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Heterologous Ad26.COV2.S booster after primary BBIBP-CorV vaccination against SARS-CoV-2 infection: 1-year follow-up of a phase 1/2 open-label trial

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ABSTRACT

Background: Inactivated whole-virus vaccination elicits immune responses to both SARS-CoV-2 nucleocapsid (N) and spike (S) proteins, like natural infections. A heterologous Ad26.COV2.S booster given at two different intervals after primary BBIBP-CorV vaccination was safe and immunogenic at days 28 and 84, with higher immune responses observed after the longer pre-boost interval. We describe booster-specific and hybrid immune responses over 1 year.

Methods: This open-label phase 1/2 study was conducted in healthy Thai adults aged ≥ 18 years who had completed primary BBIBP-CorV primary vaccination between 90–240 (Arm A1; n = 361) or 45–75 days (Arm A2; n = 104) before enrolment. All received an Ad26.COV2.S booster. We measured anti-S and anti-N IgG antibodies by Elecsys®, neutralizing antibodies by SARS-CoV-2 pseudovirus neutralization assay, and T-cell responses by quantitative interferon (IFN)-γ release assay. Immune responses were evaluated in the baseline-seronegative population (pre-booster anti-N < 1.4 U/mL; n = 241) that included the booster-effect subgroup (anti-N < 1.4 U/mL at each visit) and the hybrid-immunity subgroup (anti-N ≥ 1.4 U/mL and/or SARS-CoV-2 infection, irrespective of receiving non-study COVID-19 boosters).

Results: In Arm A1 of the booster-effect subgroup, anti-S GMCs were 131-fold higher than baseline at day 336; neutralizing responses against ancestral SARS-CoV-2 were 5-fold higher than baseline at day 168; 4-fold against Omicron BA.2 at day 84. IFN-γ remained approximately 4-fold higher than baseline at days 168 and 336 in 18–59-year-olds. Booster-specific responses trended lower in Arm A2. In the hybrid-immunity subgroup at day 336, anti-S GMCs in A1 were 517-fold higher than baseline; neutralizing responses against ancestral SARS-CoV-2 and Omicron BA.2 were 28- and 31-fold higher, respectively, and IFN-γ was approximately 14-fold higher in 18–59-year-olds at day 336. Durable immune responses trended lower in ≥ 60-year-olds.

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Conclusion: A heterologous Ad26.COV2.S booster after primary BBIBP-CorV vaccination induced booster-specific immune responses detectable up to 1 year that were higher in participants with hybrid immunity.
Clinical Trials Registration. NCT05109559.

1. Introduction

During the global COVID-19 pandemic, whole inactivated virus (WIV) COVID-19 vaccines such as BBIBP-CorV (Sinopharm) were more extensively used for primary mass vaccination than mRNA vaccines in many low- and middle-income countries [1]. Authorized for use in more than 90 countries, BBIBP-CorV was the most widely used WIV globally [2]. A heterologous booster after 2-dose primary vaccination increases immune responses and effectiveness against SARS-CoV-2 infection to a greater extent than homologous boosting [3–9]. The World Health Organization (WHO) recommends heterologous boosting with either mRNA or vectored vaccines after BBIBP-CorV to ensure immunogenicity and protective benefits similar to primary vaccination with mRNA or vectored vaccines [10]. Long-term data on the immunogenicity of an Ad26.COV2.S booster after primary WIV vaccination against SARS-CoV-2 Omicron subvariants in Asia are lacking.

WIV COVID-19 vaccines elicit an immune response to both the spike (S) and nucleocapsid (N) proteins [11,12] and anti-N responses to BBIBP-CorV can be detected for 6 months or longer [2,12,13]. Anti-N titers in BBIBP-CorV–vaccinated individuals may therefore indicate prior vaccination, prior SARS-CoV-2 infection, or both (i.e., hybrid immunity) [11]. Hybrid immunity induces higher anti-S and anti-N responses, and is associated with a greater reduction in the risk of infection and symptomatic COVID-19, than vaccine-induced immunity alone [12,14]. With the high coverage of booster vaccinations and SARS-CoV-2 infections worldwide, an increasing proportion of the global population has acquired hybrid immunity. Immune responses in those with hybrid immunity are not easily distinguished from booster-only effects, especially in longer-term studies of heterologous boosters after WIV COVID-19 vaccines. To ascertain the direct effects of a heterologous booster alone, long-term immune responses to boosters after WIV vaccines should be analyzed in a study population for which an anti-N antibody cut-off index (COI) has been defined, to exclude individuals with pre-existing and newly acquired hybrid immunity.

A phase 1/2 study in Thailand showed that the heterologous Ad26.COV2.S booster was well tolerated and induced robust humoral and cell-mediated immune responses at days 28 and 84 when given at 2 different intervals (90–240 days or 45–75 days) after primary BBIBP-CorV vaccination [15,16]. Humoral responses were highest at day 28 against ancestral SARS-CoV-2, followed by the Delta, and then the Omicron BA.2 and BA.1 variants. T-cell–produced interferon (IFN)-γ increased approximately 10-fold in both arms at day 28 [15]. We now report 1-year follow-up immunogenicity and safety data in this population. We also present the immune responses up to 1 year induced by the booster alone, and by hybrid immunity acquired during the study.

2. Methods

2.1. Trial design and participants

NCT05109559 was a prospective multicenter, open-label, non-randomized, observer-blind, phase 1/2 study of an Ad26.COV2.S booster injection given at two different time intervals after 2-dose primary BBIBP-CorV vaccination. The study design, recruitment, eligibility criteria and safety and immunogenicity data at days 28 and 84 have been previously reported [15,16]. Enrolment into the two study arms was open-label and non-random, based on whether primary vaccination had been completed, 90–240 days (Arm A1) or 45–75 days (Arm 2) before enrolment. Participants received a single Ad26.COV2.S booster after their second BBIBP-CorV dose and were followed for 1 year.

Eligible participants were ≥ 18 years old, healthy, had verified documentation of 2 BBIBP-CorV doses given 21–35 days apart, and had given written informed consent prior to study enrolment.

The study protocol was approved by the Central Research Ethics Committee of Thailand, as well as the institutional review board of each clinical site, and was conducted in compliance with Good Clinical Practices and ICH E6 guidelines.

2.2. Vaccination procedures

On day 0, participants received 5×10^{10} virus particles of Ad26.COV2.S vaccine [17] in 0.5 mL injected into the deltoid muscle. Immunogenicity analyses were done on blood samples collected from all participants during study visits at days 0 (before the booster injection), 28, 84, 168 and 336.

All serious adverse events (SAE) and adverse events of special interest (AESI) were monitored throughout the study by the investigators, collaborators, and an independent Data Safety Monitoring Board (DSMB).

2.3. Immunogenicity assays

Binding antibodies were measured using a modified enzyme-linked immunosorbent assay (ELISA) in an electro-chemiluminescence immunoassay format to detect immunoglobulin (Ig)G antibodies to the SARS-CoV-2 S and N proteins (Elecsys® Anti-SARS-CoV-2; Roche Diagnostics) [18,19]. The results, expressed in ELISA units (U)/mL, were equivalent to the WHO international standard unit, binding antibody units (BAU)/mL [18]. The threshold for anti-S positivity was 0.8 U/mL [18]. The high prevalence of SARS-CoV-2 during the study period (Supplementary Fig. 1) [20] resulted in increased anti-N titers following SARS-CoV-2 infections in many individuals. As a result, to avoid excluding many participants, all of whom had completed primary WIV vaccination, a higher threshold was selected to define positive and negative N protein values than the manufacturer's threshold of 1.0 U/mL [19] (Supplementary Fig. 2A). To distinguish and compare the immune responses to the Ad26.COV2.S booster alone from those induced by hybrid immunity acquired during the study, participants were monitored at each visit for anti N-titers ≥ 1.4 U/mL and/or a history of symptomatic SARS-CoV-2 infections confirmed by RT-PCR or antigen testing. The anti-N COI of 1.4 U/mL was used to define the baseline-seronegative group, as well as the booster-effect and hybrid immunity subgroups (Supplementary Fig. 2B). If participants in the baseline-seronegative group had anti-N titers ≥ 1.4 U/mL or confirmed SARS-CoV-2 infections at any visit, they moved from the booster-effect subgroup to the hybrid-immunity subgroup (Fig. 1).

