ORIGINAL ARTICLE

# Homologous and Heterologous Covid-19 Booster Vaccinations

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## ABSTRACT

### BACKGROUND

Although the three vaccines against coronavirus disease 2019 (Covid-19) that have received emergency use authorization in the United States are highly effective, breakthrough infections are occurring. Data are needed on the serial use of homologous boosters (same as the primary vaccine) and heterologous boosters (different from the primary vaccine) in fully vaccinated recipients.

### METHODS

In this phase 1–2, open-label clinical trial conducted at 10 sites in the United States, adults who had completed a Covid-19 vaccine regimen at least 12 weeks earlier and had no reported history of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection received a booster injection with one of three vaccines: mRNA-1273 (Moderna) at a dose of 100 $\mu$g, Ad26.COV2.S (Johnson & Johnson–Janssen) at a dose of $5\times10^{10}$ virus particles, or BNT162b2 (Pfizer–BioNTech) at a dose of 30 $\mu$g. The primary end points were safety, reactogenicity, and humoral immunogenicity on trial days 15 and 29.

### RESULTS

Of the 458 participants who were enrolled in the trial, 154 received mRNA-1273, 150 received Ad26.COV2.S, and 153 received BNT162b2 as booster vaccines; 1 participant did not receive the assigned vaccine. Reactogenicity was similar to that reported for the primary series. More than half the recipients reported having injection-site pain, malaise, headache, or myalgia. For all combinations, antibody neutralizing titers against a SARS-CoV-2 D614G pseudovirus increased by a factor of 4 to 73, and binding titers increased by a factor of 5 to 55. Homologous boosters increased neutralizing antibody titers by a factor of 4 to 20, whereas heterologous boosters increased titers by a factor of 6 to 73. Spike-specific T-cell responses increased in all but the homologous Ad26.COV2.S-boosted subgroup. CD8+ T-cell levels were more durable in the Ad26.COV2.S-primed recipients, and heterologous boosting with the Ad26.COV2.S vaccine substantially increased spike-specific CD8+ T cells in the mRNA vaccine recipients.

### CONCLUSIONS

Homologous and heterologous booster vaccines had an acceptable safety profile and were immunogenic in adults who had completed a primary Covid-19 vaccine regimen at least 12 weeks earlier. (Funded by the National Institute of Allergy and Infectious Diseases; DMID 21-0012 ClinicalTrials.gov number, NCT04889209.)

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In the United States, the significant efficacy of three candidate vaccines — mRNA-1273 (Moderna), Ad26.COV2.S (Johnson & Johnson–Janssen), and BNT162b2 (Pfizer–BioNTech) — against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resulted in the issuing of emergency use authorization (EUA) by the Food and Drug Administration (FDA) between December 2020 and February 2021.[1-3] The widespread rollout of these vaccines, as well as vaccines from other manufacturers worldwide, has resulted in the administration of more than 6.4 billion doses. As of January 1, 2022, a total of 208.2 million persons in the United States (62.7% of the population) had been fully vaccinated.[4]

Although the vaccines that are available under EUA in the United States provide high levels of protection against severe illness and death, the enhanced transmission of the B.1.617.2 (delta) variant starting in the spring of 2021 resulted in increasing numbers of breakthrough infections in fully vaccinated persons.[5-7] The delta wave was quickly followed by the B.1.1.529 (omicron) wave in November 2021. The detection of such infections coincided with evidence of waning immunity.[6-8]

Booster vaccines enhance waning immunity and expand the breadth of immunity against SARS-CoV-2 variants of concern. Since levels of binding and neutralizing antibodies correlate with vaccine efficacy for both messenger RNA (mRNA) and adenovirus-vectored vaccines, the measurement of these levels can be useful in predicting efficacy after boosting.[9-12] Homologous boosters of the 30-μg BNT162b2 and 50-μg mRNA-1273 vaccines were found to increase neutralizing antibody titers against wild-type SARS-CoV-2 virus (WA1) by a factor of 5.5 and 3.8, respectively, and against the delta variant by a factor of 5 and 2.1, respectively.[13,14] Data from Israel suggest that a third dose of the BNT162b2 vaccine was highly effective in preventing infection,[15] severe disease, hospitalization, or death.[16] These findings contributed to a recommendation for booster vaccination for the general U.S. population.[17]

Heterologous prime–boost strategies may offer immunologic advantages to extend the breadth and longevity of protection provided by the currently available vaccines. The administration of a heterologous two-dose regimen of an adenovirus-vectored vaccine (ChAdOx1, Oxford–AstraZeneca) followed by an mRNA vaccine was more immunogenic than a two-dose homologous ChAdOx1 vaccine regimen.[18-21] An option to use heterologous booster vaccines could simplify the logistics of administering such vaccines, since the booster formulation could be administered regardless of the primary series.

To assist in the development of booster strategies during an ongoing pandemic, we conducted the phase 1–2 MixNMatch Study (DMID 21-0012) to assess homologous and heterologous booster vaccinations in persons who had previously completed an EUA Covid-19 vaccination regimen at least 12 weeks earlier. Here, we report the initial results of this trial.

*A Quick Take is available at NEJM.org*

## METHODS

### TRIAL DESIGN, PARTICIPANTS, AND OVERSIGHT

This open-label, nonrandomized, adaptive-design clinical trial was performed in sequential stages at 10 sites in the United States. (The sites are listed in Table S109 in the Supplementary Appendix, available with the full text of this article at NEJM.org.) Eligible participants were healthy adults who had received a full Covid-19 vaccine regimen available under EUA at least 12 weeks earlier and who had reported no history of SARS-CoV-2 infection or monoclonal antibody infusion. To facilitate rapid enrollment in the trial, we did not screen for laboratory evidence of SARS-CoV-2 infection. Full eligibility criteria are available in the protocol, also available at NEJM.org.

