

2.6.2 Pharmacology Written Summary

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List of Abbreviations

Abbreviation	Definition
ACE2	angiotensin converting enzyme 2
BAL	bronchoalveolar lavage
CD	cluster of differentiation
COVID-19	Coronavirus Disease 2019
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
ELISA	enzyme-linked immunosorbent assay
ERD	enhanced respiratory disease
FDA	Food and Drug Administration
FFU	focus-forming unit
FRNT	focus reduction neutralization test
GLP	Good Laboratory Practice
GMT	geometric mean titer
IFN	interferon
IgG	immunoglobulin G
IL	interleukin
IM	intramuscular
IT	intratracheal
LLOQ	lower limit of quantification
LN	lymph node
LNP	lipid nanoparticle
LOD	limit of detection
MERS-CoV	Middle Eastern Respiratory Disease Coronavirus
mRNA	messenger RNA
NHP	non-human primate
NS	nasal swab(s)
PBS	phosphate-buffered saline
PEG2000-DMG	1,2 dimyristoyl-rac-glycero-3-methoxypolyethylene glycol-2000
PFU	plaque-forming unit
PSVN	pseudovirus neutralization
RBD	receptor-binding domain
S	spike
S-2P	spike protein modified with 2 proline substitutions within the heptad repeat 1 domain
S2P.529	Omicron-matched S-2P
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
sgRNA	subgenomic RNA
SM-102	a custom-manufactured ionizable lipid
Tfh	T follicular helper
Th1	T helper 1
Th2	T helper 2
TNF	tumor necrosis factor

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VOC	variant of concern
VSV	vesicular stomatitis virus
WT	wild-type

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2.6.2.1 BRIEF SUMMARY

Coronaviruses are a large family of viruses that cause illness ranging from the common cold to more severe diseases, such as Middle-Eastern respiratory syndrome-CoV and SARS-CoV. An outbreak of COVID-19 caused by SARS-CoV-2 began in Wuhan, Hubei Province, China in December 2019, and the disease quickly spread globally.

ModernaTX, Inc. (the Sponsor)'s scalable mRNA/LNP technology platform allowed for a rapid response to the pandemic and was used to develop mRNA-1273, an LNP-encapsulated mRNA-based vaccine against SARS-CoV-2. mRNA-1273 was proven highly effective against COVID-19 following SARS-CoV-2 infection and has been licensed or conditionally approved across multiple regions for the prevention of COVID-19 in individuals 18 years of age and older.

In November 2021, the SARS-CoV-2 Omicron variant (BA.1, also known as B.1.1.529) was detected in South Africa. Available evidence confirms that BA.1 has transmission advantage over prior variants, with significant antigenic change. In addition, it contains antibody escape site mutations (such as K417N, T478K, E484A, N501Y, among others). The BA.1 variant contains more than 30 amino acid substitutions. Additional sub-lineages of Omicron have also emerged with one, BA.2, demonstrating increased transmissibility versus BA.1 which subsequently became the predominant circulating variant in most geographical regions. As of May 2022, Omicron sub-lineages BA.4 and BA.5 have been identified and are increasing in circulation in certain geographical regions.

Based on sera obtained from clinical studies, one month after completing the primary series of mRNA-1273, neutralizing antibodies against Omicron were detectable, but in lower levels compared to antibody levels against ancestral SARS-CoV-2 with the D614G mutation. However, 4 weeks after an mRNA-1273 booster dose (50 µg), increased Omicron variant neutralization was observed, although titers were numerically lower than that observed against ancestral SARS-CoV-2 with the D614G variant (hereafter referred to as WA1 D614G) at 1 month after the second dose of the primary series ([Pajon et al 2022](#)).

Variant-matched booster vaccines have been suggested as a strategy to focus the antibody response against VOCs compared to the authorized, standard-of-care booster vaccines against COVID-19.

mRNA-1273 contains a single mRNA that encodes SARS-CoV-2 spike protein with 2 proline substitutions within the heptad repeat 1 domain (S-2P). In response to the need for variant-matched boosters, the Sponsor has evaluated mRNA-1273.529, which contains a single mRNA that encodes SARS-CoV-2 S-2P for BA.1 (S-2P.529) and mRNA-1273.214, a bivalent vaccine that contains mRNA-1273 and mRNA-1273.529 in a 1:1 ratio. All mRNAs are formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG.

In support of the development of mRNA-1273.214, nonclinical pharmacology evaluations were conducted in mice (BALB/c, K18-hACE2, and 129S2 strains) and NHPs (rhesus macaques) to evaluate the immunogenicity, antigen-specific B cell responses, and protection from Omicron

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challenge after administration of mRNA-1273 or Omicron-matched vaccines as primary series (mRNA-1273, mRNA-1273.529, or mRNA-1273.214) with or without boosting with mRNA-1273, mRNA-1273.529, or mRNA-1273.214. Additionally, the potential for vaccine-associated ERD after viral challenge was further evaluated in NHPs.

In the challenge evaluations, K18-hACE2 mice were challenged with a WA1/2020 recombinant variant with D614G substitution (WA1 D614G, described previously in [Plante et al 2021](#)) or the BA.1 isolate (hCoV-19/USA/WI WSLH 221686/2021). The 129S2 mice were challenged with a mouse-adapted variant (WA1 2020/N501Y/D614G), as the substitution of N501Y enables engagement of murine ACE2 and productive infection of conventional strains of laboratory mice ([Gu et al 2020](#); [Liu et al 2021](#); [Rathnasinghe et al 2021](#); [Ying et al 2021](#)). Non-human primates were challenged with SARS-CoV-2 Omicron ([Gagne et al 2022](#)).

In a non-GLP immunogenicity study using BALB/c mice, after a 2-dose primary series, mRNA-1273.214 showed robust neutralization against WA1 D614G, BA.1, and BA.2, and overall, mRNA-1273.214 provided the broadest neutralization coverage across the variants evaluated ([Section 2.6.2.2.1](#)).

In a non-GLP immunogenicity and B cell response study using BALB/c mice, a single Omicron-matched booster (monovalent mRNA-1273.529 or bivalent mRNA-1273.214) was not sufficient to dramatically increase neutralization antibody titers 2 weeks after boosting, but drove the expansion of Omicron-specific antigen-reactive B cells in the germinal centers, which were available to respond rapidly to subsequent vaccination with mRNA-1273.529 ([Section 2.6.2.2.2](#)). This was seen by the dramatic increase of BA.1 and BA.2 neutralization after the second booster (Dose 4).

In a non-GLP immunogenicity and protection study using K18-hACE2, BALB/c, and 129S2 mice, boosting with either mRNA-1273 or mRNA-1273.529 enhanced protection against BA.1 infection ([Section 2.6.2.2.3](#), [Ying et al 2022](#)). The differences in protective efficacy between the 2 mRNA boosters was limited, with both boosters offering protection against the historical WA1 D614G variant, increased neutralizing titers against BA.1 and BA.2, and increased protection against BA.1 in mice immunized with lower doses.

In a non-GLP immunogenicity and protection study in NHPs (rhesus macaques), 2 weeks after boosting with either mRNA-1273 or mRNA-1273.529 (after a primary series of mRNA-1273) comparable and significant increases in neutralization antibody responses against all VOCs, including BA.1 were observed ([Section 2.6.2.2.4](#), [Gagne et al 2022](#)). Boosting was important for enhancing mucosal antibody binding and neutralization responses and expanded cross-reactive memory B cells. Protection in the lungs of NHPs were robust regardless of the booster given.

Overall, mRNA-1273 affords similar or lower neutralization and protection against key VOCs, while mRNA-1273.529-boosted mice more potently neutralizes BA.1. However, mice administered mRNA-1273.529 as a primary series regimen have lower neutralization against other non-BA.1 variants and the ancestral SARS-CoV-2 strain. Bivalent vaccines, such as mRNA-1273.214, demonstrated greater cross-variant neutralization in nonclinical studies. Refer to mRNA-1273.214 Module 2.5 for a summary of results from clinical studies with mRNA-1273

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bivalent vaccines. Animals boosted with both mRNA-1273.529 and mRNA-1273.214 showed equivalent or better BA.1 and BA.2 neutralization, protection, and antigen-reactive B cells versus mRNA-1273. In mice, mRNA-1273.214 demonstrated increased potency when compared with monovalent vaccines.

The completed studies are listed in [Table 1](#), summarized in additional detail in [Section 2.6.2.2](#), and presented in a tabular format summary in [Module 2.6.3](#).

Table 1: Completed Nonclinical Pharmacology Studies Supporting Development of mRNA-1273.214

Study Title	Report Number	Laboratory Name and Location	eCTD Reference
Evaluation of immunogenicity of Omicron-matched mRNA vaccines as primary series in mice	MOD-5156	ModernaTX, Inc. Cambridge, MA	4.2.1.1
Evaluation of immunogenicity and antigen-reactive B cell responses of Omicron-matched mRNA vaccine boosters in mice	MOD-5019	ModernaTX, Inc. Cambridge, MA	4.2.1.1
Primary series and booster studies in mice of mRNA-1273 and mRNA-1273.529 immunogenicity and protection from Omicron challenge	WASHU-01-MOD-5020	ModernaTX, Inc. Cambridge, MA Washington University School of Medicine St. Louis, MO	4.2.1.1
mRNA-1273 primary series and mRNA-1273 versus mRNA-1273.529 booster regimen in a rhesus macaque SARS-CoV-2 Omicron challenge model	VRC-20-857	BIOQUAL, Inc. Rockville, MD	4.2.1.1

Abbreviations: eCTD = electronic common technical document; mRNA = messenger RNA; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

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2.6.2.2 PRIMARY PHARMACODYNAMICS

The preclinical mRNA-1273 and mRNA-1273.529 Drug Products used in these studies were mRNA formulations prepared with the same method as the Good Manufacturing Practice mRNA-1273 and mRNA-1273.529 clinical Drug Products. mRNA-1273 encodes the S protein of the Wuhan-Hu-1 isolate of SARS-CoV-2, whereas mRNA-1273.529 encodes the S protein of the SARS-CoV-2 BA.1 variant (Omicron); both vaccines include 2 proline mutations introduced to stabilize the S protein into the prefusion conformation. In preclinical studies, mRNA-1273.214 was a 1:1 bench side mix of mRNA-1273 and mRNA-1273.529. All vaccines were formulated into a mixture of 4 lipids: SM-102, cholesterol, DSPC, and PEG2000-DMG. A summary of the dose formulation analysis results is presented in [Table 2](#).

The preclinical mRNA-1273.529 vaccine encoded the following substitutions: A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493K, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F.

All vaccines were encapsulated in an LNP through a modified ethanol-drop nanoprecipitation process as previously described ([Hassett et al 2019](#)). Briefly, ionizable, structural, helper, and polyethylene glycol lipids were mixed with mRNA-1273 in acetate buffer, pH 5.0, at a ratio of 2.5:1 (lipids:mRNA). The mixture was neutralized with tris(hydroxymethyl)aminomethane hydrochloride, pH 7.5, sucrose was added as a cryoprotectant, and the final solution was sterile filtered. Vials were filled with formulated lipid nanoparticle and stored frozen at -20°C until further use. The preclinical vaccine product underwent analytical characterization, which included the determination of particle size and polydispersity, encapsulation, mRNA purity, double stranded RNA content, osmolality, pH, endotoxin, and bioburden, and the material was deemed acceptable for the in vivo study ([Corbett et al 2020b](#)).

A noncoding mRNA (UNFIX-01) or PBS was used as a negative control. The UNFIX-01 was synthesized and similarly formulated into LNPs as described in [Corbett et al 2021](#). The UNFIX-01 contains a short mRNA sequence formulated into the same LNP dispersion as mRNA-1273. After delivery into cells, this mRNA is not translated into protein.

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Table 2: Test Materials and Summary of Dose Formulation and Analysis

Test Material	mRNA Description	mRNA Lot No(s).	Size (nm)	PDI	EE%
mRNA-1273	mRNA SARS-CoV-2 spike protein ^a	19150	74	0.15	98
mRNA-1273.529	mRNA SARS-CoV-2 BA.1 spike protein ^b	19194	76	0.13	98
mRNA-1273.214	Bivalent mRNA SARS-CoV-2 spike protein + mRNA SARS-CoV-2 BA.1 spike protein (1:1) ^c	19150 + 19194	NA	NA	NA
UNFIX-01	Untranslating control ^d	DP-016000	84	0.14	93%

Abbreviations: BA.1 = SARS-CoV-2 Omicron variant (also known as B.1.1.529); EE% = encapsulation efficiency; LNP = lipid nanoparticle; mRNA = messenger RNA; NA = not applicable; No = number; PDI = polydispersity index; S-2P = spike protein modified with 2 proline substitutions within the heptad repeat 1 domain; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; SM-102 = a custom-manufactured ionizable lipid.

Note: All mRNA test material stocks were formulated in SM-102-containing LNPs with a final storage buffer in 10.7 mM sodium acetate; 8.7% sucrose; 20 mM tris(hydroxymethyl)aminomethane hydrochloride at pH 7.5 and stored frozen at -20°C. The EE% is the percentage of mRNA inside the LNP. It is calculated by determining the amount of free mRNA that is accessible to ribogreen in intact LNP and the total mRNA present in detergent-treated LNP; $EE\% = 100 - \text{the percentage of nonencapsulated/free mRNA}$.

^a Full-length SARS-CoV-2 S-2P for the original strain (Wuhan-Hu-1) that includes a transmembrane domain for insertion into the cell membrane after translation.

^b Sequence-optimized mRNA encoding prefusion-stabilized SARS-CoV-2 S protein containing S-2P for BA.1 (Omicron).

^c mRNA-1273.214 is a 1:1 bench side mix of separately formulated mRNA-1273 and mRNA-1273.529.

^d The UNFIX-01 contains a short mRNA sequence formulated into the same LNP dispersion as mRNA-1273 and mRNA-1273.529.

2.6.2.2.1 Evaluation of Immunogenicity of Omicron-Matched mRNA Vaccines as Primary Series in Mice (MOD-5156)

The objective of this study was to evaluate the immunogenicity of mRNA-1273 and Omicron-containing vaccines, both monovalent mRNA-1273.529 and bivalent mRNA-1273.214, in mice after 2-dose primary series.

Methods:

Detailed methods are provided in Study [MOD-5156](#).

BALB/c mice (n = 8/group) received 2 IM injections of PBS control article or 1 µg mRNA-1273, mRNA-1273.529, or mRNA-1273.214 as a primary series approximately 3 weeks apart. Blood was collected from all animals on Day 21 (before second dose) and Day 36 (2 weeks after second dose). Serum samples were analyzed for binding antibody responses via ELISA and/or neutralization antibody responses via VSV-based PSVN assay.

