Supplementary Appendix

Supplement to: Chalkias S, Feng J, Chen X, et al. Neutralization of omicron subvariant BA.2.75 after bivalent vaccination. N Engl J Med. DOI: 10.1056/NEJMc2212772

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

Chalkias et al., Neutralization of Omicron BA.2.75 After Bivalent Vaccination

Supplementary Appendix

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Supplementary Methods

Immunogenicity

Participant samples

Serum samples (n=428) obtained from participants in the per-protocol immunogenicity set (Fig. S2) of an ongoing, open-label, phase 2/3 study (NCT04927065) comparing the immunogenicity, safety, and reactogenicity of an omicron-containing bivalent booster vaccine (mRNA-1273.214) with the currently-authorized mRNA-1273 booster vaccine in adults who had previously received a 2-injection primary series (100-µg) and first booster doses (50-µg) of mRNA-1273 in the COVE trial[1,2] or under US emergency use authorization were tested in a pseudovirus neutralizing antibody assay (PsVNA).[3] As previously described, participants received single second boosters of 50-µg mRNA-1273 or 50-µg bivalent mRNA-1273.214.[3] The bivalent mRNA-1273.214 50-µg vaccine contains two mRNAs (1:1, 25-µg each) encoding the prefusion-stabilized spike glycoproteins of ancestral SARS-CoV-2 (Wuhan-Hu-1) and the omicron variant (BA.1). Monovalent mRNA-1273 50-µg vaccine contains a single mRNA encoding the spike glycoprotein of ancestral SARS-CoV-2 (Wuhan-Hu-1). mRNA-1273.214 and mRNA-1273 were administered intramuscularly at 50-µg in a 0.5 mL volume.

Statistical Analysis

Geometric mean (GM) titers (ID50) were assessed at baseline pre-booster dose and post-booster dose at day 29 (D29) in the per-protocol immunogenicity set of all participants regardless of previous SARS-CoV-2 infection (n=428) and those with (N=334) and without (n=94) evidence of previous SARS-CoV-2 infection (Fig. S2). GM prebooster and Day 29 ID50 titers and geometric mean (GM) fold-rise of post-baseline D29 titers relative to prebooster titers for each variant with 95% confidence intervals (CI) are provided. The 95% CI was calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for the GM value and GM fold-rise, respectively, then back transformed to the original scale for presentation. Fold decrease against D614 G and BA.1 with 95% CIs were also provided, the 95% CIs were based on t-distribution of differences in the log-transformed values, then back-transformed to the original scale for presentation. The lower limit of quantitation (LLOQs) for the PsVNA assay are 18.5 for ancestral SARS-CoV-2 (D614G) and 19.9 for omicron BA.1, and the upper limit of quantitation (ULOQs) are 45,118 for ancestral SARS-CoV-2 (D614G) and 15502.7 for omicron BA.1. The limit of detection (LOD) of the PsVNA assay used for BA.4/5 and BA.2.75 was 10; values below the lower limit of detection were assigned a value of 5 for calculating summary statistics.

Pseudotype Virus Neutralization Assay

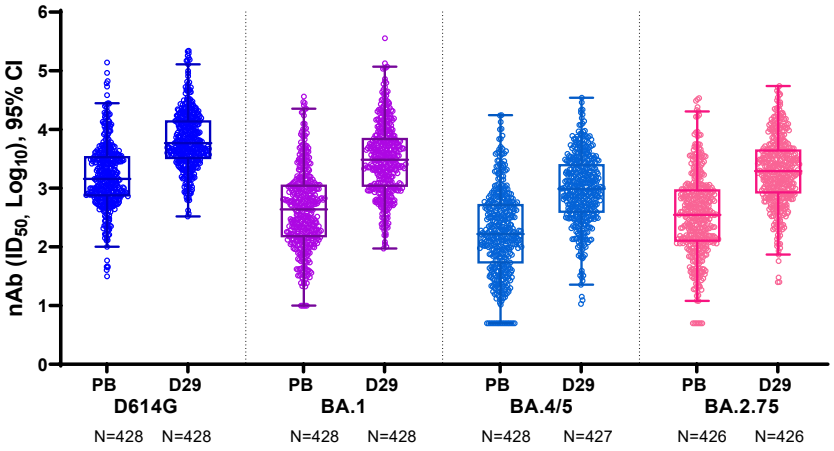
Neutralization was measured with lentiviral particles pseudotyped with SARS-CoV-2 spike and containing a firefly luciferase (Luc) reporter gene for quantitative measurements of infection by relative luminescence units (RLU). This assay is formally validated and reviewed by the FDA (MF# 026862) for SARS-CoV-2 variants D614G, beta, delta and omicron BA.1. The assay was performed in 293T/ACE2.MF provided by Drs. Michael Farzan and Huihui Mu. Pseudoviruses were prepared, titrated and used for measurements of neutralizing antibodies essentially as described previously.[4] Briefly, an expression plasmid encoding a codon-optimized full-length spike of the Wuhan-1 ancestral sequence (VRC7480) was provided by Drs. Barney Graham and Kizzmekia Corbett at the Vaccine Research Center, National Institutes of Health (USA).

