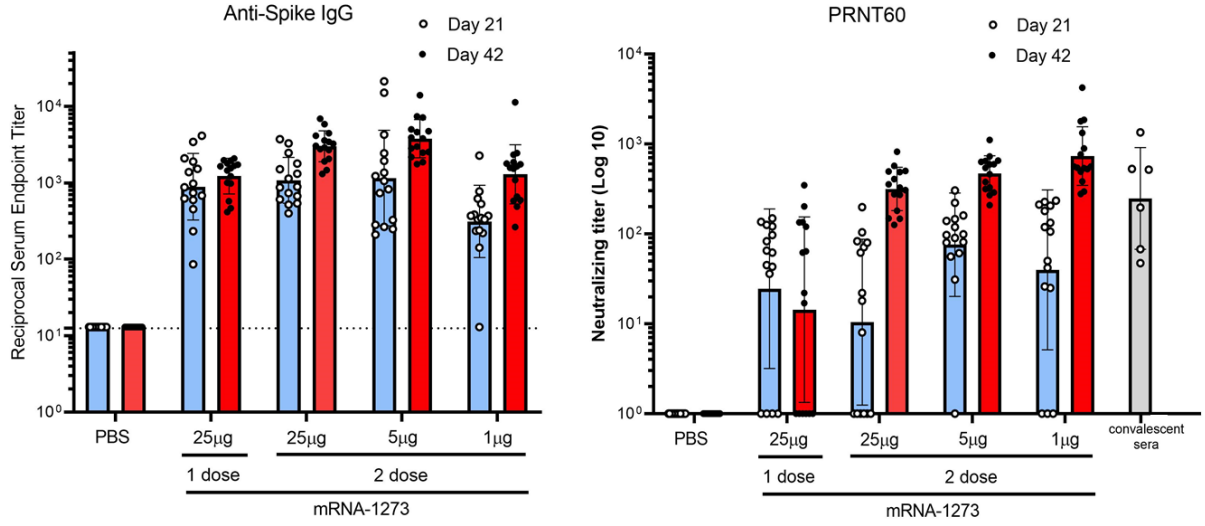
Lungs of hamsters evaluated histologically after challenge with WT SARS-CoV-2 showed that, over the 14-day infection course (Report UTMB01 [Table 3]), mRNA-1273 immunized hamsters

on a prime/boost schedule generally displayed mild to moderate inflammation with viral antigens that were either absent or minimally abundant at Day 4 post-challenge (Figure 24). Lung sections of animals immunized with a prime-only dose of 25 µg of mRNA-1273 exhibited inflammation that was largely associated with perivascular and peribronchiolar regions in both a focally diffuse or multifocal distribution, but this inflammation was generally milder than that in animals in the PBS control group at the same time point and did not persist through Day 14 (Report UTMB01 [Table 3]). One outlier animal was identified in each of 1 µg prime/boost and 25 µg prime-only groups exhibited more severe histopathological phenotypes with the highest levels of viral antigen detected among all immunized hamsters (Figure 24); however these animals did not exhibit drastic weight loss and showed live virus and virus replication in the lungs and nasal turbinates that was generally similar to that in other animals in their group.

Conclusions:

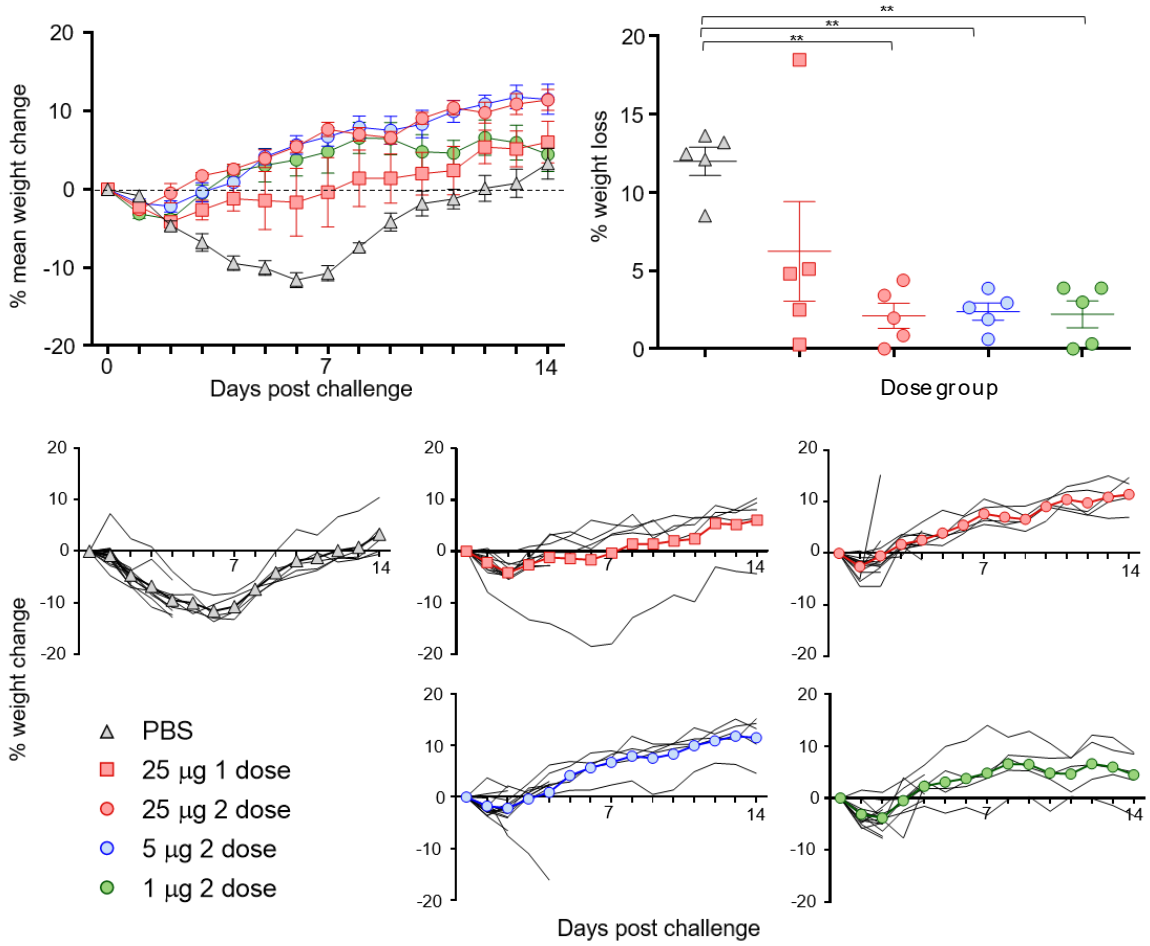
Overall, mRNA-1273 administered on a prime/boost dosing schedule is immunogenic and efficacious, and provides protection from WT SARS-CoV-2 in golden Syrian hamsters.

Figure 21: Binding and Neutralizing Antibody Titers in Hamsters Immunized With mRNA-1273 (Prime Only and Prime/Boost With 3-Week Interval) – Report UTMB01



Abbreviations: 1 dose = prime-only dose schedule; 2 dose = prime/boost with 3 week interval dose schedule; Ig = immunoglobulin ; PBS = phosphate-buffered saline; PRNT60 = titer required to reduce viral plaques by 60%.
Source: Report UTMB01 (Figure 1).

