Supplementary Appendix

Supplement to: Pajon R, Doria-Rose NA, Shen X, et al. SARS-CoV-2 omicron variant neutralization after mRNA-1273 booster vaccination. N Engl J Med 2022;386:1088-91. DOI: 10.1056/NEJMc2119912

This appendix has been provided by the authors to give readers additional information about the work.

(PDF updated February 1, 2022)

Supplement

Table of Contents

Acknowledgements
The authors thank William Hildebrand (Moderna Biomarker group), Kai Wu (Moderna Infectious Diseases Virology group), and Joanne E Tomassini and Frank Dutko (Moderna consultants) for writing and editorial support.

Supplementary Methods

Selection of immunogenicity subset

Participant samples

Serum samples for testing in the immunogenicity subset were randomly selected from healthy adult participants who completed a primary vaccination series of two doses of 100 µg mRNA-1273 in the blinded Part A of the phase 3 COVE study (NCT04470427) and were enrolled in the ongoing phase 2/3 open-label study (NCT04927065) and received 50 µg or 100 µg mRNA-1273.211 (Part A), or 100 µg mRNA-1273 (Part B) or 100 µg mRNA-1273.213 (Part D) (Table S1). Additionally, sera samples were tested from randomly selected participants who completed the two-dose vaccination regimen in the blinded Part A of the phase 2 trial (NCT04405076) and were enrolled in the open-label part B of the study and received a 50 µg dose of mRNA-1273 booster.[1,2] Samples were selected from the trials such that 20 participants would be included in each dose/booster vaccine arm, and balanced with 10 participants each in age groups of <65 and ≥65 years to avoid biasing the results for younger vs older age groups. Samples were generally balanced for characteristics as in the larger clinical trials (Table S1 and S2). Booster mRNA-1273.211 comprised 25 µg each of mRNAs encoding ancestral Wuhan-Hu-1 (mRNA-1273) and the Beta B.1.351 (mRNA-1273.351) spike proteins and mRNA-1273.213 consisted of 25 µg each of mRNA encoding for Delta B.1.617.2 (mRNA-1273.617.2) and Beta B.1.351 (mRNA-1273.351) spike proteins. The length of median time between the 2nd dose of the primary vaccination series and the booster ranged from 7 to 13 months (Table S1). All immunological time-point serum samples in these clinical studies was tested for the presence of anti-nucleocapsid (N) antibodies using the ROCHE's ELECSYS SARS-CoV-2 N assay. With two exceptions (see Figure S3), the samples used in the current study come from participants who did not contain detectable anti-N antibodies at the time of sample collection as evidence for the absence of prior infection.

Pseudovirus Neutralization Assay (VRC)

Neutralization activity against SARS-CoV-2 was measured in a single-round-of-infection assay with lentivirus-based Spike-pseudotyped virus particles (pseudoviruses) as previously described.[3] To produce SARS-CoV-2 pseudoviruses, an expression plasmid bearing codon-optimized SARS-CoV-2 full-length S plasmid was co-transfected into HEK293T/17 cells (ATCC#CRL-11268) cells with packaging plasmid pCMVDR8.2, luciferase reporter plasmid pHR′CMV-Luc and a TMPRSS2 plasmid. Mutant S plasmids were produced by Genscript. Pseudoviruses were mixed with 8 serial 4-fold dilutions of sera or antibodies in triplicate and then added to monolayers of ACE2-overexpressing 293T cells (gift of Michael Farzan and Huihui Mu), in triplicate. Three days post infection, cells were lysed, luciferase was activated with the Luciferase Assay System (Promega), and relative light units (RLU) were measured at 570 nm on a Spectramax L luminometer (Molecular Devices). After subtraction of background RLU (uninfected cells), % neutralization was calculated as 100x((virus only control)-(virus plus

antibody))/(virus only control). Dose-response curves were generated with a 5-parameter nonlinear function, and titers reported as the serum dilution or antibody concentration required to achieve 50% (50% inhibitory dilution [ID50]) neutralization. The input dilution of serum is 1:20, thus, 20 is the lower limit of detection. Samples that do not neutralize at the limit of detection at 50% are plotted at 10, and that value was used for geometric mean calculations. Each assay includes triplicates. In addition, the reported values for samples in VRC200 cohort are the geometric mean of 2 or more assays.

Pseudovirus Neutralization Assay (Duke)

The pseudovirus neutralization assay performed at Duke has been described in detail[4] and is a formally validated adaptation of the assay utilized by the VRC; the Duke assay is FDA approved for D614G. Both assays utilized identical reagents for pseudovirus production and identical cells as targets for infection. For measurements of neutralization, pseudovirus was incubated with 8 serial 5-fold dilutions of serum samples (1:20 or 1:10 starting dilution) in duplicate in a total volume of 150 µl for 1 hr at 37ºC in 96-well flat-bottom culture plates. 293T/ACE2-MF cells were detached from T75 culture flasks using TrypLE Select Enzyme solution, suspended in growth medium (100,000 cells/ml) and immediately added to all wells (10,000 cells in 100 µL of growth medium per well). One set of 8 wells received cells + virus (virus control) and another set of 8 wells received cells only (background control). After 66-72 hrs of incubation, medium was removed by gentle aspiration and 30 µl of Promega 1X lysis buffer was added to all wells. After a 10 minute incubation at room temperature, 100 µl of Bright-Glo luciferase reagent was added to all wells. After 1-2 minutes, 110 µl of the cell lysate was transferred to a black/white plate. Luminescence was measured using a GloMax Navigator luminometer (Promega). Neutralization titers are the inhibitory dilution (ID) of serum samples at which RLUs were reduced by 50% (ID50) compared to virus control wells after subtraction of background RLUs. Serum samples were heat-inactivated for 30 minutes at 56ºC prior to assay.

