Vaccine xxx (xxxx) xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Safety, reactogenicity, and immunogenicity of Ad26.COV2.S: Results of a phase 1, randomized, double-blind, placebo-controlled COVID-19 vaccine trial in Japan

Yumi Tsuchiya [a,*], Hiroshi Tamura [a], Koji Fujii [a], Hirotaka Numaguchi [a], Kiichiro Toyoizumi [b], Tina Liu [c], Mathieu Le Gars [d], Vicky Cárdenas [e], Takashi Eto [f]

[a] Research and Development, Janssen Pharmaceutical K.K., Tokyo, Japan
[b] Statistics and Decision Sciences, Janssen Pharmaceutical K.K., Tokyo, Japan
[c] Clinical and Statistical Programming, Janssen China Research and Development, Beijing, China
[d] Janssen Vaccines and Prevention, Leiden, the Netherlands
[e] Janssen Research and Development, Spring House, USA
[f] Souseikai Hakata Clinic, Fukuoka-city, Fukuoka, Japan

ARTICLE INFO

Article history:
Received 19 August 2022
Received in revised form 10 November 2022
Accepted 3 January 2023
Available online xxxx

Keywords:
COVID-19
SARS-CoV-2
Ad26.COV2.S
Vaccine
Phase 1 clinical trial
Virus neutralization assay

ABSTRACT

Background: This study evaluated safety, reactogenicity, and immunogenicity of a 2-month homologous booster regimen of Ad26.COV2.S in Japanese adults.
Methods: In this multicenter, placebo-controlled, Phase 1 trial, adults (Cohort 1, aged 20–55 years, N = 125; Cohort 2, aged ≥ 65 years, N = 125) were randomized 2:2:1 to receive Ad26.COV2.S 5×10^{10} viral particles (vp), Ad26.COV2.S 1×10^{11} vp, or placebo, followed by a homologous booster 56 days later. Safety, reactogenicity, and immunogenicity were assessed.
Results: Two hundred participants received Ad26.COV2.S and 50 received placebo. The most frequent solicited local adverse event (AE) was vaccination-site pain, and the most frequent solicited systemic AEs were fatigue, myalgia, and headache. After primary vaccination, neutralizing and binding antibody levels increased through Day 57 (post-prime) in both cohorts. Fourteen days after boosting (Day 71), neutralizing antibody geometric mean titers (GMTs) had almost reached their peak value in Cohort 1 (5×10^{10} vp: GMT = 1049; 1×10^{11} vp: GMT = 1470) and peaked in Cohort 2 (504; 651); at Day 85, GMTs had declined minimally in Cohort 2. For both cohorts, binding antibody levels peaked at Day 71 with minimal decline at Day 85.
Conclusion: A single dose and homologous Ad26.COV2.S booster increased antibody responses with an acceptable safety profile in Japanese adults (ClinicalTrials.gov Identifier: NCT04509947).

1. Introduction

Despite expanded availability of vaccines to prevent coronavirus disease 2019 (COVID-19), the pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to cause serious illness [1]. In Japan, as of July 2022, >10 million confirmed COVID-19 cases and > 31,000 related deaths have been reported [2]. Five vaccines for the prevention of COVID-19 have been approved for use in Japan, including Ad26.COV2.S [3–6]. As variant strains continue to emerge [7], further development of safe and effective vaccines is critical to control COVID-19 in Japan.

Ad26.COV2.S is a recombinant, replication-incompetent adenovirus (Ad26) vector encoding a prefusion conformation-stabilized SARS-CoV-2 spike protein [8]. A single dose of Ad26.

Abbreviations: Ad26, adenovirus serotype 26; AE(s), adverse event(s); CI, confidence interval; FAS, full analysis set; COVID-19, coronavirus disease 2019; GMC(s), geometric mean concentration(s); GMT(s), geometric mean titer(s); IC_{50}, half-maximal inhibitory concentration; IC_{90}, 90% maximal inhibitory concentration; LLOQ, lower limit of quantification; NSAID(s), non-steroidal anti-inflammatory drug(s); PPI, per-protocol immunogenicity; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S-ELISA, SARS-CoV-2 pre-spike immunoglobulin G indirect enzyme-linked immunosorbent assay; ULOQ, upper limit of quantification; vp, viral particles; VNA, virus neutralization assay; wt, wild type; wtVNA, wild-type virus neutralization assay.

* Corresponding author at: Research and Development, Janssen Pharmaceutical K.K., Tokyo, Japan.
E-mail address: ytsuchi3@its.jnj.com (Y. Tsuchiya).

https://doi.org/10.1016/j.vaccine.2023.01.006
0264-410X/

COV2.S (5×10^{10} viral particles [vp]) achieved high efficacy against severe/critical COVID-19, hospitalization, and death [9–11]. A homologous 2-month booster induced high efficacy against symptomatic disease caused by circulating variants [11]. High responder rates for both humoral and cellular immune responses have been observed post–primary vaccination with a single dose of Ad26.COV2.S at the 5×10^{10} vp dose level [12]. Pursuant to emergency use and (conditional) marketing authorization, 5×10^{10} vp Ad26.COV2.S is being administered globally.

Here, we report the results of a primary analysis of a Phase 1 study in Japanese adults with or without stable underlying conditions to assess the safety, reactogenicity, and immunogenicity of Ad26.COV2.S at 2 dose levels.

2. Methods

2.1. Study design

This Phase 1 randomized, double-blind, multicenter, placebo-controlled trial (ClinicalTrials.gov Identifier: NCT04509947) was conducted at 3 centers in Japan beginning in August 2020. Data cut off for the present analysis was 22 February 2021. The trial enrolled 2 cohorts with participants randomly assigned 2:2:1 to receive an intramuscular injection of 5×10^{10} vp Ad26.COV2.S, 1×10^{11} vp Ad26.COV2.S, or placebo. Randomization was performed using a central randomization scheme generated before the trial began. The randomization was balanced using randomly permuted blocks and stratified by study site for each cohort. The primary objective was to assess the safety and reactogenicity of Ad26.COV2.S at 2 dose levels, each administered as a single-dose primary vaccination followed by a homologous booster 56 days later. Participants were Japanese adults.

All participants provided written informed consent. The trial adhered to the principles of the Declaration of Helsinki and to the Good Clinical Practice guidelines of the International Council for Harmonisation. The protocol (available in **Supplementary Materials**) and its amendments were approved by institutional review boards.