Results:

On Day 36 (2 weeks after the second dose), robust S-2P IgG GMTs were observed in all mRNA groups, with no notable differences in S-2P IgG GMTs across treatment groups ([Figure 1A](#)).

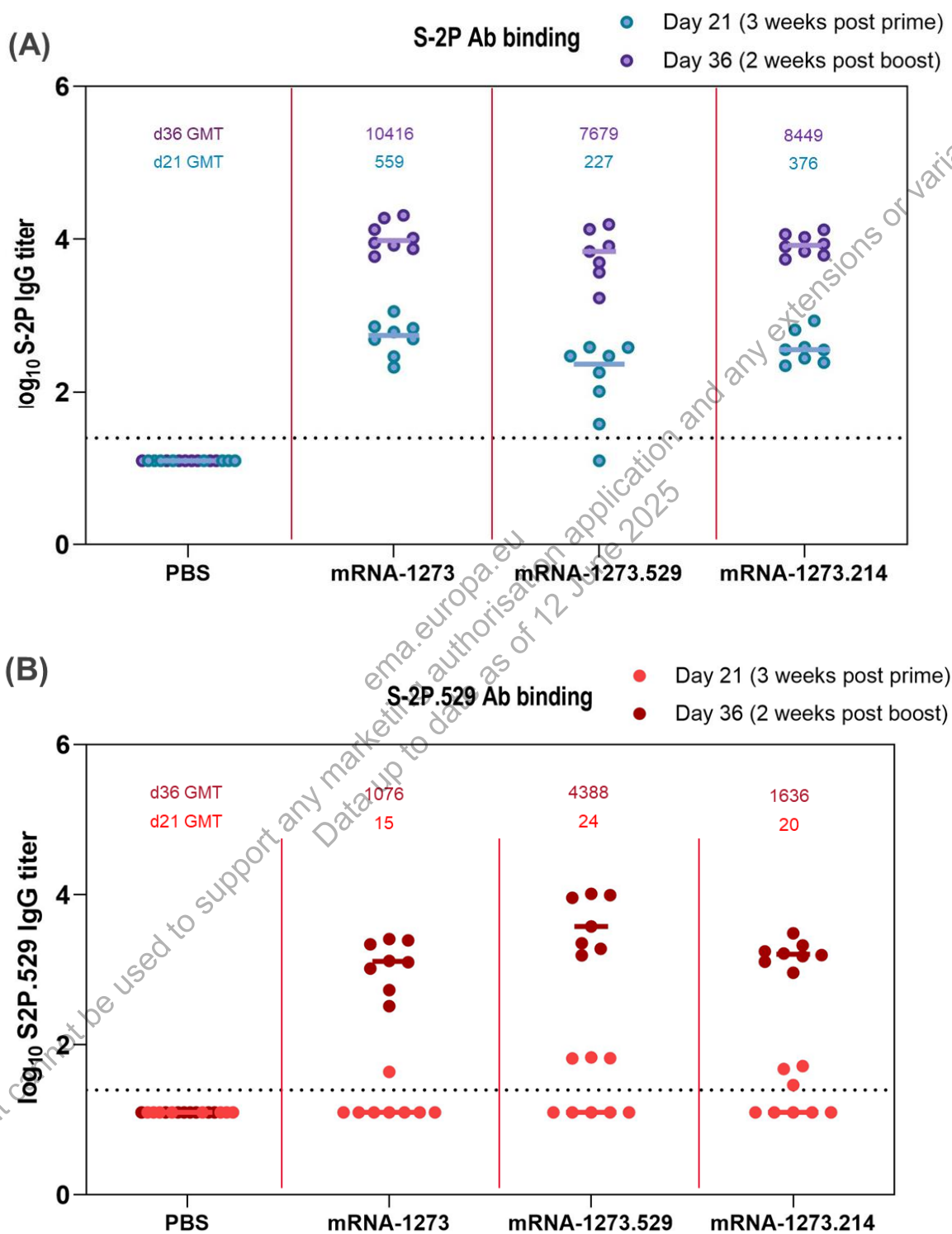
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Mice vaccinated with bivalent mRNA-1273.214 or monovalent mRNA-1273.529 achieved numerically higher S-2P.529 IgG titers than those vaccinated with monovalent mRNA-1273 (Figure 1B). Individually, mRNA-1273 showed robust neutralization responses against WA1 D614G but minimal response against BA.1 and BA.2, while mRNA-1273.529 showed high neutralization responses against BA.1 and BA.2, but minimal response against WA1 D614G (Figure 2A-B). mRNA-1273.214 showed robust neutralization against WA1 D614G, BA.1 and BA.2 (Figure 2B), and overall, mRNA-1273.214 provided the broadest neutralization coverage across the variants evaluated.

Conclusions:

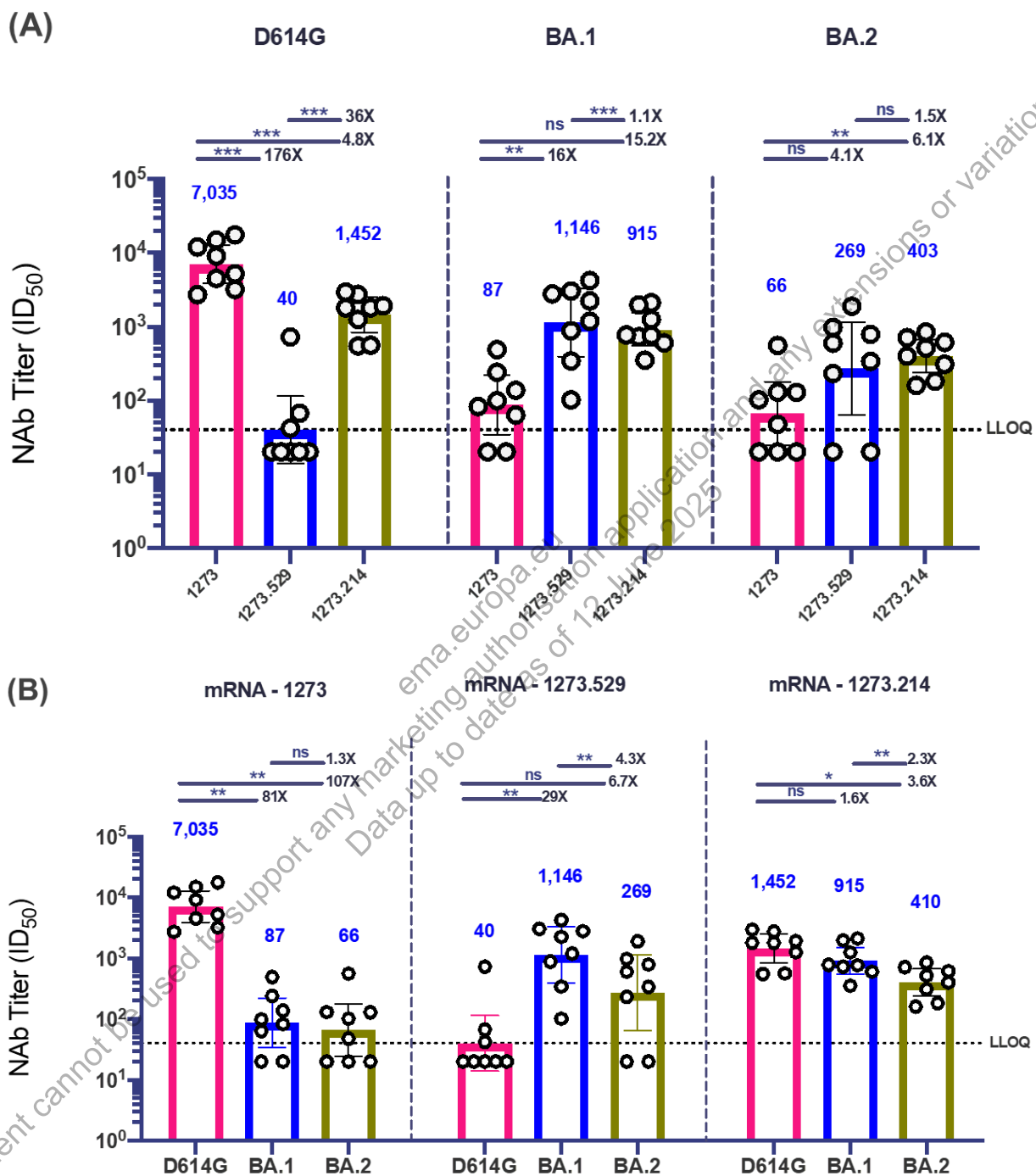
After a 2-dose primary series in mice, mRNA-1273.214 showed robust neutralization against WA1 D614G, BA.1, and BA.2, and overall, mRNA-1273.214 provided the broadest neutralization coverage across the variants evaluated.

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Figure 1: Binding Antibody Responses in BALB/c Mice After Primary Series

Abbreviations: Ab = antibody; GMT = geometric mean titer; IgG = immunoglobulin G; PBS = phosphate-buffered saline;
S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P.
Source: [MOD-5156](#).

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Figure 2: Neutralizing Antibody Responses in BALB/c Mice After Primary Series (Day 36)

Abbreviations: GMT = geometric mean titer; ID₅₀ = infectious dose 50; LLOQ = lower limit of quantification; NAb = neutralizing antibody; ns = not significant.

Note: Statistical analyses performed was nonparametric t-test; * p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001.

Source: MOD-5156.

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2.6.2.2.2 Evaluation of Immunogenicity and B Cell Responses of Omicron-Matched mRNA-Vaccine Boosters in Mice (MOD-5019)

The objectives of this study were to evaluate the immunogenicity and antigen-reactive B cell responses of mRNA-1273 and Omicron-matched boosters in mice after a 3-dose or 4-dose regimen.

Methods:

Detailed methods are provided in Study [MOD-5019](#).

BALB/c mice (n = 8/group) were administered 3 doses (2 dose primary series [Dose 1 and 2] + 1 booster [Dose 3]) or 4 doses (2 dose primary series [Dose 1 and 2] + 2 boosters [Dose 3 and Dose 4]) of 0.25 µg mRNA vaccines or PBS control intramuscularly. Animals who received the 3-dose regimen were administered 0.25 µg mRNA-1273 on Day 1 and Day 22 (primary series [Dose 1 and 2]). Approximately 4 weeks after the second dose (Day 50), these mice were boosted (Booster 1 [Dose 3]) with 0.25 µg of mRNA-1273, mRNA-1273.529, or mRNA-1273.214. Animals who received the 4-dose regimen were administered 0.25 µg mRNA-1273 on Day 1 and Day 22 (primary series [Dose 1 and 2]). Approximately 4 weeks after the second dose (Day 50), these mice were boosted (Booster 1 [Dose 3]) with 0.25 µg of mRNA-1273 or mRNA-1273.529. Approximately 4 weeks after the third dose (Day 78), these mice were boosted again (Booster 2 [Dose 4]) with 0.25 µg of mRNA-1273 or mRNA-1273.529. Animals in Groups 1 (3 doses) and 5 (4 doses) received PBS control article on the same dosing schedule as the active groups.

Blood was collected from all animals on Day 21 (before Dose 2), Day 36 (2 weeks after Dose 2), and Day 49 (before Dose 3). Blood was also collected from animals who received the 3-dose regimen on Day 64 (2 weeks after Dose 3) and from animals who received the 4-dose regimen on Day 93 (2 weeks after Dose 4). Serum samples were analyzed for binding antibody responses via ELISA and neutralization antibody responses via VSV-based PSVN assay.

Mice who received the 3-dose regimen were euthanized on Day 64, and mice who received the 4-dose regimen were euthanized on Day 93, and tissues (spleen and LN) were harvested for antigen-reactive B cell analysis.

Results:

Robust binding antibody (IgG) titers against S-2P and S-2P.529 proteins were observed after a 2-dose primary series (Dose 1 and 2) with mRNA-1273 compared with PBS control. On Day 21 and Day 36 (after primary series [Dose 1 and 2] with 0.25 µg mRNA-1273 in all groups), higher S-2P IgG GMTs (2- to 3-fold) were observed for the mRNA-1273.529 and mRNA-1273.214 booster groups compared to the mRNA-1273 booster group, but these values were considered to be within the observed variation of IgG titers in mice in this study.

On Day 64 (after boosting [Dose 3] with 0.25 µg mRNA-1273, mRNA-1273.529, or mRNA-1273.214), robust binding antibody titers against S-2P and S-2P.529 proteins were

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observed. However, the higher binding antibody titers observed on Day 64 in the mice boosted with mRNA-1273.529 and mRNA-1273.214 may be due to higher pre-boost titers observed in these groups.

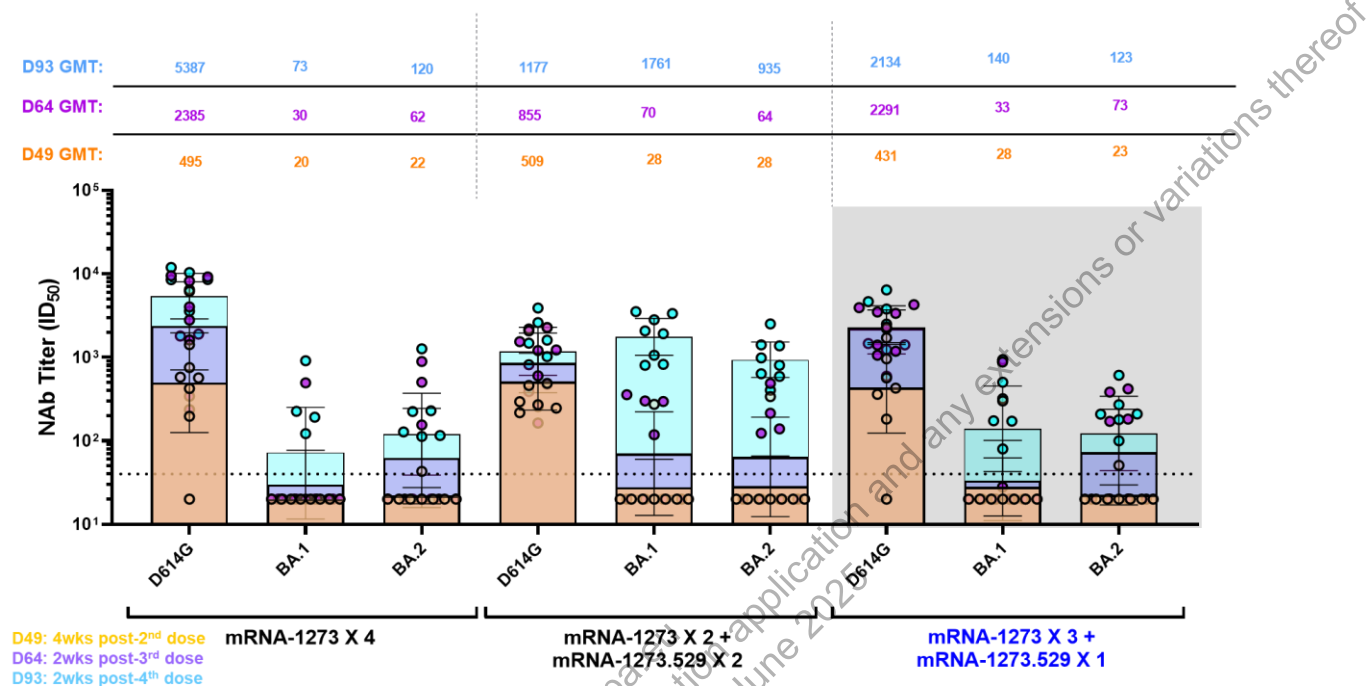
On Day 93 (after boosting [Dose 3 and 4] with 0.25 µg mRNA-1273 or mRNA-1273.529), no notable increases in S-2P or S-2P.529 IgG GMTs were noted compared with Dose 3 across all booster groups. This is likely due to the high levels of pre-existing immunity in these mice at this time point.