Live Virus Focus-Reduction Neutralization (FRNT) Assay (Emory)

VeroE6 cells were obtained from ATCC (clone E6, ATCC, #CRL-1586) and cultured in complete DMEM medium consisting of 1x DMEM (VWR, #45000-304), 10% FBS, 25mM HEPES Buffer (Corning Cellgro), 2mM L-glutamine, 1mM sodium pyruvate, 1x Non-essential Amino Acids, and 1x antibiotics. VeroE6-TMPRSS2 cells were generated and cultured as previously described.[5] nCoV/USA_WA1/2020 (WA/1), closely resembling the original Wuhan strain and resembles the spike used in the mRNA-1273 and Pfizer-BioNTech vaccine, was propagated from an infectious SARS-CoV-2 clone as previously described.[6] icSARS-CoV-2 was passaged once to generate a working stock. The B.1.351 variant isolate, kindly provided by Dr. Andy Pekosz (John Hopkins University, Baltimore, MD), was propagated once in VeroE6-TMPRSS2 cells to generate a working stock. hCoV19/EHC_C19_2811C (herein referred to as the Omicron variant) was derived from a mid-turbinate nasal swab collected in December 2021. This SARS-CoV-2 genome is available under GISAID accession number EPI_ISL_7171744. Using VeroE6-TMPRSS cells, the Omicron variant was plaque purified directly from the nasal swab, propagated once in a 12-well plate, and expanded in a confluent T175 flask to generate a

working stock. All viruses used in this study were deep sequenced and confirmed as previously described.[5]

FRNT assays were performed as previously described.[5,7,8] Briefly, samples were diluted at 3-fold in 8 serial dilutions using DMEM (VWR, #45000-304) in duplicates with an initial dilution of 1:10 in a total volume of 60 µl. Serially diluted samples were incubated with an equal volume of virus (100-200 foci per well based on the target cell) at 37ºC for 45 minutes in a round-bottomed 96-well culture plate. The antibody-virus mixture was then added to VeroE6-TMPRSS2 cells and incubated at 37ºC for 1 hour. Post-incubation, the antibody-virus mixture was removed and 100 µl of pre-warmed 0.85% methylcellulose (Sigma-Aldrich, #M0512-250G) overlay was added to each well. Plates were incubated at 37ºC for 18 hours and the methylcellulose overlay was removed and washed six times with PBS. Cells were fixed with 2% paraformaldehyde in PBS for 30 minutes. Following fixation, plates were washed twice with PBS and permeabilization buffer (0.1% BSA [VWR, #0332], Saponin [Sigma, 47036-250G-F] in PBS) was added to permeabilized cells for at least 20 minutes. Cells were incubated with an anti-SARS-CoV spike primary antibody directly conjugated to Alexaflour-647 (CR3022-AF647) for up to 4 hours at room temperature. Cells were washed three times in PBS and foci were visualized on Cytation7. Antibody neutralization was quantified by counting the number of foci for each sample using the Viridot program.[9] The neutralization titers were calculated as follows: 1 - (ratio of the mean number of foci in the presence of sera and foci at the highest dilution of respective sera sample). Each specimen was tested in duplicate. The FRNT-50 titers were interpolated using a 4-parameter nonlinear regression in GraphPad Prism 9.2.0. Samples that do not neutralize at the limit of detection at 50% are plotted at 10 and was used for geometric mean and fold-change calculations.

SARS-CoV-2 Spike Variants

This study utilized four SARS-CoV-2 spike variants. The D614G (B.1) variant contained D614G as the only spike mutation. The Beta (B.1.351) variant contained spike mutations L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, D614G, A701V. The Delta (B.1.617 AY.3) variant contained spike mutations T19R, G142D, Δ156-157, R158G, L452R, T478K, D614G, P681R, D950N. The Omicron (B.1.1.529) variant contained spike mutations A67V, Δ69-70, T95I, G142D, Δ143-145, Δ211, L212I, +214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F. The spike mutations listed here were present in corresponding pseudovirus and live virus variants used in this study. The Delta variant was only used in the pseudovirus assays.

Pilot Study

Due to the urgency of acquiring reliable information on the neutralization phenotype of Omicron, a pilot study was conducted as a preliminary assessment of the potential of Omicron to evade mRNA-1273-elicited neutralizing antibodies after 2 and 3 vaccine doses.[10] This pilot study was designed to compare Omicron to D614G and Beta and was not designed to measure the full

spectrum of vaccine-elicited neutralizing antibodies against Omicron. Serum samples from participants who received a 2-dose regimen of mRNA-1273 (100 μg) in the COVE study were tested for neutralization of Omicron in the lentivirus-based pseudovirus assay in two independent laboratories: The Vaccine Research Center (VRC lab) in the National Institutes of Health (NIH), and Duke University Medical Center (Duke lab). A live virus focus-reduction neutralization (FRNT) assay was performed at Emory University (Emory lab). One subset of samples from the COVE study was pre-selected as high titers of neutralizing antibodies against D614G to enable detection of a wide range of titer reductions against Omicron. A second subset of COVE samples having moderate titers against D614G was pre-selected to avoid potential bias of high titer sera. The three laboratories assayed D614G and Beta as comparators.