2.2. Trial participants

Cohort 1 included 125 healthy adults aged 20 to 55 years and Cohort 2 included 125 adults aged $\geq$ 65 years with or without well-controlled underlying conditions not related to an increased risk for severe COVID-19 [13]. Eligibility criteria included body mass index < 40.0 kg/m^2, normal immune system function, no prior receipt of a COVID-19 vaccine, and SARS-CoV-2 infection negative at screening. Full inclusion and exclusion criteria are detailed in **Table S1**. Randomization and vaccination of participants began following internal review of safety data 7 days after vaccination of participants enrolled in Janssen's first-in-human Ad26.COV2.S study (ClinicalTrials.gov Identifier: NCT04436276) [12].

2.3. Procedures

Participants were screened up to 28 days before vaccination. Eligible participants received vaccine (batch 20E27-04) or placebo (batch 05353DK) as an intramuscular injection into the deltoid on Days 1 and 57, with follow-up visits up to 1 year after primary vaccination. The study duration from screening until the last follow-up visit was approximately 13 months for each participant. Ad26.COV2.S and placebo were prepared as previously described [14].

Each participant was closely observed for the development of acute reactions for a minimum of 30 min after vaccination. Participants were asked to record signs and symptoms of any solicited adverse events (AEs) in a diary for 7 days after vaccination. Unsolicited AEs were reported for each vaccination until 28 days post-vaccination (Days 29 and 85 after the primary vaccination). All other serious AEs, AEs of special interest, and AEs leading to study or treatment discontinuation were reported for all participants from primary vaccination to the end of the study.

Neutralizing antibodies capable of inhibiting wild type (wt) SARS-CoV-2 infections *in vitro* were quantified using the virus neutralization assay (VNA) developed and qualified by UK Health Security Agency, Porton Down, United Kingdom [12]. The concentrations of antibodies specific for SARS-CoV-2 prefusion conformation spike protein were determined using the validated human SARS-CoV-2 pre-spike immunoglobulin G indirect enzyme-linked immunosorbent assay (S-ELISA) [12]. Collection of blood samples for immunogenicity assessments were planned on study Days 1, 15, 29, 57, 71, 85, 239, and 366.

2.4. Concomitant therapy

Use of concomitant therapies, including antipyretic or analgesic medications, non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, antihistamines, and vaccinations up to 30 days before administration of the primary vaccination were recorded at the screening visit. Use of these products was also recorded for both doses before administration on the day of vaccination until 28 days post-vaccination. Use of any other concomitant therapies was recorded if administered in conjunction with a confirmed COVID-19 case or with a new or worsening AE. The use of analgesics and NSAIDs were permitted following vaccination at the first signs of symptoms. Prophylactic use of these medications pre-vaccination was prohibited. Antipyretics were recommended by study staff post-vaccination for symptom relief as needed.

2.5. Statistical analysis

The planned total sample size was 250 participants, with 125 participants enrolled in each cohort. The number of participants chosen for this study was to provide a preliminary safety and immunogenicity assessment. Analyses for Cohorts 1 and 2 were conducted when approximately 125 participants per cohort reached Day 29, 28 days after primary vaccination, or discontinued earlier. Primary analyses for Cohorts 1 and 2 were conducted when approximately 125 participants per cohort reached Day 85, 28 days after booster vaccination, or discontinued earlier.

The full analysis set (FAS) included all participants with $\geq$ 1 documented vaccine administration. The per-protocol immunogenicity (PPI) population included all randomized and vaccinated participants for whom immunogenicity data were available, excluding participants with major protocol deviations expected to affect immunogenicity outcomes. In addition, samples obtained after missed vaccinations or from participants who became infected with SARS-CoV-2 after screening were excluded from the analysis set.

No formal statistical testing of safety and immunogenicity data was planned, and data were analyzed descriptively by vaccine group. Geometric mean and 95 % confidence interval (CI) were calculated for wild-type virus neutralization assays (wtVNA) and S-ELISA assays. The immunogenicity analyses were performed on the both the FAS and PPI populations. The ratio and correlation between neutralizing and binding antibodies as determined by wtVNA and S-ELISA, respectively, were calculated.

A baseline sample was considered positive if the wtVNA titer or S-ELISA concentration value was greater than the lower limit of quantification (LLOQ). After vaccination, a participant was considered a responder if $\geq$ 1 of the following criteria were met: 1) the baseline sample value was $\leq$ LLOQ and the post-baseline sample

was > LLOQ, or 2) the baseline sample value was > LLOQ and the post-baseline sample value was ≥ 4 times greater than the baseline sample value. Once a participant met responder criteria, the participant was considered thereafter to be a responder, regardless of the titer value.

3. Results

3.1. Participant disposition

Of the 199 participants screened in Cohort 1, 125 participants were randomized and received ≥ 1 vaccination (5×10^{10} vp group [n = 51]; 1×10^{11} vp group [n = 50]; and placebo group [n = 24]; **Fig. S1**). Of the 125 participants who received the primary vaccination, 97 (77.6 %) participants also received the booster vaccination, 28 (22.4 %) discontinued study vaccination, and 10 (8.0 %) terminated study participation prematurely. Of the 222 participants screened in Cohort 2, 125 participants were randomized and received ≥ 1 vaccination (5×10^{10} vp group [n = 50]; 1×10^{11} vp group [n = 49]; and placebo group [n = 26]; **Fig. S1**). Of the 125 participants who received the primary vaccination, 117 (93.6 %) participants also received the booster, 8 (6.4 %) discontinued study vaccination, and 2 (1.6 %) terminated study participation prematurely. The main reason for vaccine and study discontinuation in both cohorts was withdrawal by participant.

In Cohort 1, timing of the Day 57 (post–primary vaccination/pre-boost) visit ranged from 73 to 88 days (median, 78 days) due to a pause in study vaccination. Median time to Day 71 (14 days post-boost) and Day 85 (28 days post-boost) was 92 days and 106 days, respectively.

3.2. Participant demographics

Demographic and baseline characteristics, including SARS-CoV-2 seropositivity status and Ad26 VNA seropositivity status, are shown in Table 1.

The use of antipyretics or analgesics was observed more frequently in the 1×10^{11} vp group than the 5×10^{10} vp group of both cohorts. After primary vaccination in Cohort 1, 43.1 % and 74.0 % used antipyretics/analgesics in the lower- and higher-dose groups, respectively, and in Cohort 2, 8.0 % and 18.4 % used antipyretics/analgesics. After boosting, 32.6 % and 45.2 % of participants used antipyretics/analgesics in Cohort 1; in Cohort 2, 2.1 % and 4.4 % used antipyretics/analgesics. The use of antipyretics or analgesics was more frequent in Cohort 1, with 47.2 % and 28.9 % of participants reporting use of any antipyretics/analgesics ≤ 7 days after primary and booster vaccinations, respectively, compared with 11.2 % and 3.4 % in Cohort 2. Use of antipyretics or analgesics was generally less frequent after boosting than after primary vaccination in both vaccine groups in both cohorts. Paracetamol (acetaminophen) was most frequently used in the vaccine groups of both cohorts. No participants in the Cohort 1 placebo group received antipyretics or analgesics ≤ 7 days post–primary vaccination; 1 placebo recipient in Cohort 2 received paracetamol post-booster.