The trial was reviewed and approved by a central institutional review board and overseen by an independent safety monitoring committee. All the participants provided written informed consent before undergoing any trial-related activities.

The trial was supported by the National Institute of Allergy and Infectious Diseases. All trial vaccines were acquired through the government procurement process.

### VACCINES

Trial vaccines included mRNA-1273 at a dose of 100 μg (trial stage 1), Ad26.COV2.S at a dose of $5\times10^{10}$ virus particles (trial stage 2), and BNT162b2 at a dose of 30 μg (trial stage 3),[1-3] thus providing the possibility of nine different combinations of primary vaccination and booster (stage 1, groups 1–3; stage 2, groups 4–6; and stage 3, groups 7–9). For each stage, volunteers

were competitively enrolled across trial sites until targets had been met for each sequential stage. Booster doses were administered as directed in their respective EUAs.

### TRIAL PROCEDURES

Participants were enrolled in approximately equal numbers in two age strata (18 to 55 years and ≥56 years) according to their receipt of the primary vaccination regimen (50 per group). After informed consent had been obtained, participants underwent screening with a medical-history review, a targeted physical examination, and a urine pregnancy test (if indicated). (Pregnancy was among the exclusion criteria, which are detailed with the inclusion criteria in the protocol.) After screening, all eligible participants received the designated booster vaccine. Blood was collected for immunogenicity assessments on day 1 (before vaccination) and on days 15 and 29 after boosting.

We collected data regarding local and systemic solicited adverse events for 7 days and unsolicited adverse events through 28 days after vaccination. In the grading of adverse events, we used an FDA Toxicity Grading Scale.[22] Data regarding all serious adverse events, new-onset chronic medical conditions, adverse events of special interest, and related medically attended adverse events are being collected for the 12-month trial duration and are reported through day 29 in this article.

### IMMUNOGENICITY

Serum binding antibody levels against the spike (S) protein with proline modification (S-2P) were evaluated by means of the 384-well Meso Scale Discovery Electrochemiluminescence immunoassay analyzer, version 2 (4-plex ECLIA) and the 10-plex ECLIA for emerging SARS-CoV-2 variant spike proteins.[23] SARS-CoV-2 neutralization titers that were expressed as the serum inhibitory dilution required to achieve 50% and 80% neutralization ($ID_{50}$ and $ID_{80}$, respectively) were determined according to trial group, age group, and time point with the use of pseudotyped lentiviruses presenting SARS-CoV-2 spike mutation D614G and the delta and B.1.351 (beta) variants, as described previously.[24] For the beta variant, a random subset of samples (20 per group, distributed equally between age groups and among sites) was analyzed. Neutralizing activity was expressed in international units (IU50) per milliliter for the D614G neutralization assay. (The IU50 value was calculated as the $ID_{50}$ adjusted to an international standard.) SARS-CoV-2 S-specific CD4+ and CD8+ T-cell responses were evaluated after ex vivo stimulation of cryopreserved peripheral-blood mononuclear cells (PBMCs) with the use of a validated intracellular cytokine staining assay with a 27-color flow cytometry panel.[25,26]

### STATISTICAL ANALYSIS

According to the primary objectives of the trial, as stated in the protocol, the results of safety and immunogenicity analyses are descriptive. For practical reasons, a sample size of 50 participants per group and 25 per age stratum was targeted, consistent with phase 1–2 trials. No tests of hypothesis were planned for a comparison among groups. Baseline summaries (including serologic end points) are reported for all enrolled participants.

Safety and subsequent immunogenicity analyses include only participants who had received booster vaccination. In addition, we evaluated some exploratory end points in a subgroup of 180 participants who were randomly selected among those with an adequate sample of PBMCs available for testing, stratified according to age and primary vaccine. Immunogenicity end points are presented as unadjusted point estimates with 95% confidence intervals. The latter are provided as a measure of uncertainty around the estimates, have not been adjusted for multiple comparisons, and should not be used to infer statistically significant differences. In addition, we performed an analysis with trial sites as a random effect. (Details regarding this analysis are provided in the Supplementary Appendix.)

## RESULTS

### TRIAL POPULATION

From May 29 to August 13, 2021, we enrolled 458 participants (154, 150, and 154 in each of the three stages) (Figs. S1, S2, and S3). The last visit (trial day 29) occurred on September 13, 2021. One participant (in group 7) did not receive a booster vaccination. The demographic characteristics of the participants were similar across trial groups (Table 1). The interval between primary and booster vaccinations was shortest among participants who were boosted with mRNA-1273, a finding that reflected the temporal progression of enrollment across the

sequential trial stages. Two participants (one each in group 4 and group 6) who had serologic evidence of previous SARS-CoV-2 infection (the presence of antibody against nucleocapsid protein) and 1 participant (in group 5) who was found to have Covid-19 2 days before trial day 29 were included in the analyses.

### VACCINE SAFETY

Two serious adverse events that were deemed by the investigators to be unrelated to trial vaccination were reported. One event (acute renal failure caused by rhabdomyolysis associated with a fall) was reported 30 days after the mRNA-1273 booster, and the other (acute cholecystitis) occurred 24 days after the Ad26.COV2.S booster. No prespecified trial-halting rules were met, and no new-onset chronic medical conditions occurred through trial day 29. One related adverse event of special interest (severe vomiting that led to a medically attended visit the day after vaccination) occurred in group 5 (Ad26.COV2.S booster). Participants with unsolicited adverse events of any grade that were deemed by investigators to be related to a trial vaccine were reported in 24 of 154 participants (16%) who received mRNA-1273, in 18 of 150 participants (12%) who received Ad26.COV2.S, and in 22 of 153 participants (14%) who received BNT162b2 (Tables S4, S5, and S6). Most adverse events were mild or moderate. Four participants had severe adverse events that were deemed by the investigators to be related to a trial vaccine: in 1 participant with vomiting who had received mRNA-1273 (group 1) and in 3 participants who had received Ad26.COV2.S (1 with vomiting and 1 with fatigue in group 5 and 1 with an abnormal feeling and insomnia in group 6).