The two pseudovirus-based assay laboratories also tested serum samples obtained from participants who received a 2-dose regimen of mRNA-1273 (100 μg), 4 weeks apart, under Emergency Use Authorization (EUA) and later received a 50 μg boost of mRNA-1273, although here the two laboratories assayed samples from different participants. The VRC lab tested serum samples from participants who received a third dose at 9 months after the second dose, all under EUA. Specimens were obtained after participants provided written informed consent under institutional review board approved protocols at the National Institutes of Health Clinical Center (VRC200 protocol; NCT0006705). The Duke lab tested serum samples from a clinical study (NIAID heterologous boost study DMID 21-0012) designed to assess a vaccine boost at least 4 months after primary EUA-dosed vaccine recipients. The latter set specifically included the highest D614G responders.

Compared to D614G, ID50 geometric mean titers (GMTs) after 2 doses of vaccine were 84-fold and 49-fold lower against Omicron in the VRC and Duke laboratories, respectively (Fig S1), and were 41-fold lower against Omicron in the Emory laboratory (Fig S2). This substantial decrease compared to the 13.6-fold, 9.2-fold, and 5.4-fold lower ID50 GMTs, respectively, against Beta. An approximate 12-fold improvement in Omicron neutralization was seen after the 50 μg boost such that the variant was now only 6.5- and 4.2-fold less susceptible to neutralization, respectively, than D614G in the pseudovirus assay (Fig S1). A post-boost improvement in neutralization also was seen for Beta (3.4- and 2.6-fold less susceptible, respectively, than D614G). Thus, the boost provided improvement in neutralization of both the Omicron and Beta variants. Minor differences in titer reductions between the two pseudovirus assay laboratories may be explained by sample sets that were not completely identical (see Fig S1 legend). Post-boost samples were not available to be assayed at the Emory laboratory.

In a separate analysis, sequential serum samples from the 7 participants who were vaccinated and boosted under EUA were tested against D614G, Beta and Omicron in the pseudovirus assay (VRC lab). These samples were obtained 2 weeks-post second dose (already assayed above), pre-boost, and 2 weeks post-boost. Two participants were infected 5-6 months after the second dose (prior to boost) at a time when Alpha and Delta were dominant variants in the USA. ID50 titers 2 weeks after the second dose were highest against D614G and lowest against Omicron, where titers against Omicron were 35.1-fold lower and titers against Beta were 8.9-fold lower than D614G (Fig S3). Titers for the 5 uninfected participants decreased substantially by the day of boosting and rose sharply 2 weeks post-boost to titers exceeding those after the second dose; this was true for all three variants. The increase in ID50 GMT post-boost compared with a post-

second dose was 12.6-fold for Omicron and 6.7-fold for Beta in these 5 participants. In contrast to the dramatic reduction in titers seen after the second dose, the post-boost ID50 GMT against Omicron showed a more modest 6.5-fold reduction compared to D614G, and the post-boost titers against Beta showed a 3.4-fold reduction compared to D614G, for all 7 participants combined. Results for the two participants who were infected between dose 2 and the boost demonstrate that infection with an earlier variant can enhance the vaccine-response to all three variants, and that additional vaccine boosting can further increase this response (Fig. S3).

The Duke and VRC laboratories assayed an identical subset of serum samples obtained 4 weeks after 2nd inoculation with mRNA-1273 in the phase 3 COVE study. The samples were assayed against Omicron by each of two technicians in the Duke laboratory, and were assayed against Omicron twice by a single technician in the VRC laboratory, thus permitting an assessment of inter-operator, inter-run and inter-laboratory concordance. The results were highly concordant in all cases (Fig. S4).

We also compared results among the two pseudovirus assay to results obtained in a live-virus FRNT assay performed at Emory University. Neutralization titers against D614G and Omicron were lower in the FRNT assay than in either pseudovirus assay (Fig. S5). Nonetheless, both assays showed a substantial reduction on Omicron neutralization compared to D614G neutralization (40-fold, 49-fold and 84-fold reduction in the Emory, Duke and VRC assays, respectively).

Together, these pilot results indicated that neutralizing titers to Omicron are 40-84-fold lower than neutralization titers to D614G after 2 doses of mRNA-1273 (100 μg) , and that a booster dose of mRNA-1273 (50 or 100 μg) increases Omicron neutralization titers. The pilot study was limited by: 1) relatively small sample sets that do not reflect the complete range of neutralization titers in diverse demographic groups of individuals, 2) limited longitudinal data, 3) lack of data on the durability of Omicron neutralizing antibody responses post-boost and variations in intervals between 2nd-dose of primary series and booster dose and 4) lack of efficacy data.