Mutations were introduced into VRC7480 either by site-directed mutagenesis using the QuikChange Lightning Site-Directed Mutagenesis Kit from Agilent Technologies (Catalog # 210518), or were created by spike gene synthesized by GenScript using the spike sequence in VRC7480 as template. All mutations were confirmed by full-length spike gene sequencing by Sanger Sequencing, using Sequencher and SnapGene for sequence analyses. Pseudovirions were produced in HEK293T/17 cells (ATCC cat. no. CRL-11268) by transfection using Fugene 6 (Promega Cat#E2692) and a combination of spike plasmid, lentiviral backbone plasmid (pCMV ΔR8.2) and firefly Luc reporter gene plasmid (pHR' CMV Luc) 36 in a 1:17:17 ratio in Opti-MEM (Life Technologies). Transfection mixtures were added to pre-seeded HEK 293T/17 cells in T-75 flasks containing 12 ml of growth medium and incubated for 16-20 h at 37°C. Medium was removed and 15 ml of fresh growth medium added. Pseudovirus-containing culture medium was collected after an additional 2 days of incubation, clarified of cells by low-speed centrifugation and 0.45 µm micron filtration and stored at -80ºC in 1 ml aliquots.TCID50 assays were performed on freshly thawed pseudovirus as described previously.[4] For measurements of neutralization, a pre-titrated dose of virus was incubated with 8 serial 5-fold dilutions of serum samples (1:10 starting dilution) in duplicate in a total volume of 150 µl for 1 h at 37°C 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 h 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). Serum samples were heat-inactivated for 30 minutes at 56ºC prior to assay. 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.

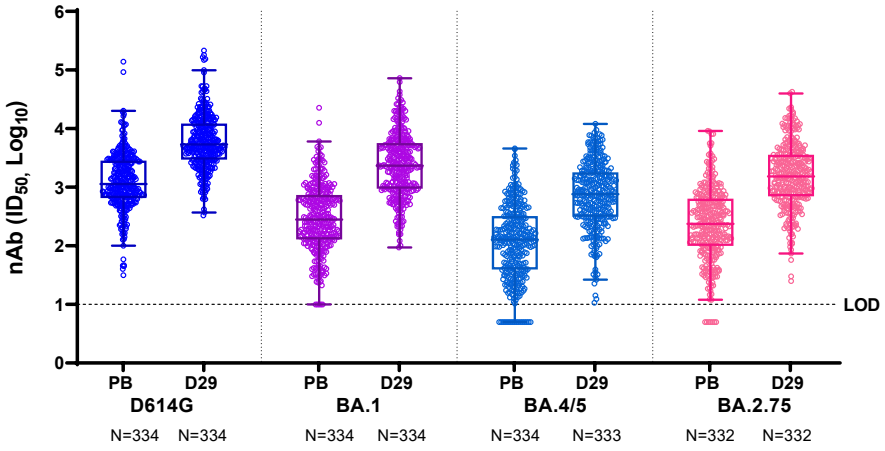
Omicron BA.4/BA.5 and BA.2.75 were assessed using a pseudovirus lentivirus neutralization assay containing full-length spike proteins for omicron subvariants BA.4 and BA.5 (designated BA.4/BA.5 for identical spike sequences between BA.4 and BA.5 [T191, L24S, ΔP25, ΔP26, ΔA27, G142D, V213G, G339D, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, L452R, S477N, T478K, E484A, F486V, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681G, N764K, D796Y, Q954H, N969K]) and for BA2.75 (T19I, L24S, P25-, P26-, A27-, G142D, K147E, W152R, F157L, I210V, V213G, G257S, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, G446S, N460K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K). Differences in RBD spike mutations of BA.2.75 and BA.4/5 include N460K present in BA.2.75 and absent in BA4/5, and L452R and F686V present in BA.4/5 and absent in BA.2.75, and a G339H in BA.2.75 instead of G339D in BA.4/5.[5] The spike protein mutations for omicron sublineages are provided in Fig S3. The reproducibility of the omicron BA.4/BA.5 neutralization assay was validated based on variability testing of samples in triplicate by multiple operators and at different times (week-to-week). The percent coefficient of variation (% CV) was similar to the neutralization assay specific for the original Wuhan strain (D614G assay).