3.3. Safety

After primary or booster vaccination with Ad26.COV2.S, the most frequently reported solicited local AE in both cohorts was vaccination-site pain (Fig. 1), with a median duration of 2 to 4 days. In Cohort 1 (20–55 years), vaccination-site pain after primary vaccination was reported by 87 participants (vaccine, n = 85; placebo, n = 2) whereas 60 participants reported pain after boosting (vaccine, n = 60; placebo, n = 0) (Table 2). In Cohort 2 (≥ 65 years),

Table 1
Summary of participant demographics and characteristics.

Characteristic	Cohort 1 (20–55 years of age)				Cohort 2 (≥ 65 years of age)			
	5×10^{10} vp	1×10^{11} vp	Placebo	Cohort 1, all participants	5×10^{10} vp	1×10^{11} vp	Placebo	Cohort 2, all participants
N	51	50	24	125	50	49	26	125
Age in years, median (range)	42.0 (21–55)	40.0 (20–54)	43.5 (21–55)	42.0 (20–55)	70.0 (65–79)	70.0 (65–79)	70.5 (65–85)	70.0 (65–85)
Male, n (%)	23 (45.1)	17 (34.0)	14 (58.3)	54 (43.2)	28 (56.0)	23 (46.9)	11 (42.3)	62 (49.6)
Body mass index, kg/m^2, median (range)	21.8 (17.4–32.5)	20.9 (15.3–29.2)	21.6 (17.9–30.6)	21.8 (15.3–32.5)	25.1 (18.9–31.7)	22.6 (17.4–31.7)	23.1 (16.2–28.6)	23.8 (16.2–31.7)
SARS-CoV-2 seropositive at baseline,[a] n (%)	0	2 (4.0)	0	2 (1.6)	0	1 (2.0)	1 (3.8)	2 (1.6)
Ad26 VNA seropositive at baseline, n (%)	2 (3.9)	2 (4.0)	0	4 (3.2)	15 (30.0)	11 (22.4)	10 (40.0)[b]	36 (29)[c]
After primary vaccination	51	50	24	125	50	49	26	125
Any systemic use of analgesics/antipyretics,[d] n (%)	22 (43.1)	37 (74.0)	0	59 (47.2)	4 (8.0)	9 (18.4)	1 (3.8)	14 (11.2)
After booster vaccination	43	31	23	97	48	45	24	117
Any systemic use of analgesics/antipyretics,[d] n (%)	14 (32.6)	14 (45.2)	0	28 (28.9)	1 (2.1)	2 (4.4)	1 (4.2)	4 (3.4)

Ad26, adenovirus serotype 26; S-ELISA, SARS-CoV-2 pre-spike immunoglobulin G indirect enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VNA, virus neutralization assay; vp, viral particles.

[a] Baseline SARS-CoV-2 serostatus is based on the S-ELISA result, with seropositivity defined as positivity.

[b] Denominator is 25.

[c] Denominator is 124.

[d] Prophylactic use of antipyretics was not permitted, but investigators/study staff were permitted to advise participants to take antipyretics at the first sign of solicited symptoms after vaccination and document medication usage.

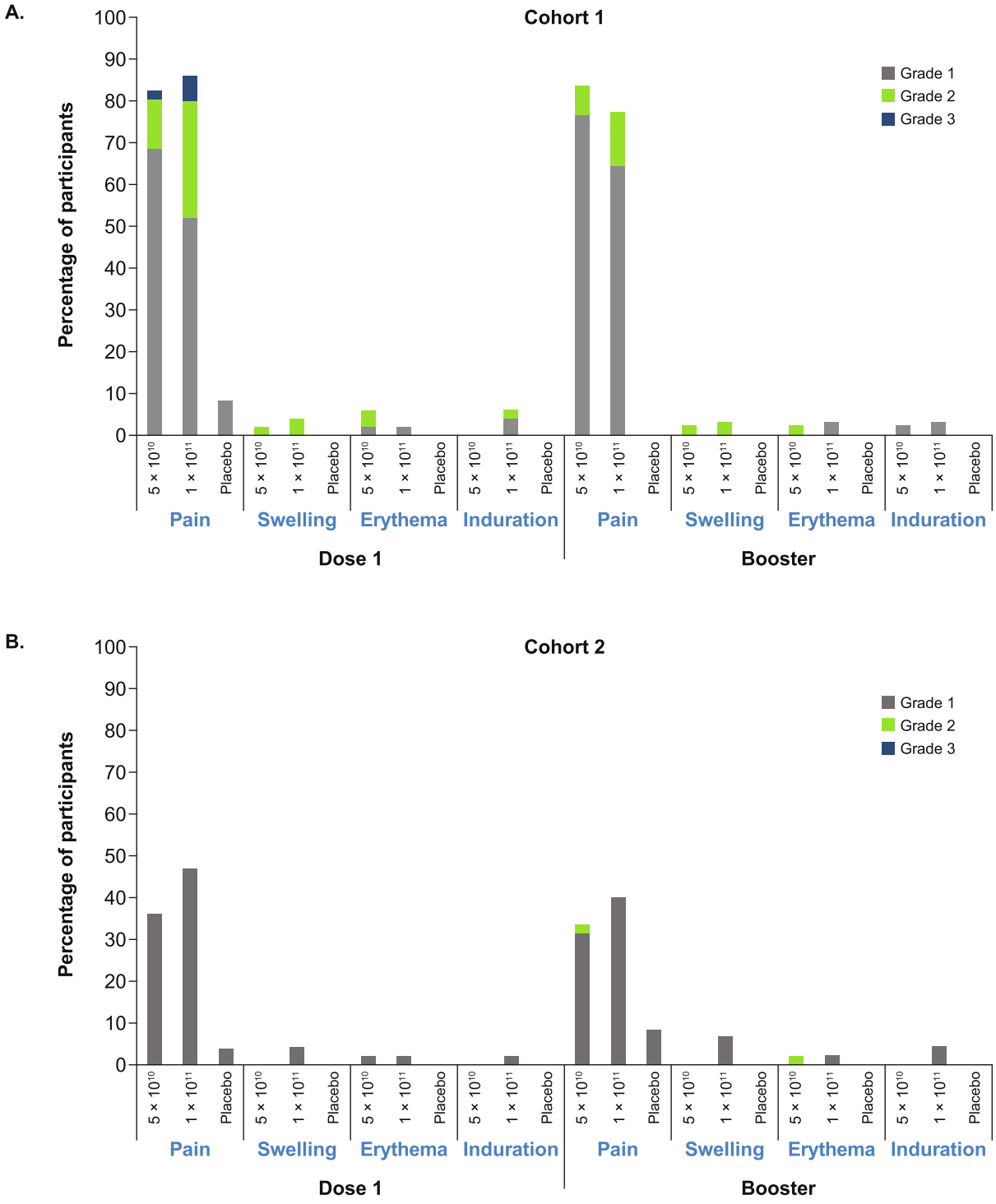

Fig. 1. Summary of solicited local AEs after primary and booster vaccination with Ad26.COV2.S or placebo in (A) Cohort 1 and (B) Cohort 2. AE, adverse event.