On Day 49 (before Dose 3 and after a 2 dose primary series with 0.25 µg mRNA-1273), robust neutralizing antibody responses against D614G (WA1) were observed, but most of the mice had minimal to no neutralizing antibody responses against BA.1 and BA.2 across all groups (Figure 3).

On Day 64 (2 weeks after Dose 3), mice boosted with mRNA-1273 showed a 4-fold increase in neutralizing antibody titers against D614G compared to pre-boost values, but neutralizing antibody titers against BA.1 and BA.2 remained near or below the LLOQ in the majority of mice (Figure 3). Mice boosted with mRNA-1273.529 showed minimal increases in neutralizing antibody titers against BA.1 and BA.2; however, values in most animals remained near or below the LLOQ. Mice boosted with mRNA-1273.214 had higher neutralizing antibody titers against all 3 variants (D614G, BA.1, and BA.2) compared with the mice boosted with mRNA-1273 and mRNA-1273.529, with the majority of mice having BA.1 and BA.2 neutralizing antibody titers above the LLOQ, suggesting that boosting with bivalent mRNA-1273.214 results in more robust immunogenicity against SARS-CoV-2 variants compared to mRNA-1273 or mRNA-1273.529.

On Day 93 (2 weeks after Dose 4), a single booster of mRNA-1273.529 (Dose 4) after 3 doses of mRNA-1273 resulted in similar neutralizing antibody responses against BA.1 and BA.2 to those observed after Dose 3 with mRNA-1273.529, where no marked increase in neutralization titers was observed (Figure 3). Two booster doses of mRNA-1273.529 (Dose 3 and 4) resulted in markedly increased BA.1 and BA.2 neutralization antibody titers. These results likely indicate that the Omicron-specific memory B cells measured after the third dose of mRNA-1273.529 responded to the fourth dose of mRNA-1273.529, resulting in a dramatic increase in neutralizing antibodies against BA.1 and BA.2.

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Figure 3: Neutralizing Antibody Responses in BALB/c Mice on After 3-Dose or 4-Dose Regimen

Abbreviations: D = day; GMT = geometric mean titer; ID₅₀ = infectious dose 50; mRNA = messenger RNA; NAb = neutralizing antibody; wks = weeks.

Notes: Neutralizing antibody titers on Day 49 (2 weeks post Dose 2), Day 64 (2 weeks post Dose 3) and Day 93 (2 weeks post Dose 4) overlaid. The dotted line represents the lower limit of quantification.

Source: MOD-5019.

On Day 64 (2 weeks after Dose 3), robust antigen-specific B cell responses were observed in iliac LN of mice boosted with mRNA-1273, mRNA-1273.529, and mRNA-1273.214. High frequencies of S-2P WT-specific B cells were observed in all 3 booster groups (range: 7.66% to 8.23%; [Figure 4A](#)), while high frequencies of S-2P.529 specific B cells were observed in the iliac LN of mice boosted with mRNA-1273.529 and mRNA-1273.214 (3.04% and 4.48%, respectively), but were not observed in mice boosted with mRNA-1273 (0.052%; [Figure 4B](#)). High frequencies of WT+ .529+ cross-reactive B cells were observed in all 3 booster groups in both assays (range: 3.18% to 7.79%), with the highest percentages observed in mice boosted with bivalent mRNA-1273.214 vaccine ([Figure 4A-B](#)). These results indicate that boosting with Omicron-matched mRNA-1273.529 and mRNA-1273.214 induces antigen-reactive B cells to S-2P.529 in the draining LN, while boosting with mRNA-1273 does not.

In the spleen, minimal antigen-specific B cell responses (< 1%) were observed across all groups, indicating that high frequencies of antigen-reactive B cells were not in the systemic circulation. This result is likely due to the low dose of mRNA (0.25 µg) administered, and the short evaluation period after the booster dose was administered.

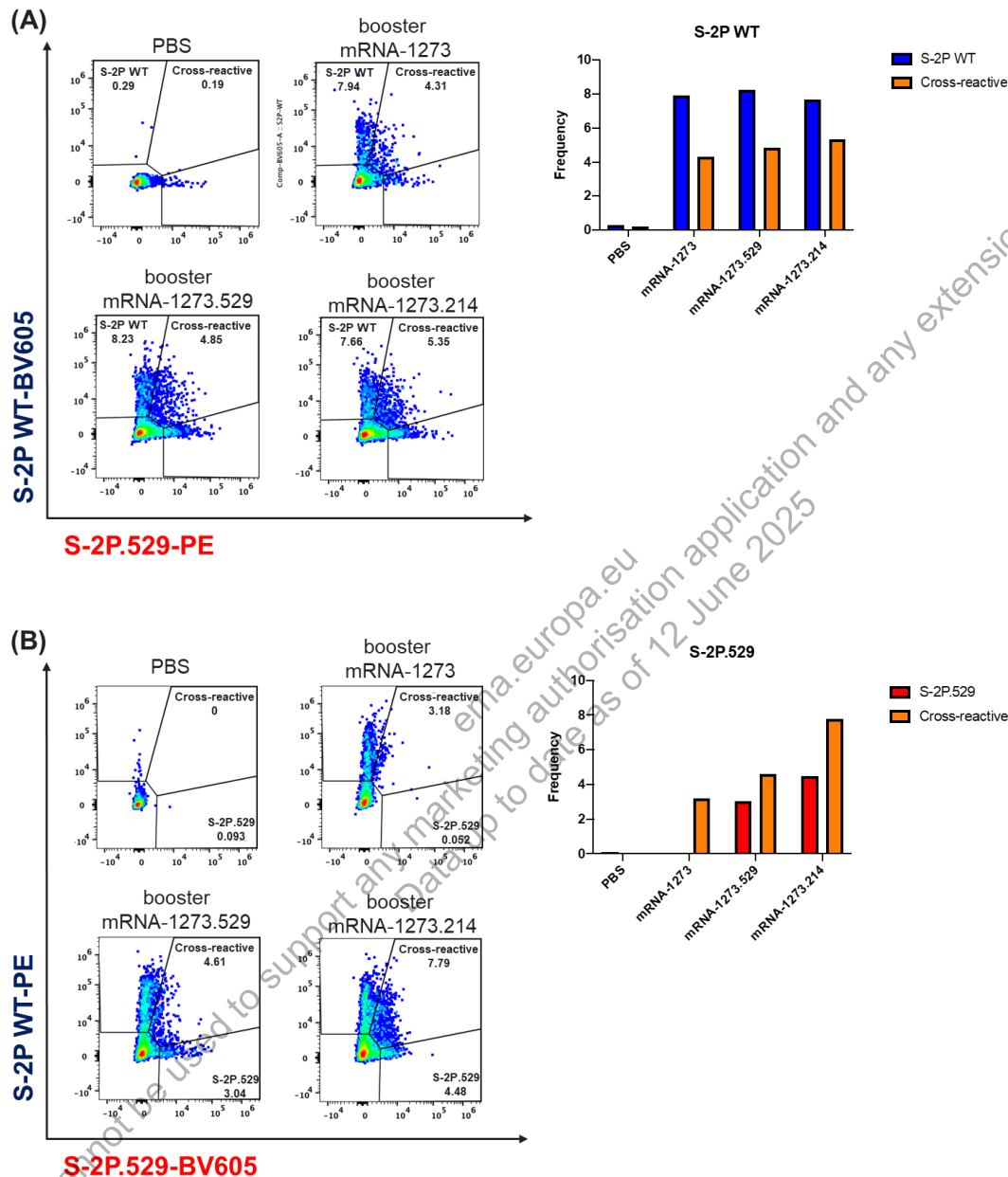
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Overall, these results indicate that while there is an increase in overall SARS-CoV-2 immunity provided by the third dose of mRNA-1273.529 or mRNA-1273.214, BA.1-specific neutralizing antibodies did not increase substantially. However, BA.1 antigen-reactive B cells were produced and were seen in the draining LN, suggesting that the BA.1-specific immunity that is being elicited after the third dose can react quickly upon subsequent vaccination or exposure to virus, as demonstrated by the dramatic increase in neutralizing antibodies against BA.1 and BA.2 after the fourth dose of Omicron-matched mRNA vaccine.

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Figure 4: Frequencies of S-2P and S-2P.529 and Cross-Reactive (WT+ .529+) B Cells in Iliac Lymph Nodes in Mice 2 Weeks After Boost (Dose 3) With mRNA-1273 or Omicron-Matched mRNA Vaccines



Abbreviations: Ig = immunoglobulin; mRNA = messenger RNA; PBS = phosphate-buffered saline; PE = phycoerythrin; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; S-2P.529 = Omicron-specific S-2P; WT = wild-type.

Notes: Iliac lymph nodes were pooled by groups (8 mice/group) and cells isolated were probed using recombinant S-2P WT and Omicron-matched S-2P.529 proteins. Frequencies of antigen-reactive B cells are expressed as a percentage of all IgD⁺ and IgM⁺ class-switched B cells.

Biotinylated S-2P WT and S-2P.529 were conjugated to BV605-labeled streptavidin or to PE-labeled streptavidin. Due to non-specific binding of PE fluorophore in non-immunized control group, frequencies of antigen-reactive cells were calculated based on conjugated BV605-labeled streptavidin only.

2.6.2 Pharmacology Written Summary

(A) Flow cytometry plots and bar graphs showing S-2P WT-specific (top left quadrant) and cross-reactive (top right quadrant) B cells in assay 1. Frequency of S-2P.529 specific B cells (bottom right quadrant) was not calculated due to non-specific binding of S-2P.529-PE probe showing in PBS control group. Bar graph showing the frequencies in the flow cytometry plots. (B) Flow cytometry plots and bar graphs showing S-2P.529-specific (bottom right quadrant) and cross-reactive (top right quadrant) B cells in assay 2. Frequency of S-2P WT-specific B cells (top left quadrant) was not calculated due to non-specific binding of S-2P WT-PE probe showing in PBS control group. Bar graph showing the frequencies in the flow cytometry plots. Source: [MOD-5019](#).

Conclusions:

In conclusion, boosting with Omicron-matched vaccines show equivalent or better BA.1 and BA.2 variant-specific neutralization response than mRNA-1273, but have lower neutralization activity against the WT WA1 strain. The bivalent mRNA-1273.214 vaccine demonstrated higher percentages of WT+ .529+ cross reactivity than the monovalent mRNA vaccines. A single Omicron-matched booster (Dose 3) is not sufficient to dramatically increase neutralization antibody titers, but it is driving the expansion of Omicron antigen-reactive B cells which are available to respond rapidly to subsequent vaccination with mRNA-1273.529. This was seen by the rapid expansion of BA.1 and BA.2 neutralization after Dose 4 with Omicron-matched mRNA vaccine.

2.6.2.2.3 Primary Series and Booster Studies in Mice of mRNA-1273 and mRNA-1273.529 Immunogenicity and Protection From Omicron Challenge (WASHU-01-MOD-5020)

The objectives of this study were to evaluate the antibody response and protection against BA.1 viral challenge, to evaluate the effects of an mRNA-1273 booster dose on antibody responses and protection, to evaluate the immunogenicity of an Omicron-matched vaccine, and to evaluate the protective efficacy of mRNA-1273 and Omicron-matched boosters ([WASHU-01-MOD-5020](#)).

Methods:

Detailed methods are provided in Study [WASHU-01-MOD-5020](#).

To evaluate the antibody response and protection against BA.1, K18-hACE2 female mice, 7 weeks old, received 2 IM injections of 0.1 µg or 5 µg of mRNA control or mRNA-1273 approximately 3 weeks apart. Blood was collected on Day 42 to measure IgG responses against S and RBD (Wuhan-1 and BA.1) via ELISA and neutralizing antibodies (WA1 D614G and BA.1) via FRNT. Five weeks after the second dose, mice were challenged with 10⁴ FFU of WA1 D614G or BA.1 SARS-CoV-2 variants. Mice were sacrificed 6 days post infection, and tissue (nasal wash, nasal turbinates, and lung) was harvested for virological, immunological, and pathological analysis.

To evaluate the effects of an mRNA-1273 booster dose on antibody responses and protection against BA.1, K18-hACE2 female mice, 7 weeks old, received 2 IM injections of 0.25 µg or 5 µg of mRNA control or mRNA-1273 approximately 3 weeks apart (primary series). Approximately 17 to 19 weeks after the second dose, mice were boosted with 1 µg of mRNA control or mRNA-1273. Four weeks after the boost dose, mice were challenged with 10⁴ FFU of BA.1.

2.6.2 Pharmacology Written Summary

Blood samples were collected before the boost dose and at 4 weeks post-boost dose (before challenge), and samples were analyzed for serum neutralizing antibodies via FRNT. Mice were euthanized 6 days post infection, and tissue (nasal wash, nasal turbinates, and lung) was harvested for virological analysis.

To evaluate the immunogenicity of an Omicron-matched vaccine, female BALB/c mice received 0.1 µg or 1 µg of mRNA-1273, mRNA-1273.529, or PBS approximately 3 weeks apart. Blood was collected on Day 21 (before 2nd dose) and at Day 36 (2 week after primary series), and samples were collected to measure IgG responses against S and RBD (Wuhan-1 and BA.1) via ELISA and neutralizing antibodies (1 µg dose only) via VSV-based PSVN.

To evaluate the response to T cells to peptide pools, BALB/c mice received 1 µg of mRNA-1273 or mRNA-1273.529 approximately 3 weeks apart. Spleens were collected on Day 36 to measure SARS-CoV-2 S glycoprotein specific T cell responses.