Solicited injection-site adverse events were common, with local pain or tenderness being reported in 75 to 86% of mRNA-1273 recipients, in 71 to 84% of Ad.26COV2.S recipients, and in 72 to 92% of BNT162b2 recipients (Fig. 1 and Tables S7, S8, and S9). Most injection-site reactions were graded as mild, with only 2 (1 in an mRNA-1273 recipient and 1 in an Ad.26COV2.S recipient) that were reported as severe. Malaise, myalgias, and headaches were also commonly reported (Tables S10, S11, and S12). The proportions of all 457 participants in all three stages who reported having a severe systemic solicited event were as follows: malaise or fatigue, 2.0 to 4.5%; myalgia, 0 to 3.3%; headache, 0.7 to 3.3%; nausea, 0 to 2.7%; chills, 0 to 3.3%; arthralgia, 0.6 to 2.0%; and fever, 0.7 to 2.7%. Solicited adverse events were most likely to occur within 3 days after booster vaccination; no clear patterns of frequency were noted for solicited or unsolicited adverse events according to the primary vaccine or age group (Tables S4 through S12).

### BINDING ANTIBODY RESPONSE

All the participants but one (who had been Ad26.COV2.S primed) had evidence of binding antibody against the SARS-CoV-2 full-length spike glycoprotein trimer (S-2P) in the WA1 strain before booster vaccination (Fig. 2). The binding antibody titers against S-2P were lower by a factor of 3 to 15 in participants who had received primary vaccination with single-dose Ad26.COV2.S than in those who had received either of the mRNA vaccines (mRNA-1273 or BNT162b2) (Table 2 and Tables S13 through S30). All the groups had an increase in the binding antibody level after boosting. Among the participants who had received an mRNA booster, an increase in the binding antibody titer by a factor of 2 or more occurred in 98 to 100% of participants who were Ad26.COV2.S primed, in 96 to 100% of those who were mRNA-1273 primed, and in 98 to 100% of those who were BNT162b2 primed. By day 15, the geometric mean binding antibody titer had increased by a factor of 5 to 55; increases were greatest in the participants who had received a BNT162b2 or an mRNA-1273 booster after Ad26.COV2.S primary vaccination (by factors of 34 and 55, respectively). The Ad26.COV2.S booster increased binding antibody titers in all the participants, but the Ad26.COV.2-primed recipients had a level that was lower by a factor of 7 to 10 than those in participants who had received an mRNA vaccine as the priming regimen. Binding antibody levels peaked at day 15 for mRNA-boosted groups and were similar or declining on day 29, whereas binding antibody levels in the Ad26.COV2.S-boosted groups on day 29 were similar to or higher than those measured on day 15.

Before booster administration, binding antibody levels against the delta variant were 34 to 45% lower than levels against WA1 S-2P according to the same 10-plex assay (Tables S31 through S36). After receiving a booster, all the participants had detectable binding antibody against the delta variant at a level that was 15 to 36% lower than that against the WA1 strain. Binding

**Table 1. Characteristics of the Participants at Enrollment.***

| Characteristic | mRNA-1273 Booster | | | Ad26.COV2.S Booster | | | BNT162b2 Booster | | |
|---|---|---|---|---|---|---|---|---|---|
| | Primary Ad26.COV2.S | Primary mRNA-1273 | Primary BNT162b2 | Primary Ad26.COV2.S | Primary mRNA-1273 | Primary BNT162b2 | Primary Ad26.COV2.S | Primary mRNA-1273 | Primary BNT162b2 |
| Group no. | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| No. of participants | 53 | 51 | 50 | 50 | 49 | 51 | 53† | 51 | 50 |
| Sex — no. (%) | | | | | | | | | |
| Female | 26 (49) | 32 (63) | 29 (58) | 27 (54) | 16 (33) | 23 (45) | 29 (55) | 26 (51) | 23 (46) |
| Male | 27 (51) | 19 (37) | 21 (42) | 23 (46) | 33 (67) | 28 (55) | 24 (45) | 25 (49) | 27 (54) |
| Age — yr | | | | | | | | | |
| Mean | 57±14 | 53±16 | 55±17 | 50±14 | 50±17 | 50±15 | 48±14 | 54±17 | 50±18 |
| Range | 24–81 | 24–76 | 22–85 | 24–77 | 20–75 | 20–76 | 22–74 | 23–75 | 19–80 |
| Race and ethnic group — no. (%)‡ | | | | | | | | | |
| Asian | 4 (8) | 5 (10) | 4 (8) | 3 (6) | 5 (10) | 6 (12) | 1 (2) | 2 (4) | 1 (2) |
| Hawaiian or Pacific Islander | 0 | 0 | 0 | 0 | 0 | 0 | 1 (2) | 0 | 0 |
| Black | 1 (2) | 2 (4) | 3 (6) | 0 | 0 | 2 (4) | 0 | 2 (4) | 1 (2) |
| White | 46 (87) | 41 (80) | 43 (86) | 44 (88) | 43 (88) | 40 (78) | 50 (94) | 47 (92) | 43 (86) |
| Multiracial | 1 (2) | 3 (6) | 0 | 3 (6) | 1 (2) | 2 (4) | 1 (2) | 0 | 4 (8) |
| Hispanic or Latino | 4 (8) | 4 (8) | 3 (6) | 2 (4) | 0 | 3 (6) | 2 (4) | 2 (4) | 5 (10) |
| Other | 1 (2) | 0 | 0 | 0 | 0 | 1 (2) | 0 | 0 | 1 (2) |
| Ethnic group not reported | 0 | 1 (2) | 0 | 1 (2) | 0 | 0 | 0 | 0 | 0 |
| Interval between first and second primary doses — days | | | | | | | | | |
| Mean | NA | 29±3 | 23±8 | NA | 28±3 | 22±3 | NA | 28±2 | 22±5 |
| Range | NA | 24–40 | 16–71 | NA | 19–36 | 18–42 | NA | 24–32 | 11–46 |
| Interval between second dose and booster — wk§ | | | | | | | | | |
| Mean | 14±1 | 16±2 | 17±2 | 18±2 | 19±4 | 21±6 | 20±2 | 23±5 | 24±5 |
| Range | 12–16 | 12–20 | 12–21 | 14–21 | 13–26 | 12–41 | 11–23 | 13–29 | 14–32 |