The SARS-CoV-2 pseudovirus neutralization assay (pNA) [21] was used to quantify the neutralizing activity of antibodies induced by booster vaccination against ancestral SARS-CoV-2 (Wuhan-Hu-1), and Omicron (B.1.1.529.1/BA.1 and B.1.1.529.2/BA.2) variants, and the T-cell response to the SARS-CoV-2 S protein was measured using a quantitative interferon (IFN)-γ release assay in whole blood, as previously reported [15]. Antibodies to the Adenovirus type 26 vector (Ad26) were measured using an Ad26 live virus neutralizing assay.

Suspected SARS-CoV-2 infections were confirmed by RT-PCR or antigen tests of nasopharyngeal swab samples.

2.4. Immunogenicity and safety endpoints

The primary immunogenicity endpoints measured at day 0 (pre-

booster baseline), and at study visits on days 28, 84, 168 and 336 were: (i) anti-S IgG geometric mean concentrations (GMC) measured by ELISA; (ii) the geometric mean fold rise (GMFR) in anti-S IgG geometric mean titers (GMT) compared with pre-boost titers; (iii) the GMT 50 % neutralization titer (NT_{50}) measured by pNA; and (iv) the GMFR in NT_{50} measured by pNA compared with baseline against ancestral SARS-CoV-2 Omicron BA.1 and BA.2 variants. The primary-endpoint binding and neutralizing antibody response data in the per-protocol population are presented in the supplement, but the focus of this report is on the immune responses elicited by the booster alone, and on hybrid immunity acquired during the study.

The study period coincided with a surge in SARS-CoV-2 infections in

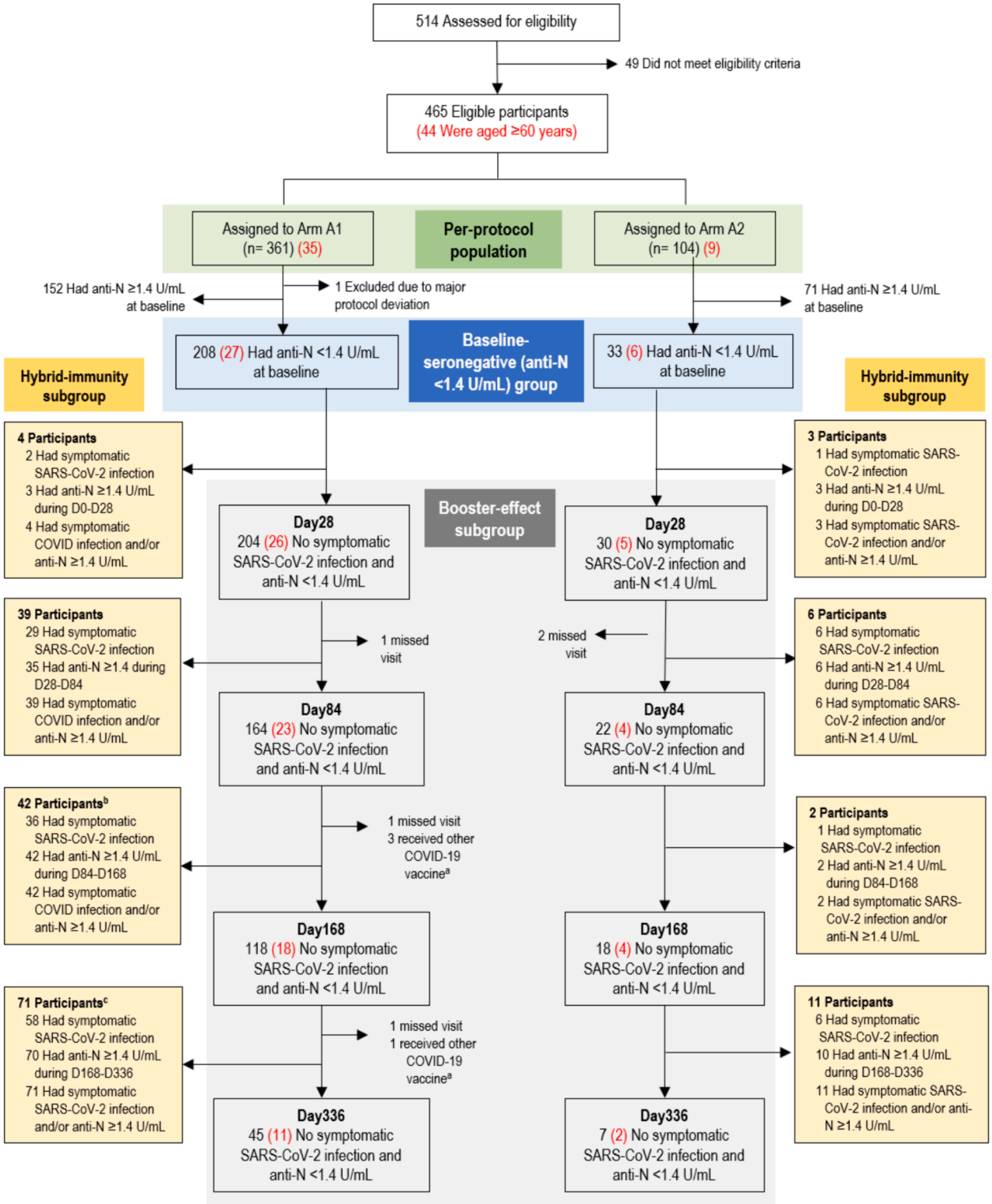


Fig. 1. Study profile and participant disposition [a] And did not meet criteria for hybrid immunity (ie., symptomatic SARS-CoV-2 infection and/or anti-N ≥ 1.4 U/mL). [b]Included 4 participants who met the criteria for hybrid immunity and had also received non-study COVID-19 vaccine boosters. [c]Included 10 participants who met the criteria for hybrid immunity and had also received non-study COVID-19 vaccine boosters.

Thailand dominated by the emergence of Omicron BA.1 and BA.2 subvariants and the disappearance of the Delta variant by March 2022 (Supplementary Fig. 1; [20]). Hence, immunogenicity against the Delta variant was not investigated further after the previously reported primary analysis [15] and day 84 results [16].

Secondary endpoints (neutralizing antibody titers, GMFR and the seroresponse rates measured by live virus microneutralization assay [mNA]) were not analyzed beyond day 84 [16] for practical purposes, because the results obtained by mNA were very similar to those obtained by pNA.

Exploratory endpoints included analyses of IFN-γ expression levels as a proxy indicator of S protein-specific T-cell responses, and the correlation between neutralizing antibodies against the Ad26 at baseline and binding and neutralizing antibodies against SARS-CoV-2 at day 28.

Post hoc subgroup analyses of immunogenicity by age and immune status were conducted in participants who were defined as seronegative at baseline (anti-N titers < 1.4 U/mL).

Primary safety endpoints not already reported at the primary analysis [15] were the frequency, severity and relatedness of SAEs and AESIs throughout the study period. Sponsor-defined AESIs included potential immune-mediated medical conditions, adverse events (AEs) associated with COVID-19, and thrombosis with thrombocytopenia syndrome.