Figure 22: Body Weight Changes Post-Challenge in Hamsters Immunized With mRNA-1273 (Prime Only and Prime/Boost With 3-Week Interval) – Report UTMB01

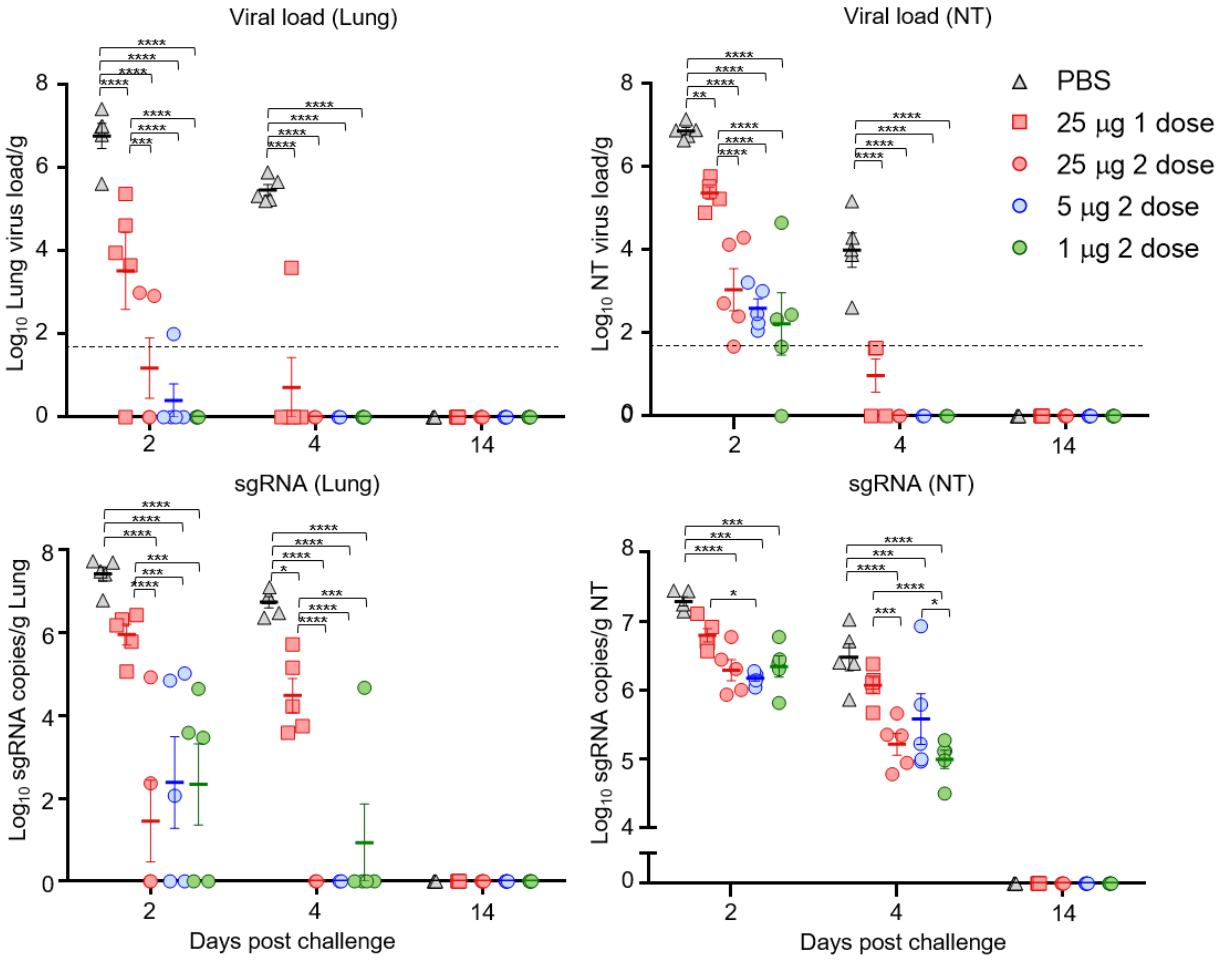


Abbreviation: 1 dose = prime-only dose schedule; 2 dose = prime/boost with 3 week interval dose schedule; PBS = phosphate-buffered saline.

Note: ** $p \leq .01$.

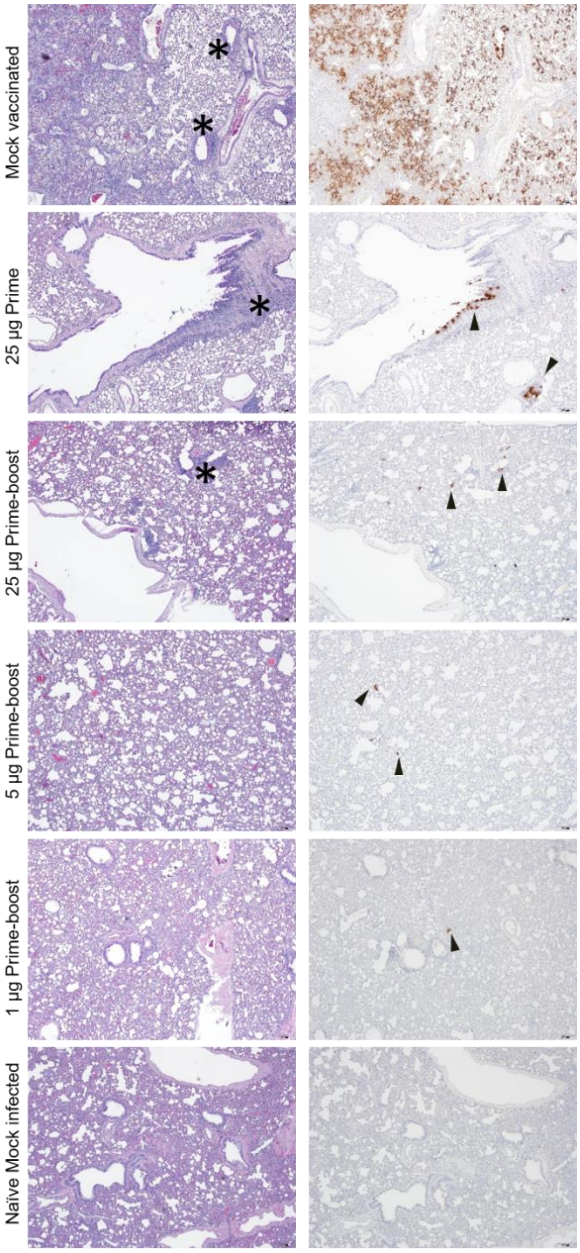
Source: Report UTMB01 (Figure 3).

Figure 23: Lung and Nasal Turbinate Viral Loads 2 Days and 4 Days Post-Viral Challenge in Hamsters Immunized With mRNA-1273 (Prime Only and Prime/Boost With 3-Week Interval) – Report UTMB01



Abbreviation: PBS = phosphate-buffered saline; NT = nasal turbinates; sgRNA = subgenomic RNA.
Note: *$p < .05$, **$p < .01$, ***$p < .001$, ****$p < .0001$
Source: Report UTMB01 (Figure 5).

Figure 24: Lung Histopathology and Immunohistochemistry Analysis 4 Days Post-Viral Challenge in Hamsters Immunized With mRNA-1273 (Prime Only and Prime/Boost With 3-Week Interval) – Report UTMB01



Note: The first column shows hematoxylin and eosin staining of lung section to show severity of inflammation and the * reflects areas of perivascular/peribronchiolar inflammation (mostly mononuclear). The second column shows immunohistochemical staining (representative photomicrographs with original magnification ×4 [scale bars, 200 µm]) with virus antigen (arrowhead) in lungs.

Definitions: Naïve Mock infected = non-immunized hamsters mock challenged with media to mimic virus inoculum; Mock vaccinated = PBS control group; prime = to animals immunized with mRNA-1273 prime-only schedule; Prime-boost = animals immunized with mRNA-1273 prime/boost with 3-week interval.

Source: Report UTMB01 (Figure 6).

2.6.2.2.9 Immunogenicity and Protective Efficacy of mRNA-1273 in Rhesus Macaques

The objectives were to evaluate the immunogenicity of mRNA-1273 and protection from SARS-CoV-2 challenge in rhesus macaques (Report VRC04).

Methods:

Indian-origin rhesus macaques of 3 to 6 years of both sexes were treated according to the study design presented in Table 10. Sera were collected at various time points pre- (Week -1 and Week 0) and post-immunization (Week 2, Week 4, and Week 6 and Week 8) with either 10 µg or 100 µg of mRNA-1273 and assessed for SARS-CoV-2 S-2P binding antibodies (total IgG), as measured by ELISA as well as for neutralizing antibodies, as measured by pseudotyped lentivirus. Sera collected at Week 8 (4 weeks post-boost) were assessed for RBD-specific, NTD domain of S1 (S1_NTD)-specific binding antibodies, as measured by ELISA, for neutralizing antibodies, as measured by live virus neutralization assay, inhibition of ACE-2 binding to RBD as measured by an electrochemiluminescence binding ACE-2 binding assay and T-cell cytokine responses to in vitro restimulation with pools of overlapping peptides from S proteins (S1 and S2 peptides), as measured by flow cytometry analysis of cell surface and intracellular immunostaining.

Peripheral blood mononuclear cells collected 4 weeks post-boost, (before viral challenge) were restimulated in vitro with pools of peptides from S1 proteins to analyze T-cell cytokine responses, as measured by flow cytometry analysis of cell surface and intracellular immunostaining.

SARS-CoV-2 S-2P binding IgG and IgA antibodies were measured by ELISA from Day 2, Day 4, and Day 7 post-challenge BAL samples. SARS-CoV-2 S-2P S-specific and N-specific binding IgG antibodies were measured by ELISA from Day 0, Day 7, and Day 14 post-challenged sera samples.