* Plus–minus values are means ±SD. NA denotes not applicable because these participants received a single dose.
† Listed in this group is one participant who withdrew from the trial during the day 1 visit and did not receive a booster vaccination.
‡ Race and ethnic group were reported by the participant and were collected as two categories, so percentages in each category do not total 100%.
§ For the participants who received the one-dose Ad26.COV2.S primary vaccine, data are listed for the interval after the single dose.

antibody levels in serum samples obtained from participants in the older age group were similar to those in the younger age group. Serologic responses to WA1 and beta S-2P on 4-plex ECLIA (Tables S13 through S24 and S37 through S42) and WA1 and delta S-2P proteins on the 10-plex ECLIA are reported in Tables S25 through S36.

#### NEUTRALIZING ANTIBODY RESPONSE

All serum samples obtained from participants who had received mRNA-1273 as the primary vaccine had prebooster neutralizing activity against D614G S-2P, whereas serum samples obtained from 24 participants (16%) who had received Ad26.COV2.S and from 5 (3%) who had received BNT162b2 had no detectable neutralizing activity against the D614G mutation. Serum neutralization levels (as measured in IU50 per milliliter) before booster vaccination were lower than levels in mRNA-1273–primed recipients by a factor of 10 for Ad26.COV2.S-primed recipients and by a factor of 3 for BNT162b2-primed recipients, regardless of the interval between primary and booster vaccination (Table 2 and Tables S43 through S48).

The kinetics of postbooster neutralizing antibody responses were similar to those observed for binding antibody responses. On day 15, postbooster neutralization titers ranged from 676 to 902 IU50 per milliliter for participants boosted with mRNA-1273, 31 to 382 IU50 per milliliter for those boosted with Ad26.COV2.S, and 344 to 694 IU50 per milliliter for those boosted with BNT162b2. The factor increases in the geometric mean neutralization titers were greatest for Ad26.COV2.S-primed recipients, followed by recipients of primary BNT162b2 and mRNA-1273. In general, postbooster titers were highest in recipients of primary mRNA-1273, followed by primary BNT162b2 and Ad26.COV2.S, regardless of the booster vaccine administered. Recipients of an mRNA booster had a neutralization response that was higher by a factor of 4 than those who were boosted with Ad26.COV.S (Tables S55 through S60).

In general, prebooster serum neutralization levels were lower against the delta and beta variants than those against the D614G mutation and were below the limit of detection in many participants (Tables S67 through S72 and S79 through S84). All but 2 participants who had received Ad26.COV2.S as both the primary and booster vaccine had measurable neutralizing antibody against the delta variant after booster vaccination; similar findings were observed when $ID_{80}$ neutralization levels were assessed (Tables S73 through S78 and S85 through S90).

#### T-CELL RESPONSE

SARS-CoV-2 spike-specific Th1 (interferon-γ, interleukin-2, or both) CD4+ T cells were detected in 69% of participants at baseline, with higher response rates and amounts among the mRNA-primed participants (Fig. 3). At day 15, an increase in the spike-specific Th1 responses occurred after boosting in all groups except those receiving the homologous Ad26.COV2.S regimen. Spike-specific Th2 (interleukin-4, interleukin-5, or interleukin-13) CD4+ T cells were infrequent (and at low levels) or absent in most subgroups. Th1-type CD4+ T-cell responses were predominant before and after homologous or heterologous boosting.

Spike-specific Th1 CD8+ cells were detected in 74 to 90% of the Ad26.COV2.S-primed recipients, as compared with levels of 10 to 30% in mRNA-primed recipients. Booster immunization increased the response rate and amount of spike-specific CD8+ T cells in all groups, except for the Ad26.COV2.S-primed participants who received homologous Ad26.COV2.S boosting, in whom no appreciable change above the already high prebooster response was noted. The highest amounts of spike-specific CD8+ T cells were observed in the Ad26.COV2.S-primed group, regardless of booster, both before boosting and at 15 days.