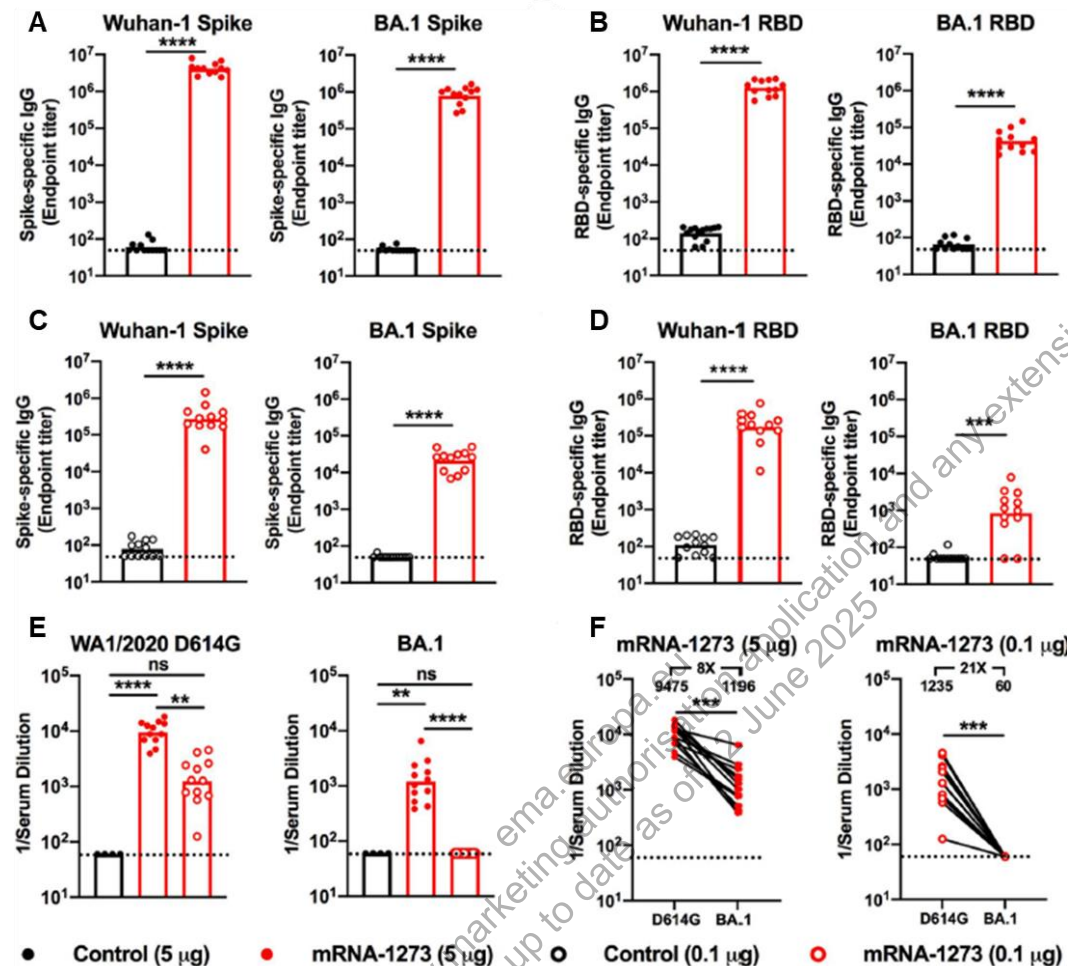
To evaluate the protective efficacy of an Omicron-matched vaccine, 129S2 mice received 0.25 µg or 5 µg of mRNA control or mRNA-1273 on Day 0 and Day 21. Approximately 10 to 11 weeks after the second dose, mice were boosted homologously or heterologously with 1 µg of mRNA control, mRNA-1273, or mRNA-1273.529. Blood was collected before the boost dose and at 3 to 4 weeks post-boost dose to assess neutralizing antibodies (WA1 D614G and BA.1 via FRNT). Three or 4 days after the post-boost blood collection, mice were challenged with 10^5 FFU of WA1/2020 N501Y/D614G or BA.1. Mice were euthanized 3 days post infection, and tissues (nasal wash, nasal turbinates, and lung) were harvested for virological, cytokine, and chemokine analysis.

Results:

Antibody Responses and Protection Against BA.1 in K18-hACE2 Mice

Robust binding antibody (IgG) response was observed against both the Wuhan-1 and BA.1 S (Figure 5A and Figure 5C) and RBD (Figure 5B and Figure 5D) proteins after 2-doses primary series of mRNA-1273. In both mRNA-1273 groups, the Wuhan-1 S- and RBD-specific IgG titers increased in a dose-dependent manner and were higher than the respective BA.1 IgG titers at each dose level. Strong serum neutralizing antibody responses against WA1 D614G and BA.1 were observed after primary series of 5 µg mRNA-1273 compared with mRNA control ($p < 0.0001$ for WA1 D614G and $p < 0.001$ for BA.1, Figure 5E), with significantly reduced (approximately 8 fold, $p < 0.001$) neutralizing antibody GMTs against BA.1 compared with WA1 D614G. After primary series with 0.1 µg mRNA-1273, WA1 D614G neutralizing antibody GMTs were approximately 8-fold ($p < 0.01$) less compared to the 5 µg mRNA-1273 dose, with all BA.1 GMTs being assigned to the LOD. (Figure 5E). In both dose groups, serum BA.1 neutralizing activity was significantly reduced compared with WA1 D614G ($p < 0.0001$; Figure 5F), suggesting that a 2-dose primary series with mRNA-1273 does not produce a strong immunogenicity response against Omicron.

2.6.2 Pharmacology Written Summary

Figure 5: Antibody Responses of mRNA Vaccines in K18-hACE2 Mice

Abbreviations: ANOVA = analysis of variance; IgG = immunoglobulin G; mRNA = messenger RNA; ns = not significant; RBD = receptor-binding domain.

Notes: Statistical analyses performed were as follows: Mann-Whitney test (A-D); 1-way ANOVA with Dunn's post-test (E); and Wilcoxon signed-rank test (F); ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Source: WASHU-01-MOD-5020.

Five weeks after completion of the primary series of mRNA-1273 vaccines (0.1 µg or 5 µg), mice were challenged with WA1 D614G or BA.1. Weight loss was prevented 6 days post infection with WA1 D614G in the mRNA-1273 groups compared with mRNA control ($p < 0.001$), while weight loss was not observed in mice challenged with BA.1 in either the mRNA-1273 or mRNA control groups (Figure 6A-B).

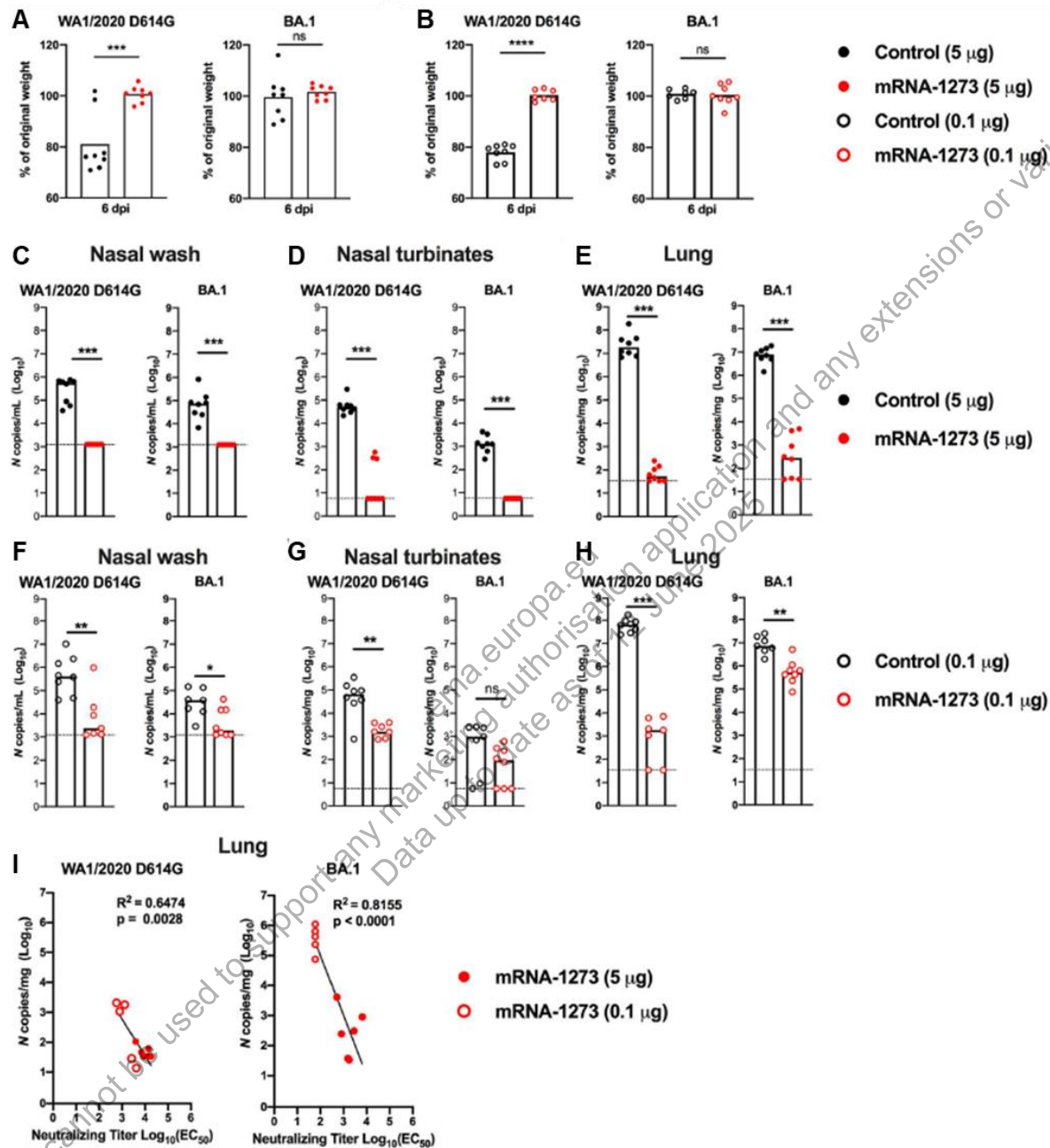
Viral load was measured 6 days post infection in nasal washes, nasal turbinates, and lungs of mice challenged with WA1 D614G or BA.1. In mRNA control mice, BA.1 generally resulted in lower levels of infection when compared with WA1 D614G (Figure 6C-H). Mice vaccinated with 5 µg of mRNA-1273 had robust protection against both WA1 D614G and BA.1, with no detection of WA1 D614G in nasal wash and low levels detected in nasal turbinates and lung. There was no detection of BA.1 in nasal wash or nasal turbinates in the 5 µg group, and low

2.6.2 Pharmacology Written Summary

detection in the lungs (Figure 6C-E). In the 0.1 µg group, protection was less robust against both viral challenges. All animals had detectable WA1 D614G and BA.1 in nasal wash, nasal turbinates, and lungs, although the amount was significantly reduced when compared with the mRNA control group ($p < 0.001$; Figure 6F-H). Serum neutralizing antibody titers were inversely correlated with the viral load in the lung for both WA1 and BA.1 variants (Figure 6I), where the burden of infection generally decreased as the neutralizing antibody titers increased.

Viral challenge resulted in an increased inflammatory response (increased expression of pro-inflammatory cytokine and chemokines) as measured in lung homogenates 6 days post infection. In the 5 µg mRNA-1273 group, the inflammatory response was diminished compared with mRNA controls for both WA1 D614G and BA.1. Mice that received 0.1 µg mRNA-1273 also showed diminished levels of cytokines and chemokines after WA1 D614G challenge; however, no protection was observed in mice challenged with BA.1 where levels of pro-inflammatory cytokines and chemokines in the lung were similar to those observed in the mRNA control group. Lung pathology revealed severe pneumonia in mRNA control mice challenged with WA1 D614G, whereas mice vaccinated with 0.1 µg or 5 µg mRNA-1273 did not develop lung pathology after challenge. In mice challenged with BA.1, the 5 µg group was protected against mild pathological changes, however, pathological findings in the mRNA control and 0.1 µg mRNA-1273 group were similar.

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Figure 6: Protection Against SARS-CoV-2 Infection After mRNA Vaccination in K18-hACE2 Mice

Abbreviations: dpi = days postinfection; EC₅₀ = half maximal effective concentration; mRNA = messenger RNA; ns = not significant; SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2.

Notes: Statistical analyses were performed as follows: unpaired t-test (A, B); Mann-Whitney test (C-H); * p < 0.05;

** p < 0.01; *** p < 0.001; **** p < 0.0001).

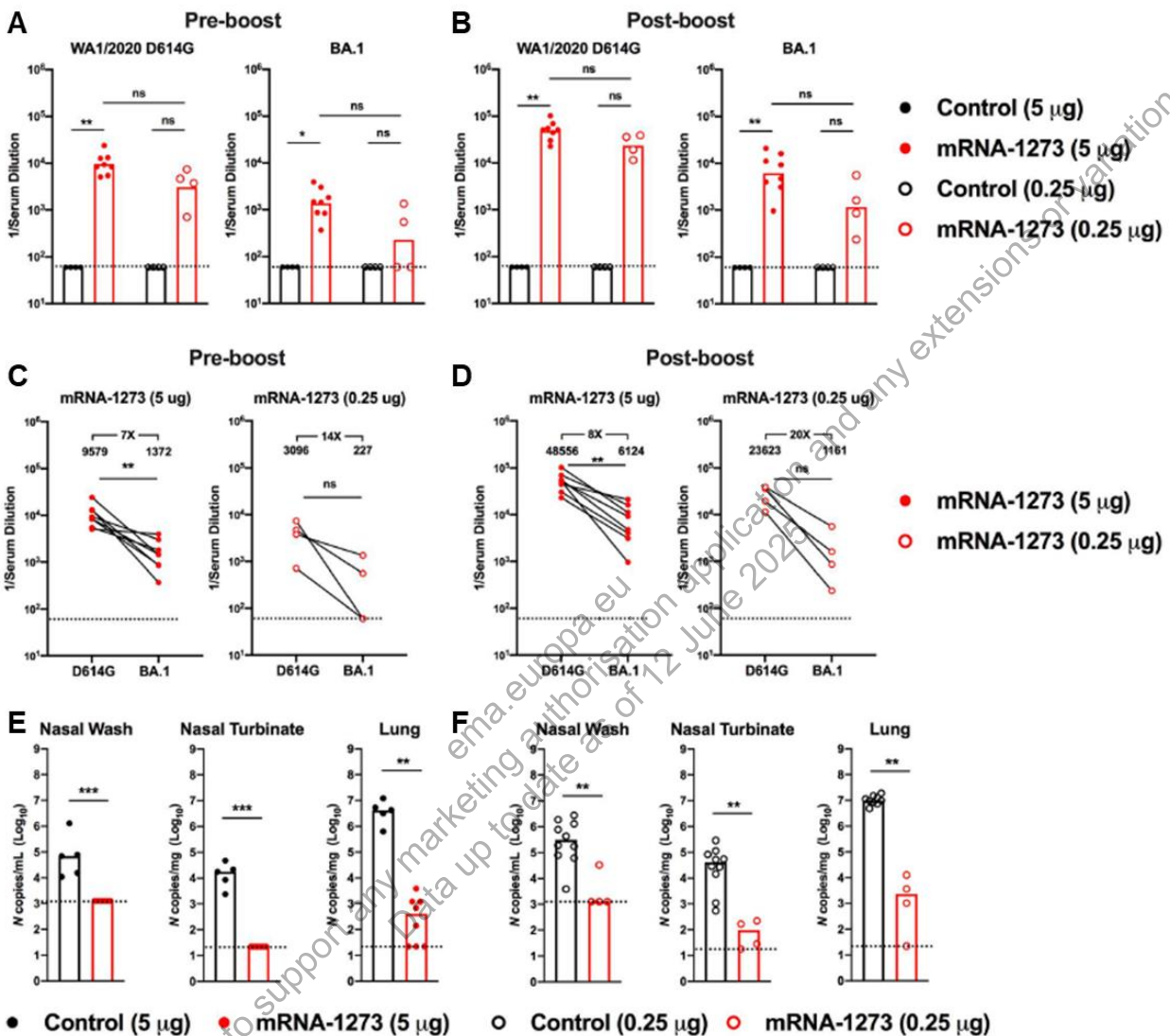
Source: WASHU-01-MOD-5020.