2.5. Statistical analysis

Sample size assumptions were made based on an 80 % seroresponse rate in each study arm, with an expected two-sided 95 % CI of 71.1–86.7 [15]. To account for an estimated 20 % loss of participants, a sample size of 100 was needed in each group. Increased enrolment of up to 360 participants was planned in one arm (Arm A1) for safety analysis, to increase the probability of AE detection. This sample size would provide a 97 % probability of observing at least one AE occurring at 1 % frequency, even with 20 % loss to follow-up. This 1-year follow-up analysis was intended to be descriptive, and not powered to compare formally the immunogenicity between participants in Arms A1 and 2.

Safety was analyzed in all enrolled participants who received a study booster and had available safety data (the safety population). Descriptive analyses of immunogenicity were conducted in participants with no major protocol deviations that could interfere with immunogenicity assessment (the per-protocol population). Immune responses were also analyzed in participants who had pre-booster anti-N titers < 1.4 U/mL (the baseline-seronegative group). Within this group, subgroup analyses of immunogenicity were evaluated by age group (18–59 and ≥ 60 years) and immune status as follows (Fig. 1). The booster-effect subgroup included the baseline-seronegative participants who had not developed symptomatic SARS-CoV-2 infection, nor received any other COVID-19 vaccine after the booster, and whose anti-N titers remained < 1.4 U/mL at each study visit. We defined the hybrid-immunity subgroup to represent a real-world heterogenous population of participants who developed SARS-CoV-2 infection and/or had anti-N titers ≥ 1.4 U/mL at any study visit, irrespective of whether they had received a non-study COVID-19 vaccine.

GMCs, GMTs, and GMFR from baseline (all with 95 % CI) were calculated by study arm and subgroup. Analyses incorporated censoring where appropriate, and age-adjusted log-scale coefficients were back-transformed to compute the estimate and corresponding confidence limits. Missing immunogenicity data were not imputed and were analyzed as if participants were randomly missing.

The correlation between neutralizing antibodies against Ad26 at baseline (day 0) and SARS-CoV-2 antibody titers at day 28 was calculated for total anti-S IgG, and for anti-N IgG against the ancestral SARS-CoV-2 and Omicron BA.1 and BA.2 variants.

3. Results

3.1. Study population and immune status

Between 20 December 2021 and 4 February 2022, 514 community members who had been contacted by study site personnel to determine their interest in participating, or who had volunteered after seeing recruitment posters, were assessed for eligibility [15]. Forty-nine were not eligible to participate. The remaining 465 who met the eligibility criteria were enrolled at three sites in Thailand: 361 into Arm A1 and 104 into Arm A2 (Fig. 1). Participants in the per protocol population were aged 18–81 years with a median age of 40 years (IQR, 30–50); 465 (100 %) were Asian and 203 (43.8 %) were male (Supplementary Table 1). Study retention was high, with 452 participants (97.2 %) completing the day 336 visit: 355 (98.3 %) in Arm A1 and 97 (93.3 %) in Arm A2. Twelve participants (2.6 %) did not complete the study: 4 (0.9 %) moved from the study area, 6 (1.3 %) were lost to follow-up and 2 (0.4 %) were unavailable to attend study visits (Supplementary Table 2).

Overall, 241 participants had anti-N < 1.4 U/mL at baseline and were included in the baseline-seronegative group: 208 in Arm A1 and 33 in Arm A2 (Fig. 1). Their baseline characteristics are summarized in Table 1. The booster-effect subgroup analyzed to determine the immune responses to the Ad26.COV2.S booster alone, included 204 of 208 participants (98.1 %) in Arm A1 and 30 of 33 (91.0 %) in Arm A2 at day 28.

By day 336, 45 of 204 (22 %) 18–59-year-old adults in Arm A1 had reported no symptomatic SARS-CoV-2 infections and had anti-N < 1.4 U/mL, along with 11 of 26 (42 %) of individuals aged ≥ 60 years (Fig. 1). In Arm A2, 7 of 30 (23 %) 18–59-year-old adults and 2 of 5 (40 %) aged ≥ 60 years had anti-N < 1.4 U/mL. Four participants in Arm A1 received non-study COVID-19 boosters after day 84 and did not have SARS-CoV-2 infection or anti-N ≥ 1.4 U/mL during the study, hence they were excluded from further analysis.

The number of participants in the hybrid immunity subgroup at each timepoint is shown in Fig. 1. Hybrid immunity developed in 178 of 241 baseline-seronegative participants (73.9 %) by day 336: 156 (75.0 %) in Arm A1 and 22 (66.7 %) in Arm A2 (Table 2). Symptomatic SARS-CoV-2 infections occurred in 139 participants (i.e., 29.9 % of 465 participants in the safety population and 57.3 % of 241 participants in the baseline-seronegative subgroup): 125 (60.1 %) in Arm A1 and 14 (42.4 %) in Arm A2 (Table 2). Fourteen participants in Arm A1 had also received non-study BNT162b2 (Pfizer-BioNTech) and/or mRNA-1273 (Moderna) vaccines after day 84. They were included in the hybrid-immunity subgroup because their anti-N levels increased to ≥ 1.4 U/mL and/or because they had a symptomatic SARS-CoV-2 infection confirmed by RT-PCR or rapid antigen test, or from the patient's history.

Table 1
Baseline characteristics in the baseline-seronegative group, which included the booster-effect and the hybrid-immunity subgroups.

	All(n = 241)	Arm A1(n = 208)	Arm A2(n = 33)
Median age (IQR), years	43.0 (32.0–51.0)	43.5 (32.5–52.0)	38.0 (21.0–48.0)
18–59	210 (87.1 %)	182 (87.5 %)	28 (84.8 %)
≥60	31 (12.9 %)	26 (12.5 %)	5 (15.2 %)
Sex, n (%)			
Female	122 (50.6 %)	108 (51.9 %)	14 (42.4 %)
Male	119 (49.4 %)	100 (48.1 %)	19 (57.6 %)
Race, n (%)			
Asian	241 (100 %)	208 (100 %)	33 (100 %)
Median BMI (IQR), kg/m^2	25.0 (22.2–29.0)	25.0 (22.7–28.9)	24.9 (21.1–29.3)
Median time since dose 2 of primary series (IQR), days	107.0 (96.0–122.0)	110.0 (102.0–123.0)	56.0 (50.0–64.0)

Table 2
Factors contributing to hybrid immunity.

	All(n = 241)	Arm A1(n = 208)	Arm A2(n = 33)
Developed hybrid immunity during study, n (%)[a]			
No	56 (23.2 %)	49[a] (23.6 %)	7 (21.2 %)
Yes (Had SARS-CoV-2 symptomatic[b] infection and/or had anti-N ≥ 1.4 U/ml)	178 (73.9 %)	156 (75.0 %)	22 (66.7 %)
Missed visits	7 (2.9 %)	3 (1.4 %)	4 (12.1 %)
Anti-N increased to ≥ 1.4 U/mL during study, n (%)	170 (70.5 %)	150 (72.1 %)	20 (60.6 %)
Had symptomatic SARS-CoV-2 infection,[b] n (%)	139 (57.3 %)	125 (60.1 %)	14 (42.4 %)

[a] Four participants had received other COVID-19 vaccine during the study and were excluded from the booster effect group.
[b] Confirmed by RT-PCR or antigen test.

3.2. Humoral immune responses in the per protocol population and the baseline-seronegative group

The per protocol study population was immunologically heterogeneous because it included participants with and without anti-N antibodies from primary vaccination or prior infection (Table 1). Per-protocol population binding and neutralizing antibody response data through day 336 are summarized by study arm in Supplementary Tables 3–5 as the primary immunogenicity endpoints.