## DISCUSSION

We report the interim findings from this open-label clinical trial examining the safety, reactogenicity, and immunogenicity of SARS-CoV-2 booster vaccines in healthy adults who had previously received an EUA vaccine series. All booster vaccines were immunogenic in the participants regardless of which primary EUA regimen they had received, and the results were broadly in agreement with recent results from the United Kingdom.[27] The factor increases from prebooster levels in both binding and neutralizing antibody titers were similar or greater after heterologous boosting than after homologous boosting. Reactogenicity was similar to that

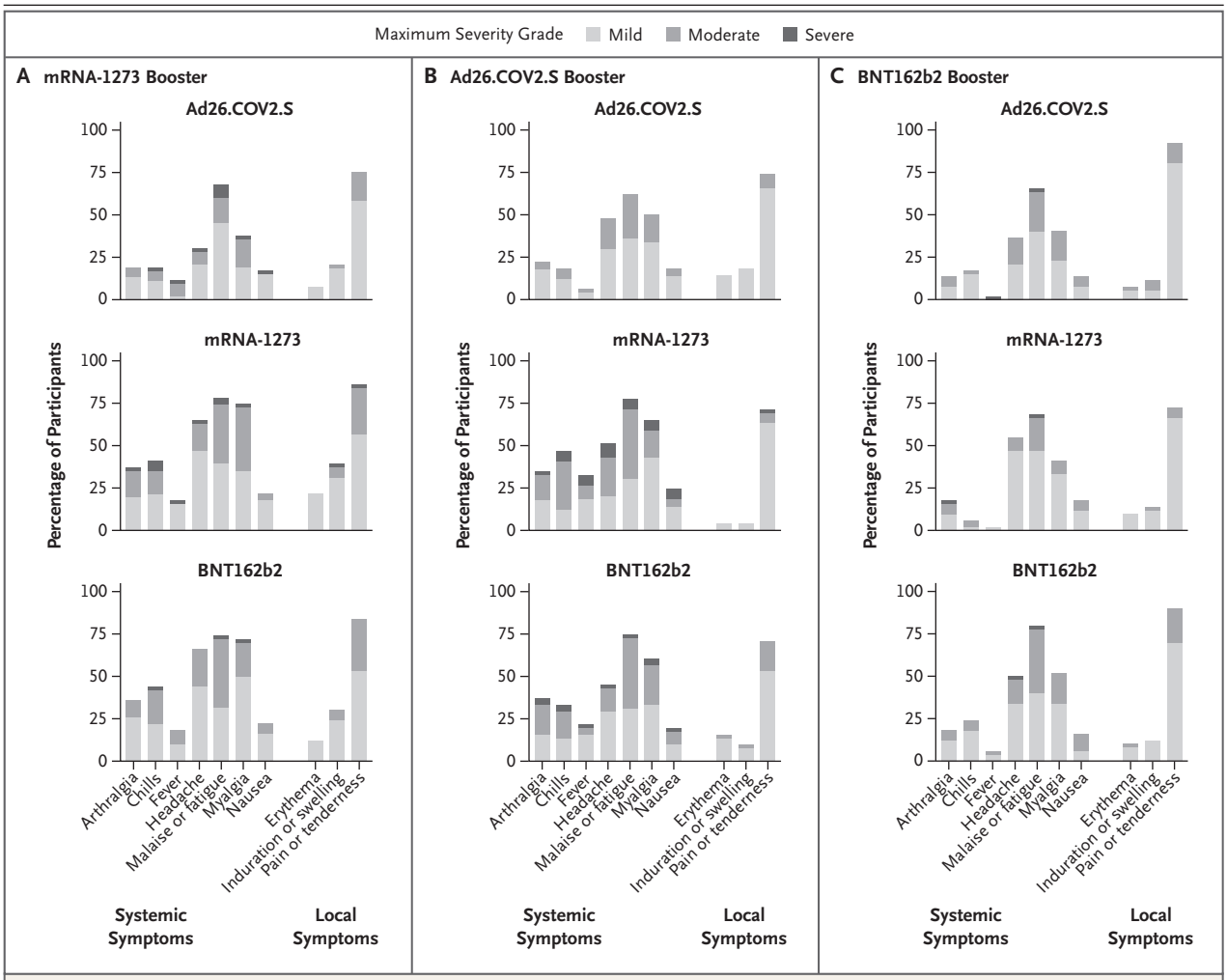


**Figure 1. Reactogenicity of the Three Booster Vaccines against Covid-19, According to Primary Vaccine Regimen.**

Shown are local (injection-site) and systemic reactions that were reported within 7 days after the administration of the mRNA-1273 (Panel A), Ad26.COV2.S (Panel B), and BNT162b2 (Panel C) boosters, according to the primary immunization regimen. Local and systemic reactions after the booster injection were graded as mild (does not interfere with activity), moderate (interferes with activity), or severe (prevents daily activity).

described in previous evaluations of mRNA-1273, Ad26.COV2.S, and BNT162b2 vaccines[1-3] and did not differ between heterologous and homologous boosters. No safety concerns were identified.

Previous studies have shown correlations between serum binding antibody and neutralizing antibody levels with protection from Covid-19 after both mRNA and adenovirus-vectored vaccination.[9,11] However, these correlates of protection were calculated on the basis of data collected before the widespread circulation of the delta and omicron variants, although a recent analysis showed that neutralizing antibody levels also correlated with protection from variants of concern, including delta,[12] before the outbreak of the omicron variant. A substantial increase in neutralizing antibody titers was observed in all trial participants after booster vaccination regardless of the booster and primary vaccines that were used.