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Effects of an mRNA-1273 Booster Dose on Antibody Responses and Protection Against BA.1 in K18-hACE2 Mice

Prior to administration of a booster dose (mRNA-1273 or mRNA control), neutralizing antibody titers in mice that received the 0.25 µg mRNA-1273 primary series were lower against both WA1 D614G and BA.1 when compared with the 5 µg mRNA-1273 group (Figure 7A). In the 5 µg mRNA-1273 group, serum neutralizing activity was significantly reduced against BA.1 compared with WA1 D614G ($p < 0.01$; Figure 7C). One month after boosting with 1 µg mRNA-1273, serum neutralizing titers rose against both WA1 D614G and BA.1, with a more robust response against WA1 D614G (Figure 7B). At 1 month after boosting with 1 µg mRNA-1273, mice were challenged with BA.1, and viral load was measured in the upper and lower respiratory tract. Viral load in the upper respiratory tract was significantly lower in mice that received the primary series of mRNA-1273 at either dose compared with placebo ($p < 0.001$; Figure 7E-F).

2.6.2 Pharmacology Written Summary

Figure 7: A Booster Dose of mRNA-1273 Enhances Neutralizing Antibody Responses and Confers Protection in K18-hACE2 Mice

Abbreviations: ANOVA = analysis of variance; mRNA = messenger RNA; N = nucleocapsid; ns = not significant.

Notes: Statistical analyses were performed as follows: 1 way ANOVA with Kruskal-Wallis post-test (A, B). Wilcoxon signed-rank test (C, D); Mann-Whitney test (E,F); ** p < 0.01; *** p < 0.001.

Source: [WASHU-01-MOD-5020](#).

2.6.2 Pharmacology Written Summary

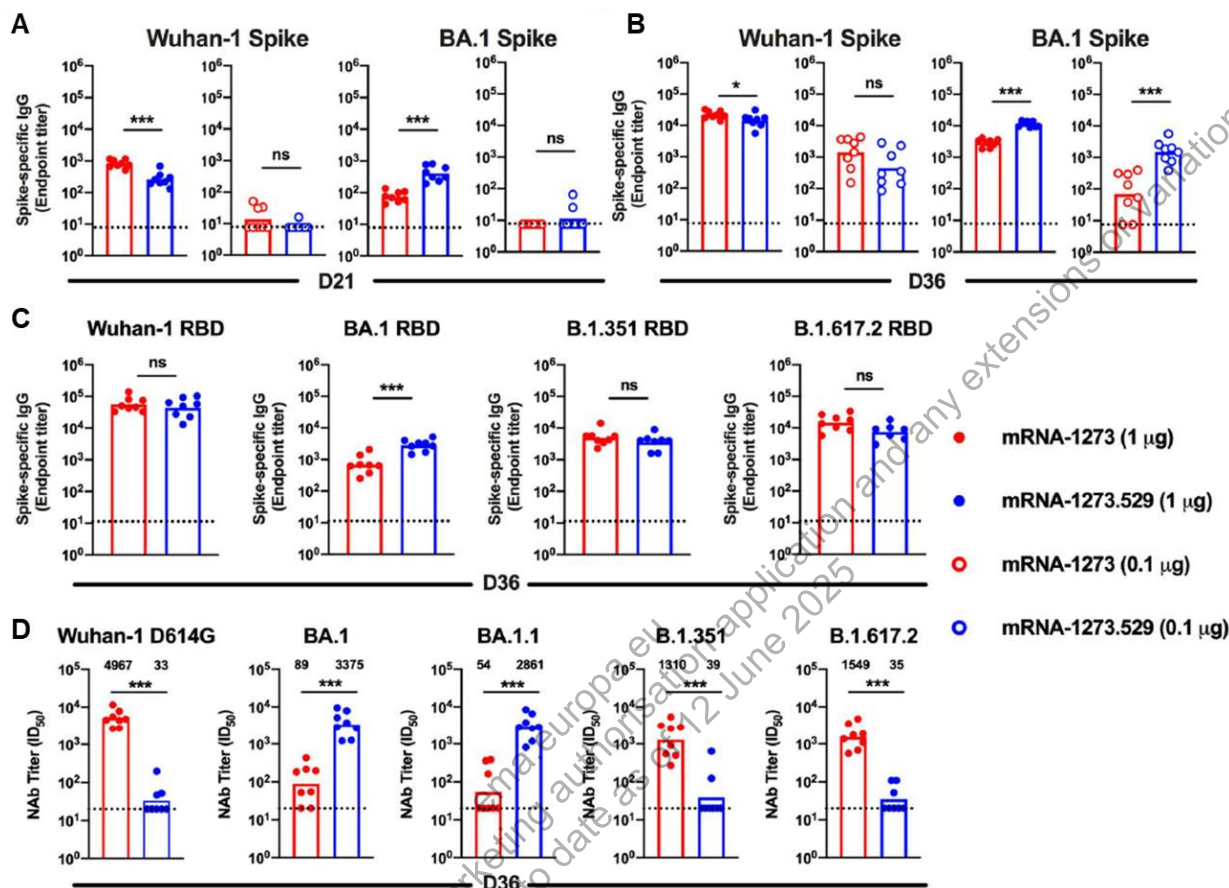
Immunogenicity of an Omicron-matched mRNA Vaccine in BALB/c Mice

On Day 21 (3 weeks after the first dose), Wuhan-1 S-specific IgG titers were higher than the BA.1 S-specific IgG titers in mice that received of 1 µg mRNA-1273, while Wuhan-1 and BA.1 S-specific IgG titers were similar in mice that received 1 µg of mRNA-1273.529 (Figure 8A). At the lower dose of either mRNA-1273 or mRNA-1273.529, IgG titers against both Wuhan-1 and BA.1 were near the LOD.

On Day 36 (2 weeks after the second dose), either dose of mRNA-1273 produced higher IgG titers against Wuhan-1 S than BA.1 S (Figure 8B). In comparison, mice that received 0.1 µg mRNA-1273.529 produced higher IgG titers against BA.1 S than Wuhan-1 S, while Wuhan-1 and BA.1 S-specific IgG titers were similar after 2 doses of 1 µg mRNA-1273.529 (Figure 8B). Additionally, 2 doses of 1 µg mRNA-1273 or mRNA-1273.529 produced similar IgG titers against Wuhan-1 RBD, B.1.351 (Beta) RBD, or B.1.617.2 (Delta) RBD (Figure 8C). The BA.1 RBD-specific titers were higher in mice vaccinated with mRNA-1273.529 (1 µg) than those vaccinated with mRNA-1273 (Figure 8C).

Robust serum neutralizing antibody responses were observed against Wuhan-1 D614G after a primary series of 1 µg mRNA-1273, with slightly lower neutralizing antibody titers against B.1.351 (Beta) or B.1.617.2 (Delta) (Figure 8D). Consistent with data in K18-hACE2 mice, the serum neutralizing activity was significantly reduced against BA.1 and BA.1.1 compared with WA1 D614G. Mice immunized with 2 doses of 1 µg mRNA-1273.529 had high neutralization antibody titers against BA.1 and BA.1.1, but lower levels against WA1 D614G, B.1.351 (Beta), and B.1.617.2 (Delta) (Figure 8D). Overall, these data suggest that a primary series with an Omicron-matched vaccine (mRNA-1273.529) induces robust neutralizing activity against BA.1 and BA.1.1, but not against other previous SARS-CoV-2 variants.

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Figure 8: Antibody Responses in BALB/c Mice After Immunization with mRNA-1273 and mRNA-1273.529 Vaccines

Abbreviations: D = day; ID₅₀ = 50% infectious dose; IgG = immunoglobulin G; mRNA = messenger RNA; NAb = neutralizing antibody; ns = not significant; RBD = receptor-binding domain.

Notes: Statistical analyses were performed as follows: Mann-Whitney test; * p < 0.05; ** p < 0.01; *** p < 0.001.

Source: [WASHU-01-MOD-5020](#)

SARS-CoV-2 Spike Glycoprotein-specific T cell Responses in BALB/c Mice

At Day 36, 2 weeks after the second dose, no significant differences in detected CD4 (IFN- γ , IL-2, TNF- α , IL-4, IL-5, and IL-13) and CD8 (CD107a, IFN- γ , IL-2, and TNF- α) cytokine production to Wuhan-1 S glycoprotein in splenocytes from mice dosed with 1 µg mRNA-1273 versus mRNA-1273.529. The CD4 and CD8 responses matched historical mRNA-1273 T cell mouse data ([Corbett et al 2020a](#)), and there was no evidence of CD4 Th 2 skewed responses in either group.

Protective Effects of an Omicron-matched mRNA Vaccine Booster in 129S2 Mice

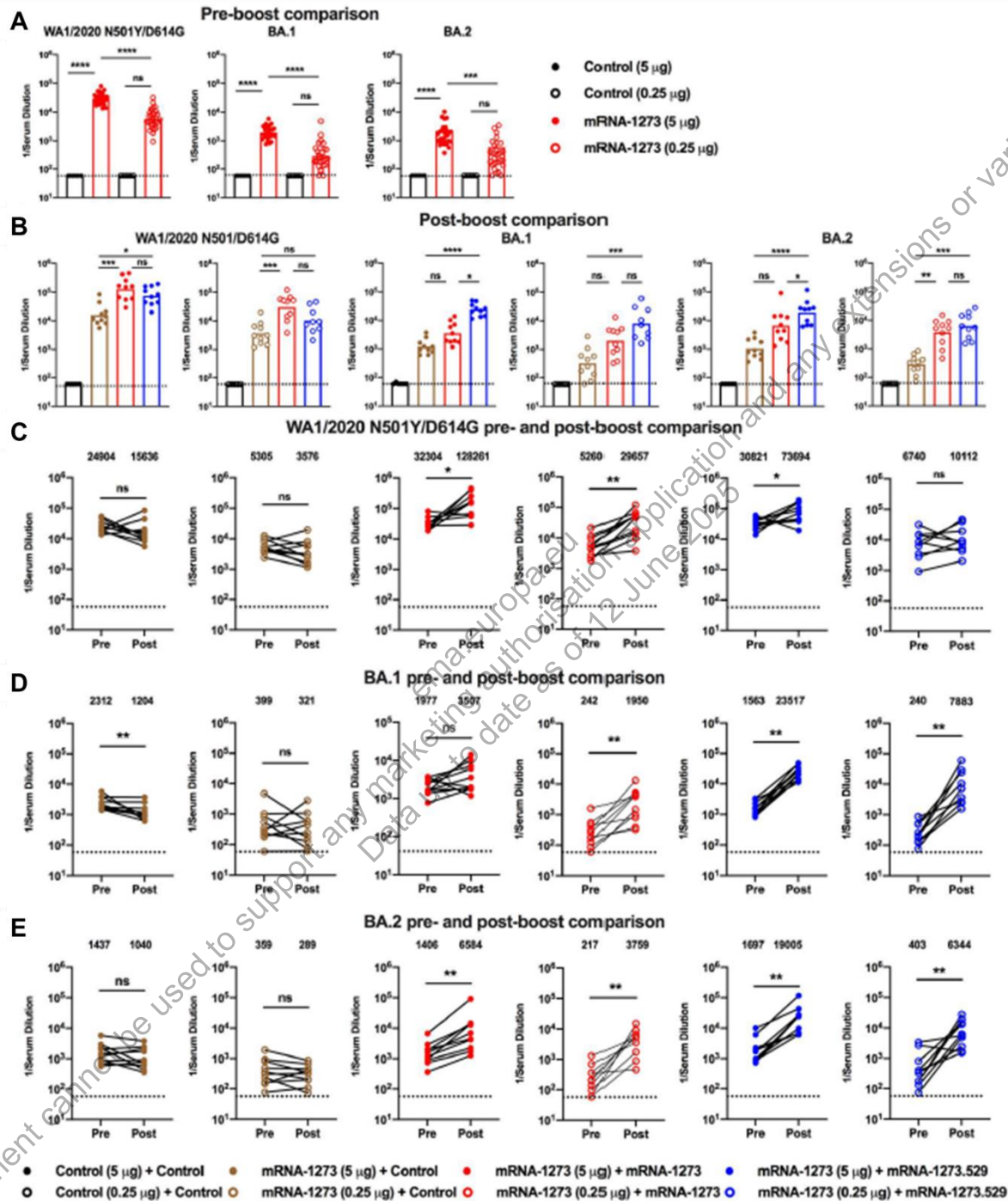
After a 2-dose primary series of 0.25 µg or 5 µg mRNA-1273 or mRNA control vaccines, blood was collected (before boosting), and animals were boosted with 1 µg mRNA control, mRNA-1273, or mRNA-1273.529. Mice that were immunized with 0.25 or 5 µg mRNA-1273

2.6.2 Pharmacology Written Summary

had high levels of pre-boost neutralizing antibody titers against WA1/2020 N501Y/D614G, compared with lower serum pre-boost neutralizing antibody titers against BA.1 or BA.2. (Figure 9A).