In the baseline-seronegative group, the point estimates for GMFR in anti-S titers were highest at day 28 in both arms before waning until day 168 (Fig. 2A, top panel). The GMFRs of anti-S titers were lower in Arm A2 than A1 until day 168, as suggested by non-overlapping 95 % CIs between arms. The wide 95 % CIs in Arm A2 reflect the small sample size. The trend suggesting a possible increase in anti-S titers after day 168 may correspond to the increase in participants who developed hybrid immunity as the study progressed.

. The neutralizing responses appeared to be the highest at day 28; at this point and the GMFRs for NT_{50} were highest against the ancestral SARS-CoV-2 strain (49.6 [95 % CI, 38.7–63.6] and 30.1 [95 % CI 18.3–49.7] in Arms A1 and A2, respectively; Fig. 2B, top panel). Neutralizing responses in Arm A1 were lower against Omicron BA.2 (9.5 [95 % CI, 6.9–13.1]) followed by BA.1 (4.0 [95 % CI 2.9–5.5]. Thereafter, the neutralizing antibody response waned until day 168. A trend towards an increase at day 336 was seen as hybrid immunity developed. Arm A2 showed similar patterns at lower levels than A1 until day 168, although the 95 % CIs overlapped between arms and variants.

The neutralizing responses by age group in Arm A1 suggested a trend towards lower GMTs of NT_{50} against ancestral SARS-CoV-2 and the Omicron BA.1 and BA.2 variants in participants aged ≥ 60 years compared to those aged 18–59 years, although with substantial overlap between 95 % CIs at all timepoints (Fig. 3A). Omicron BA.1 was only analyzed to day 84 because this variant was no longer circulating within 3 months after enrolment was completed (i.e., by May 2022; Supplementary Fig. 1).

3.3. Humoral responses in the booster-effect subgroup

In the booster-effect subgroup, the binding antibody response, which waned after day 28, was higher in Arm A1 than Arm A2 without overlapping 95 % CIs until day 168 (Fig. 2A, middle panel). The binding antibody response remained detectable at day 336, when the GMFRs from baseline in anti-S IgGs were 131.2 (95 % CI, 87.4–196.9) in Arm A1 and 45.3 (95 % CI, 18.0–114.4) in Arm A2 (Fig. 2A, middle panel).

Neutralizing antibody responses were substantially higher against the ancestral SARS-CoV-2 than the Omicron BA.1 and BA.2 variants in Arm A1 at days 28 (49.1 [95 % CI 38.6–62.5], 9.8 [95 % CI 7.1–13.5]) and 4.1 [95 % CI 3.0–5.7], respectively) and 84 (20.4 [95 % CI 14.9–27.9], 3.8 [95 % CI 2.4–6.0]) and 1.6 [95 % CI 1.0–2.3], respectively) (Fig. 2B, middle panel)). GMFR increases in NT_{50} from baseline in Arm 1 were detectable to day 168 against ancestral SARS-CoV-2 (5.4 [95 % CI, 3.5–8.4]) and to day 84 against Omicron BA.2 (3.8 [95 % CI, 2.4–6.0]). The sample size in Arm A2 was small, but the data suggested a trend towards lower neutralizing responses against the variants that appeared to follow a similar pattern.

Analyses of GMTs of NT_{50} by age group in Arm 1 of the booster-effect subgroup suggested a trend towards lower neutralizing antibody responses in ≥ 60-year-olds than in younger adults over the study period, although the 95 % CIs overlapped between age groups (Fig. 3B). The sample sizes were small at the later timepoints, but the data suggest that booster-induced neutralizing responses were detected for 6 months. At day 168, the GMTs were 92.4 (95 % CI, 58.1–147.0) and 78.5 (95 % CI, 37.9–162.5) against ancestral SARS-CoV-2 in younger and older adults, respectively. Mean GMTs of NT_{50} against Omicron BA.2 were 18.8 (95 % CI, 11.8–29.9) and 22.8 (95 % CI, 12.9–40.2) in younger and older adults, respectively, at day 168. Arm A2 showed similar trends in smaller sample numbers (data not shown).

3.4. Humoral responses in the hybrid-immunity subgroup

In the hybrid-immunity subgroup, the small sample numbers at day 28 led to very wide 95 % CIs for the GMFR of anti-S antibodies: 5766.6 (95 % CI 127.4–260,973.1) in Arm A1 and 331.3 (95 % CI 20.62–5,322.8) in Arm A2 (Fig. 2A, bottom panel). Nevertheless, these high GMFRs demonstrated the strength of the hybrid binding antibody response among the participants who likely contracted SARS-CoV-2 infections (symptomatic or asymptomatic) during the 28 days following the booster (Fig. 1). Binding antibody responses remained substantially higher than in the booster-effect subgroup throughout the study in both arms: on day 336, the GMFRs were 517.0 (95 % CI, 408.0–655.1) in Arm A1 and 431.2 (95 % CI, 193.8—959.7) in Arm A2 among 155 and 21 patients, respectively (Fig. 2A, bottom panel). The 95 % CIs did not overlap with those of booster-effect anti-S responses at any timepoint.

The GMFRs of NT_{50} in the hybrid-immunity subgroup had wide 95 % CIs, likely due to the small sample sizes and variable times relative to study visits when participants developed symptomatic or asymptomatic SARS-CoV-2 infections (Fig. 2B bottom panel). By day 336, these neutralizing responses remained substantially higher than in the booster-effect subgroup (28.0 [95 % CI, 20.6–38.1] and 39.8 [95 % CI, 21.7–72.8] against ancestral SARS-CoV-2 and 31.2 [95 % CI, 21.3–45.9] and 48.5 [95 % CI, 22.7–103.6] against Omicron BA.2 in Arms A1 and A2, respectively).

Analyses of GMTs of NT_{50} by age group in Arm 1 of the hybrid-immunity subgroup suggest that neutralizing antibody responses appeared to be lower in ≥ 60-year-old than 18–59-year-old adults up to 1 year, although 95 % CIs overlapped considerably between age groups (Fig. 3C). These responses against ancestral SARS-CoV-2 and the Omicron BA.2 variant remained higher than in the booster-effect subgroup over 1 year in both age groups. On day 336, the GMFRs of NT_{50} against ancestral SARS-CoV-2 were 443 (95 % CI, 329.9–595.0) in 18–59-year-olds and 370.5 (95 % CI, 168.1–816.3) in participants aged ≥ 60 years, and against Omicron BA.2 they were 475.9 (95 % CI, 337.2–671.7) and 256.1 (95 % CI, 94.8–691.6) in the respective age groups.

3.5. T-cell Responses in the baseline-negative group

As with humoral responses, the GMCs of T-cell-generated IFN-γ in Arm A1 of the baseline-seronegative subgroup reflect the heterogeneous group of participants who had booster-specific T-cell responses and those who acquired hybrid immune responses at later timepoints (Fig. 4A, top panel). T-cell responses in Arm A2, in which the sample size was small, showed similar trends (Supplementary Fig. 3, top panel).