Main Study Booster Neutralization of D614G, Beta, Delta and Omicron Variants
Omicron neutralization titers in serum sample obtained on the day of boost (BD1) and one month after boosting (BD29) with either mRNA-1273 (50 μg), mRNA-1273.211 (50 and 100 μg) or mRNA-1273.213 (100 μg) in Figure 1B of the main text were compared to corresponding neutralization titers of the same serum samples assayed against prototypic D614G and either the Delta or Beta variant of SARS-CoV-2 (Fig S6). Comparison with D614G neutralization is described in the main text. The strong boosting of Omicron neutralization was matched by similar strong boosting of Delta and Beta neutralization. For the mRNA-1273 boost (100 μg), GMT of omicron neutralization was only 1.3-fold lower than for Delta (GMT 2294 vs. 2228). For the mRNA-1273.211 boost, Omicron neutralization was nearly equivalent to Beta neutralization, although titers against both variants after the 50 μg boost were 2.4-2.6 fold lower than after the 100 μg boost. Boosting with either of the two bivalent vaccines (100 μg of either mRNA-1273.211 or mRNA1273-213) generated Omicron neutralization titers that were very similar to those seen after boosting with mRNA-1273 (100 μg).

Pilot Study Figures

Figure S1. Neutralization of D614G, Beta and Omicron Pseudoviruses by Serum Samples Obtained from Moderna mRNA-1273 Vaccine Recipients

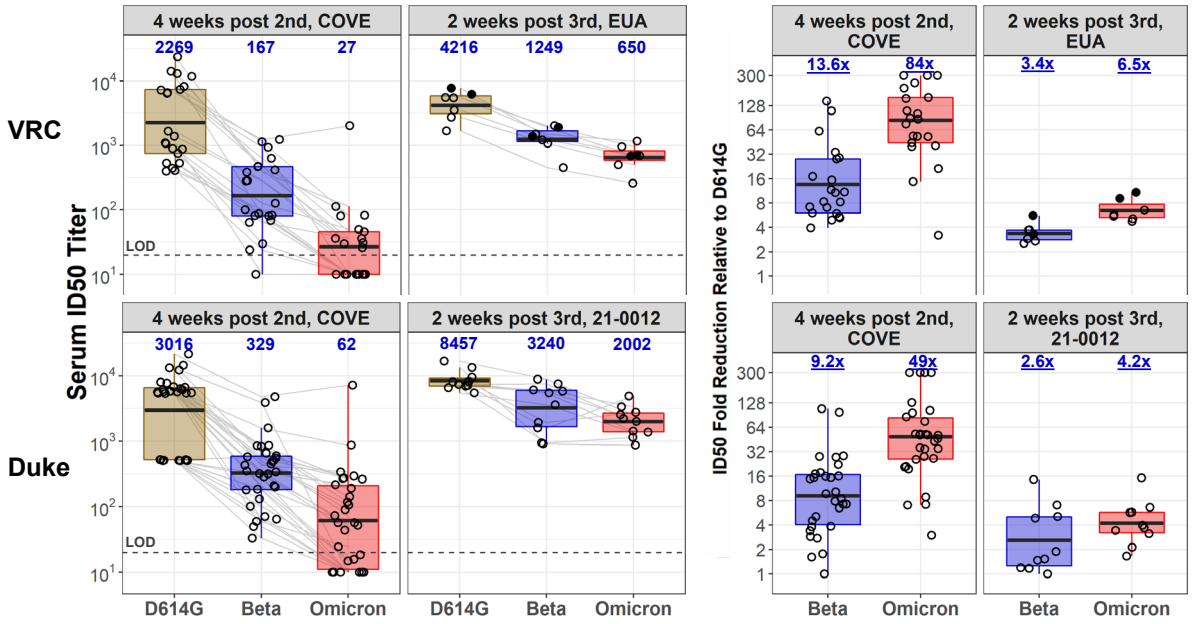


Figure S1. Neutralization of D614G, Beta and Omicron Pseudoviruses by Serum Samples Obtained from Moderna mRNA-1273 Vaccine Recipients. Serum samples obtained from 30 vaccine recipients 4 weeks after 2nd inoculation with mRNA-1273 in the phase 3 COVE study (NCT04470427) were assayed against pseudoviruses bearing either the D614G, Beta or Omicron spike of SARS-CoV-2 in two independent laboratories (VRC: Vaccine Research Center, National Institutes of Health; Duke: Duke University Medical Center). Identical samples from the COVE study were assayed in both laboratories and were pre-selected for possessing either high (ID50 = 5,408 - 22,573, n=20) or medium (ID50 = 506 - 526, n=10) neutralization titers against prototypic D614G. The Duke laboratory assayed 20 high titer and 10 medium titer samples. The VRC laboratory assayed 11/20 high titer samples and all 10 medium titer samples. Both laboratories also assayed serum samples from participants who received the primary series of two mRNA-1273 inoculations (100 µg) and a late boost (50 µg mRNA-1273). The late boost samples in the VRC laboratory were obtained 2 weeks after boosting in 7 participants who received the primary series under EUA (VRC200 protocol); the same 7 participants are shown in Fig 2. The late boost samples in the Duke laboratory were obtained 2 weeks after boosting in a phase 1/2 “Mix and Match” study (DMID 21-0012, NCT04889209) and were preselected for possessing high neutralization titers against D614G (ID50 = 5,489 - 16,760); these participants were boosted at least 4 months after the second dose. Shown are ID50 titers (A) and fold reduction in ID50 geometric mean titers (GMT) compared to D614G (B). Values below the limit of detection (ID50 = 20) were assigned a value of ID50 = 10. Positive titers below the LOD are explained by assays that were performed twice and the results averaged for plotting purposes. In these cases, one assay result tested weakly positive, while the other tested negative and was assigned a value of one-half the LOD, thus resulting in an average titer below the LOD. Bars extend from the 25th to 75th percentile. Horizontal lines and values above each bar in the left panels are GMT. Values above each bar in the right panels are the geometric mean fold change relative to D614G. Solid circles, two participants infected 5-6 months after second dose. Open circles, uninfected participants.