One month after boosting with mRNA-1273 or mRNA-1273.529, neutralizing antibody titers against WA1/2020 N501Y/D614G were higher than pre-boost values (Figure 9B-C). Approximately 1 month after boosting with mRNA-1273.529, neutralizing antibody titers against BA.1 and BA.2 were substantially higher than pre-boost values; while mice boosted with mRNA-1273 had a less substantial increase in neutralizing antibody titers against BA.1 and BA.2 (Figure 9D-E). These data suggest that boosting with mRNA-1273 or mRNA-1273.529 both result in enhanced neutralizing antibody responses against BA.1 and BA.2, with higher neutralizing antibodies produced after boosting with an Omicron-matched vaccine.

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Figure 9: Booster Doses of mRNA-1273 or mRNA-1273.529 Enhance Neutralizing Antibody Responses in 129S2 Mice

Abbreviations: ANOVA = analysis of variance; mRNA = messenger RNA; ns = not significant.

Notes: Statistical analyses were performed as follows: 1-way ANOVA with Dunn's post-test (A, B); Wilcoxon signed-rank test (C, D); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Source: WASHU-01-MOD-5020.

2.6.2 Pharmacology Written Summary

After boosting with 1 µg mRNA control, mRNA-1273, or mRNA-1273.529, mice were challenged with WA1/2020 N501Y/D614G or BA.1. At 3 days postinfection, BA.1 viral RNA levels in the upper and lower respiratory tract of mice that received mRNA control was substantially lower than WA1/2020 N501Y/D614G, consistent with known lower pathogenicity of BA.1 in rodents ([Halfmann et al 2022](#)).

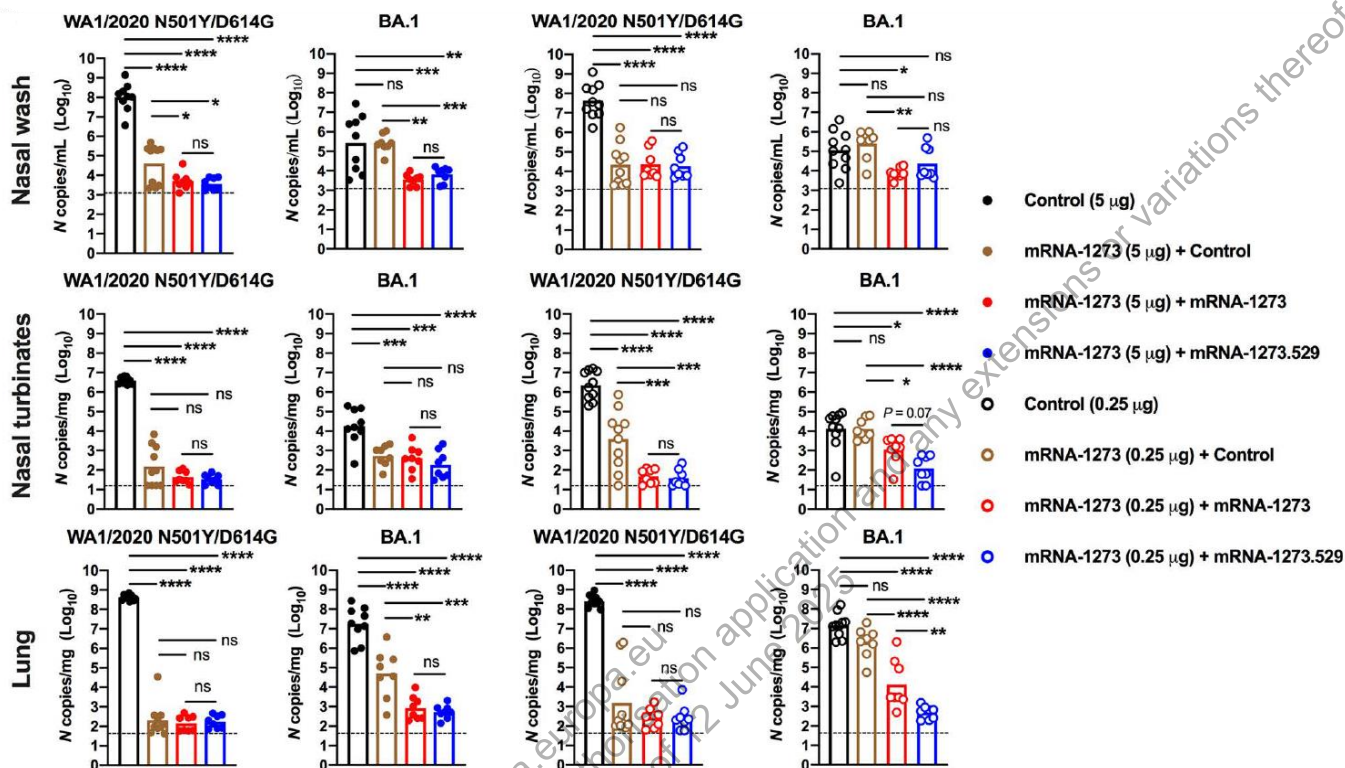
Mice that received a primary series of 0.25 or 5 µg mRNA-1273 and boosted with mRNA-1273 or mRNA-1273.529, WA1/2020 N501Y/D614G viral load was significantly reduced compared with mRNA control ($p < 0.0001$; [Figure 10](#)). In mice primed with 5 µg mRNA-1273 and boosted with either mRNA-1273 or mRNA-1273.529, BA.1 viral RNA levels were low (near LOD), with no significant difference between groups, indicating robust and equivalent protection against BA.1 infection in these animals. However, differential protection against BA.1 was noted in mice primed with 0.25 µg mRNA-1273 and boosted with mRNA-1273 or mRNA-1273.529. While BA.1 viral load in the upper and lower respiratory tract was generally lower with either boost compared to mRNA control, there was a trend toward lower BA.1 viral load ($p = 0.07$) in the nasal turbinate in mice boosted with mRNA-1273.529. Furthermore, BA.1 viral load in the lungs was significantly reduced ($p < 0.01$) in mice boosted with mRNA-1273.529 compared to that in mice boosted with mRNA-1273 ([Figure 10](#)).

Cytokine and chemokine responses in lung homogenates were evaluated 3 days after challenge with WA1/2020 N501Y/D614G or BA.1 in mice that were primed and boosted with mRNA vaccines. After WA1/2020 N501Y/D614G challenge, mice primed with mRNA-1273 and boosted with either mRNA control, mRNA-1273, or mRNA-1273.529 generally had lower levels of pro-inflammatory cytokines and chemokines compared with mice that received 3 doses of mRNA control.

In mRNA control-vaccinated mice, the inflammatory response after BA.1 challenge was diminished compared with WA1/2020 N501Y/D614G infection, consistent with known lower pathogenicity of BA.1 in 129S2 mice ([Halfmann et al 2022](#)). After BA.1 challenge, mice immunized with 5 µg mRNA-1273, and boosted with any of the 3 mRNA vaccines, showed reduced levels of chemokines and cytokines compared to those that had 3 doses of mRNA control, indicating that boosting with homologous mRNA-1273 or heterologous mRNA-1273.529 results in increased protection against BA.1-induced inflammation. Furthermore, BA.1-induced inflammatory response mice primed with 0.25 µg mRNA-1273 and boosted with mRNA control was similar to the response in mice that had 3 doses of mRNA control; however, lower levels of pro-inflammatory cytokines and chemokines were observed in mice primed with 0.25 µg mRNA-1273 and boosted with mRNA-1273.529 compared to those boosted with mRNA-1273.

Overall, boosting with an Omicron-matched vaccine (mRNA-1273.529) resulted in modestly enhanced protection against BA.1 induced inflammatory response in 129S2 mice, consistent with the virology data.

2.6.2 Pharmacology Written Summary

Figure 10: Booster Doses of mRNA-1273 or mRNA-1273.529 Enhance Protection Against BA.1 Infection in 129S2 Mice

Abbreviations: ANOVA = analysis of variance; mRNA = messenger RNA; N = nucleocapsid; ns = not significant.

Notes: 1-way ANOVA with Tukey's post-test; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Source: WASHU-01-MOD-5020.

Conclusions

The low dose vaccines induced levels of neutralizing antibodies in mice similar to those measured in human sera after completion of a 2-dose primary series with mRNA-1273 (Anderson et al 2020; Widge et al 2021; Wu et al 2021). The high-dose vaccines induced neutralizing antibodies that inhibited cell culture of both WA1 D614G and BA.1, although reduced efficacy was observed against BA.1. Mice showed robust protection against both SARS-CoV-2 variants with the high-dose primary series of mRNA-1273. Neutralizing antibodies induced by the low dose primary series of mRNA-1273 showed less inhibitory activity against BA.1, which was reflected in breakthrough infections in the upper and lower respiratory tracts. Cytokine and histology analyses confirmed the lower levels of protection against BA.1 in mice by the low dose series of mRNA-1273. The serum neutralizing titers 1 month after boosting with mRNA-1273 were increased, although the response to BA.1 was lower than the response to WA1 D614G.

Using mRNA-1273.529 in a primary series robustly induced neutralizing antibodies against BA.1; however, neutralizing antibody titers against WA1 D614G, B.1.351 (Beta), and B.1.617.2 (Delta) were lower. The CD4 and CD8 cytokine responses match historical mRNA-1273 T cell

2.6.2 Pharmacology Written Summary

mouse data ([Corbett et al 2020a](#)), and there was no evidence of CD4 Th-2-skewed responses in either group.

Administering mRNA-1273.529 as a booster following a primary series of mRNA-1273 enhanced neutralizing responses against BA.1 and BA.2, with enhanced neutralizing antibody production after boosting. Boosting with either mRNA vaccine resulted in enhanced neutralizing antibody responses against WA1 D614G and was associated with protection in the lower and upper respiratory tract against both viral challenges.

Overall, boosting with either mRNA-1273 or mRNA-1273.529 enhanced protection against BA.1 infection. The differences in protection efficacy between the 2 mRNA boosters was limited, with either booster offering protection against the historical WA1 D614G variant, increased neutralizing titers against BA.1 and BA.2, and increased protection against BA.1 in mice immunized with lower doses.

2.6.2.2.4 mRNA-1273 Primary Series and mRNA-1273 Versus mRNA-1273.529 Booster Regimen in a Rhesus Macaque SARS-CoV-2 Omicron Challenge Model (VRC-20-857)

The objectives of this study were to determine whether boosting with mRNA-1273.529 (matched to Omicron S) approximately 9 months after a 2-dose regimen of mRNA-1273 (4 weeks apart) in rhesus macaques (NHPs) elicits increased/enhanced immunity and protection against Omicron challenge, when compared with a booster of mRNA-1273 ([VRC-20-857](#)).

Methods:

Detailed methods are provided in Study [VRC-20-857](#).

Eight NHPs were immunized with 100 µg mRNA-1273 at Week 0 and Week 4, delivered IM into the right quadriceps. At Week 41, the 8 NHPs were split into 2 groups (N = 4/group) and boosted with 50 µg mRNA-1273 or 50 µg mRNA-1273.529. Eight animals in the mRNA control group were immunized with 50 µg mRNA control at the time of boost and had never been vaccinated with the primary series regimen.

Sera was collected before boosting (Week 6 [peak] and Week 41 [memory]) and after boosting (Week 43) and assessed for S- and RBD-specific IgG binding to WA1 and a panel of VOCs and were measured by a mean square displacement V-plex. Neutralizing antibodies were measured by a live virus assay and substantiated using a lentiviral pseudovirus neutralization assay. Antibody avidity over time following immunization was also measured.

Nasal washes and BAL were collected before boosting at Week 8 (4 weeks after the second dose of the primary series regimen), Week 39, and after boosting at Week 43, to assess upper and lower airway antibody response (binding antibody as measured by ELISA) and antibody function as a surrogate for neutralization capacity as measured by ACE2 receptor inhibition assay.

2.6.2 Pharmacology Written Summary

B cell binding to pairs of fluorochrome-labeled S-2P probes was measured using different VOCs, including Omicron, at Week 6, Week 41, and Week 43 (2 weeks after boosting). To further explore the effect of boosting on anamnestic B cell responses, the activation status of S-binding memory B cells was phenotyped. In addition, T cell responses were measured.

Four weeks after administration of either boost (Week 45), animals were challenged with 1×10^6 PFU via both intratracheal and intranasal routes. Two days after challenge, oral swabs were collected; 2, 4, and 8 days after challenge, BAL fluid was collected; 1, 2, 4, and 8 days after challenge, NS were collected. SARS-CoV-2 sgRNA copy numbers were measured to determine the extent of viral replication. To assess lung pathology in the NHPs, 2 animals/group were euthanized on Day 8.

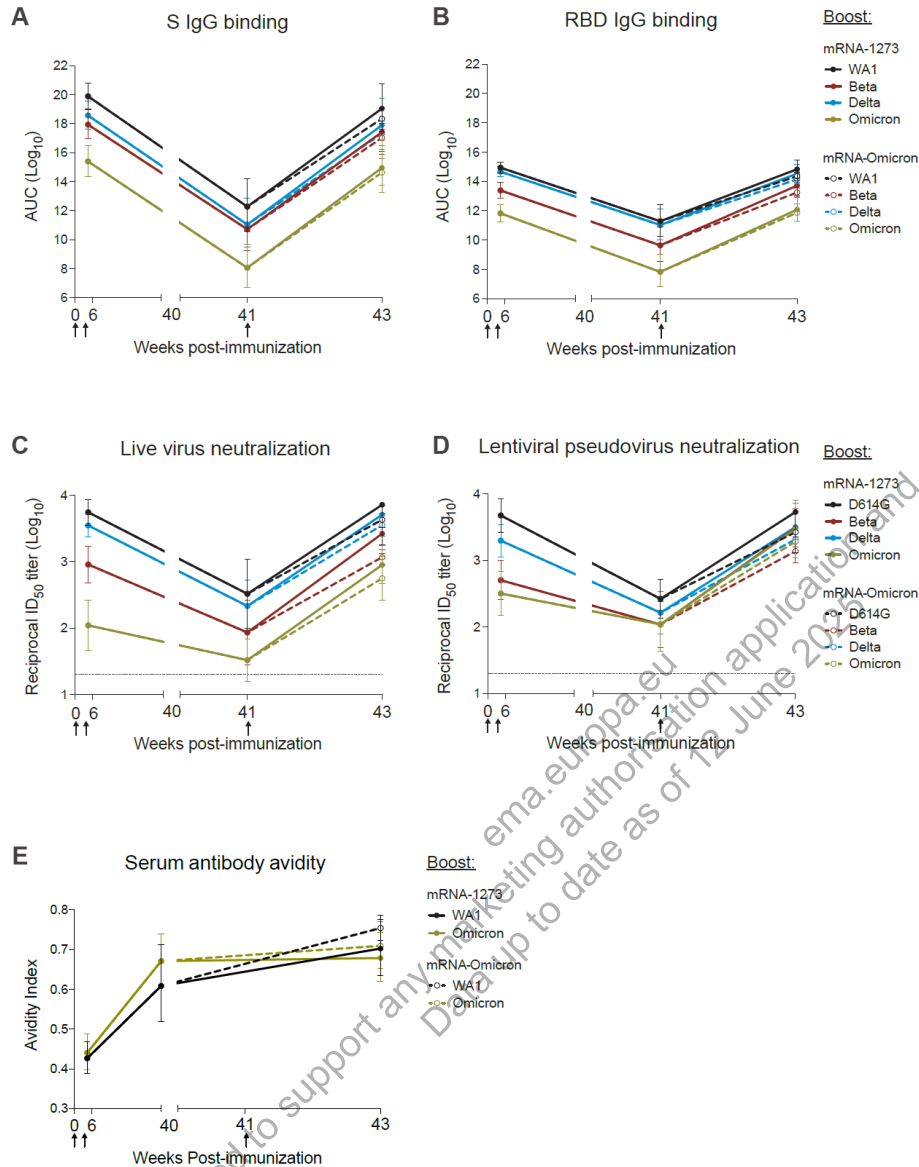
Results:

At Week 6 (2 weeks after the second dose), S and RBD-specific IgG GMTs were highest for WA1 and followed by Delta, Beta, and Omicron (Figure 11A-B). The GMTs were markedly decreased by Week 41 (before boosting) for all VOCs. At Week 43 (2 weeks after boosting with either mRNA-1273 or mRNA-1273.529) IgG GMTs rebounded to close to the same level as those observed at Week 6. Overall, titers to Omicron were lower than the other VOCs.