vaccination-site pain was reported by 42 participants after primary vaccination (vaccine, n = 41; placebo, n = 1) and by 36 participants after boosting (vaccine, n = 34; placebo, n = 2). After primary vaccination, a small proportion of vaccine recipients reported vaccination-site erythema, swelling, and induration (Table 2). These local AEs were reported by a similar number of participants in the vaccine groups following the booster dose. No placebo recipients in either cohort reported erythema, swelling, or induration after the primary or booster dose. The majority of all solicited local AEs were grade 1 or 2 and were transient in nature. Grade 3 vaccination-site pain was reported in 4 participants in Cohort 1 (1 in the 5×10^{10} vp group and 3 in the 1×10^{11} vp group). No grade 4 solicited local AEs were reported in any group in either cohort.

After primary or booster vaccination with Ad26.COV2.S in both cohorts, the most frequently reported solicited systemic AEs were fatigue, headache, and myalgia (Fig. 2; Table 2). In Cohort 1, a higher proportion of participants in the 1×10^{11} vp group experienced AEs after primary vaccination compared with the 5×10^{10} vp group. After the booster, similar proportions of participants in

Table 2
Summary of solicited local and systemic AEs.

AE, n (%)	Cohort 1 (20–55 years of age)			Cohort 2 (≥ 65 years of age)		
	5×10^{10} vp	1×10^{11} vp	Placebo	5×10^{10} vp	1×10^{11} vp	Placebo
Post–primary vaccination, N	51	50	24	50	49	26
Participants with ≥ 1 local solicited AE, n (%)	42 (82.4)	43 (86.0)	2 (8.3)	18 (36.0)	25 (51.0)	1 (3.8)
Vaccination-site erythema	3 (5.9)	1 (2.0)	0	1 (2.0)	1 (2.0)	0
Vaccination-site swelling	1 (2.0)	2 (4.0)	0	0	2 (4.1)	0
Vaccination-site induration	0	3 (6.0)	0	0	1 (2.0)	0
Vaccination-site pain	42 (82.4)	43 (86.0)	2 (8.3)	18 (36.0)	23 (46.9)	1 (3.8)
Participants with ≥ 1 systemic solicited AE, n (%)	45 (88.2)	48 (96.0)	2 (8.3)	13 (26.0)	24 (49.0)	4 (15.4)
Fatigue	37 (72.5)	44 (88.0)	1 (4.2)	11 (22.0)	17 (34.7)	4 (15.4)
Headache	27 (52.9)	37 (74.0)	0	8 (16.0)	12 (24.5)	0
Myalgia	34 (66.7)	37 (74.0)	2 (8.3)	7 (14.0)	13 (26.5)	0
Nausea	8 (15.7)	7 (14.0)	0	4 (8.0)	3 (6.1)	0
Pyrexia	13 (25.5)	37 (74.0)	0	2 (4.0)	5 (10.2)	0
Post–booster vaccination, N	43	31	23	48	45	24
Participants with ≥ 1 local solicited AE, n (%)	36 (83.7)	24 (77.4)	0	16 (33.3)	20 (44.4)	2 (8.3)
Vaccination-site erythema	1 (2.3)	1 (3.2)	0	1 (2.1)	1 (2.2)	0
Vaccination-site swelling	1 (2.3)	1 (3.2)	0	0	3 (6.7)	0
Vaccination-site induration	1 (2.3)	1 (3.2)	0	0	2 (4.4)	0
Vaccination-site pain	36 (83.7)	24 (77.4)	0	16 (33.3)	18 (40.0)	2 (8.3)
Participants with ≥ 1 systemic solicited AE, n (%)	28 (65.1)	23 (74.2)	0	13 (27.1)	13 (28.9)	5 (20.8)
Fatigue	20 (46.5)	19 (61.3)	0	5 (10.4)	8 (17.8)	3 (12.5)
Headache	14 (32.6)	10 (32.3)	0	2 (4.2)	4 (8.9)	1 (4.2)
Myalgia	20 (46.5)	14 (45.2)	0	11 (22.9)	7 (15.6)	3 (12.5)
Nausea	5 (11.6)	4 (12.9)	0	0	1 (2.2)	0
Pyrexia	3 (7.0)	10 (32.3)	0	0	0	0

AE, adverse event; vp, viral particle.

both groups of Cohort 1 reported headache, myalgia, and nausea; more participants in the higher-dose group reported fatigue and pyrexia. Solicited systemic AEs were generally less common in Cohort 2 compared with Cohort 1. In the placebo groups of both cohorts, fatigue and myalgia were generally the most common systemic AEs after each dose. Most solicited systemic AEs were grade 1 or 2 in severity. In Cohort 1, the frequency of grade 3 solicited systemic AEs was higher after primary vaccination than after boosting; there were no grade 3 events after boosting in Cohort 2. Following primary vaccination with 1×10^{11} vp, pyrexia was the most frequently reported solicited systemic AE of grade ≥ 3, with all events occurring in Cohort 1 (**Table S2**). Three of the pyrexia events in Cohort 1 were grade 4, all of which resolved in ≤ 4 days following vaccination and were considered related to the study vaccine. One grade 3 event of pyrexia occurred in Cohort 1 after the booster; no events of grade 3 pyrexia were reported in the 5×10^{10} vp group and no events of grade 4 were reported at either dose level after the booster.