Figure S1. Distribution of Neutralizing Antibody Titers Against SARS-CoV-2 (D614G) and Omicron Variants After Receipt of mRNA-1273.214 As a Second Booster Dose

A. All participants



B. No Previous Infection



C. Previous Infection

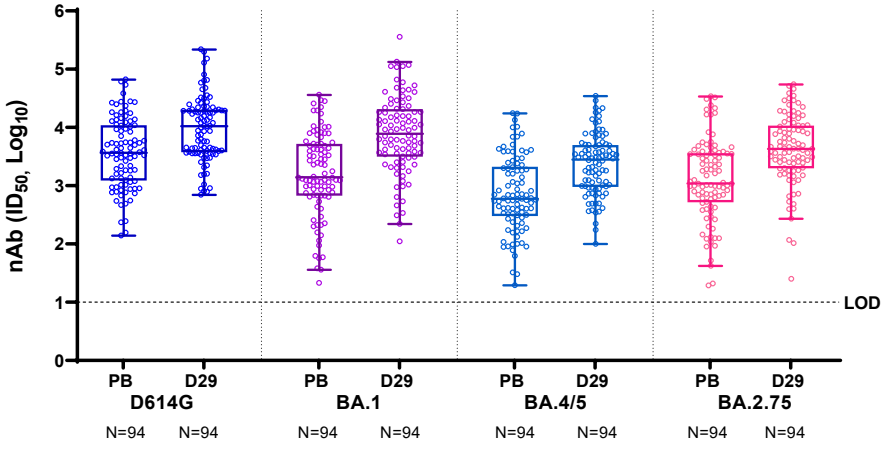


Figure S1. PB=prebooster, D29=day 29. Distribution of pseudovirus neutralizing antibody titers (ID50) against ancestral SARS-CoV-2 (D614G) and omicron sublineages (BA.1, BA.4/5, BA.2.75) in serum samples from adult participants in the per-protocol immunogenicity set regardless of previous SARS-CoV-2 infection (panel A), and with and without SARS-CoV-2 infection (panels B and C) who received a 2-injection primary vaccination series and a single 50 μg booster dose of mRNA-1273 in a phase 2/3 trial (NCT04927065) and a second booster dose of bivalent mRNA-1273.214.[3] Geometric mean titers (GMT) ID50 were assessed at day 29 post-booster dose. The LLOQs for the PsVNA assay are 18.5 for ancestral SARS-CoV-2 (D614G) and 19.9 for omicron BA.1, and the ULOQs are 45,118 for ancestral SARS-CoV-2 (D614G) and 15502.7 for omicron BA.1. Corresponding log10 values for LLOQs for the PsVNA assay are 1.3 for both ancestral [D614G] and omicron BA.1, and ULOQs are 4.7 for ancestral SARS-CoV-2 [D614G] and 4.2 for omicron BA.1. Boxes and horizontal bars denote interquartile (IQR) ranges and median endpoint titers; whisker endpoints are the maximum and minimum values within ±1.5 times the IQR above the 75% and below the 25% percentiles. The limit of detection of the PsVNA assay for BA.4/5 and BA.2.75 was 10; values below the lower limit of detection were assigned a value of 5.

Figure S2. Trial Profile of Immunogenicity Analysis Sets in mRNA-1274.214 Study Arm