Bronchoalveolar lavage (BAL) fluid and nasal swabs (NS) obtained at Day 1 (only NS), Day 2, Day 4, and Day 7 post-challenge were assessed for active viral replication and viral load by PCR, and viral genomes by qRT-PCR). Innate immune system cytokines and chemokines were analyzed in BAL fluid obtained on Day 2 and Day 4 post-challenge to further investigate viral infection in the lung, as measured by (b) (4)

Histopathological analysis as assessed by IHC (using anti-SARS-CoV-2 staining) and chromogenic in situ hybridization (cISH) analysis as well as inflammation (using H&E staining)

were evaluated on lung-tissue specimens collected after animals were euthanized on Day 7/8 or Day 14/15 post-challenge.

Table 10: Study Design - Report VRC04

Treatment Dose (µg)[a]	Immunization Schedule		Challenge	Sample Collection and Experimental Assessments	
	Prime	Boost		Pre-Challenge	Post-Challenge
PBS	Week 0	Week 4	Week 8[b]	Week -1, Week 0, Week 2, Week 4, Week 6, and Week 8; Sera for SARS-CoV-2 S-2P bAbs (total IgG), nAbs (pseudovirus) Week 8; Sera for bAbs (SARS-CoV-2 S-2P [total IgG], S1_NTD- and RBD-specific IgG), nAbs (pseudovirus and live virus), ACE-2 binding and cytokine analysis	Day 0, Day 7, and Day 14 post-challenge; Sera for bAbs (N-specific and S-specific IgG) Day 2, Day 4, and Day 7 post-challenge; BAL for SARS-CoV-2 S-2P bAbs (total IgG, IgG, and IgA) Day 2 and Day 4 post-challenge; BAL for cytokine analysis Day 1 (NS only), Day 2, Day 4, and Day 7 post-challenge; NS and BAL for viral replication (sgRNA), viral load and genomes Day 7/8 and Day 14/15 post-challenge; lungs for histopathology analysis[c]: IHC[d] and cISH[e]
10 µg mRNA-1273					
100 µg mRNA-1273					

Abbreviations: ACE-2 = angiotensin-converting enzyme-2; bAbs = binding antibodies; BAL = bronchoalveolar lavage; cISH = chromogenic in situ hybridization; CoV = coronavirus; ELISA = enzyme-linked immunosorbent assay; ICS = intracellular cytokine staining; IHC = immunohistochemistry; NA = not applicable, nAbs = neutralizing antibodies; NS = nasal swab; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; RBD = Receptor binging domain; S1_NTD = N-terminal domain of S1; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; Th1 = T helper 1; Th2 = T helper 2.

[a] Groups composed of female and male Indian-origin rhesus macaques of 3 to 6 years old (Total n = 24: male [n = 12] and female [n = 12]).

[b] Animals were challenged intratracheally and intranasally with a total dose of 7.6×10^5 PFU from a stock of 1.9×10^5 PFU/mL SARS-CoV-2 (USA-WA1/2020). The challenge was administered in a total volume of 4 mL (3 mL intratracheal and 1 mL intranasally [0.5 mL per nostril]) SARS-CoV-2.

[c] Inflammation assessed by H&E staining in lung samples.

[d] IHC performed using anti-SARS-CoV-2 staining in lung samples.

[e] cISH for detecting positive-sense SARS-CoV-2 RNA in lung samples.

Source: Report VRC04 (Table 2).

Results:

When compared to human convalescent sera, mRNA-1273 elicited a more potent RBD- and S1_NTD-specific serum antibody responses in rhesus macaques than natural infection in humans (Report [Figure 2]). In the 100 µg group, inhibition of ACE-2 binding to RBD was significantly higher than inhibition in the human convalescent serum and PBS control groups, and 4 weeks post-boost, potent neutralizing capacity was observed using the live SARS-CoV-2 reporter virus and the pseudovirus neutralization assay in both the 10 µg and 100 µg groups (Figure 25).

Intracellular cytokine staining revealed that, overall, Th1-directed CD4+ T-cell and IL-21 producing T follicular helper (Tfh)-cell response levels were driven by immunization with mRNA-1273, with responses higher in the 100 µg group than in the 10 µg mRNA-1273 group. Th2-directed CD4+ T-cell responses were low to undetectable in both mRNA-1273 groups (Figure 26).

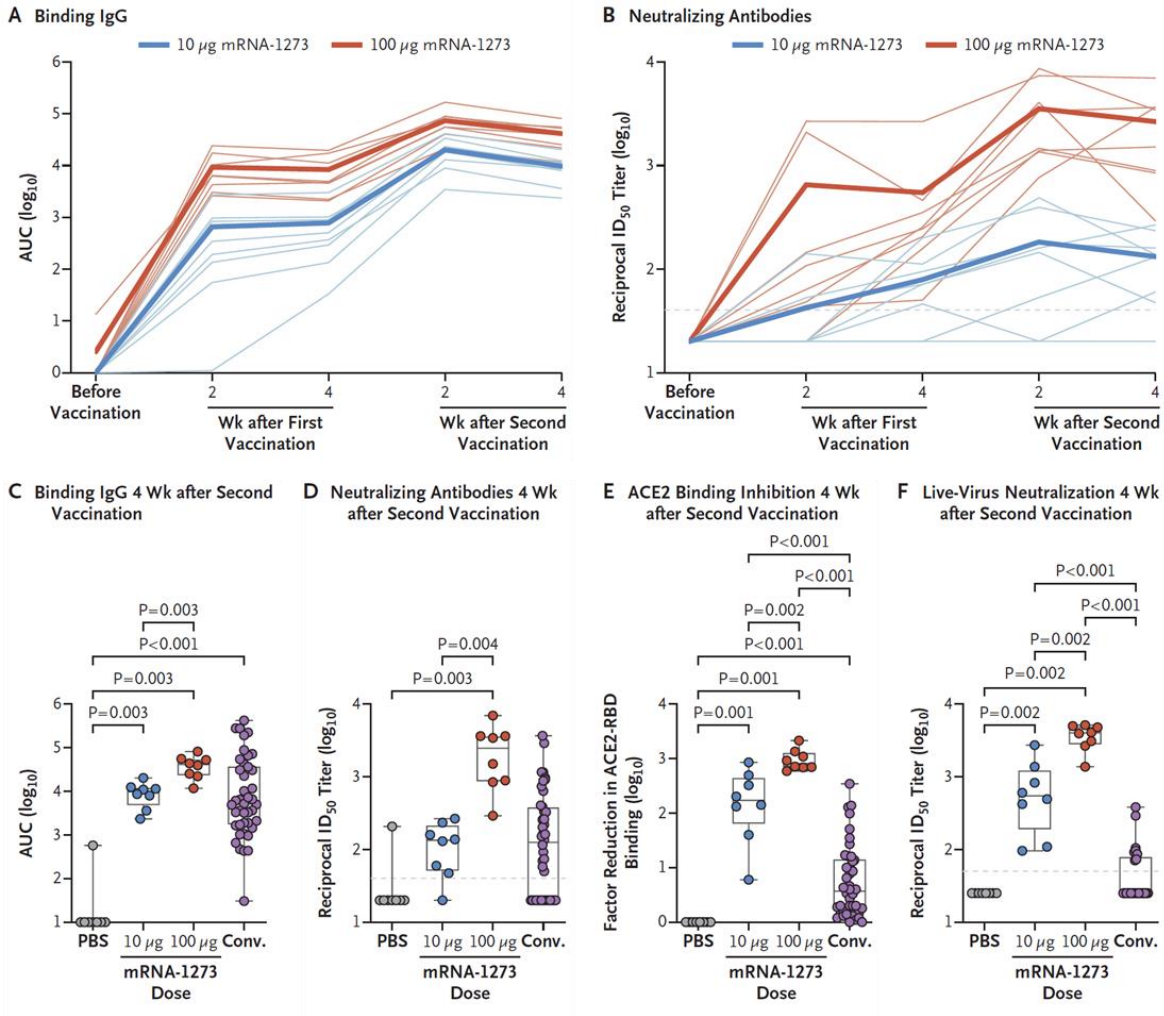
Evaluation of the protection against WT SARS-CoV-2 infection in the upper and lower airways revealed a reduction in detectable sgRNA in BAL fluid (7 out of 8 animals in both mRNA-1273 groups) and NS samples (all animals in 100 µg group) as early as 2 days post-challenge. Specifically, no viral replication was detectable in the nose of any of the 8 animals in the 100 µg group by Day 2 after challenge (Figure 27). Additionally, qRT-PCR showed a decrease in total viral RNA in BAL fluid and NS samples 2 days post-challenge (Report VRC04 [Figure 5]). There was limited inflammatory cytokine induction in the upper and lower airway of rhesus macaques within 2 days (Report VRC04 [Figure 6]), suggesting that the rapid control of the virus was sufficient to limit innate immune activation. There was also a dose-dependent increase in S-specific IgG antibody titers in BAL fluid compared with the titers in control animals (Report VRC04 [Figure 8]). S-specific IgA responses in BAL fluid were lower than the IgG responses, and the IgA responses were higher in the 100 µg group than in the 10 µg and control group (Report VRC04 [Figure 7]).