The majority of unsolicited AEs were grade 1 or 2 in severity in both cohorts (**Table S3**). In Cohort 1, the most frequently reported unsolicited AE was arthralgia (3/51 [5.9 %] in the 5×10^{10} vp group; 5/50 [10.0 %] in the 1×10^{11} group). The most frequently reported unsolicited AE in Cohort 2 was administration-site pruritus (4/50 [8.0 %] in the 5×10^{10} vp group; 1/49 [2.0 %] in the 1×10^{11} vp group; and 2/26 [7.7 %] in the placebo group). For participants who received Ad26.COV2.S, the frequency of unsolicited AEs was lower in Cohort 2 than in Cohort 1. In both cohorts, the frequency of unsolicited AEs was generally higher after primary vaccination than after boosting. After primary vaccination in the 1×10^{11} vp group, 3 unsolicited AEs of grade ≥ 3 were reported (Cohort 1, n = 2; Cohort 2, n = 1). In the 5×10^{10} vp group of Cohort 2, there was one grade 3 unsolicited AE reported after boosting. Cohort 1 participants in the 1×10^{11} vp group reported 2 grade 3 unsolicited AEs considered related to vaccination, both of which occurred post–primary vaccination (1 AE each of arthralgia and myalgia). No grade ≥ 3 unsolicited AEs related to vaccination were reported in either Cohort 1 or Cohort 2 post-boost with either dose. No AEs of special interest were reported in either cohort.

3.4. Immunogenicity

By Day 15 after a single dose of Ad26.COV2.S (14 days post–primary vaccination), neutralizing antibody titers against wt SARS-CoV-2 were detected in ≥ 90 % of participants irrespective of vaccine dose or age (Fig. 3). In Cohort 1 (20–55 years), geometric mean titers (GMTs) were 277 (95 % CI, 225–342) in the 5×10^{10} vp group and 375 (95 % CI, 277–509) in the 1×10^{11} vp group. In Cohort 2 (≥ 65 years), GMTs were 152 (95 % CI, 120–193) in the 5×10^{10} vp group and 204 (95 % CI, 156–267) in the 1×10^{11} vp group.

By Day 57 (56 days post–primary vaccination/pre-boost), responder rates increased to 98 % to 100 % in both cohorts. In Cohort 1, GMTs increased to 456 (95 % CI, 373–559) in the 5×10^{10} vp group and to 699 (95 % CI, 533–916) in the 1×10^{11} vp group. In Cohort 2, GMTs were 281 (95 % CI, 204–386) and 470 (95 % CI, 354–624) in the 5×10^{10} vp group and the 1×10^{11} vp group, respectively.

After boosting in both cohorts, GMTs increased and had almost reached peak value by 14 days post-boost (Day 71), with responder rates reaching 100 %. In Cohort 1, GMTs remained stable from Day 71 (5×10^{10} vp: GMT = 1049; 95 % CI, 828–1329; 1×10^{11} vp: GMT = 1470; 95 % CI, 1156–1870) to Day 85 (5×10^{10} vp: GMT = 1088; 95 % CI, 817–1449; 1×10^{11} vp: GMT = 1671; 95 % CI, 1155–2418). Among the older adults in Cohort 2, there was a trend towards lower GMTs at Day 85 (5×10^{10} vp: GMT = 429; 95 % CI, 335–550; 1×10^{11} vp: GMT = 478; 95 % CI, 374–610) compared with Day 71 (5×10^{10} vp: GMT = 504; 95 % CI, 404–627; 1×10^{11} vp: GMT = 651; 95 % CI, 501–847).

By Day 57 in both cohorts, responder rates of binding antibodies to SARS-CoV-2 S protein reached 100 %. The binding antibody geometric mean concentrations (GMCs) peaked at Day 71 with a minimal decline at Day 85 (**Fig. S2**). At Day 71, GMCs in Cohort 1 were

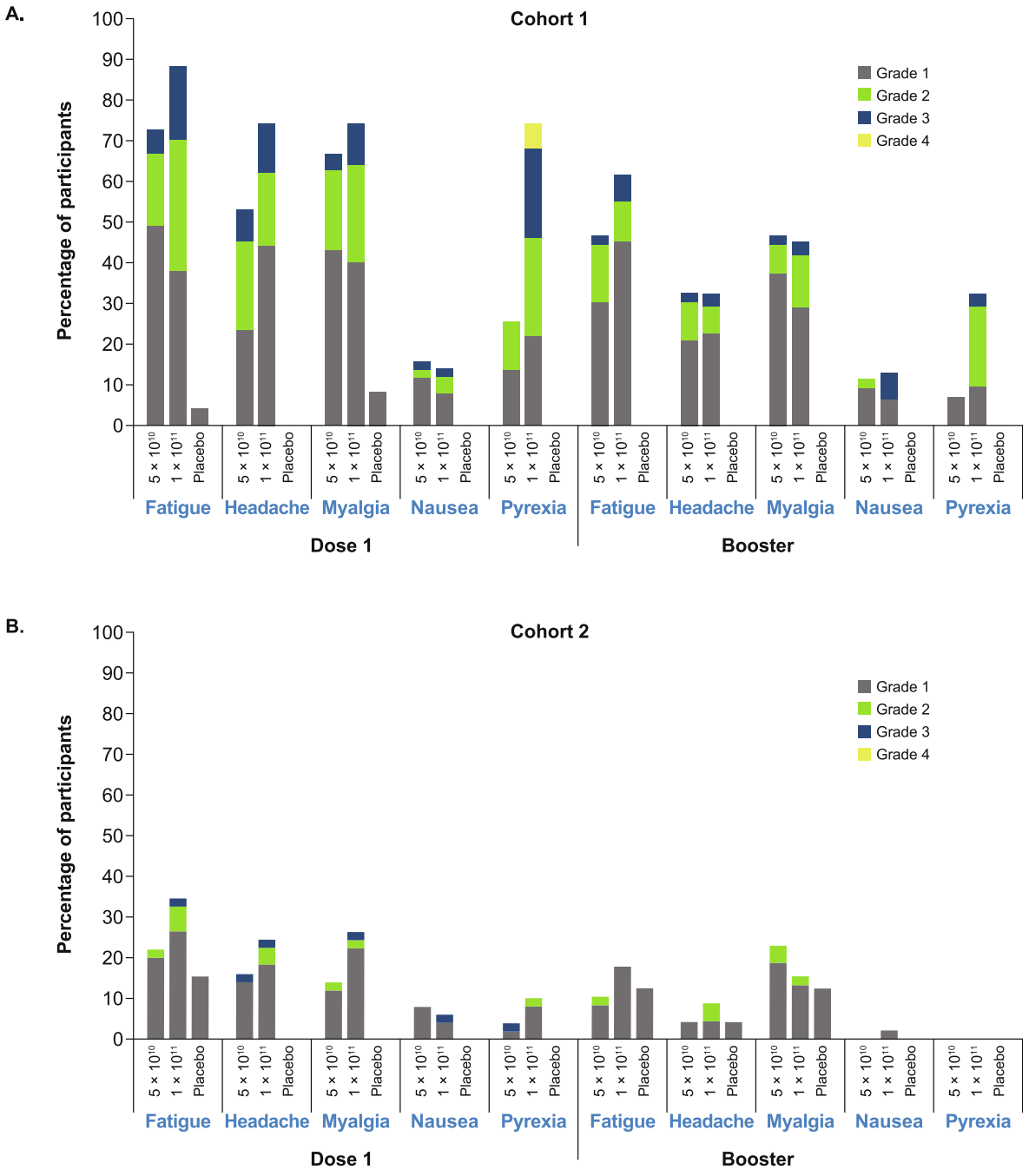

Fig. 2. Summary of solicited systemic AEs after primary and booster vaccination with Ad26.COV2.S or placebo in (A) Cohort 1 and (B) Cohort 2. AE, adverse event.