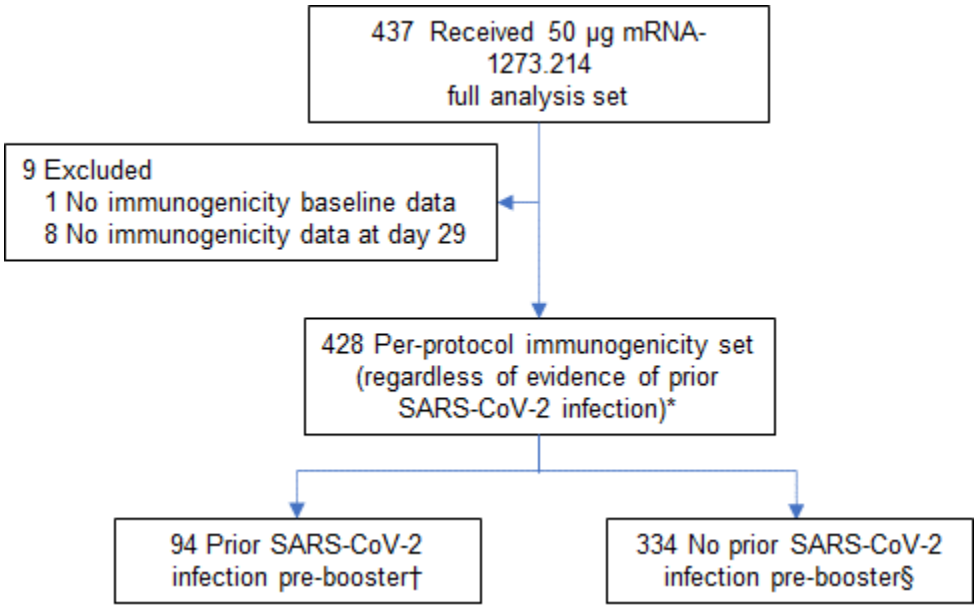


Figure S2. The full analysis set consists of all participants who received study vaccine. *The per-protocol set for immunogenicity consists of all participants in the full analysis set who received the planned dose of study vaccination and had no major protocol deviations that impact key or critical data. §Prior SARS-CoV-2-infection based on positive RT-PCR and/or serology test at baseline. ¶No serologic or virologic evidence of SARS-CoV-2 infection at baseline, i.e., who are SARS-CoV-2 negative, defined by both negative RT-PCR test for SARS-CoV-2 and negative serology test based on binding antibody specific to SARS-CoV-2 nucleocapsid.

Figure S3. Spike Mutations in SARS-CoV-2 Omicron Sublineages

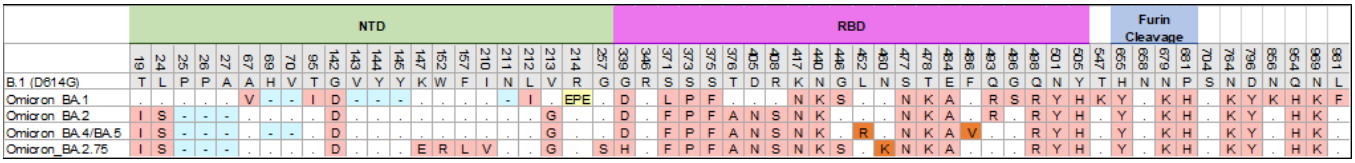


Figure S3. Spike Mutations in SARS-CoV-2 Omicron Sublineages. Spike amino acid residues are shown for prototypic SARS-CoV-2 B.1 (D614G) and four sublineages of the omicron variant. Only sites that exhibit amino acid substitutions, deletions or insertions are shown. Residues that remain unchanged from Wuhan B.1 (D614G) are indicated by small, dashed lines. Deletions are indicated by large, dashed lines and are shaded blue. A single insertion of EPE is highlighted in yellow. Key residues in omicron sublineages BA.4/BA.5 and BA.2.75 discussed in the text are highlighted in dark orange. Also shown are the regions encompassing the N-terminal domain (NTD), receptor binding domain (RBD) and furin cleavage site.

Table S1. Observed Neutralizing Antibodies by SARS-CoV-2 Infection Status After Receipt of mRNA-1273.214 As a Second Booster Dose