At Week 6, neutralizing antibody titers (as measured by live virus neutralization assay) were highest for D614G and followed by Delta, Beta, and Omicron; Figure 11C). The neutralizing antibody titers to all VOCs declined by Week 41 (before boosting). At Week 43 (2 weeks after boosting with mRNA-1273 or mRNA-1273.529) the neutralizing antibody titers rebounded to levels similar to those observed at Week 6. The neutralizing antibody titers (as measured by PSVN) followed a similar pattern, with Beta and Omicron titers exceeding levels observed at Week 6 (Figure 11D).

Antibody avidity to Omicron S-2P significantly increased ($p < 0.0001$), and no other changes were observed (Figure 11E).

2.6.2 Pharmacology Written Summary

Figure 11: Serum Antibody Responses Following mRNA-1273 Primary Series and Boost

Abbreviations: AUC = area under the curve; CI = confidence interval; ID₅₀ = reciprocal 50% inhibitory duration; IgG = immunoglobulin G; LOD = limit of detection; mRNA = messenger RNA; NHP = non-human primates; RBD = receptor-binding domain; S = spike.

Note: Circles represent geometric means (A-D) or arithmetic means (E). Error bars represent 95% CI. Assay LOD is indicated by dotted lines. The break in the X-axis indicates a change in scale without a break in the range depicted. Responses to variants are color-coded as WA1 or D614G (black), Beta (red), Delta (blue), and Omicron (green). Arrows represent timepoints of immunizations. Following the boost at Week 41, mRNA-1273-boosted NHP is indicated by solid lines and mRNA-1273.529-boosted NHP is indicated by dashed lines. Four NHP were included per boost group.

Source: [VRC-20-857](#).

2.6.2 Pharmacology Written Summary

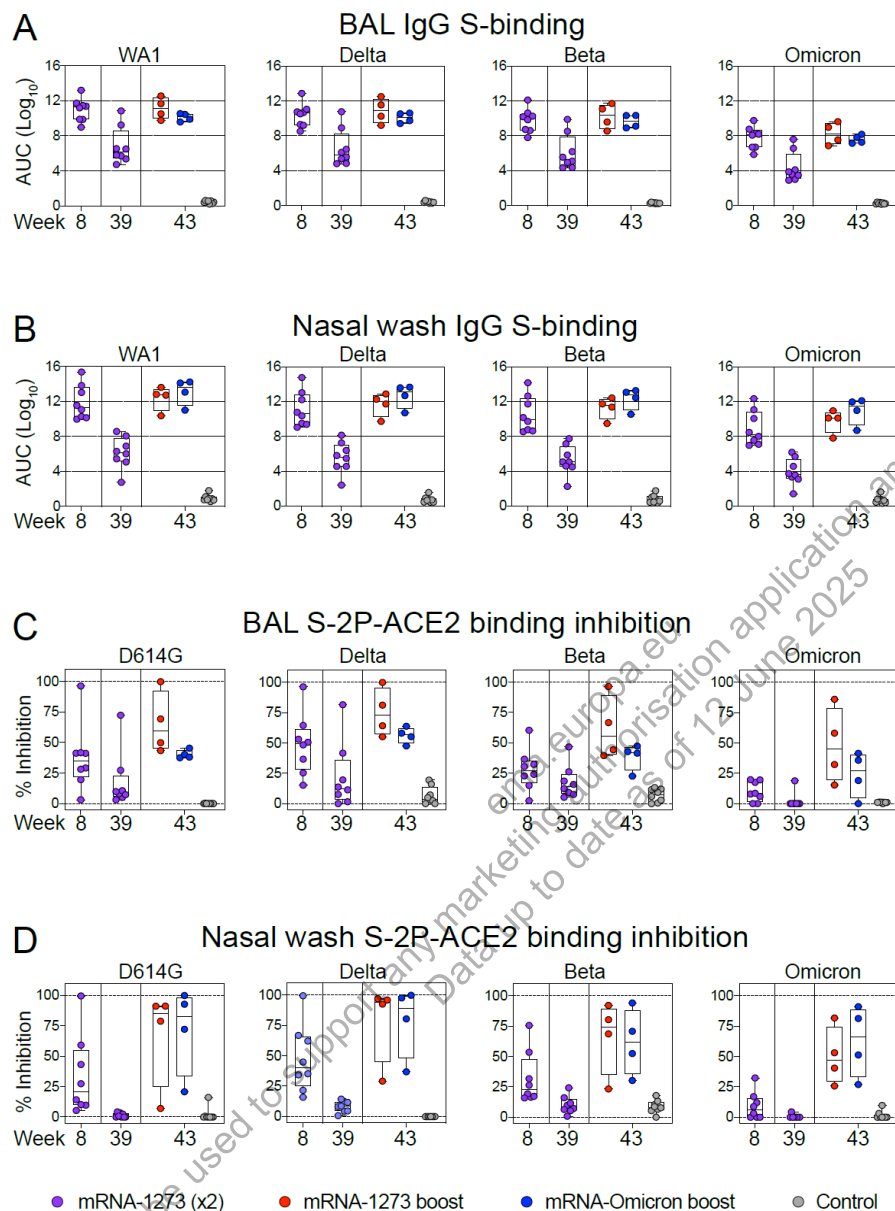
At Week 8, IgG S-specific IgG titers in BAL were highest for WA1 and followed by Delta, Beta, and Omicron (Figure 12A). The IgG titers were decreased for all VOCs by Week 39 (before boosting). At Week 43 (2 weeks after boosting with mRNA-1273 or mRNA-1273.529), titers were increased 3 to 4 logs for all VOCs with values higher than those observed at Week 8.

In an ACE2-binding inhibition assay, BAL expressed 25% to 50% median binding inhibition for all VOCs at Week 8, except for Omicron S-2P (in which binding inhibition was low to undetectable; Figure 12C). There was a decline in ACE2 binding inhibition in all VOCs by Week 39, which increased by Week 43 after boosting with mRNA-1273 or mRNA-1273.529 in all VOCs. Despite an increase in ACE2 inhibition of Omicron S-2P after boosting, it was still lower than all the other variants.

At Week 8, IgG S-specific IgG titers in nasal wash were highest for WA1 and followed by Delta, Beta, and Omicron (Figure 12B). The IgG titers were decreased for all VOCs by Week 39 (before boosting). At Week 43 (2 weeks after boosting with mRNA-1273 or mRNA-1273.529), titers were increased for all VOCs with values higher than those observed at Week 8 (Figure 12B). In nasal wash, ACE2 inhibition was low to undetectable at Week 39 (before boosting) and was increased at Week 43 (2 weeks after boosting) in all VOCs (Figure 12D).

Collectively, these data show that boosting with homologous mRNA-1273 or mRNA-1273.529 leads to comparable and significant increases in neutralizing antibody responses against all VOCs, including Omicron, at Week 43 (2 weeks after boosting). Additionally, boosting was important for enhancing mucosal antibody binding and neutralization responses.

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Figure 12: Mucosal Antibody Response to Boosting with mRNA-1273 or mRNA-1273.529

Abbreviations: ACE2 = angiotensin-converting enzyme 2; AUC = area under the curve; BAL = bronchoalveolar lavage; IgG = immunoglobulin G; NHP = non-human primate; mRNA = messenger RNA; S = spike; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain.

Note: Circles indicate individual NHP. Boxes represent the interquartile range with the median denoted by a horizontal line. Dotted lines are for visualization purposes and denote 4-log₁₀ increases in binding titers (A-B) or 0% and 100% inhibition (C-D). Eight controls and 8 vaccinated NHP were split into 2 cohorts after boosting.

Source: [VRC-20-857](#).

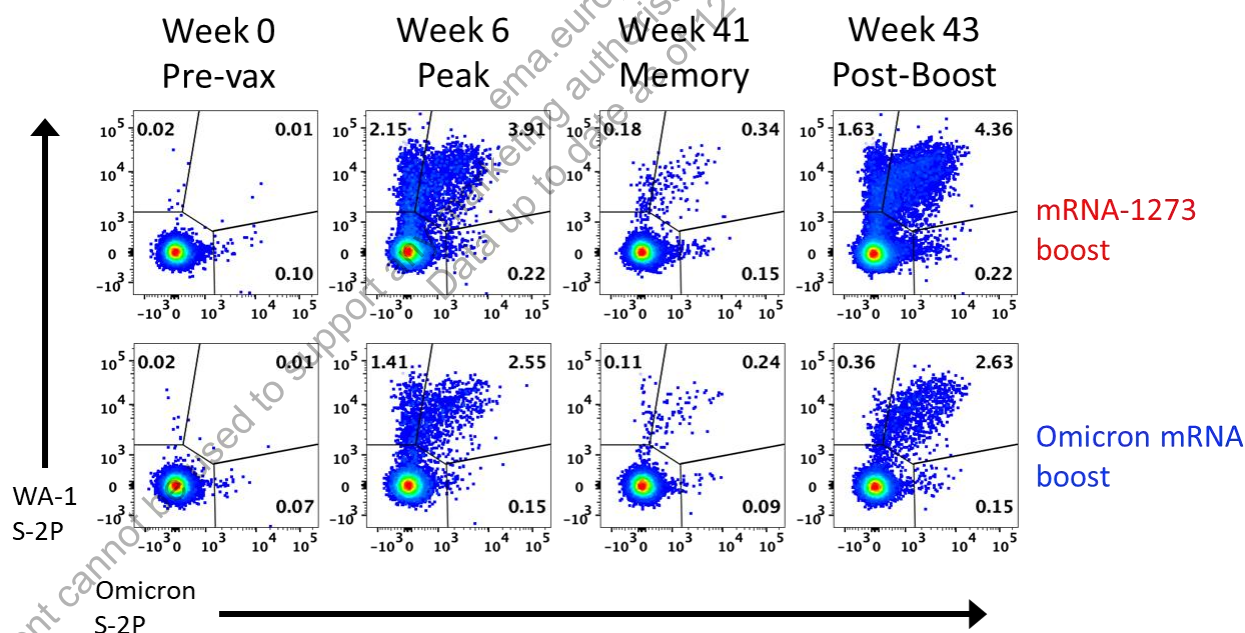
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At Week 6, 63% of the total S-2P-specific memory B cell responses were dual-specific and capable of binding both WA1 and Omicron probes, with 33% binding to WA1 alone and 4% binding to Omicron alone (Figure 13). The total S-specific memory B cell compartment decreased approximately 90% (as a fraction of all class-switched memory B cells) by Week 41. The dual-specific population remained the largest group within the S-binding pool.

At Week 43 (2 weeks after boosting), an expansion of the total S-specific memory B cell compartment (similar to that observed at Week 6) was observed. After boosting with mRNA-1273, 71% of all S-2P-specific memory B cells were dual-specific for WA1 and Omicron. After boosting with mRNA-1273.529, 81% of S-2P-specific B cells were cross-reactive against both WA1 and Omicron. Both the mRNA-1273 and mRNA-1273.529 boosters expanded cross-reactive memory B cells (76% for mRNA-1273 and 85% for mRNA-1273.529), but only the mRNA-1273 boost expanded B cells specific for epitopes unique to the ancestral strain (95% dual-specific for WA1 and/or Delta).

These results indicate that either boost expanded cross-reactive dual-specific (WA1- and Omicron-positive) B cells. Boosting with mRNA-1273 alone also expanded WA1 only B cell responses. This increase was consistent with the similar and high level of neutralizing antibody titers against D614G and Omicron after either booster.