All groups, with the exception of the homologous Ad26.COV2.S prime–boost group, had post-booster geometric mean titers of neutralizing antibody levels of more than 100 IU50 per milliliter, a level that correlated with 90.7% vaccine efficacy at preventing symptomatic Covid-19 in a previous study.[9] These data strongly suggest that homologous and heterologous booster vaccine doses will increase protective efficacy against symptomatic SARS-CoV-2 infection. This speculation is supported by emerging data showing the effectiveness of the BNT162b2 booster against

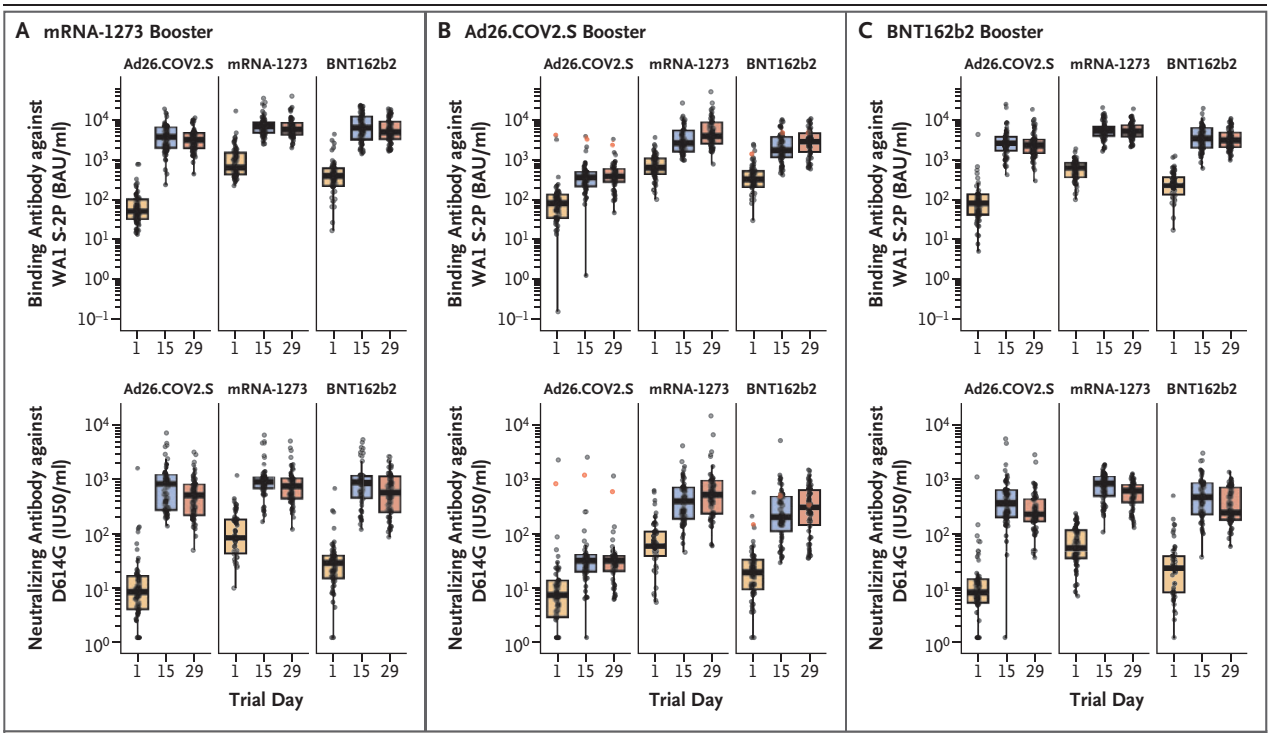


**Figure 2. Binding Antibody and Neutralizing Antibody Responses.**

Shown are box plots of IgG binding antibody titers against SARS-CoV-2 and pseudovirus neutralizing antibody titers on day 1 (prebooster) and on days 15 and 29, according to whether the participant received the mRNA-1273 (Panel A), Ad26.COV2.S (Panel B), or BNT162b2 (Panel C) booster vaccine. The primary vaccination regimens are listed above the box plots. Binding antibody responses were measured against the wild-type (WA1 S-2P) control variant on a 4-plex electrochemiluminescence immunoassay analyzer (ECLIA), and neutralizing antibody titers were measured against the D614G mutation of the SARS-CoV-2 spike protein. Titers were bridged to international standards and reported as binding antibody units per milliliter and international units for the 50% inhibitory dose (IU50) per milliliter. Data points for individual participants are shown as gray circles. In each box plot, the horizontal line represents the median value, with the top and bottom of the box indicating the 75th percentile and 25th percentile, respectively; the whiskers indicate values that are within 1.5 times the interquartile range. The red dots represent participants who had detectable antibody against the SARS-CoV-2 nucleocapsid protein at enrollment, indicative of previous SARS-CoV-2 infection.

symptomatic disease in the United States[28] and against severe disease, hospitalization, or death in Israel.[15,16] Predicting vaccine efficacy against severe SARS-CoV-2 infection, hospitalization, or death is considerably more challenging than predicting efficacy against symptomatic disease, and cellular immunity may contribute to the protection associated with humoral response for these more important outcomes.[12]

In group 2, the geometric mean titer of neutralizing antibody of 902 IU50 per milliliter after homologous boosting in mRNA-1273 vaccine recipients was substantially higher than that achieved approximately 4 weeks after completion of the primary two-dose mRNA-1273 vaccine series (geometric mean titer, 247 IU50 per milliliter) among participants in whom Covid-19 did not develop,[9] a finding that indicates a robust anamnestic response after booster vaccination. Similar responses have been reported after homologous boosting with mRNA-1273, BNT162b2, and Ad26.COV2.S vaccines.[13,14,29] Neutralizing activity against the delta and beta variants also increased substantially after mRNA booster vaccination, in line with previous reports.[30] In many of these studies, participants did not have detectable neutralizing activity against these variants before boosting, but the majority had detectable responses after booster vaccination. The postbooster neutralizing activity against the delta variant was lower than that against the D614G mutation by a factor of approximately 3, a decrement that was similar regardless of the primary vaccine series. This magnitude of lower response is similar to the lower neutralizing activity against delta than against the D614G mutation that was observed after primary vaccination with mRNA-1273.[23] Despite the lower titer against