	mRNA-1273.214			
	Ancestral D614G	**Omicron BA.1**	**Omicron BA.4/5**	**Omicron BA.2.75**
All Participants, N	428	428	428	428
Pre-booster, n	428	428	428	426
Observed GMT (95% CI)	1603.4 (1420.3-1810.0)	432.1 (372.5-501.2)	172.7 (147.4-202.3)	341.0 (292.0-398.3)
Day 29, n	428	428	427	426
Observed GMT (95% CI)	6619.0 (5941.7-7373.5)	3070.4 (2685.4-3510.6)	940.6 (826.3-1070.6)	1946.7 (1711.3-2214.6)
GMFR (95% CI)	4.1 (3.8-4.4)	7.1 (6.5-7.8)	5.4 (5.0- 5.9)	5.7 (5.2-6.2)
No Previous Infection, N	334	334	334	334
Pre-booster, n	334	334	334	332
Observed GMT (95% CI)	1266.7 (1120.2-1432.5)	298.1 (258.8-343.5)	115.6 (98.5-135.6)	235.4 (201.7-274.6)
Day 29, n	334	334	333	332
Observed GMT (95% CI)	5977.3 (5321.9-6713.3)	2372.4 (2070.6-2718.2)	727.4 (632.8-836.1)	1561.8 (1363.7-1788.7)
GMFR (95% CI)	4.7 (4.4-5.1)	8.0 (7.2-8.8)	6.3 (5.7-6.9)	6.6 (6.0-7.3)*
Previous Infection, N	94	94	94	94
Pre-booster, n	94	94	94	94
Observed GMT (95% CI)	3704.0 (2793.2-4911.7)	1614.6 (1149.7- 2267.7)	719.5 (531.6-973.9)	1264.0 (907.4-1760.6)
Day 29, n	94	94	94	94
Observed GMT (95% CI)	9509.7 (7345.9-12310.9)	7676.2 (5618.2-10488.1)	2337.4 (1825.5-2992.9)	4238.5 (3179.5-5650.2)
GMFR (95% CI)	2.6 (2.2-2.9)	4.8 (4.0-5.7)	3.2 (2.8-3.8)	3.4 (2.8-4.0)*

CI = Confidence interval, GMT=geometric mean titer; GMFR=geometric mean fold rise (post-baseline/pre-booster baseline titers). LLOQ=lower limit of quantification ULOQ=upper limit of quantification LOD=limit of detection. Neutralizing antibody titers assessed in all participants and those with and without evidence of previous SARS-CoV-2 infection in the per-protocol immunogenicity set. *number of participants/total day 29 participants=424/426. Antibody values assessed by pseudovirus neutralizing antibody assay reported as below the LLOQ (18.5 for ancestral SARS-CoV-2 [D614G] and 19.9 for omicron B.1.1.529) are replaced by 0.5 × LLOQ. Values greater than ULOQ (45,118) for ancestral SARS-CoV-2 [D614G] and 15,502.7 for omicron B.1.1.529) are replaced by the ULOQ if actual values are not available. The limit of detection (LOD) of the PsVNA assay for BA.4/5 and BA.2.75 was 10; values below LOD were assigned a value of 5. 95% CI is calculated based on the t-distribution of the log-transformed values or the difference in the log-transformed values for GM value and GM fold-rise, respectively, then back transformed to the original scale.

Table S2. Fold Decreases in Variants Relative to Ancestral SARS-CoV-2 (D614G) and Omicron BA.1 at Day 29 After Receipt of mRNA-1273.214 as a Second Booster Dose

Analysis population	Assay	Fold decrease in relation to ancestral SARS-CoV-2 (D614G)	Fold decrease in relation to omicron BA.1
All Participants	D614G	1 (NE-NE)	0.46 (0.43-0.51)
	BA.1	2.16 (1.98-2.35)	1 (NE-NE)
	BA.4/5	7.04 (6.46-7.67)	3.26 (3.03-3.51)
	BA.2.75	3.41 (3.13-3.71)	1.58 (1.49-1.67)
No Previous Infection	D614G	1 (NE-NE)	0.40 (0.36-0.43)
	BA.1	2.52 (2.31-2.75)	1 (NE-NE)
	BA.4/5	8.22 (7.47-9.04)	3.26 (3.00-3.53)
	BA.2.75	3.83 (3.50-4.20)	1.52 (1.43-1.62)
Previous Infection	D614G	1 (NE-NE)	0.81 (0.66-0.99)
	BA.1	1.24 (1.01-1.51)	1 (NE-NE)
	BA.4/5	4.07 (3.51-4.72)	3.28 (2.78-3.88)
	BA.2.75	2.24 (1.85-2.72)	1.81 (1.57-2.09)

NE=not estimable, CI=Confidence interval. Fold decreases in antibody titers relative to ancestral SARS-CoV-2 (D614G) and omicron BA.1 in all participants and those with and without evidence of previous SARS-CoV-2 infection in the per-protocol immunogenicity set. The fold decrease relative to ancestral SARS-CoV-2 (D614G) and omicron BA.1 for each variant was obtained by first calculating the mean difference in log10 scale, then back transformation to the original scale for presentation and the 95% CIs for fold decreases are based on the differences in the log-transformed values, then back-transformed to the original scale for presentation.

Supplement References

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