



Safety and immunogenicity of Ad26.COV2.S in adults: A randomised, double-blind, placebo-controlled Phase 2a dose-finding study

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ARTICLE INFO

Keywords:

Ad26.COV2.S

COVID-19

Immunogenicity

Safety

SARS-CoV-2

Vaccine

ABSTRACT

Background: A single dose of Ad26.COV2.S is well-tolerated and effective in preventing moderate-to-severe disease outcomes due to COVID-19. We evaluated the impact of dose level, number of doses, and dose interval on immunogenicity, reactogenicity, and safety of Ad26.COV2.S in adults. Anamnestic responses were also explored.

Methods: This randomised, double-blind, placebo-controlled, Phase 2a study was conducted in adults aged 18–55 years and ≥ 65 years (NCT04535453). Four dose levels (1.25×10^{10} , 2.5×10^{10} , 5×10^{10} , and 1×10^{11} viral particles [vp], single and 2-dose schedules, and dose intervals of 56 and 84 days, were assessed. Four or 6 months post-primary vaccination, Ad26.COV2.S 1.25×10^{10} vp was given to evaluate anamnestic responses. Humoral and cell-mediated immune responses were measured. Reactogenicity and safety were assessed in all participants.

Results: All Ad26.COV2.S schedules induced humoral responses with evidence of a dose response relationship. A single dose of Ad26.COV2.S (5×10^{10} vp) induced antibody and cellular immune responses that persisted for up to at least 6 months. In the 2-dose regimens, antibody responses were higher than 1-dose regimens at comparable dose levels, and the magnitude of the immune response increased when the interval between doses was increased (84 days vs 56 days). Rapid, marked immune responses were observed in all groups after vaccine antigen exposure indicating immune memory. Durable immune responses were observed in all groups for up to at least 6 months post-antigen exposure. Strong and consistent correlations between neutralising and binding antibodies were observed. CD4⁺ and CD8⁺ T cell responses were similar after all regimens. Reactogenicity within 7 days post-vaccination tended to be dose-related.

Conclusion: The study supports the primary, single dose schedule with Ad26.COV2.S at 5×10^{10} vp and homologous booster vaccination after a 6 month interval. Rapid and marked responses to vaccine antigen exposure indicate induction of immune memory by 1- and 2-dose primary vaccination.

Abbreviations: AE, adverse event; AESI, adverse event of special interest; CMI, cell mediated immunity; COVID-19, coronavirus disease-2019; GMC, geometric mean concentration; GMI, geometric mean increase; GMT, geometric mean titre; LLOQ, lower limit of quantification; S, Spike; SAE, serious adverse event; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S-bAbs, Spike- binding antibodies; TTS, thrombosis with thrombocytopenia syndrome; VNA, virus neutralising antibodies.

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<https://doi.org/10.1016/j.vaccine.2024.04.059>

Received 19 January 2024; Received in revised form 19 April 2024; Accepted 19 April 2024

Available online 4 May 2024

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1. Introduction

Coronavirus disease-2019 (COVID 19) vaccines were instrumental in saving millions of lives during the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic and continue to play a role in long term disease control [1]. Yet, in April 2024 around 30 % of the global population remains unvaccinated against COVID-19 and further development of safe and effective vaccines should continue [2]. Ad26.COV2.S is a monovalent vaccine composed of a recombinant, replication-incompetent human adenovirus type 26 vector, constructed to encode the Wuhan SARS-CoV-2 spike (S) protein stabilised in its prefusion conformation [3]. A single dose of Ad26.COV2.S protects against COVID-19 and is effective in preventing moderate-to-severe COVID-19 and associated hospitalisations and deaths [4–6].

Many of the clinical trials that commenced early on during the pandemic are only now reaching completion. In August 2020, we initiated a Phase 2a study designed to answer questions about dose levels, number of doses, and dose intervals to optimise immune responses to the Ad26.COV2.S vaccine. The study also evaluated reactogenicity and safety of different vaccine regimens and assessed anamnestic responses to vaccine antigen exposure 4 or 6 months after the last primary vaccination dose. Ad26.COV2.S was granted emergency use authorisation by the US Food and Drug Administration on 27 February 2021, conditional marketing authorisation by the European Commission on 11 March 2021, and authorisation or conditional approval in more than 120 countries and territories worldwide. The final approved formulation of Ad26.COV2.S contains 5×10^{10} virus particles (vp) administered as a single priming dose to individuals aged 18 years and older [7]. More than 50 million doses of Ad26.COV2.S were administered worldwide by April 2022 [8].