Figure S2. Neutralization of D614G, Beta and Omicron Live Viruses by Serum Samples Obtained from Moderna mRNA-1273 Vaccine Recipients

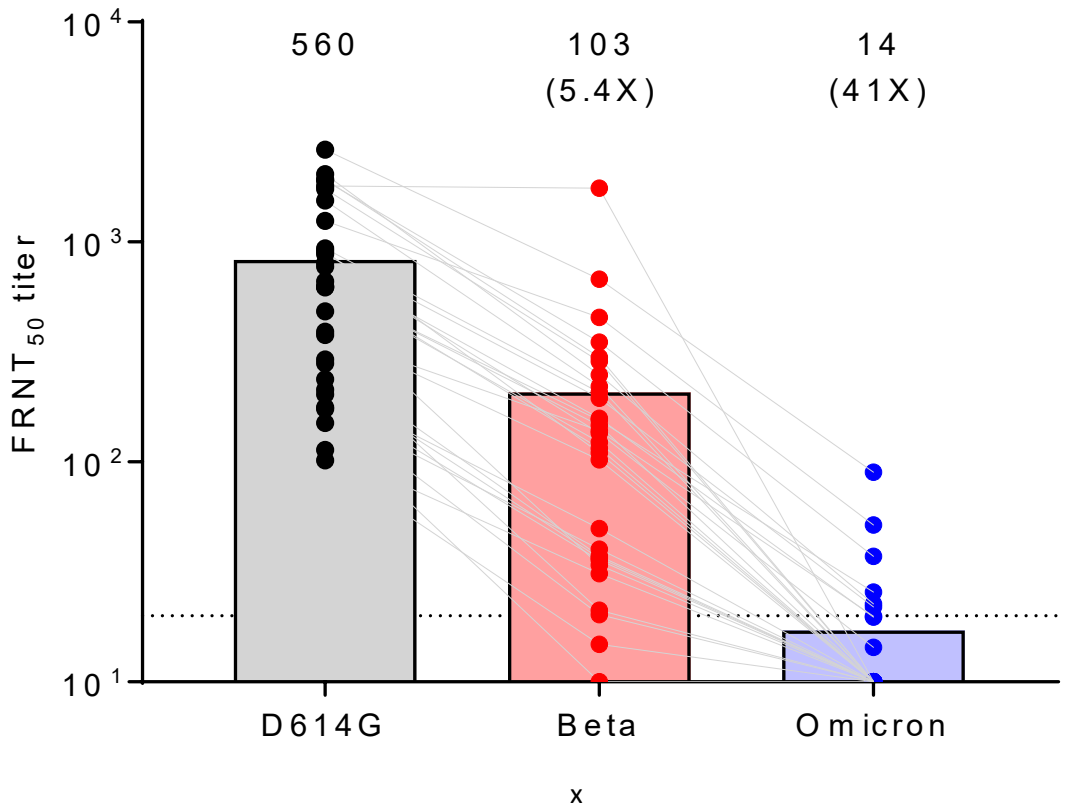


Figure S2. Neutralization of D614G, Beta and Omicron Live Viruses by Serum Samples Obtained from Moderna mRNA-1273 Vaccine Recipients. Serum samples obtained from the same 30 vaccine recipients 4 weeks after 2nd inoculation with mRNA-1273 in the phase 3 COVE study in Figure 1 were assayed in the live virus focus-reduction neutralization (FRNT) assay in the Emory laboratory. Shown are individual $FRNT_{50}$ titers as solid dots, with GMT (values above each plot) and fold reduction in geometric mean titers (GMT) compared to D614G (values in parentheses). Values below the limit of detection ($FRNT_{50}$ = 20) were assigned a value of $FRNT_{50}$ = 10. Positive titers below the LOD are explained by assays that were performed twice and the results averaged for plotting purposes. In these cases, one assay result tested weakly positive, while the other tested negative and was assigned a value of one-half the LOD, thus resulting in an average titer below the LOD. Bars extend to the GMT.