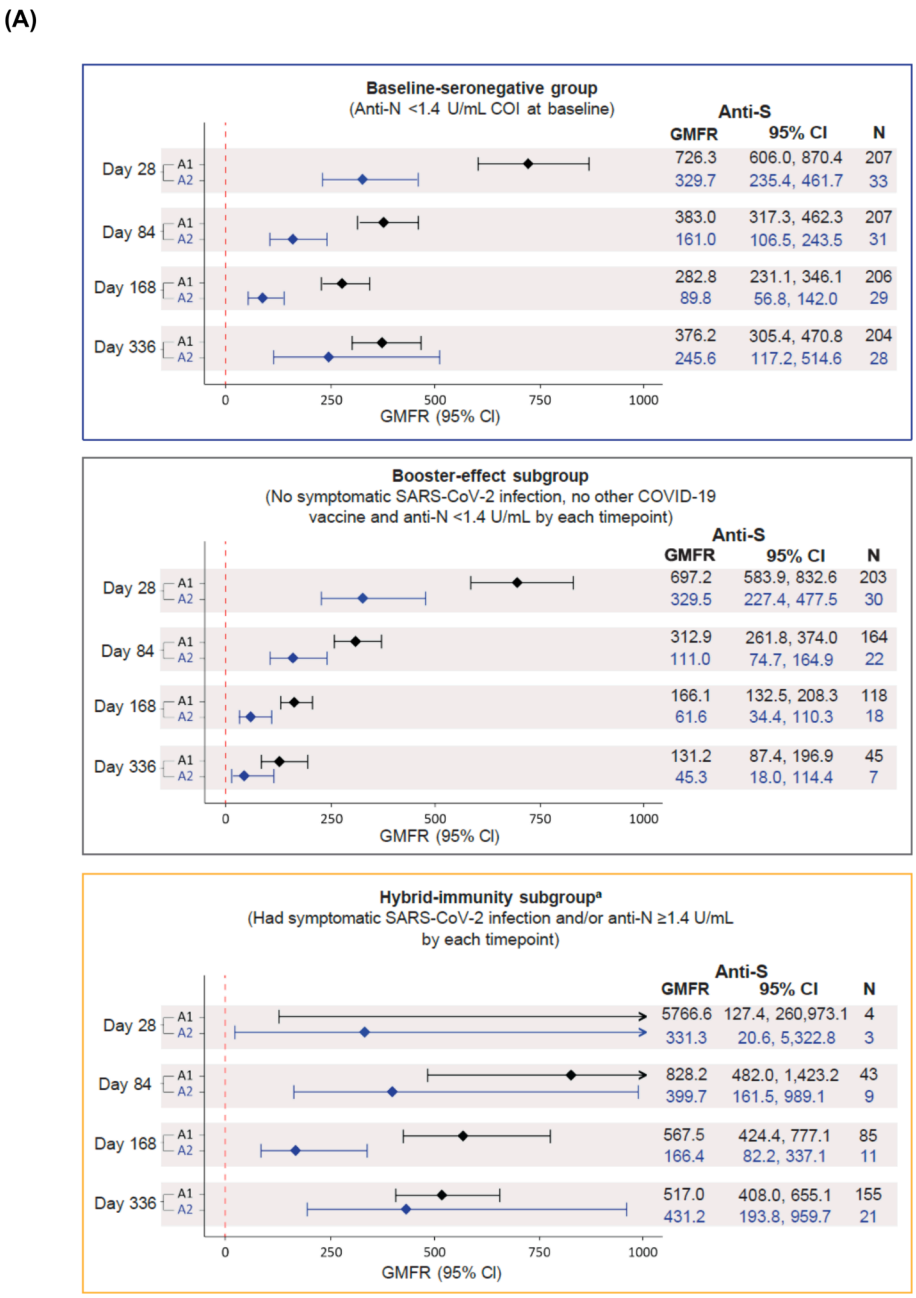


Fig. 2. Geometric Mean Fold Rise (GMFR) from baseline of (A) anti-Spike IgG and (B) NT_{50} against ancestral SARS-CoV-2 and Omicron BA.1 and BA.2 variants by study arm and subgroup. [a] Included 14 participants who received non-study COVID-19 vaccine boosters.

3.6. T-cell Responses in the booster-effect subgroup

IFN-γ GMCs in the booster-effect subgroup suggest that in participants aged 18–59 years, T-cell responses to the booster appeared to be highest at day 28 (GMC 1205 [95 % CI 969–1498]), then waned to 464 (95 %CI, 258–836) at day 336 (Fig. 4, middle panel). In adults aged ≥ 60 years, the GMC was 886 (95 %CI, 318–2465) at day 28 and 467(95 % CI, 32–6786) at day 336 in the limited number of participants evaluated. Arm 2 of the booster-effect subgroup suggested a similar trend in 18–59-year-old adults (Supplementary Fig. 3, middle panel). In the 2 adults aged ≥ 60 years in this subgroup at day 28, the GMC was 1330 and a similar GMC was detected in the 1 participant evaluated at days 84, 168 and 336.

3.7. T-cell Responses in the hybrid-immunity subgroup

In the hybrid-immunity subgroup, the IFN-γ GMCs in 18–59-year-old participants were higher than in the booster-effect subgroup at all timepoints, with no overlap between these subgroups' 95 % CIs from day 84 onwards (Fig. 4, bottom panel vs middle panel). The GMCs were 5642 (95 %CI, 155–205300) and 1708 (95 %CI, 1357–2149) at days 28 and 336, respectively. From day 84 onwards, T-cell responses trended lower in the few participants aged ≥ 60 years than in younger adults (810 [95 % CI, 139–4716] at day 84 and 628 [95 % CI, 245–1610] at day 336). Trends in T-cell responses appeared to be similar in the ≤ 10 participants evaluated in Arm A2 (Supplementary Fig. 3, bottom panel).

Fig. 3. Neutralizing antibody response to SARS-CoV-2 and Omicron BA.1 and BA.2 variants in Arm A1 in (A) participants with anti-N < 1.4 U/mL at baseline, (B) the booster-effect subgroup, and (C) the hybrid-immunity subgroup, by age group. [a] Included 14 participants who received non-study COVID-19 vaccine boosters.

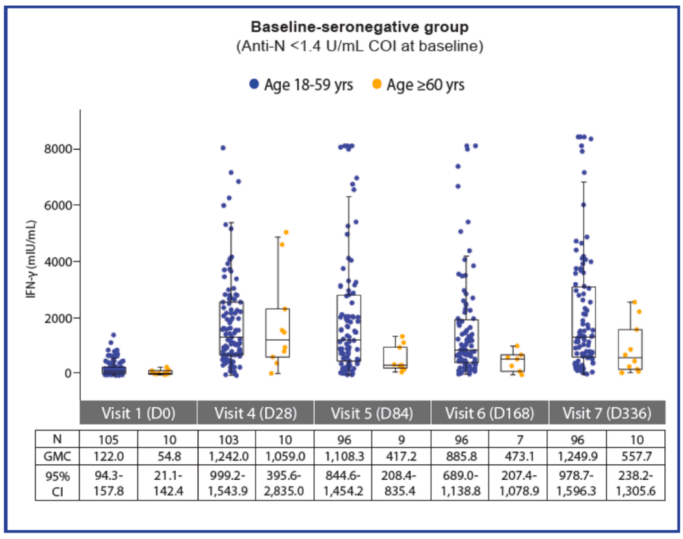
3.8. Impact of pre-existing anti-Adenovirus 26 immunity on humoral responses to the Ad26.COV2.S booster

Baseline anti-Ad26 seropositivity was observed in 76 of 243 study participants (32.5 %): 61 of 181 (33.7 %) in Arm A1 and 15 of 53 (28.3 %) in Arm A2. There was no significant correlation with day 28 total binding anti-S ($R = -0.15$; $P = 0.09$) or neutralizing antibodies against ancestral SARS-CoV-2 ($R = -0.20$; $P = 0.18$) (Fig. 5). A weak negative correlation between baseline anti-Ad26 neutralizing titers and day 28 neutralizing antibodies against the Omicron BA.1 and BA.2 variants were found ($R = -0.31$; $P = 0.04$ and $R = -0.29$; $P = 0.05$, respectively).