Histopathology assessment at 7 or 8 days post-challenge in animals immunized with 100 µg of mRNA-1273 revealed no significant inflammation in the lungs, and no detectable viral RNA or antigens was observed in the lungs (Figure 28) (Report VRC04 [Figure 10] and Report VRC04 [Table 3]). Furthermore, there was no evidence of vaccine-associated immunopathology.

Conclusions:

Immunization of rhesus macaques with mRNA-1273 induced robust SARS-CoV-2 binding antibodies and neutralizing activity and protection in the upper and lower airways after challenge, with no pathologic changes observed in the lungs.

Figure 25: Binding and Neutralizing Antibody Titers in Rhesus Macaques Immunized With mRNA-1273 (Prime /Boost With 4-Week Interval) – Report VRC04

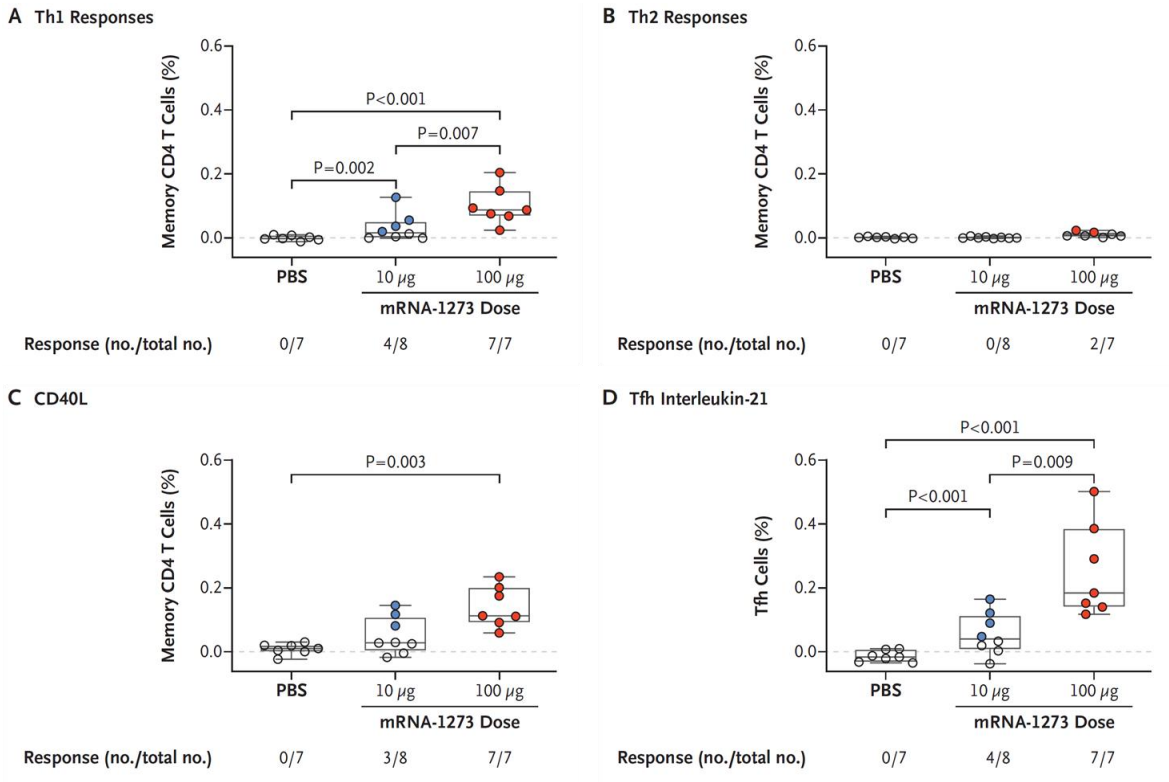


Abbreviations: ACE-2 = angiotensin-converting enzyme 2; AUC = area under the curve; Conv, convalescent human serum; ID_{50} = 50% inhibitory dilution; Ig = immunoglobulin; PBS = phosphate-buffered saline; Wk = week(s).

Note: In Panels A and B faint lines represent individual animals, and bold lines represent the geometric mean titer for each group. In Panels C, D, E, and F, each dot represents an individual rhesus macaque. Dotted line indicates the assay limit of detection. Results were compared with the antibody responses in a panel of convalescent-phase human serum specimens (42 specimens in Panels C, D, and E and 26 specimens in Panel F).

Source: Report VRC04 (Figure 1).

Figure 26: T-Cell Cytokine Responses in Rhesus Macaques Immunized With mRNA-1273 (Prime /Boost With 4-Week Interval) – Report VRC04

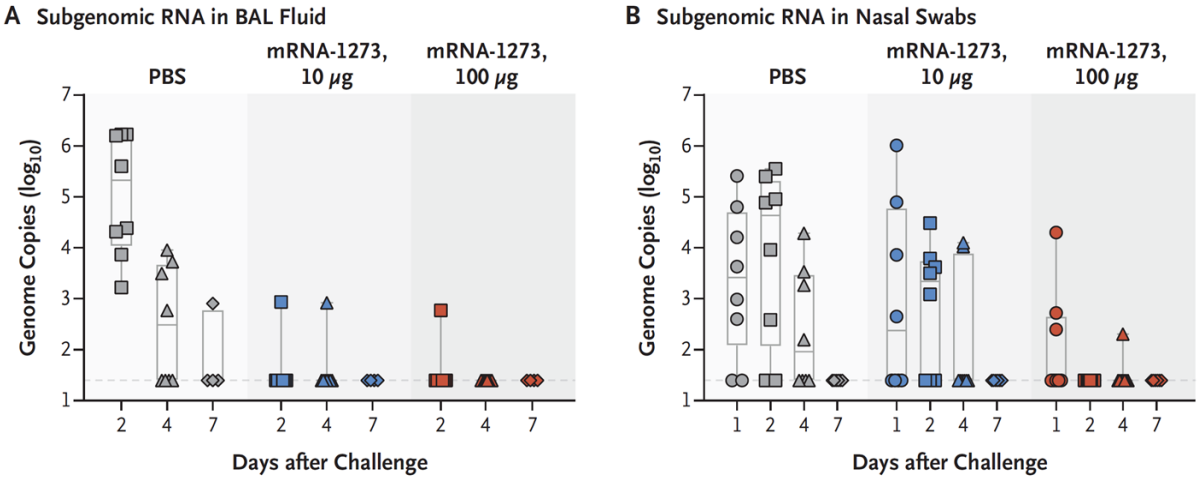


Abbreviations: PBS = phosphate-buffered saline; Tfh = T follicular helper; Th = T-helper.

Note: Each dot represents an individual rhesus macaque. Dotted line indicates the assay limit of detection. Response rates are displayed as fractions above each group. Open symbols represent animals with a probable nonresponse, and solid symbols represent animals with a probable response.