Figure S3. Longitudinal Assessment of Waning and Recall Neutralizing Antibody Responses in Recipients of Three Doses of mRNA-1273

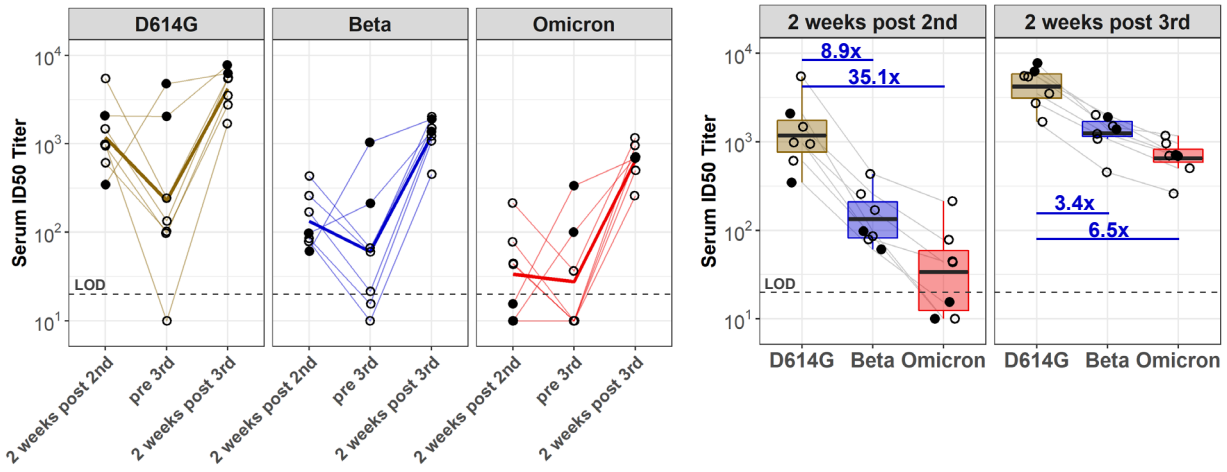


Figure S3. Longitudinal assessment of waning and recall neutralizing antibody responses in recipients of three doses of mRNA-1273. A. Shown are ID50 neutralization titers of serum samples from 7 recipients of three doses of mRNA-1273 assayed against D614G, Beta and Omicron (VRC: Vaccine Research Center, National Institutes of Health). Samples were obtained 2 weeks after second dose, day of boost and 2 weeks post boost from participants who received the vaccine under EUA. Thin lines are individual samples. Heavy lines are geometric mean titers (GMTs) of all 7 values per time point. B. Aggregate ID50 titers for the two peak immune time points. Values below the limit of detection (ID50 = 20) were assigned a value of ID50 = 10. Positive titers below the LOD are explained by assays that were performed twice and the results averaged for plotting purposes. In these cases, one assay result tested weakly positive, while the other tested negative and was assigned a value of one-half the LOD, thus resulting in an average titer below the LOD. Bars extend from the 25th to 75th percentile. Horizontal lines within the bars, and values above each bar, are GMT. Also shown are the geometric mean fold reduction in ID50 compared to D614G. Solid circles, two participants who were infected 5-6 months after second dose. Open circles, uninfected participants.

Figure S4. Inter-operator, -Run and -Laboratory Concordance of Omicron Neutralization Assays (COVE Samples)

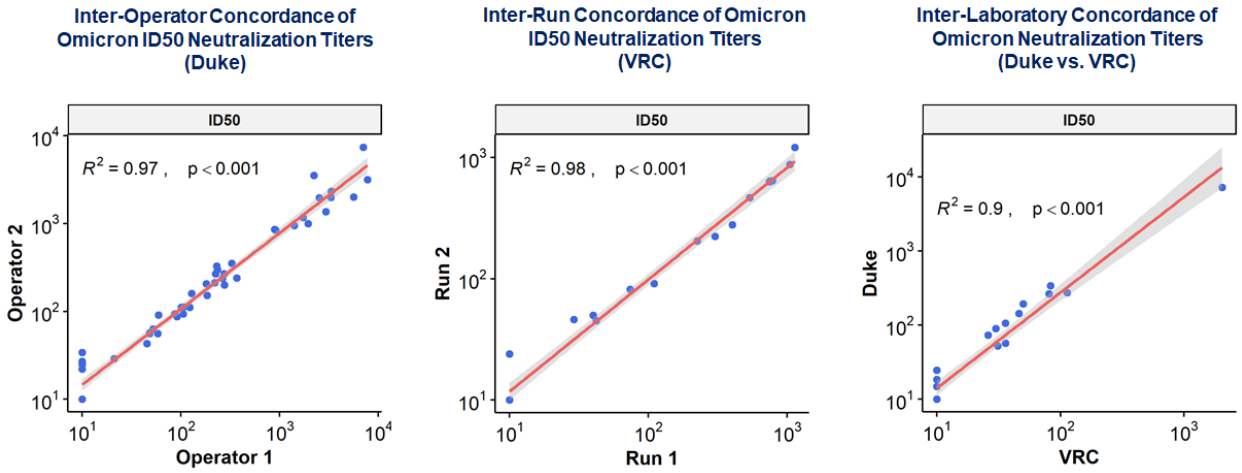
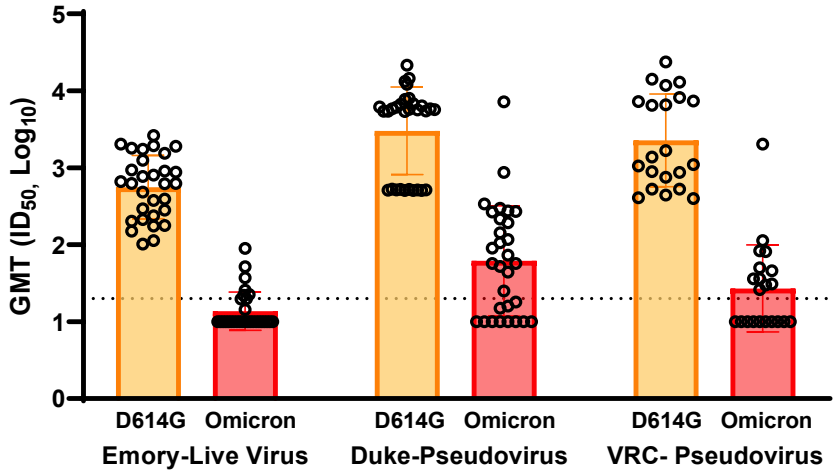