2488 EU/mL (95 % CI, 1923–3219) and 3435 EU/mL (95 % CI, 2744–4300) in the 5×10^{10} vp group and 1×10^{11} vp group, respectively. By Day 85, GMCs in Cohort 1 were 2097 EU/mL (95 % CI, 1633–2692) and 3033 EU/mL (95 % CI, 2493–3689). In Cohort 2, Day 71 GMCs were 1030 EU/mL (95 % CI, 783–1354) and 1471 EU/mL (95 % CI, 1112–1945) in the 5×10^{10} vp group and 1×10^{11} vp group, respectively. Day 85 GMCs in Cohort 2 were 885 EU/mL (95 % CI, 673–1162) and 1242 EU/mL (95 % CI, 945–1633) in the 5×10^{10} vp group and 1×10^{11} vp group, respectively.

From Day 15 through Day 85, neutralizing antibody titers correlated with binding antibody concentrations irrespective of age. In Cohort 1, Spearman's correlation coefficients were 0.705 and 0.769 at Days 57 and 85, respectively (Fig. 4**A**). In Cohort 2, Spearman's correlation coefficients were 0.708 and 0.847 at Days 57 and 85, respectively (Fig. 4**B**).

Baseline positivity for Ad26 VNA was detected in 4.0 % (4/101) of vaccine recipients in Cohort 1 and 26.3 % (26/99) of vaccine recipients in Cohort 2. Up to 14 days post-boost in Cohort 2, neutralizing antibody responses against wt SARS-CoV-2 were similar in Ad26.COV2.S recipients who were Ad26 VNA positive at baseline versus those who were Ad26 VNA negative at baseline. By 28 days post-boost in Cohort 2, although the 95 % CI of the GMTs largely overlapped, neutralizing antibody responses against wt SARS-CoV-2 tended to be lower in participants who were Ad26 VNA positive at baseline versus those who were Ad26 VNA negative at baseline (**Fig. S3**). In both cohorts, correlation was low between

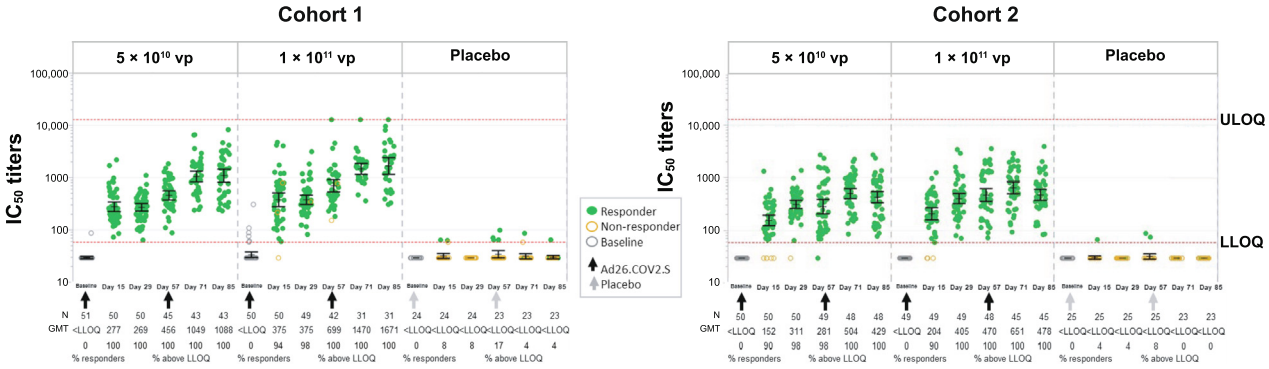

Fig. 3. wtVNA titers after Ad26.COV2.S primary and booster vaccinations in both Cohort 1 (20–55 years old) and Cohort 2 (≥65 years old). Vaccinations were administered at Day 1 and Day 57 (56 days post–primary vaccination) with wtVNA titers analyzed 14 and 28 days following both primary and booster vaccinations. GMT, geometric mean titer; IC_{50}, half-maximal inhibitory concentration; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification; vp, viral particles; wtVNA, wild-type virus neutralization assay.

baseline Ad26 titers and immune response to Ad26.COV2.S measured by wtVNA titers against SARS-CoV-2 through Day 85 (Fig. S4). Overall, no impact of pre-existing Ad26 humoral immunity on Ad26.COV2.S humoral immunogenicity was observed.

4. Discussion

This Phase 1 trial conducted in Japanese adults demonstrated that a single dose of Ad26.COV2.S followed by a booster 56 days later had an acceptable safety and reactogenicity profile at the 5×10^{10} vp dose level. Higher reactogenicity was observed with the higher dose (1×10^{11} vp) than the lower dose level, supporting use of Ad26.COV2.S at the 5×10^{10} vp dose level under emergency use authorization and (conditional) marketing approval. Overall, in healthy adults aged 20–55 years and adults aged ≥ 65 years without underlying conditions or with well-controlled underlying conditions, the homologous booster was generally well tolerated. No safety signals were identified in this study.

At both dose levels, reactogenicity was less frequent in older adults. Reactogenicity was also lower following the booster than primary vaccination. Generally, higher frequencies of solicited local AEs, solicited systemic AEs, and unsolicited AEs were observed at the higher dose level compared with the lower dose level in both cohorts. Overall, the 5×10^{10} vp dose level safety data evaluated in this study align with safety data reported in a phase 1/2a study [12] and two global phase 3 studies of Ad26.COV2.S [9,11], although direct comparisons cannot be made between the studies owing to differences in study design. These other studies reported a similar frequency of solicited AEs to those observed in our study [9,11,12], demonstrated a lower reactogenicity profile for older adults compared with younger adults [10,11], and showed lower reactogenicity after a booster dose compared with primary vaccination [11].

Pyrexia (systemic solicited AE) was reported most frequently in the higher-dose group in younger adults after primary vaccination (74.0 %, 37/50; 14 events were grade ≥ 3). In the lower-dose group of younger adults, pyrexia occurred in 25.5 % (13/51) of participants after primary vaccination and 7.0 % (3/51) after boosting; no grade ≥ 3 pyrexia events were reported in the lower-dose group. The median duration of pyrexia was 1 day in both dose groups. Antipyretics and analgesics were used more by the higher-dose group than the lower-dose group.