**Table 2. Binding and Neutralizing Antibody Responses.***

| Variable | mRNA-1273 Booster | | | Ad26.COV2.S Booster | | | BNT162b2 Booster | | |
|---|---|---|---|---|---|---|---|---|---|
| | Primary Ad26.COV2.S | Primary mRNA-1273 | Primary BNT162b2 | Primary Ad26.COV2.S | Primary mRNA-1273 | Primary BNT162b2 | Primary Ad26.COV2.S | Primary mRNA-1273 | Primary BNT162b2 |
| **Group no.** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| No. of participants on day 15 | 53 | 51 | 50 | 50 | 49 | 50 | 52 | 51 | 49 |
| **IgG serum binding antibody titer†** | | | | | | | | | |
| GMT (95% CI) | | | | | | | | | |
| Day 1 | 59 (46–76) | 872 (680–1117) | 357 (262–484) | 71 (48–106) | 639 (514–794) | 321 (251–410) | 75 (55–103) | 534 (445–642) | 224 (177–282) |
| Day 15 | 3244 (2540–4142) | 6865 (5840–8070) | 6155 (4895–7739) | 326 (236–451) | 3029 (2433–3772) | 1905 (1498–2422) | 2563 (2052–3201) | 5256 (4513–6120) | 3345 (2711–4127) |
| Day 29 | 2986 (2478–3598) | 6224 (5282–7333) | 5231 (4274–6402) | 369 (291–467) | 4560 (3544–5867) | 2600 (2086–3240) | 2277 (1833–2828) | 5273 (4567–6088) | 3164 (2649–3779) |
| Percent with ≥2 factor increase from baseline titer on day 15 (95% CI) | 100 (93–100) | 96 (86–100) | 98 (89–100) | 86 (73–94) | 84 (70–93) | 92 (81–98) | 98 (90–100) | 100 (93–100) | 100 (93–100) |
| Increase in GMT from baseline (95% CI) | 55 (40–75) | 8 (6–10) | 17 (13–22) | 5 (4–6) | 5 (4–6) | 6 (5–8) | 34 (26–45) | 10 (8–12) | 15 (12–19) |
| **Pseudovirus neutralizing antibody‡** | | | | | | | | | |
| GMT (95% CI) | | | | | | | | | |
| Day 1 | 9 (6–13) | 89 (68–116) | 25 (18–34) | 8 (5–12) | 62 (45–85) | 19 (13–26) | 9 (6–14) | 58 (45–74) | 21 (15–30) |
| Day 15 | 676 (518–883) | 902 (728–1118) | 786 (596–1035) | 31 (22–44) | 382 (290–503) | 216 (158–297) | 344 (244–484) | 694 (578–832) | 437 (334–573) |
| Day 29 | 432 (323–578) | 700 (569–862) | 496 (370–663) | 30 (22–40) | 528 (383–729) | 267 (196–362) | 242 (190–309) | 515 (436–609) | 306 (244–384) |
| Percent with ≥4 factor increase from baseline titer on day 15 (95% CI) | 100 (93–100) | 84 (71–93) | 100 (93–100) | 50 (36–64) | 61 (46–75) | 82 (69–91) | 98 (90–100) | 94 (84–99) | 98 (89–100) |
| Increase in GMT from baseline (95% CI) | 73 (52–101) | 10 (8–13) | 32 (24–42) | 4 (3–6) | 6 (4–9) | 12 (9–18) | 36 (25–53) | 12 (9–15) | 20 (15–27) |

* The confidence intervals (CI) have not been adjusted for multiplicity, so the intervals should not be used to infer definitive treatment effects for secondary outcomes.
† The IgG serum binding antibody response was measured as the geometric mean titer (GMT) of the binding antibody units per milliliter against the wild-type SARS-CoV-2 variant (WA1 S-2P).
‡ The pseudovirus neutralizing antibody response was measured with the use of a pseudovirus expressing the D614G mutation of the SARS-CoV-2 spike protein and reported as the titer in international units of the 50% inhibitory dose (IU50) per milliliter.