Figure S4. Inter-operator, -Run and -Laboratory Concordance of Omicron Neutralization Assays (COVE Samples). Inter-operator, inter-run, and inter-laboratory concordance of Omicron neutralization assays (COVE and DMID21-0012 samples) (A). Duke lab tested 30 COVE samples and 11 DMID21-0012 samples in parallel by 2 operators. VRC lab tested 21 COVE samples in 2 separate runs by one operator. Inter-operator and inter-run concordance was assessed using the Spearman correlation test. Inter-laboratory concordance was similarly assessed with ID50 titers for the 21 COVE samples that were tested by both laboratories.

Figure S5. Comparison of Three Neutralizing Assays for Serum Samples Obtained from Moderna mRNA-1273 Vaccine Recipients



GMT:	560	14	3016	62	2269	27
% positive:	100	30	100	73	100	52

Figure S5. Comparison of Three Neutralizing Assays for Serum Samples Obtained from Moderna mRNA-1273 Vaccine Recipients. Serum samples obtained from 30 vaccine recipients 4 weeks after 2nd inoculation with mRNA-1273 in the phase 3 COVE study (NCT04470427) were assayed against pseudoviruses bearing either the D614G, Beta or Omicron spike of SARS-CoV-2 in two independent laboratories (Vaccine Research Center, National Institutes of Health [VRC]; Duke University Medical Center [Duke]), and a live-virus FRNT assay (Emory). Samples are the same as in Figure S1. Bars, geometric mean; whiskers, geometric standard deviation. The assay lower limit of detection (LOD) was 20, indicated by the dotted line. Values below the LOD are assigned a value of 10. Positive titers below the LOD are explained by assays that were performed twice and the results averaged for plotting purposes. In these cases, one assay result tested weakly positive, while the other tested negative and was assigned a value of one-half the LOD, thus resulting in an average titer below the LOD.

Main Study Figure

Figure S6. Booster Neutralization of D614G, Beta, Delta and Omicron Variants

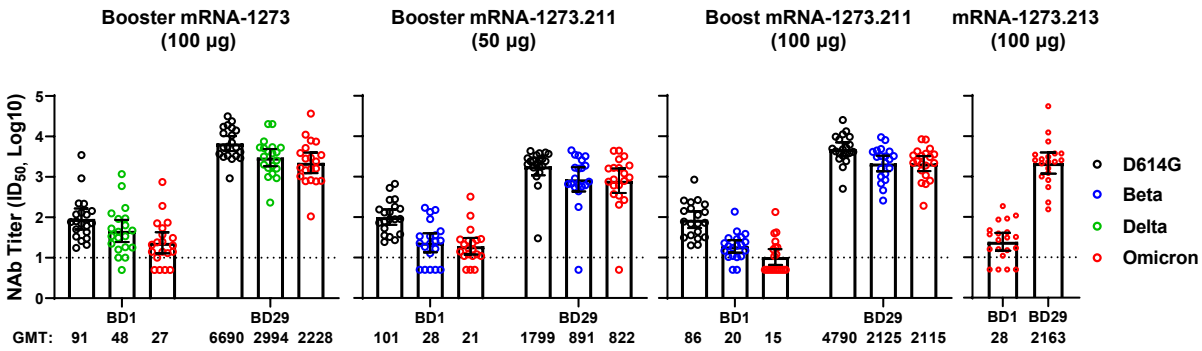


Figure S6. Booster neutralization of D614G, Beta, Delta and Omicron Variants. Serum samples were obtained from vaccine recipients of 2 doses of mRNA-1273, who were then randomly selected to receive one booster dose of mRNA-1273 (100 µg), or of multivalent mRNA-1273.211 (50 or 100 ug) boosters. Twenty participants were selected per each dose/vaccine. Neutralizing antibody (nAb) titers ID50 were assayed against pseudoviruses containing the spike protein of D614G, Beta, Delta and the Omicron variants (Duke laboratory). Whiskers represent 95% confidence intervals. The assay lower limit of detection was 10. BD=booster dose, GMT =geometric mean titers. The median time (months) between 2nd dose of primary vaccination and booster doses were 10.7 for 100 µg mRNA-1273, 9.0 for 50 µg mRNA-1273.211, 9.9 for 100 µg mRNA-1273.211 and 13.0 for mRNA-1273.213. Booster samples for mRNA-1273 were not tested for Beta, samples for mRNA-1273.211 were not tested for Delta and mRNA-1273.213 was not tested for Beta or Delta. The assay lower limit of detection (LOD) was 10, indicated by the dotted line. Values below the LOD are assigned a value of 5. BD1=day 1 prior to boost. BD29=29 days after boost.