In this study, a single dose of Ad26.COV2.S elicited robust humoral responses in a large majority of vaccine recipients, with neutralizing antibodies present in > 90 % of participants by Day 15 post–primary vaccination, irrespective of age group or vaccine dose level. Neutralizing and binding antibody levels and responder rates increased up to Day 57 (56 days post–primary vaccination/pre-boost), with a trend for higher antibody levels in those aged 20 to 55 versus ≥ 65 years. Increased humoral immunogenicity was observed after the booster in both cohorts, albeit with different kinetics. The younger adults had almost reached peak immunogenicity 14 days after boosting and remained stable up to 28 days after boosting. These results are consistent with observations in ongoing Phase 1/2a (ClinicalTrials.gov Identifier: NCT04436276 [COV1001]) and Phase 2 (ClinicalTrials.gov Identifier: NCT04535453 [COV2001]) studies [12,15], and suggest that a single dose and a homologous booster of Ad26.COV2.S further increased SARS-CoV-2–specific antibody responses.

The booster elicited a modest increase in neutralizing antibody titers for older adults compared with younger adults. The older adults reached peak immunogenicity at 14 days post-boost with a trend towards decline by 28 days post-boost. Detectable baseline levels of Ad26 titers, indicative of previous Ad26 exposure, were observed in 36 of 124 older adults. Although no critical significant impact of pre-existing Ad26 humoral immunity on Ad26.COV2.S humoral immunogenicity was observed based on pre-existing Ad26 neutralizing antibodies at baseline in older adults, age, potential comorbidities, vaccination interval and/or pre-existing Ad26 immunity could play a role in both the lower responses observed and a trend for faster waning in post-boost neutralizing titers observed in older adults versus younger adults. The small number of participants with pre-existing Ad26 immunity in Cohort 1 (4/125) precluded any meaningful conclusions regarding immunogenicity of Ad26.COV2.S in younger adults.

Results of the humoral immune response correlation analysis (wtVNA vs S-ELISA) indicated that the high correlation observed between the 2 assays was independent of time, and that S-ELISA can be considered for future use as a surrogate for wtVNA.

In conclusion, we have demonstrated that a single dose of Ad26.COV2.S in Japanese participants induced humoral immune responses and had an acceptable safety and reactogenicity profile at the 5×10^{10} vp dose level, and that a booster dose increased immunogenicity while maintaining an acceptable safety profile. The results of this study indicated that the protective effect observed after primary vaccination with Ad26.COV2.S in the large Phase 3 ENSEMBLE trial (COV3001, NCT04505722) [9,10] and after an Ad26.COV2.S booster in the Phase 3 ENSEMBLE2 trial (COV3009, NCT04614948) [16] can also be expected in the Japanese population. These findings support the continued development of Ad26.COV2.S for the prevention of COVID-19.

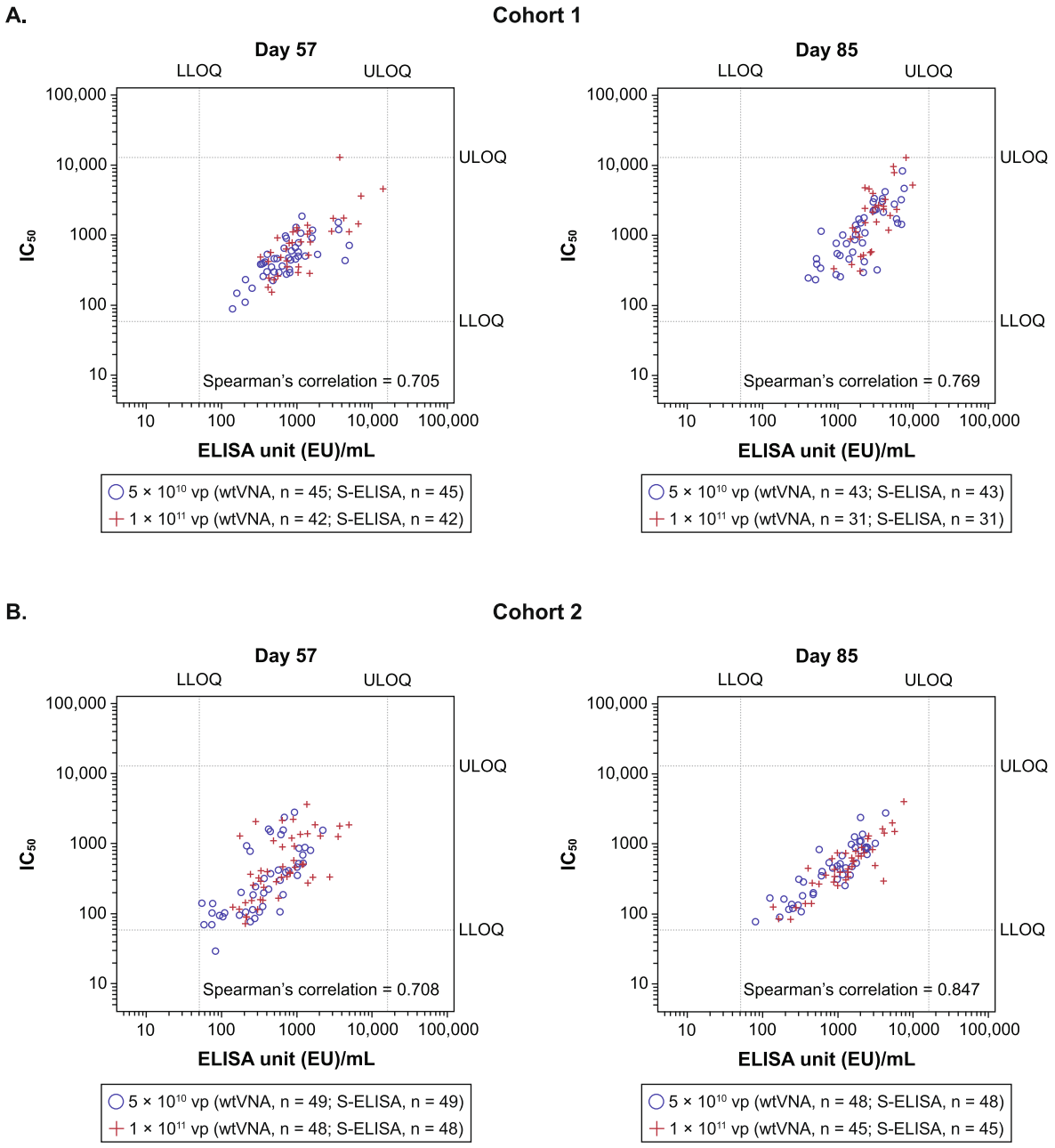

Fig. 4. Correlation analysis of SARS-CoV-2 neutralizing antibody titers (wtVNA) versus S protein binding antibody titers (S-ELISA) in (A) Cohort 1 and (B) Cohort 2. IC_{50}, half-maximal inhibitory concentration; LLOQ, lower limit of quantification; S-ELISA, SARS-CoV-2 pre-spike immunoglobulin G indirect enzyme-linked immunosorbent assay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; ULOQ, upper limit of quantification; vp, viral particles; wtVNA, wild-type virus neutralization assay.