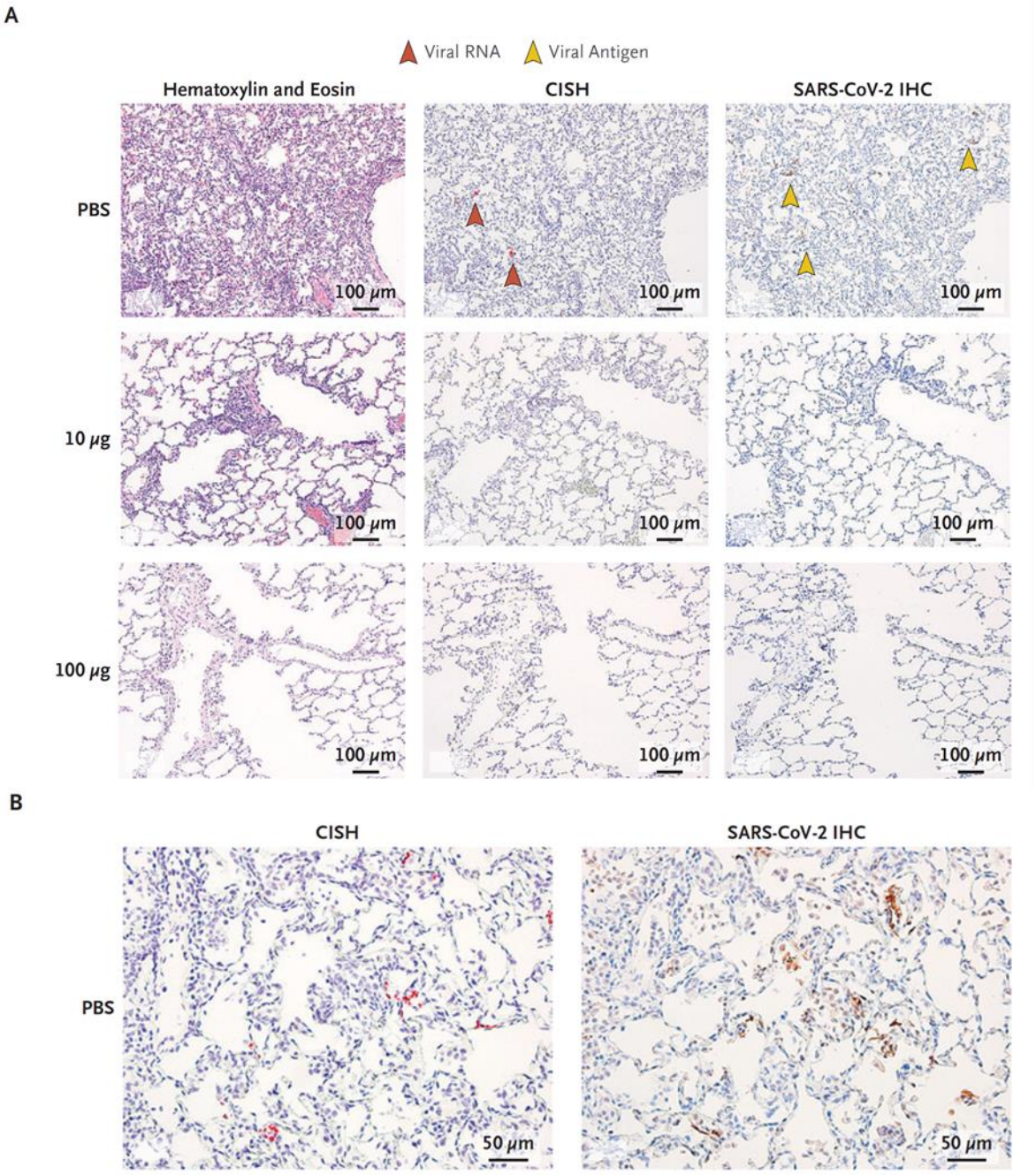
Source: Report VRC04 (Figure 3).

Figure 27: Viral Replication in the Upper and Lower Airways of Rhesus Macaques Immunized With mRNA-1273 (Prime/Boost With 4-Week Interval) – Report VRC04



Abbreviations: BAL = bronchoalveolar lavage; PBS = phosphate-buffered saline; PCR = polymerase chain reaction.
Note: Each symbol represents an individual rhesus macaque. Dotted line indicates the assay limit of detection.
Source: Report VRC04 (Figure 4).

Figure 28: Lung Histopathology and Viral Detection 7 Days Post-Challenge in Rhesus Macaques Immunized With mRNA-1273 (Prime /Boost With 4-week Interval) – Report VRC04



Abbreviations: cISH = chromogenic in situ hybridization; IHC = immunohistochemistry; PBS = phosphate-buffered saline; SARS-CoV-2 = 2019 novel coronavirus.

Source: Report VRC04 (Figure 9).

2.6.2.2.10 Evaluation of Immunogenicity and Efficacy From Expanded Dose Range of mRNA-1273 in Rhesus Macaques

The objective was to evaluate the protective efficacy of mRNA-1273 against WT SARS-CoV-2 isolate USA-WA1/2020 challenge using rhesus macaques (Report VRC07).

Methods:

Indian-origin rhesus macaques of 3 to 6 years of both sexes were treated according to the study design presented in Table 11. Sera collected pre-immunization (Week -1 and Week 0), post-prime (Week 2 and Week 4) and post-boost (Week 6 and Week 7) were assessed for SARS-CoV-2 S-2P antibody binding, as measured by ELISA and neutralizing antibodies, as measured by the pseudotyped lentivirus assay. Sera collected in Week 7 (3 weeks post-boost) were assessed for S-, RBD-, and S1_NTD-specific IgG antibody binding, as measured by ELISA. Inhibition of ACE-2 binding to RBD was assessed, as measured by an electrochemiluminescence ACE-2 binding assay. Additionally, sera collected at Week 7 and on Day 2, Day 4, and Day 7 post-challenge was tested for neutralizing antibodies as measured by pseudotyped lentivirus and live-virus neutralization assays.

Peripheral blood mononuclear cells collected 6 weeks post-prime (2 weeks before viral challenge) were restimulated in vitro with pools of overlapping peptides from S proteins (S1 and S2 peptides) to analyze T-cell cytokine responses via intracellular cytokine staining, as measured by flow cytometry analysis of cell surface and intracellular immunostaining.

Four weeks post-boost (8 weeks post-prime for the prime-only group) active viral replication and viral load were assessed by PCR and viral genomes were assessed by qRT-PCR were assessed in post-challenge BAL (Day 2, Day 4, and Day 7 post-challenge) and NS samples (Day 1, Day 2 , Day 4, and Day 7 post-challenge).

Histopathological analysis and viral quantification were performed on lung-tissue specimens on Day 7, Day 8, or Day 9 post-challenge.

Table 11: Study Design – Report VRC07

Treatment Dose (µg)[a]	Immunization Schedule		Challenge[b]	Sample Collection and Experimental Assessments	
	Prime	Boost		Pre-Challenge	Post-Challenge
PBS[c]	Week 0	Week 4	Week 8	Week -1, Week 0, Week 2, Week 4, Week 6, and Week 7; Sera for SARS-CoV-2 S-2P bAbs (total IgG), nAbs (pseudovirus[d])	Day 2, Day 4, and Day 7 Post-challenge; Sera for nAbs (pseudovirus[d] and live virus)
100 µg NTFIX mRNA					
2.5 µg mRNA-1273				Week 7; Sera for SARS-CoV-2 S-2P bAbs (S-specific, RBD-specific and S1_NTD-specific IgG), ACE-2 binding and nAbs (pseudovirus[c] and live virus)	Day 1 (NS only), Day 2, Day 4, and Day 7 post-challenge; BAL and NS for viral replication, viral load, and viral genomes
30 µg mRNA-1273					Day 7, Day 8, and Day 9 post-challenge; lungs for histopathology analysis[e]: IHC[f]
100 µg mRNA-1273	Week 0	-	Week 8	Week 6; Sera for cytokine analysis	

Abbreviations: ACE-2 = angiotensin-converting enzyme-2; bAbs = binding antibodies; BAL = bronchoalveolar lavage; CoV = coronavirus; ELISA = enzyme-linked immunosorbent assay, H&E = hematoxylin and eosin; IHC = immunohistochemistry; ICS = intracellular cytokine staining; nAbs = neutralizing antibodies; NS = nasal swabs; NTFIX = Noncoding mRNA (NTFIX) formulated into the same lipid nanoparticle dispersion as mRNA-1273; PBS = phosphate-buffered saline; PFU = plaque-forming units; RBD = Receptor binging domain; S1_NTD = N-terminal domain of S1; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = 2019 novel coronavirus; Th1 = T-helper 1; Th2 = T-helper 2; WT = wild type.

[a] Groups (n = 6) composed of female and male Indian-origin rhesus macaques of 3 to 6 years old (n = 10 female and n = 20 male).

[b] Animals were challenged with WT SARS-CoV-2 at total dose of 7.6×10^5 PFU from a stock of 1.9×10^5 PFU/mL SARS-CoV-2 (USA-WA1/2020). The challenge was administered in a total volume of 4 mL (3 mL intratracheal and 1 mL intranasal [0.5 mL per nostril]) of SARS-CoV-2.

[c] One group of rhesus macaques was treated with PBS, but the data was not included as PBS and control mRNA were consistent and control mRNA was considered a more relevant comparator.

[d] Pseudovirus neutralization assay in this study utilized D614G S gene polymorphic variant of the SARS-CoV-2. This D614G S gene strain has become predominant in both United States and Worldwide (Korber et al 2020).

[e] Inflammation assessed by H&E staining in lung samples.

[f] IHC performed using anti-SARS-CoV-2 staining in lung samples.