Table S1. Sample Selection for Immunogenicity and Characteristics

n (%)	mRNA-1273 primary vaccination series	50 µg mRNA-1273 booster	100 µg mRNA-1273 booster	50 µg mRNA-1273.211 booster	100 µg mRNA-1273.211 booster	100 µg mRNA-1273.213 booster
N	20	20	20	20	20	20
Study	Phase 3 COVE Part A (blinded, primary series)	Phase 2 Part A|B	Phase 2/3 Part B	Phase 2/3 Part A	Phase 2/3 Part A	Phase 2/3 Part D
Age (yrs)						
Mean (range)	57.8 (22-75)	55.3 (19-73)	61.2 (45-77)	55.8 (29-74)	62.3 (34-78)	57.5 (33-79)
<65	10 (50)	10 (50)	10 (50)	10 (50)	10 (50)	10 (50)
≥65	10 (50)	10 (50)	10 (50)	10 (50)	10 (50)	10 (50)
Sex						
Female	10 (50)	10 (50)	8 (40)	13 (65)	11 (55)	13 (65)
Male	10 (50)	10 (50)	12 (60)	7 (35)	9 (45)	7 (35)
Race						
Asian	1 (5)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)
Black or African American	2 (10)	1 (5)	1 (5)	2 (10)	0 (0)	1 (5)
Native Hawaiian or other pacific islander	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
American Indian or Alaska Native	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)
Multiple	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)	0 (0)
White	16 (80)	19 (95)	19 (95)	16 (80)	20 (100)	18 (90)
Ethnicity						
Hispanic or Latino	6 (30)	0 (0)	8 (40)	1 (5)	3 (15)	1 (5)
Not Hispanic or Latino	14 (70)	20 (100)	12 (60)	19 (95)	17 (85)	18 (90)
Not Reported	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)
Median time (days) post-dose 2 to booster	-	221	325	272.5	300.5	403
Median time (days) post-vaccination serum sample	29*	29.5	28.5	25.5	26.5	NA

*Median time of sample one month (day 57) after dose 2 of primary series. NA=not available; 29 day booster sample data is not yet available. Variant-matched vaccine mRNA-1273.211 booster (1:1 mix of mRNA-1273 and Beta mRNA-1273.351) and mRNA-1273.213 (1:1 mix of Delta mRNA-1273.617.2 and Beta mRNA-1273.351). NA=not available.

Table S2. Representativeness of the Study Sample

Category	Description
Age	Older individuals generally have increased susceptibility to severe disease and weaker immune responses to vaccines than younger individuals.
Sex and gender	Males tend to have greater susceptibility to COVID-19 infections than women and women report greater side effects to COVID-19 vaccines than males.
Race or ethnicity	COVID-19 affects communities of color disproportionately in the United States.
Overall representativeness of this trial	The participants across the 3 trials in this study were balanced and demonstrated expected ratios of men to women. Participants completed questionnaires regarding their assigned sex at birth and gender identity at study entry in each of the trials. Older adults generally have greater susceptibility to severe disease and weaker immune responses to vaccines, including COVID-19. While the majority of participants were white in this study, the proportions of racial and ethnic representations in the larger study populations were consistent with US demographics.

References

1. El Sahly HM, Baden LR, Essink B, et al. Efficacy of the mRNA-1273 SARS-CoV-2 Vaccine at Completion of Blinded Phase. N Engl J Med 2021;385:1774-85.
2. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med 2021;384:403-16.
3. Jackson LA, Anderson EJ, Rouphael NG, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med 2020;383:1920-31.
4. Gilbert PB, Montefiori DC, McDermott AB, et al. Immune correlates analysis of the mRNA-1273 COVID-19 vaccine efficacy clinical trial. Science 2021:eab3435.
5. Edara VV, Pinsky BA, Suthar MS, et al. Infection and Vaccine-Induced Neutralizing-Antibody Responses to the SARS-CoV-2 B.1.617 Variants. N Engl J Med 2021;385:664-6.
6. Xie X, Muruato A, Lokugamage KG, et al. An Infectious cDNA Clone of SARS-CoV-2. Cell Host Microbe 2020;27:841-8 e3.
7. Edara VV, Norwood C, Floyd K, et al. Infection- and vaccine-induced antibody binding and neutralization of the B.1.351 SARS-CoV-2 variant. Cell Host Microbe 2021;29:516-21 e3.
8. Vanderheiden A, Edara VV, Floyd K, et al. Development of a Rapid Focus Reduction Neutralization Test Assay for Measuring SARS-CoV-2 Neutralizing Antibodies. Curr Protoc Immunol 2020;131:e116.
9. Katzelnick LC, Coello Escoto A, McElvany BD, et al. Viridot: An automated virus plaque (immunofocus) counter for the measurement of serological neutralizing responses with application to dengue virus. PLoS Negl Trop Dis 2018;12:e0006862.
10. Doria-Rose NA, Shen X, Schmidt SD, et al. Booster of mRNA-1273 Vaccine Reduces SARS-CoV-2 Omicron Escape from Neutralizing Antibodies. medRxiv 2021:2021.12.15.21267805.