Funding

This work was supported by Janssen Pharmaceutical K.K. and Janssen Research and Development, affiliates of Janssen Vaccines and Prevention and part of the Janssen pharmaceutical companies of Johnson & Johnson.

Prior Presentation

Data reported in this manuscript were previously presented at the 25th Annual Meeting of the Japanese Society for Vaccinology; 3-5 December 2021; Nagano, Japan.

Data Availability

The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency. As noted on this site, requests for access to the study data can be submitted through Yale Open Data Access (YODA) Project site at http://yoda.yale.edu.

Declaration of Competing Interest

YT is an employee of Janssen Pharmaceutical K.K. KF, HN, KT, and HT are employees of Janssen Pharmaceutical K.K. and are share-

holders in Johnson & Johnson. TL is an employee of Janssen Pharmaceuticals, China. MLG and VC are employees of Janssen Pharmaceuticals and shareholders in Johnson & Johnson. TE has no conflicts of interest to disclose.

Acknowledgments

We thank all the participants in this clinical trial, the staff members and all the investigators at the clinical sites (Tatsuya Yoshihara, MD, PhD, and Miwa Haranaka, MD); the members of data review committee; the COV1002 study team (Miki Kusano, Yuichi Nagata, Ryohei Iizuka, Yoko Ono, Hiroko Shimizu, Ryosuke Goto, and Maaike Ligthart); the members of the neutralization assay testing team of the UK Health Security Agency; and the Nexelis team for providing laboratory testing services. Medical writing and editorial assistance were provided by Sadie van Dyne, PhD, and Jill E. Kolesar, PhD, of Lumanity Communications Inc., and were funded by Janssen Global Services, LLC.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2023.01.006.

References

[1] Rosenberg ES, Holtgrave DR, Dorabawila V, Conroy MB, Greene D, Lutterloh E, et al. New COVID-19 cases and hospitalizations among adults, by vaccination status — New York, 3 May-25 July 2021. MMWR Morb Mortal Wkly Rep 2021;70:1150–5. https://doi.org/10.15585/mmwr.mm7034e1.

[2] World Health Organization. WHO coronavirus (COVID-19) dashboard, https://covid19.who.int/; [accessed 1 June 2022].

[3] McGill COVID19 Vaccine Tracker Team. COVID19 vaccine tracker Japan, https://covid19.trackvaccines.org/country/japan/; 2021 [accessed 3 December 2021].

[4] Pharmaceuticals and Medical Devices Agency. Report on the deliberation results vaxzevria intramuscular injection, https://www.pmda.go.jp/files/000243208.pdf; 2021 [accessed 5 April 2022].

[5] Pharmaceuticals and Medical Devices Agency. Report on the deliberation results COVID-19 vaccine Moderna intramuscular injection, https://www.pmda.go.jp/files/000245381.pdf; 2021 [accessed 5 April 2022].

[6] Pharmaceuticals and Medical Devices Agency. Report on the deliberation results comirnaty intramuscular injection, https://www.pmda.go.jp/files/000245075.pdf; 2021 [accessed 5 April 2022].

[7] Latif AA, Mullen JL, Alkuzweny M, Tsueng G, Cano M, Haag E, et al. Japan variant report, https://outbreak.info/location-reports?loc=JPN&dark=true&selected=Delta&selected=Alpha&selected=Beta&selected=Gamma; [accessed 23 February 2022].

[8] Bos R, Rutten L, van der Lubbe JEM, Bakkers MJG, Hardenberg G, Wegmann F, et al. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 spike immunogen induces potent humoral and cellular immune responses. npj Vaccines 2020;5:91. https://doi.org/10.1038/s41541-020-00243-x.

[9] Sadoff J, Gray G, Vandebosch A, Cardenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against Covid-19. N Engl J Med 2021;384:2187-201. https://doi.org10.1056/NEJMoa2101544.

[10] Sadoff J, Gray G, Vandebosch A, Cardenas V, Shukarev G, Grinsztejn B, et al. Final analysis of efficacy and safety of single-dose Ad26.COV2.S. N Engl J Med 2022;386:847-60. https://doi.org10.1056/NEJMoa2117608.

[11] Hardt K, Vandebosch A, Sadoff J, Le Gars M, Truyers C, Lowson D, et al. Efficacy, safety, and immunogenicity of a booster regimen of Ad26.COV2.S vaccine against COVID-19 (ENSEMBLE2): results of a randomised, double-blind, placebo-controlled, phase 3 trial. Lancet Infect Dis 2022. https://doi.org/10.1016/S1473-3099(22)00506-0.

[12] Sadoff J, Le Gars M, Shukarev G, Heerwegh D, Truyers C, de Groot AM, et al. Interim results of a phase 1-2a trial of Ad26.COV2.S COVID-19 vaccine. N Engl J Med 2021;384:1824-35. https://doi.org10.1056/NEJMoa2034201.

[13] Centers for Disease Control and Prevention (CDC). COVID-19. People with certain medical conditions. Updated 14 December 2021, https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html; 2021 [accessed 27 December 2021].

[14] Stephenson KE, Le Gars M, Sadoff J, de Groot AM, Heerwegh D, Truyers C, et al. Immunogenicity of the Ad26.COV2.S vaccine for COVID-19. JAMA 2021;325:1535-44. https://doi.org10.1001/jama.2021.3645.

[15] Sadoff J, Le Gars M, Brandenburg B, Cardenas V, Shukarev G, Vaissiere N, et al. Durable antibody responses elicited by 1 dose of Ad26.COV2.S and substantial increase after boosting: 2 randomized clinical trials. Vaccine 2022;40:4403–11. https://doi.org/10.1016/j.vaccine.2022.05.047.

[16] Janssen. COVID-19 vaccine Ad26.COV2.S VAC31518 (JNJ-78436735). Janssen's briefing materials: vaccines and related biological products advisory committee. https://www.fda.gov/media/152954/download; 2021. [accessed 5 August 2022].