Source: Report VRC07 (Table 2).

Results:

Results from this study showed that mRNA-1273 elicits robust binding and neutralizing antibody responses in rhesus macaques, especially in the 30 µg prime/boost group. At all dose levels and dosing schedules (prime-only and prime/boost), these responses increased in a dose-dependent manner (Figure 29). Robust neutralizing activity was observed in rhesus macaques immunized with 30 µg of mRNA-1273 on a prime/boost schedule as measured by pseudovirus neutralization assay (Figure 29). A single dose of 100 µg of mRNA-1273 elicited binding antibody titers that remained steady for 7 weeks but had low or undetectable neutralizing activity in either the pseudovirus or live virus assay (Figure 29).

All mRNA-1273-vaccinated treatment groups had S-specific binding antibody and ACE-2 competing antibody levels that were similar to or higher than that of human convalescent sera (Figure 30). Neutralizing activity against SARS-CoV-2 pseudovirus and live virus was only evident in the 30 µg prime/boost group and the 100 µg prime/boost group (Figure 30). Higher anti-RBD and anti-NTD responses were observed in the 100 µg prime-only and 30 µg prime/boost groups, compared with the 2.5 µg prime/boost group (Report VRC07 [Figure 3]). Neutralizing antibodies were substantially higher in the 30 µg prime/boost group 2 days, 4 days, and 7 days post-challenge than in the NTFIX control (a noncoding mRNA formulated into the same lipid nanoparticle dispersion as mRNA-1273) and the 2.5 µg prime/boost groups, and were somewhat higher in the 100 µg prime-only group 7 days post-challenge (Report VRC07 [Figure 4]).

Vaccination with mRNA-1273 induced Th1-directed T-cell and IL-21-producting Tfh cell responses, a profile that is not predicted to promote ERD (Report VRC07 [Figure 5]).

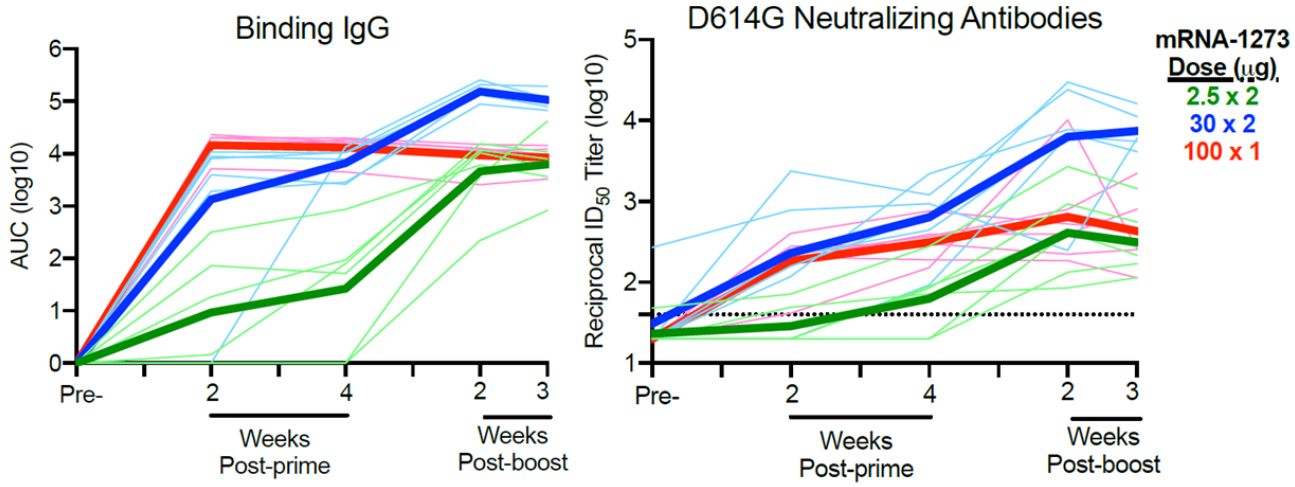
All mRNA-1273 groups demonstrated lower viral loads in BAL fluid than the control group (Report VRC07 [Figure 6]). mRNA-1273 was protective in the lungs and nose of animals in the 30 µg prime/boost and 100 µg prime-only groups, as measured by sgRNA. No animals in the 100 µg prime-only group and only 1 of 6 animals in the 30 µg prime/boost group had detectable sgRNA in the BAL fluid (Figure 31). In NS samples, no animals in the 30 µg prime/boost or the 100 µg prime-only group had detectable sgRNA 1 day and 4 days post-challenge (Figure 31).

Inflammation in mRNA-1273-treated animals ranged from absent to occasional, whereas moderate to severe inflammation was observed in the lungs of control animals with a greater number of affected lung sections. No evidence of vaccine-associated immunopathology was found in histopathological analysis of lung tissue and no viral antigen was detected in the 30 µg prime/boost group post-challenge (Figure 32; Report VRC07 [Table 3]).

Conclusions:

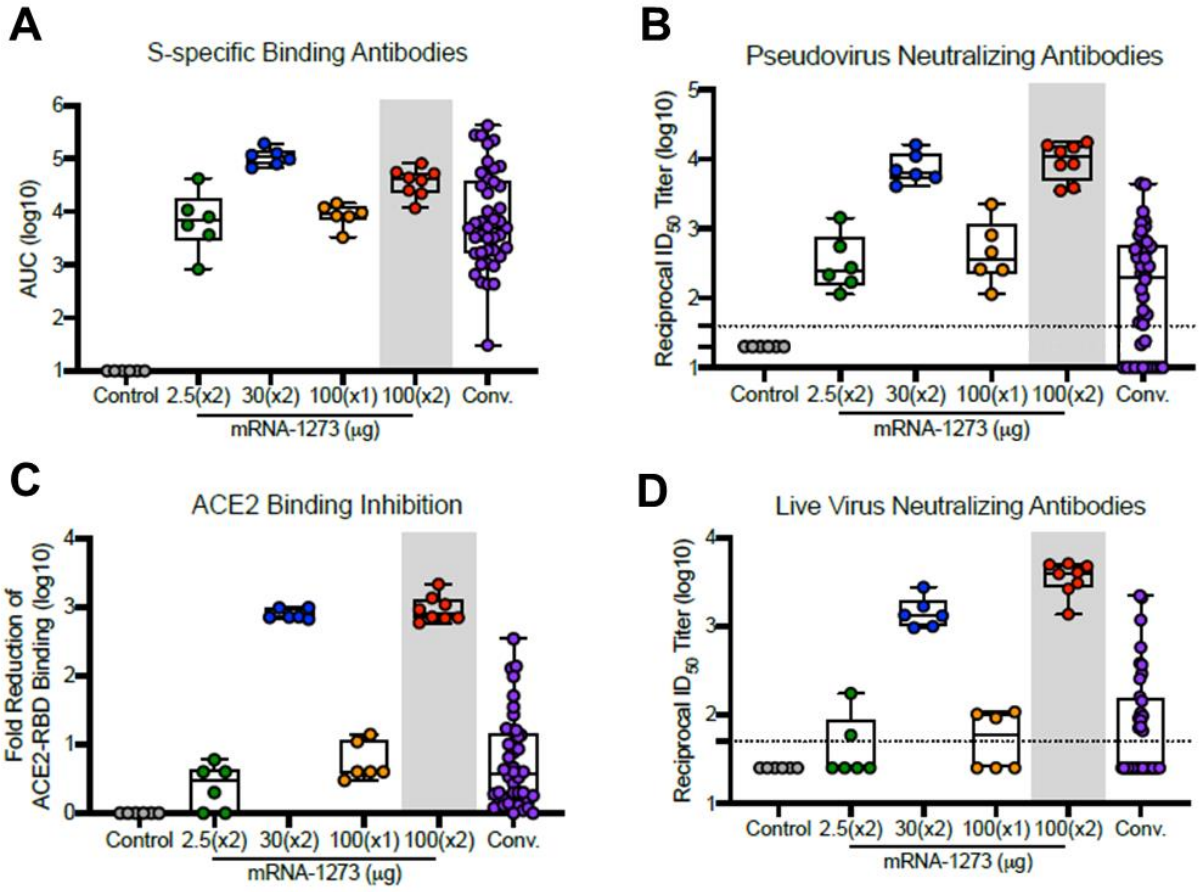
Overall, immunization of rhesus macaques with mRNA-1273 induced robust SARS-CoV-2 binding antibodies and neutralizing activity and protection in the upper and lower airways from WT SARS-CoV-2 challenge, with no pathologic changes observed in the lungs or signs of disease enhancement.