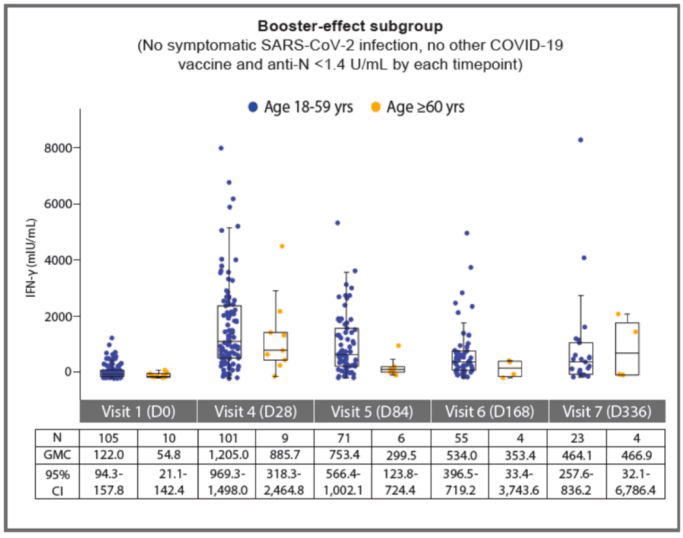
3.9. Safety

Study vaccine-related local and systemic AEs occurring within 28 days of receiving the booster were reported previously at the primary analysis [15].

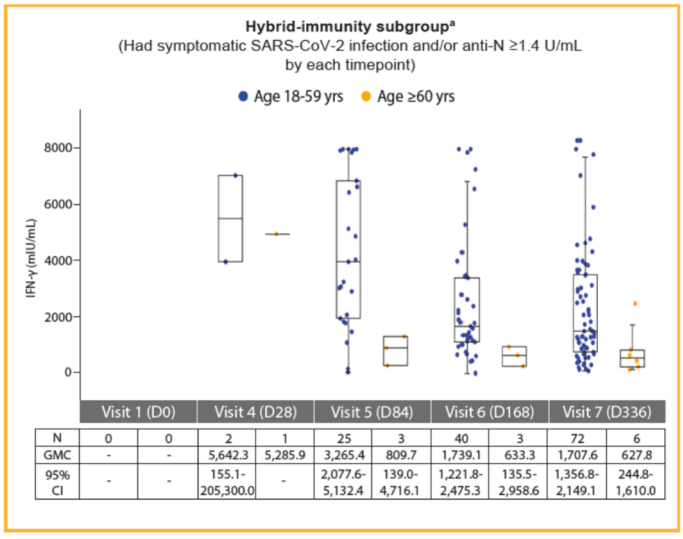
During the entire study period, grade 1–3 SAEs occurred in 64 of 465 participants (13.8 %) in the safety analysis population: 52 participants (14.4 %) in Arm A1 and 12 (11.5 %) in Arm A2) (Table 3). No SAEs were medically assessed as related to the study booster. No SAEs were fatal or resulted in persistent disability or incapacity. The SAEs in 60 participants resolved completely; in the remaining 4 participants (0.9 %), the SAEs (pemphigus vulgaris, prostate cancer, herniated lumbar disc and



	Visit 1 (D0)		Visit 4 (D28)		Visit 5 (D84)		Visit 6 (D168)		Visit 7 (D336)	
N	105	10	103	10	96	9	96	7	96	10
GMC	122.0	54.8	1,242.0	1,059.0	1,108.3	417.2	885.8	473.1	1,249.9	557.7
95% CI	94.3-157.8	21.1-142.4	999.2-1,543.9	395.6-2,835.0	844.6-1,454.2	208.4-835.4	689.0-1,138.8	207.4-1,078.9	978.7-1,596.3	238.2-1,305.6



	Visit 1 (D0)		Visit 4 (D28)		Visit 5 (D84)		Visit 6 (D168)		Visit 7 (D336)	
N	105	10	101	9	71	6	55	4	23	4
GMC	122.0	54.8	1,205.0	885.7	753.4	299.5	534.0	353.4	464.1	466.9
95% CI	94.3-157.8	21.1-142.4	969.3-1,498.0	318.3-2,464.8	566.4-1,002.1	123.8-724.4	396.5-719.2	33.4-3,743.6	257.6-836.2	32.1-6,786.4



	Visit 1 (D0)		Visit 4 (D28)		Visit 5 (D84)		Visit 6 (D168)		Visit 7 (D336)	
N	0	0	2	1	25	3	40	3	72	6
GMC	-	-	5,642.3	5,285.9	3,265.4	809.7	1,739.1	633.3	1,707.6	627.8
95% CI	-	-	155.1-205,300.0	-	2,077.6-5,132.4	139.0-4,716.1	1,221.8-2,475.3	135.5-2,958.6	1,356.8-2,149.1	244.8-1,610.0

Fig. 4. Geometric mean concentrations (GMC) of T-cell IFN-γ in Arm A1 by study group and age. [a] Included 7 participants who received non-study COVID-19 vaccine boosters.

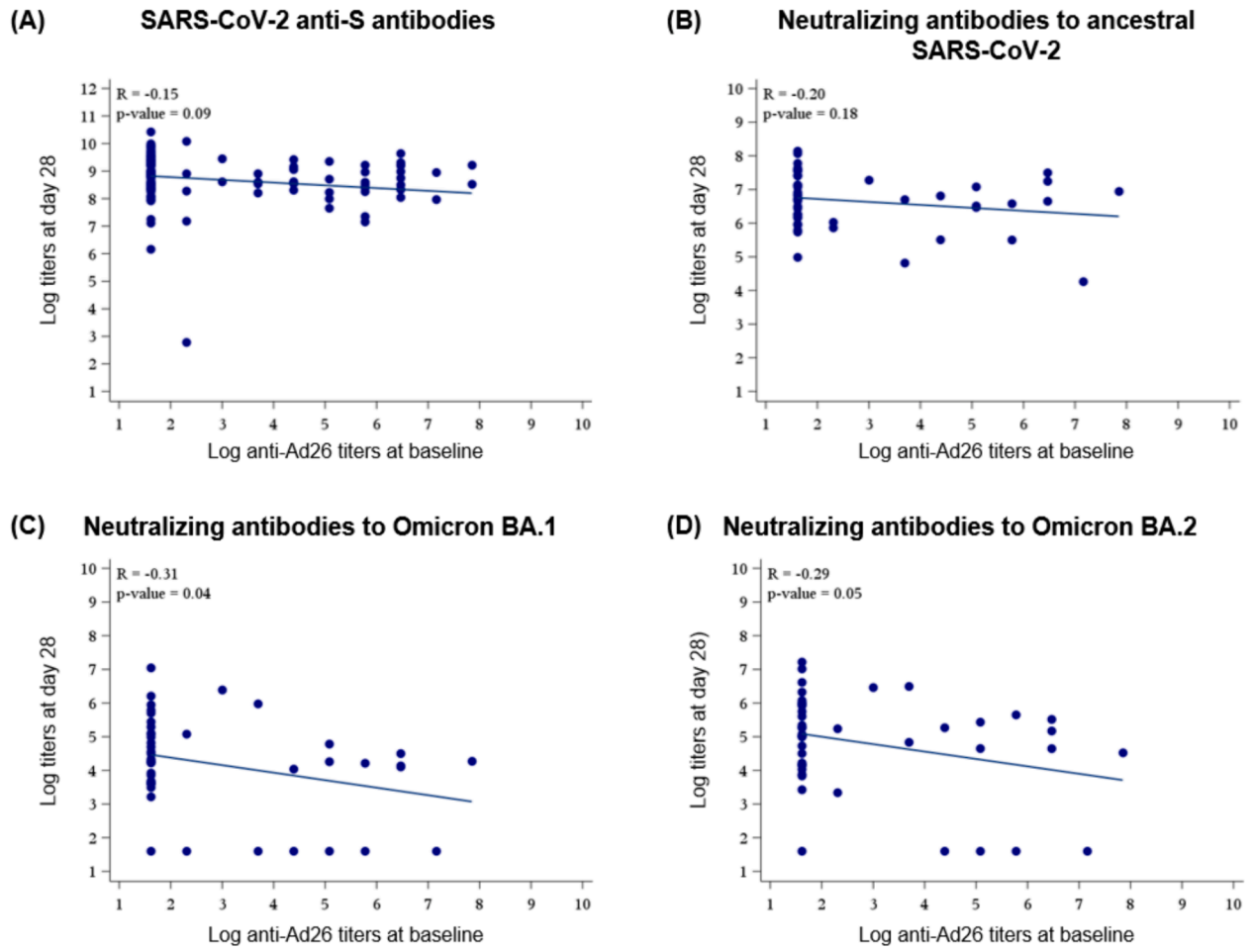


Fig. 5. Correlation between GMTs of baseline anti-Ad26 neutralizing antibodies and day 28 (A) SARS-CoV-2 anti-S antibodies; and neutralizing antibodies against (B) ancestral SARS-CoV-2 and (C) Omicron BA.1 and (D) Omicron BA.2 in the baseline seronegative group.