Figure 13: B cell Cross Reactivity



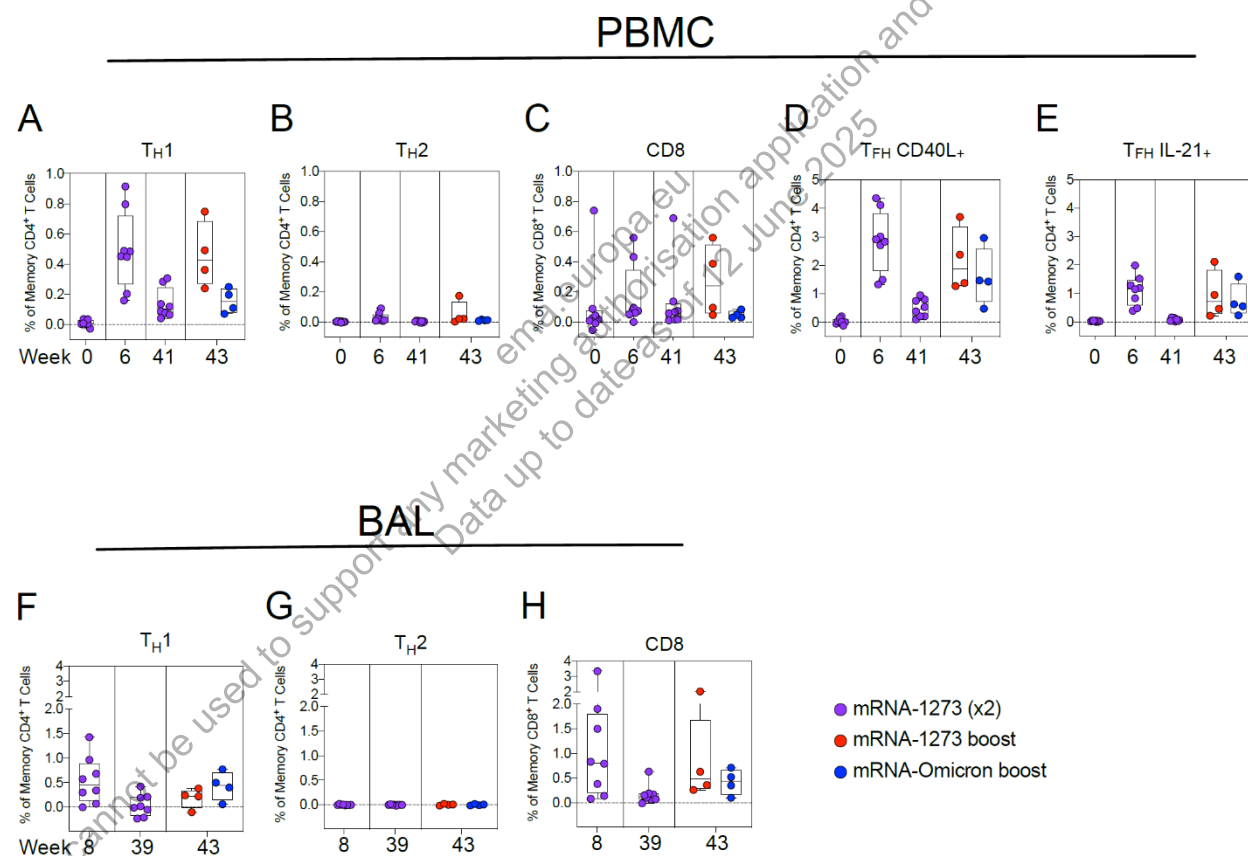
Abbreviations: mRNA = messenger RNA; S-2P = spike protein with 2 proline substitutions within the heptad repeat 1 domain; vax = vaccination.
Source: [VRC-20-857](#).

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mRNA-1273 elicited Th1, Tfh, and low-level CD8 T cell responses at the peak of the response (Week 6) that significantly decreased over time (Figure 14). There was little to no response from Th2 cells. Administration of either mRNA-1273 or mRNA-1273.529 boost significantly increased Tfh responses (Figure 14D and Figure 14E). T helper 1 and CD8 T cells in BAL fluid were detected at Week 8, which decreased to undetectable levels at Week 39 (before boosting) (Figure 14F and Figure 14H). These responses were increased with either the mRNA-1273 or mRNA-1273.529 boost (Week 43).

A primary series with mRNA-1273 and boost with either mRNA-1273 or mRNA-1273.529 induced Th1-directed T cell and IL-21–promoting Tfh cell responses, a profile that is not predicted to promote ERD.

Figure 14: Both mRNA-1273 and mRNA-1273.529 Boost T Cell Responses to Spike Peptides



Abbreviations: BAL = bronchoalveolar lavage; CD = cluster of differentiation; IL = interleukin; mRNA = messenger RNA; PBMC = peripheral blood mononuclear cell; Tfh = T follicular helper; Th1 = T helper 1; Th2 = T helper 2.
Source: [VRC-20-857](#).

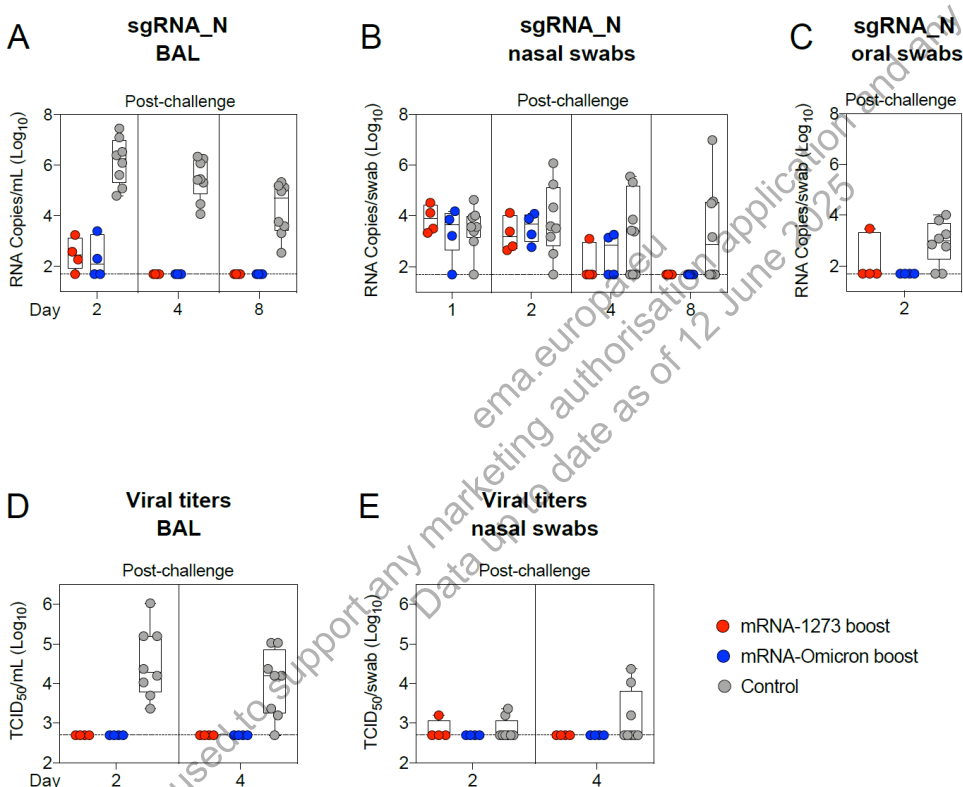
Four days after viral challenge with 1×10^6 PFU of SARS-CoV-2 Omicron, sgRNA (an indication of viral load; sgRNA) showed that unvaccinated NHP had a higher viral load in BAL (2 days post infection) than NHP vaccinated with either mRNA-1273 or mRNA-1273.529. All

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vaccinated NHP had undetectable levels of sgRNA by Day 4 postinfection (Figure 15A). In NS, vaccinated NHP had less sgRNA 4 days post infection compared with unvaccinated NHP, and by Day 8, no vaccinated NHPs had detectable sgRNA (Figure 15B). No viral titers were detected in the BAL of vaccinated NHP on Day 2 and Day 4 post infection (Figure 15D), and in only 1 NHP's NS (Day 2 post infection; Figure 15E).

In lung tissue collected at euthanasia, no viral antigen was detected in lung samples of vaccinated NHPs. Inflammation in unvaccinated NHPs was moderate to severe, whereas vaccinated NHPs exhibited mild to moderate lung pathology.

Figure 15: Boosting Provides Equivalent Protection in the Lungs Against Omicron Challenge



Abbreviations: BAL = bronchoalveolar fluid; LOD = limit of detection; mRNA = messenger RNA; NHP = non-human primate; sgRNA_N = subgenomic RNA encoding for the N gene; TCID₅₀ = median tissue culture infectious dose.

Note: Circles indicate individual NHP. Boxes represent the interquartile range with the median denoted by a horizontal line.

Assay LOD is indicated by dotted lines. Eight controls and 4 vaccinated NHPs were included per boost cohort.

Source: [VRC-20-857](#).

Conclusions:

Results show that boosting with homologous mRNA-1273 or mRNA-1273.529 leads to comparable and significant increases in neutralizing antibody responses against all VOC, including Omicron, at Week 43 (2 weeks after boosting). Additionally, boosting was important for enhancing mucosal antibody binding and neutralization responses. The data showed that

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cross-reactive B cells were expanded following a boost with either mRNA-1273 or mRNA-1273.529, while only mRNA-1273 was capable of boosting memory B cells specific for WA1 alone. Significant and equivalent control of virus replication in lower airways was observed following either boost. There was no evidence of viral antigen in the lung samples of any vaccinated NHP, and boosted animals displayed histopathologic alterations that were classified as minimal to mild or moderate. The data indicated that protection against Omicron was robust in the lungs regardless of boost selection at the timepoint evaluated.

2.6.2.3 SECONDARY PHARMACODYNAMICS

No secondary pharmacodynamic studies have been performed with mRNA-1273.214.

2.6.2.4 SAFETY PHARMACOLOGY

No safety pharmacology studies have been performed with mRNA-1273.214.

2.6.2.5 PHARMACODYNAMIC DRUG INTERACTIONS

No pharmacodynamic drug interaction studies have been performed with mRNA-1273.214.

2.6.2.6 DISCUSSION AND CONCLUSIONS

In November 2021, the SARS-CoV-2 Omicron variant (BA.1) was detected in South Africa. Available evidence confirms that BA.1 has transmission advantage over prior variants, with significant antigenic change. In addition, it contains antibody escape site mutations (such as K417N, T478K, E484A, N501Y, among others). The BA.1 variant contains more than 30 amino acid substitutions. Additional sub-lineages of Omicron have also emerged with one, BA.2, demonstrating increased transmissibility versus BA.1 which subsequently became the predominant circulating variant in most geographical regions.

Variant-matched booster vaccines have been suggested as a strategy to focus the antibody response against VOCs compared to the authorized, standard-of-care booster vaccines against COVID-19.

In support of the development of mRNA-1273.214, the Sponsor has conducted nonclinical primary pharmacology studies with mice and NHPs to test the immunogenicity, antigen-reactive B cell response, and protection offered by a combination of mRNA-1273 or Omicron-matched vaccines as primary series (mRNA-1273, mRNA-1273.529, or mRNA-1273.214) with or without boosting with mRNA-1273, mRNA-1273.529, or mRNA-1273.214. The results are summarized as follows:

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Primary Series – Mice

- After a 2-dose primary series in mice, mRNA-1273.214 showed robust neutralization against WA1 D614G, BA.1, and BA.2, and overall, mRNA-1273.214 provided the broadest neutralization coverage across the variants evaluated ([Section 2.6.2.2.1](#)).
- After primary series with mRNA-1273, robust binding antibody (IgG) titers against S-2P and S-2P.529 were seen, along with robust neutralizing antibody responses against WA1 D614G. However, most mice had minimal to no neutralizing antibody responses against BA.1 and BA.2 across all groups ([Section 2.6.2.2.2](#)).
- A low dose mRNA-1273 primary series (0.1 µg or 0.25 µg) induced levels of neutralizing antibodies in mice similar to those measured in human sera. However, there was less inhibitory activity against BA.1, which was reflected in breakthrough infections in the upper and lower respiratory tracts after viral challenge. Cytokine and histology analyses confirmed the low-to-minimal protection against BA.1 ([Section 2.6.2.2.3](#)).
- A high-dose mRNA-1273 primary series (5 µg) induced antibodies that neutralized both WA1 D614G and BA.1, although reduced neutralization was observed against BA.1. Mice showed robust protection against both WA1 D614G and BA.1 viral challenge ([Section 2.6.2.2.3](#)).
- Using mRNA-1273.529 as a primary series induced robust neutralizing antibodies against BA.1; however, neutralizing antibody titers against WA1 D614G, B.1.351 (Beta), and B.1.617.2 (Delta) were lower ([Section 2.6.2.2.3](#)).

Primary Series Plus Booster(s) – Mice and NHP

- Mice boosted with mRNA-1273.214 had higher neutralizing antibody titers against all 3 variants (WA1 D614G, BA.1, and BA.2) compared with the mice boosted with mRNA-1273 and mRNA-1273.529 ([Section 2.6.2.2.2](#)).
- Boosting mice with mRNA-1273.529 or mRNA-1273.214 induced antigen-reactive B cells to S-2P.529 in the draining LN, while boosting with mRNA-1273 did not. In the spleen, minimal antigen-specific B cell responses were observed across all groups, indicating that antigen-reactive B cells were not in sufficient quantity to be measured in the systemic circulation ([Section 2.6.2.2.2](#)).
- The serum neutralizing titers in mice 1 month after boosting with mRNA-1273 were increased, although the response to BA.1 was lower than the response to WA1 D614G. Boosting mice with mRNA-1273.529 following a primary series of mRNA-1273 enhanced neutralizing responses against BA.1 and BA.2 ([Section 2.6.2.2.3](#)).
- Boosting mice with either mRNA vaccine resulted in enhanced neutralizing antibody responses against WA1 D614G and was associated with protection in the lower and upper

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respiratory tract against WA1/2020 N501Y/D614G or BA.1 viral challenge along with lower levels of pro-inflammatory cytokines ([Section 2.6.2.2.3](#)).

- Boosting NHPs with mRNA-1273 or mRNA-1273.529 led to comparable and significant increases in neutralizing antibody responses against all VOCs, including Omicron, and was important for enhancing mucosal antibody binding and neutralization responses ([Section 2.6.2.2.4](#)).
- Cross-reactive B cells in NHPs were expanded following a boost with mRNA-1273 or mRNA-1273.529, while only mRNA-1273 was capable of boosting memory B cells specific for WA1 alone ([Section 2.6.2.2.4](#)).
- Significant and equivalent control of virus replication in lower airways was observed in NHPs following either boost. There was no evidence of viral antigen in the lung samples of any vaccinated NHP, and boosted animals displayed histopathologic alterations that were classified as minimal to mild or moderate ([Section 2.6.2.2.4](#)).
- Two booster doses of mRNA-1273.529 resulted in markedly increased BA.1 and BA.2 neutralization antibody titers in mice, likely indicating that the Omicron-specific memory B cells measured after the third dose of mRNA-1273.529 responded to the fourth dose of mRNA-1273.529 ([Section 2.6.2.2.2](#)).

Additionally, in NHPs, a primary series with mRNA-1273 and a boost with either mRNA-1273 or mRNA-1273.529 drives a predominant Th1-directed immune response, which is not predicted to drive vaccine associated ERD. Prior studies with similar designs to the booster studies described here by the Sponsor also showed no vaccine associated ERD in mice, hamsters, and NHPs, as was demonstrated by balanced Th1/Th2 directed immune responses to immunization (mRNA-1273 Biologics License Application).

Overall, mRNA-1273 affords lower neutralization and protection against key VOCs, while mRNA-1273.529 more potently neutralizes BA.1. However, mRNA-1273.529 has lower neutralization against other non-BA.1 variants or the ancestral SARS-CoV-2 strain. Bivalent vaccines, such as mRNA-1273.214, demonstrated greater cross-variant neutralization in nonclinical studies. Refer to mRNA-1273.214 Module 2.5 for a summary of results from clinical studies with mRNA-1273 bivalent vaccines. Both mRNA-1273.529 and mRNA-1273.214 showed equivalent or better BA.1 and BA.2 neutralization, protection, and antigen-reactive B cells versus mRNA-1273. In mice, mRNA-1273.214 demonstrated increased potency when compared with monovalent vaccines.

2.6.2.7 TABLES AND FIGURES

Tables and figures are included in their respective sections.

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2.6.2.8 REFERENCES

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