Figure 29: S-specific Binding Antibody Titers and Neutralizing Antibody Titers in Rhesus Macaques Immunized With mRNA-1273 (Prime/Boost With 4-Week Interval) – Report VRC07



Abbreviations: AUC = area under the curve; D614G = a D614G S variant of the SARS-CoV-2 pseudovirus. missense mutation in the spike protein of SARS-CoV-2; ID_{50} = 50% inhibitory dilution; Ig = immunoglobulin.
Note: Faint lines represent individual animals, and bold lines represent the geometric mean titer for each group. Dotted line indicates the assay limit of detection.
Source: Report VRC07 (Figure 1).

Figure 30: S-Specific and ACE-2 Specific Binding Antibody Titers and Neutralizing Antibody Titers in Rhesus Macaques Immunized With mRNA-1273 (Prime/Boost With 4-Week Interval) – Report VRC07

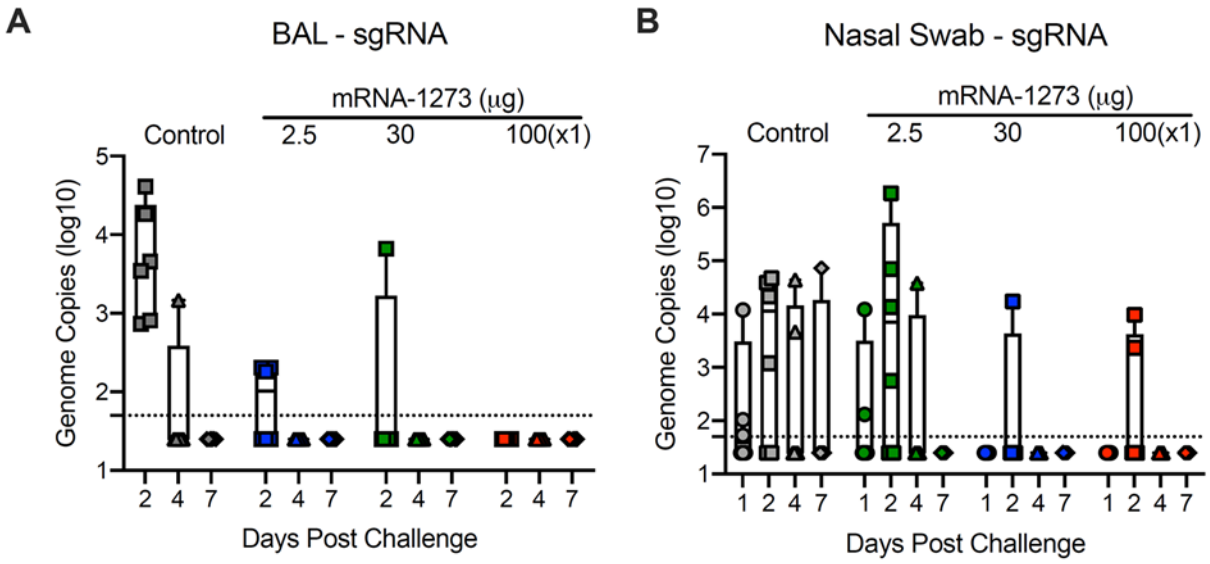


Abbreviations: ×1 = prime-only schedule; ×2 = prime/boost schedule with 3-week interval; ACE2 (ACE-2) = angiotensin-converting enzyme 2; AUC = area under the curve; Conv. = human convalescent; ID_{50} = reciprocal 50% inhibitory dilution; RBD = receptor binding domain; S = spike.

Note: Noncoding mRNA (NTFIX) formulated into the same lipid nanoparticle dispersion as mRNA-1273 was used as the control. Each symbol represents an individual rhesus macaque. Dotted line indicates the assay limit of detection.

Source: Report VRC07 (Figure 2).

Figure 31: Viral Replication in the Upper and Lower Airways of Rhesus Macaques Immunized With mRNA-1273 (Prime/Boost With 4-Week Interval) – Report VRC07

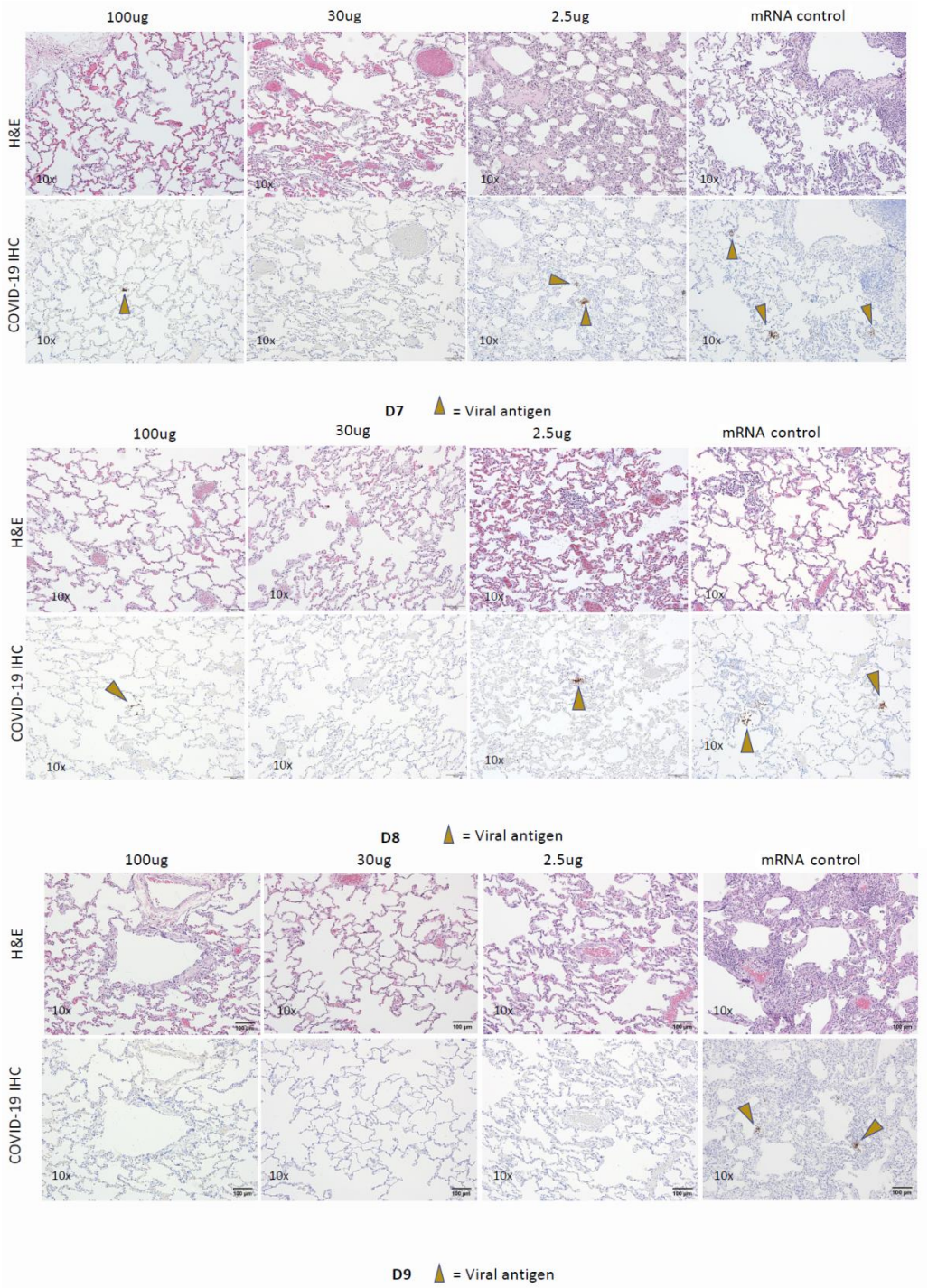


Abbreviations: BAL = bronchoalveolar lavage; sgRNA = subgenomic RNA.

Note: Noncoding mRNA (NTFIX) formulated into the same lipid nanoparticle dispersion as mRNA-1273 was used as the control. Each symbol represents an individual rhesus macaque. Dotted line indicates the assay limit of detection.

Source: Report VRC07 (Figure 7).

Figure 32: Lung Histopathology and Viral Detection 7, 8, and 9 Days Post Challenge in mRNA-1273-Immunized Rhesus Macaques – Report VRC07



Abbreviations: D = Day; COVID-19 = coronavirus disease 2019; H&E = hematoxylin and eosin; IHC = immunohistochemistry.

Note: Noncoding mRNA (NTFIX) formulated into the same lipid nanoparticle dispersion as mRNA-1273 was used as the control.