Table 3
Summary of SAEs throughout the study period in the safety population.

	All(N = 465)	Arm A1(n = 361)	Arm A2(n = 104)
Patients with ≥ 1 SAE, n (%)	64 (13.8 %)	52 (14.4 %)	12 (11.5 %)
Study booster-related	0 (0 %)	0 (0 %)	0 (0 %)
Not related to study booster	64 (13.8 %)	52 (14.4 %)	12 (11.5 %)
Grade 1 (Mild)	45 (9.7 %)	37 (10.25 %)	8 (7.69 %)
Grade 2 (Moderate)	8 (1.7 %)	8 (2.2 %)	0 (0 %)
Grade 3[a] (Severe)	11 (2.4 %)	7 (1.9 %)	4 (3.9 %)
Required in-patient hospitalization	23 (4.9 %)	18 (5.0 %)	5 (4.8 %)
Pregnancy resulting in congenital anomaly or birth defect[b]	1 (0.2 %)	1 (0.3 %)	0 (0 %)
Leading to study discontinuation	0 (0 %)	0 (0 %)	0 (0 %)

SAE, serious adverse event.
[a] No grade 4 or 5 SAEs occurred.
[b] Congenital micro-cleft lip not requiring surgical intervention.

liver cancer) were ongoing at the study cut-off. In 48 participants (10.3 %), the SAEs were confirmed SARS-CoV-2 infections, for which 6 participants (1.3 %) required hospitalization; all 48 affected participants recovered fully. All SARS-CoV-2 infections were mild or moderate except in 1 participant (0.2 %), whose infection was classified as severe because they were treated with favipiravir. The individual was treated as an outpatient and recovered after 10 days.

AESIs occurred in 3 participants (0.6 %) in Arm 1 and all were medically assessed as not related to the study booster (Table 4). Grade 2 ageusia in the context of SARS-CoV-2 infection occurred in 2 participants, and resolved within 9 and 10 days, respectively. Grade 3 pemphigus vulgaris occurred in 1 participant 2.5 months after the booster, required hospitalization and improved. The participant's condition remained stable at the study cut-off.

Seven pregnancies occurred during the study, which resulted in 6 normal deliveries at term. These pregnancies were confirmed 2.5 weeks

Table 4
Summary of AESIs throughout the study period in the safety population.

	All(N = 465)	Arm A1(n = 361)	Arm A2(n = 104)
Participant with ≥ 1 any-cause AESI by severity[a]	3 (0.4 %)	3 (0.6 %)	0 (0 %)
Study booster-related	0 (0 %)	0 (0 %)	0 (0 %)
Not related to study booster	3 (0.4 %)	3 (0.6 %)	0 (0 %)
Grade 1 (Mild)	0 (0 %)	0 (0 %)	0 (0 %)
Grade 2[b] (Moderate)	2 (0.4 %)	2 (0.6 %)	0 (0 %)
Grade 3[c,d] (Severe)	1 (0.2 %)	1 (0.3 %)	0 (0 %)
Leading to study discontinuation	0 (0 %)	0 (0 %)	0 (0 %)

AESI, adverse event of special interest.
[a] Maximum severity grade was counted.
[b] Ageusia.
[c] Pemphigus vulgaris.
[d] No grade 4 or 5 AESIs occurred.

to 9 months after the booster injection. One pregnancy that was confirmed almost 8 months after the booster injection resulted in spontaneous abortion after *in vitro* fertilization. Five newborns were healthy; 1 had a minor congenital anomaly (micro-cleft lip, requiring no surgical intervention) that was medically assessed as unrelated to the study booster given to a participant in her first trimester of pregnancy.

4. Discussion

Anti-N antibodies can persist for 6 months or longer after a second BBIBP-CorV dose [2,12]. Conducting these post hoc analyses in subgroups of a 'baseline-seronegative' (anti-N < 1.4 U/mL) study population allowed us to distinguish the Ad26.COV2.S booster-specific immune responses after primary BBIBP-CorV vaccination from those induced by newly acquired hybrid immunity over 1 year. The immune responses in the total baseline-seronegative group reflected the combined booster-induced and hybrid immunity over the study period. To reflect the real-world situation in which not only SARS-CoV-2 infections, but also COVID-19 booster coverage has increased worldwide, the hybrid-immunity subgroup included a heterogenous population of participants who developed SARS-CoV-2 infection and/or had anti-N titers ≥ 1.4 U/mL at any study visit, regardless of whether or not they had received a non-study COVID-19 vaccine booster. The hybrid-immunity subgroup results demonstrated how humoral and T-cell responses evolved after a heterologous booster given during the COVID-19 pandemic under ongoing exposure to different circulating SARS-CoV-2 viruses.

In the booster-effect subgroup, binding antibody responses were detectable up to 1 year in both arms, and neutralizing antibody responses in Arm A1 were detectable for 6 months against ancestral SARS-CoV-2, and 3 months against Omicron BA.2. In participants aged ≥ 60 years, booster-induced neutralizing antibody and T-cell responses trended lower than in younger adults but were detectable for 1 year. Consistent with the primary analysis results [15], binding and neutralizing antibody responses induced by the Ad26.COV2.S booster showed a trend for being higher in Arm A1 (which had a longer interval before the booster [90–240 days]) than in Arm A2 until day 168, and neutralizing antibody responses in Arm A1 tended to be higher against ancestral SARS CoV-2 than the Omicron variants. The difference in pre-boost intervals did not impact the 1-year durability of binding antibody responses. Our findings are consistent with those observed with another adenovirus-vectored vaccine, ChAdOx-1, given as a booster at different intervals after primary WIV CoronaVac vaccination: immune responses against ancestral SARS-CoV-2 and Omicron BA.1 showed similar trends at days 28 and 90, and highest responses with the longest pre-boost interval (120–180 days) [13]. Participants aged ≥ 60 years showed a trend towards lower booster-induced neutralizing antibody and T-cell responses than younger adults in Arm A1; sample sizes in Arm A2 were too small to draw firm conclusions.

In the hybrid-immunity subgroup, humoral responses were substantially higher than those induced by the booster alone and were detectable among both younger and older adults throughout the 1-year follow-up, even against Omicron BA.2. T-cell responses induced in the hybrid-immunity subgroup were durable over 1 year and were substantially higher than those induced in the booster-effect subgroup after day 28, as indicated by non-overlapping 95 % CIs between subgroups in younger adults. T-cell responses appeared to be lower among older participants after the longer pre-boost interval, but were still detected over 1 year. Although these results should be interpreted with caution because of the small number of older individuals evaluated, the trend towards lower immune responses in participants aged ≥ 60 years may suggest that older adults might need more frequent boosters.