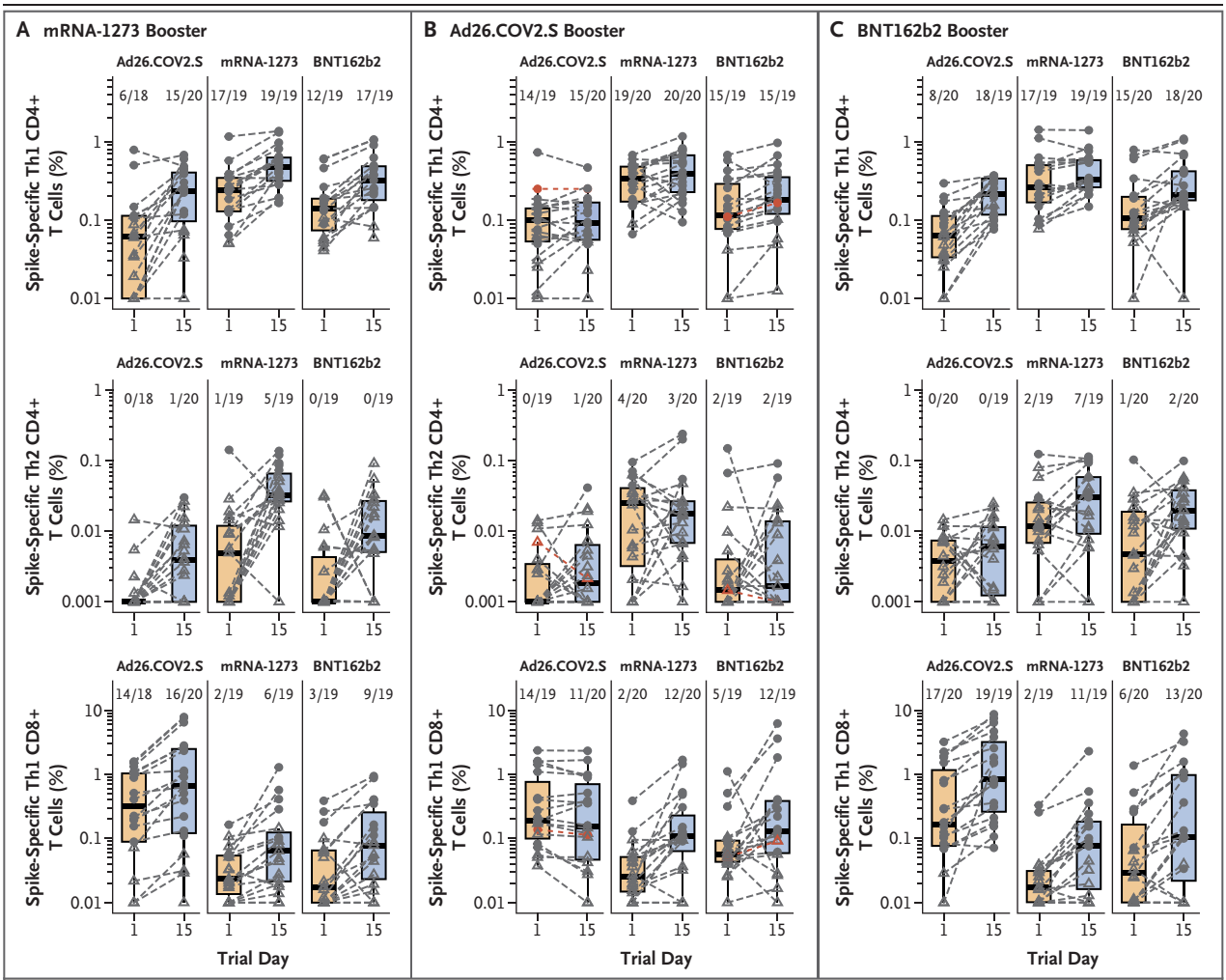


**Figure 3. CD4+ and CD8+ T-Cell Responses.**

Spike-specific T cells are shown in box plots before the administration of a homologous or heterologous booster vaccine on day 1 and after boosting on day 15. The boosters are shown at the top of each column, and the primary vaccines that each participant received are listed directly above each box plot. Circles indicate positive responses, and triangles indicate negative responses. Red symbols denote participants who had detectable antibody against the SARS-CoV-2 nucleocapsid protein at enrollment, indicative of previous SARS-CoV-2 infection. The responses are depicted as the background-subtracted percentage of spike-specific Th1 (interferon-γ, interleukin-2, or both) CD4+ T cells (top row), spike-specific Th2 (interleukin-4, interleukin-5, or interleukin-13) CD4+ T cells (middle row), and Th1 CD8+ T cells (bottom row). (Background subtraction refers to the subtraction of the values of the negative control sample from the peptide-stimulated sample.) The number of participants with a positive response among those tested is indicated as a fraction above each plot. Dashed lines link individual responses before and after the administration of the booster vaccine. The horizontal bar in each box indicates the median of all responses tested.

delta, all three vaccines were protective against hospitalization or death from infection with the delta variant.[31,32] These data suggest that boosting can maintain or increase protection against variants of concern, as has been reported previously.[13,14,29]

Vaccine-elicited spike-specific CD4+ and CD8+ T-cell responses may contribute to the durability of the antibody response and prevention of severe disease in cases of breakthrough infection.[33,34] Our findings indicate that the three primary Covid-19 vaccines induced a predominantly Th1 CD4+ T-cell response that persisted in the majority of recipients for up to 6 months, which is similar to the kinetics seen in SARS-CoV-2 natural infection.[35] By contrast, CD8+ T cells were more durable in recipients of the primary Ad26.COV2.S vaccine, and heterologous boosting with the Ad26.COV2.S vaccine substantially increased spike-specific CD8+ T cells in the re-

cipients of primary mRNA vaccines. Thus, the heterologous boost immunization strategy provides an immune response that may prove to be beneficial for durable prevention and control of Covid-19.

Our trial has limitations. It was not designed to directly compare responses among different booster regimens and did not include an unboosted control group. The sample size is insufficient for comparisons among groups, and the demographic characteristics of the participants are not representative of the U.S. population. Volunteers were not randomly assigned to trial groups or stratified according to population characteristics or the interval since the last vaccination. Similarly, the sample size and interim follow-up period were not sufficient to identify rare or late adverse events after booster vaccination, and the interval between completion of the primary series and the booster vaccination was variable and shorter than the 6 months authorized for recipients of primary mRNA vaccines. The dose of the mRNA-1273 booster that we evaluated was higher than that authorized by the FDA for boosting. Results for a half-dose (50-$\mu$g) mRNA-1273 booster are currently being evaluated. The immunogenicity data are limited to antibody responses through trial day 29 and T-cell responses through trial day 15. The different homologous and heterologous vaccination regimens also varied in terms of cellular and humoral immune responses, which may affect the durability of protection.

In this preliminary trial, we found that boosting with any of the three vaccines that are currently authorized for emergency use in the United States will stimulate an anamnestic response in persons who have previously received a primary series of any of these vaccines. Homologous boosting provided a wide range of immunogenicity responses, and heterologous boosting provided similar or higher levels. Reactogenicity and adverse events were similar across booster groups. These data suggest that an immune response will be generated for each of these vaccines used as a booster regardless of the primary Covid-19 vaccination regimen.

The results presented here are interim data from an ongoing trial, so the database has not been locked. Data have not been subjected to source verification or standard quality-check procedures that would ordinarily occur at the time of database lock. All sites are monitored by independent contractors.

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## APPENDIX

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