Source: Report VRC07 (Figure 8, Figure 9, and Figure 10).

2.6.2.3 SECONDARY PHARMACODYNAMICS

No secondary pharmacodynamics studies have been performed with mRNA-1273.

2.6.2.4 SAFETY PHARMACOLOGY

No safety pharmacology studies have been performed with mRNA-1273.

2.6.2.5 PHARMACODYNAMIC DRUG INTERACTIONS

No pharmacodynamic drug interactions studies have been performed with mRNA-1273.

2.6.2.6 DISCUSSION AND CONCLUSIONS

Expression of SARS-CoV-2 S-2P Encoded by mRNA (In Vitro) or mRNA-1273 (In Vivo)

The expression of the mRNA-encoded SARS-CoV-2 S-2P antigen was confirmed in vitro and in vivo. HEK293T cells transfected with a dose range (0.003125 through 0.2 μg) of mRNA expressed the encoded SARS-CoV-2 S-2P antigen, as demonstrated by surface-protein staining with mAbs specific to the RBD (CR3022) or the NTD (4A8) epitopes of the SARS-CoV-2 S protein. The expression of these epitopes was similarly confirmed in spleen and dLN immune cells (cDCs and pDCs) in BALB/c mice administered a single IM dose (2 or 10 μg) of mRNA-1273.

Immunogenicity – Antibody Response:

Immunization with mRNA-1273 drives dose-dependent increases in full length S protein, RBD, and NTD binding antibody titers and SARS-CoV-2 neutralizing antibody titers in mice and rhesus macaques (NHPs), and consistent S protein and RBD binding and neutralizing antibody titers in hamsters.

Prime-only Immunization Schedule

- Prime-only immunization schedule generated high binding antibody titers in all mRNA-1273 doses tested and robust neutralizing antibody responses in mice (1 and 10 μg), hamsters (25 μg), and NHPs (100 μg).

Prime/Boost Immunization Schedule

- Boosting with a second dose of mRNA-1273 led to a significant increase in binding antibody and neutralizing antibody titers in all animal species.
- S protein binding and neutralizing antibody titers were statistically correlated in young mice immunized with mRNA-1273 on a prime/boost schedule.
- ACE-2/RBD competing antibody titers were measured in NHPs immunized with mRNA-1273 on a prime/boost schedule.
- Binding and neutralizing antibody titers observed in NHPs immunized with 30 or 100 μg of mRNA-1273 on a prime/boost schedule were similar to or higher than titers measured in a panel of 42 human convalescent sera from subjects with mild and severe disease.

Immunogenicity – T-cell Response:

Prime/boost immunization with mRNA-1273 induced Th1-directed CD4+ T-cell responses with no evidence of Th2-directed CD4+ T-cell responses in mice and NHPs.

- A Th1-directed CD4+ T-cell response was observed in immunized young and aged mice and NHPs, with no Th2-directed CD4+ T-cell response measured.
- A robust and dose-dependent CD8+ T-cell response was observed in mice after immunization with mRNA-1273. NHPs showed a low CD8+ T-cell response following immunization with mRNA-1273.

Protection From Viral Infection:

mRNA-1273 protects against high-dose SARS-CoV-2 challenge in mice (10^4 or 10^5 PFU in young mice, 10^3 PFU in aged mice), hamsters (10^5 PFU), and NHPs (7.6×10^5 PFU).

Prime/Boost Immunization Schedule

- In young and aged mice, a prime/boost immunization with 1 μg of mRNA-1273 fully protected animals challenged with mouse-adapted SARS-CoV-2 (4 weeks [aged mice] and 5 or 13 weeks [young mice] post-boost) in both the upper (nasal turbinates) and lower (lungs) airways. The lower 0.1 μg mRNA-1273 dose provided partial

protection the and lower airways, decreasing the viral load versus PBS control-dosed animals post-challenge.

- In NHPs, a prime/boost immunization with 30- or 100-µg of mRNA-1273 fully protected animals challenged with SARS-CoV-2 (4 weeks post-boost) in both the upper and lower airways. A 10 µg prime/boost dose of mRNA-1273 fully protected NHPs from viral replication in the lungs, and partially protected against viral replication in the nose turbinates post-challenge.

- In hamsters, a prime/boost immunization with 1, 5, or 25 µg of mRNA-1273 fully protected animals challenged with SARS-CoV-2 (3 weeks post-boost) in both the upper and lower airways.

- In hamsters and aged mice, a prime/boost immunization with 1 µg of mRNA-1273 protected the animals from body weight loss after SARS-CoV-2 challenge.

Prime-only Immunization Schedule

- In young mice, a prime-only immunization with 1 or 10 µg of mRNA-1273 fully protected the animals against viral load in the lungs after mouse-adapted SARS-CoV-2 challenge 7 weeks post-prime dose.

- In NHPs, a prime-only immunization of 100 µg of mRNA-1273 provided protection from SARS-CoV-2 WT challenge both in the upper and lower airways.

- In hamsters, a prime-only dose regimen with 25 µg of mRNA-1273 provided protection from SARS-CoV-2 challenge, with full viral clearance on Day 4 post-challenge.

Vaccine-Associated Enhanced Respiratory Disease:

In mice and NHPs, mRNA-1273 drives a predominant Th1-directed immune response, which is not predicted to drive vaccine-associated ERD.

- Immunization of mice with mRNA-1273 drives a balanced ratio of IgG1 to IgG2a/c, indicating a Th1-directed CD4+ T-cell response is induced. Robust Th1-directed CD4+ T-cell responses were observed post-immunization in mice and NHPs, with minimal or no Th2 cytokines measured.

- A robust neutralizing antibody response was measured in mice, hamsters, and NHPs, demonstrating that mRNA-1273 is unlikely to promote vaccine-associated ERD, which is typically associated with high binding antibody titers and poor neutralizing antibody titers.

After viral challenge, there was no evidence of vaccine-associated ERD in mice, hamsters, and NHPs immunized with optimal (protective) and suboptimal (subprotective) doses of mRNA-1273 as assessed by viral loads, lung pathology, and body weight measurements.

- At both optimal and suboptimal mRNA-1273 dose levels in mice, hamsters, and NHPs, no increased viral titers were measured in lungs or nasal turbinates versus PBS control animals. At higher doses, mice, hamsters, and NHPs were fully protected from viral replication in both lungs and nasal turbinates. At subprotective dose levels, animals either remained fully protected in the lungs or had reduced viral burden post-challenge versus control animals. There were no observations of increased viral load in immunized animals at protective or subprotective dose levels.

- Lung histopathology analysis of mice and NHPs immunized with optimal or suboptimal doses of mRNA-1273 showed no evidence of vaccine-associated ERD after challenge, as demonstrated by minimal inflammation and lack of significant neutrophilic-associated alveolar disease or eosinophil-dominant inflammatory response compared to control animals, where moderate to severe inflammation of small airways and adjacent alveolar interstitium was observed.

- Inflammatory cytokine induction in the lung was limited in NHP immunized with either 10 or 100 μg of mRNA-1273, suggesting a rapid control of virus sufficient to limit innate immune activation.

- In aged mice immunized with suboptimal dose levels of mRNA-1273, the body weight loss measured after viral challenge was lower than in control animals (SARS-CoV DIV and PBS groups).

The results from nonclinical pharmacology studies demonstrated that mRNA-1273 is well tolerated, immunogenic, and provides protection from SARS-CoV-2 challenge. In mice, hamsters, and NHPs, a prime-only immunization schedule induced robust SARS-CoV-2-specific binding and neutralizing antibody responses that significantly increased after boosting with a second dose of mRNA-1273. A prime/boost immunization schedule elicited a substantial

dose-dependent binding antibody response in rats. In addition, Th1-directed antigen-specific CD4+ and CD8+ T-cell responses were observed in mice and a Th1-directed antigen-specific CD4+ T-cell response was observed in NHPs. mRNA-1273 was fully protective from viral challenge in immunized mice and hamsters when administered as a prime-only or prime/boost schedule at ≥ 1 µg/dose and in immunized NHPs when administered as a prime/boost schedule at ≥ 30 µg/dose. Furthermore, mRNA-1273 did not promote vaccine-associated ERD in mice, hamsters, and NHPs as demonstrated by balanced Th1/Th2-directed immune responses to immunization, the absence of increased lung pathology, and controlled viral replication after viral challenge when administered at doses predicted to be fully (optimal dose) or partially (suboptimal dose) protective.

2.6.2.7 TABLES AND FIGURES

The tables and figures are included in the body of the document.

2.6.2.8 REFERENCES

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