In a US study, an Ad26.COV2.S heterologous booster after primary mRNA vaccination also resulted in higher neutralizing responses to ancestral SARS-CoV-2 than other variants, with similar mean titers against Omicron BA.1 and BA.2, and Omicron-specific CD8 + T-cell responses [22]. In our Thai study of an Ad26.COV2.S heterologous booster after WIV vaccination, the booster-specific neutralizing antibody response to ancestral SARS-Co-V2 was durable for 6–12 months, with a lower 3–6-month response against Omicron BA.1 and BA.2. Booster-specific IFN-γ concentrations peaked after 1 month, but persisted for 1 year. These findings are consistent with other studies showing that cellular immune responses can have greater durability and cross-reactivity than neutralizing antibody responses, even in older individuals [23,24]. While vaccine-induced T-cell responses play a role in immune longevity and preventing severe SARS-CoV-2 infections [25], vaccine policy and booster recommendations for adequate protection have been largely based on humoral immune responses and durability.

In the hybrid-immunity subgroup, it appears that most participants' immune responses were related to SARS-CoV-2 infections given that ≥ 75 % had a history of symptomatic infections at each visit after day 28. Neutralizing immune responses against Omicron BA.1 and BA.2 increased substantially between days 28 and 84, unlike in the booster-effect group. These findings are consistent with previous reports that immune responses after natural infection contracted post-vaccination are higher than after vaccination alone [12,14]. A large prospective Dutch cohort study showed that hybrid immunity was more protective than vaccine-induced immunity against infection with Omicron BA.1, BA.2 and BA.5 for up to 30 weeks after the last immunizing event (i.e. vaccine or infection), and was associated with higher levels of anti-S antibodies [14]. Another study from Portugal showed that hybrid immunity after infection with Omicron BA.1 or BA.2 resulted in durable, substantially greater protection against Omicron BA.5 infection than vaccination alone [26].

The previously reported favorable safety and tolerability profile of this booster [15,16] continued over 1-year follow-up. None of the SAEs reported during the study period was medically assessed as related to the booster. The participant with pemphigus vulgaris received specialist care and the DSMB assessed the event as not related to the vaccination. The condition developed 2.5 months after the study booster, whereas most known vaccine-related AEs occur within 4–6 weeks following vaccination [15]. In other case reports and reviews, pemphigus vulgaris developed within 3–21 days following COVID-19 vaccination [27]. In this study, a SARS-CoV-2 infection was classified as a SAE if the participant was admitted to hospital or received favipiravir, and 75 % of the SAEs reported were SARS-CoV-2 infections. All SARS-CoV-2 infections were mild or moderate except in 1 participant (0.2 % of the total safety population). This may suggest that the Ad26.COV2.S booster after primary BBIBP vaccination could protect against severe SARS-CoV-2 infections for up to 1 year, with additional protection likely from hybrid immunity.

A weak negative correlation between the baseline level of anti-Ad26 titers and day 28 neutralizing antibodies against Omicron BA.1 and BA.2 was seen. The clinical significance of these findings is unknown. Importantly, among samples randomly selected from the phase 3 ENSEMBLE trial of Ad26.COV2.S, 31 % from Brazilian and 66 % from South African participants had Ad26 neutralizing antibodies [28]. However, the correlation between pre-vaccination anti-Ad26 titers and post-vaccination anti-S titers in those samples was low to negligible. Studies of Ad26-vectored Ebola and HIV-1 vaccines in which a high proportion of participants had pre-existing Ad26-specific antibodies showed no impact on vaccine-induced immune responses [29–31].

This study's strengths include the 1-year follow up with a high retention rate of 97 %, evaluation of immune responses after different time intervals between primary vaccination and boosting, separate analyses of booster-specific and hybrid immunity responses, and analyses of immune responses by age group. Study limitations include the post hoc nature of the booster-effect and hybrid immunity analyses, and the small sample sizes at later timepoints and in Arm 2. As reported previously, the sample size in Arm A2 was smaller than in Arm A1 because during enrolment, fewer patients were eligible for the Arm A2-prespecified 45–75-day interval from primary vaccination [15]. The

study was designed to provide descriptive information about immunogenicity over time following 2 different Ad26.COV2.S booster schedules. The sample size was not designed to power formal statistical analyses over 1 year of differences between arms, variants, or age groups, and P values were not calculated. However, 95 % CIs were provided for all point estimates. The degree of difference between arms, subgroups, variants, and age groups can be inferred from whether or not the 95 % CIs overlapped between the variables compared. The selection of the anti-N COI of 1.4 instead of 1.0 U/mL used in other studies measuring anti-N responses [14,32] may have resulted in fewer participants in the booster-effect subgroup at baseline, but may also have been more likely to reduce potential confounding effects from prior WIV vaccination or asymptomatic SARS-CoV-2 infections.

In conclusion, Ad26.COV2.S booster-induced humoral responses appeared to peak at 1 month. Binding responses were higher when given after the longer than the shorter interval following primary BBIBP-CorV vaccination and neutralizing responses showed a similar trend. Booster-induced binding and neutralizing antibody responses against ancestral SARS-CoV-2 were detectable for 12 and 6 months, respectively; neutralizing responses against Omicron BA.2 showed a trend for being lower, and lasted 3 months. By contrast, hybrid immunity, conferred mostly by SARS-CoV-2 infections, induced higher humoral and T-cell responses measured up to 12 months after the heterologous booster, consistent with findings from other studies. T-cell responses were detected 1 year after the booster in both subgroups. The Ad26.COV2.S booster remained well tolerated with long-term follow-up, with no new safety concerns.

CRediT authorship contribution statement

Sant Muangnoicharoen: Data curation, Investigation, Methodology, Project administration, Resources, Validation, Writing – review & editing. **Rakpong Wiangcharoen:** Data curation, Investigation, Methodology, Project administration, Resources, Writing – review & editing. **Saranath Lawpoolsri:** Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing – review & editing. **Sira Nanthapisal:** Investigation, Data curation, Project administration, Resources, Supervision, Writing – review & editing. **Anan Jongkaewwattana:** Data curation, Investigation, Supervision, Validation, Visualization, Writing – review & editing. **Chatnapa Duangdee:** Data curation, Validation. **Supitcha Kamolratanakul:** Investigation, Data curation, Resources, Writing – review & editing. **Viravarn Luvira:** Investigation, Supervision, Writing – review & editing. **Narumon Thanthamnu:** Data curation, Resources, Validation. **Narisara Chantratita:** Data curation, Supervision, Validation, Writing – review & editing. **Arunee Thitithanyanont:** Data curation, Supervision, Validation, Writing – review & editing. **T. Anh Wartel:** Conceptualization, Investigation, Methodology, Supervision, Writing – review & editing. **Jean-Louis Excler:** Conceptualization, Methodology, Supervision, Visualization, Writing – review & editing. **Martin F. Ryser:** Conceptualization, Funding, Data curation, Writing – review & editing. **Chloe Leong:** Project administration, Resources, Supervision. **Tippi K. Mak:** Conceptualization, Data curation, Writing – review & editing, Supervision. **Punnee Pitisuttithum:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: S. M., R.W., S.K., S.L., A.J., A.T., V.L., P.C., N.T., N.C. report no potential conflicts of interest. S.N. has received honoraria for lectures from GSK, Zuellig Pharma, Astra Zeneca, Novartis, Abbot, Sanofi, Organon and Takeda and has participated in advisory boards for GlaxoSmithKline and Takeda. J.K.L. and T.A.W. and received institutional funding from Mahidol University to support study conduct, and T.A.W. supported management of Data Safety Monitoring Board activities and meetings. J-L.E. has no competing interests to declare.. M.F.R. is an employee of Janssen Pharmaceuticals and holds restricted stock units in the company. C.L. is an employee of Janssen Pharmaceuticals. T.K.M. has received consulting fees from Janssen Pharmaceuticals. P.P. has received funding from Mahidol University and Johnson & Johnson for study support.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.05.010.

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