Adenovirus vectors continue to be investigated as platforms for prophylactic vaccines, particularly because of their adaptability, and their ability to induce strong and durable cellular immune responses which are important for clearing virus and developing memory [9–11]. The present study provides valuable data describing the impact of dose level, dose number, and dose interval on the safety and immunogenicity of Ad26.COV2.S. Anamnestic responses and correlations between post-vaccination levels of virus neutralising antibodies (VNAs) and Spike-binding antibodies (S-bAbs) were also explored. These data contribute to our overall understanding of the immunogenicity and safety of adenovirus-vector vaccines.

2. Materials, Methods and patients

2.1. Study design and participants

This was a randomised, double-blind, placebo-controlled, multicentre, Phase 2a study conducted in healthy adults aged 18–55 years and aged ≥ 65 years who were in good or stable health (NCT04535453). Adult participants were enrolled at 9 sites in Germany, Spain, and the Netherlands. A group of 12–17-year-old adolescents was enrolled in this same study; those results have been reported previously and are not included here (manuscript in preparation).

Eligible adults were healthy or medically stable individuals who had not received a prior COVID-19 vaccine. Individuals were excluded if they had a history of polymerase chain reaction (PCR)-confirmed SARS-CoV-2 infection, severe acute respiratory syndrome or Middle East respiratory syndrome; if they had comorbidities associated with an increased risk of progression to severe COVID-19; or if they were working in an occupation with a high risk of exposure to SARS-CoV-2 (eg, health care workers or emergency response personnel). Pregnant and breast-feeding women were also excluded, and women of child-bearing potential were required to practice contraception until 3 months after last vaccination.

Randomisation was performed centrally using a computer-generated randomisation schedule. Randomisation was balanced using randomly

permuted blocks and was stratified by study site and age group (approximately two-thirds of participants 18–55 years and one-third ≥ 65 years of age, per vaccine group). The appearance of Ad26.COV2.S vaccine was slightly different from placebo. Blinding was guaranteed by masking of syringes after preparation by an unblinded pharmacist/qualified study-site personnel.

Four dose levels of Ad26.COV2.S were investigated: 1×10^{11} vp, 5×10^{10} vp, 2.5×10^{10} vp, and 1.25×10^{10} vp. Participants were randomised to one of 10 study groups to receive 1 or 2 doses of Ad26.COV2.S or placebo (isotonic saline solution) at 28-day, 56-day or 84-day intervals (Table 1). All vaccinations were of 0.5 ml volume and administered intramuscularly into the deltoid. A single injection with 1.25×10^{10} vp Ad26.COV2.S (Groups 1–5, 7, 9) or placebo (Groups 6, 8, 10) to mimic virus exposure was administered 4 months after the second vaccination (i.e., 4 months after the second active vaccination in the 2-dose regimens and 6 months after active vaccination in the 1-dose regimens [Groups 4 and 5]).

COVID-19 vaccines were authorised for emergency use during the course of the study, and unblinding of participants and investigators was permitted to offer participants who initially received placebo a single dose of Ad26.COV2.S.

Study oversight was provided by an independent data monitoring committee. The study adhered to the principles of the Declaration of Helsinki and to the Good Clinical Practice guidelines and the protocol was approved by all applicable institutional ethics review boards. All participants provided written informed consent prior to enrolment.

2.2. Objectives

The primary study objectives were 1) to assess the humoral immune responses 28 days after two doses of Ad26.COV2.S administered at one of three dose levels (1.25×10^{10} vp, 2.5×10^{10} vp, 5×10^{10} vp) with a 56-day interval between doses; 2) to assess the humoral immune responses 28 days after a single dose of Ad26.COV2.S containing either 1×10^{11} vp or 5×10^{10} vp; 3) to assess the humoral immune responses 28 days after two doses of Ad26.COV2.S 5×10^{10} vp given either 28-days or 84-days apart; 4) to assess the safety and reactogenicity of Ad26.COV2.S in the administered regimens.

Secondary objectives were to assess the anamnestic response 7 days after vaccine antigen exposure, and to assess safety and reactogenicity of the antigen exposure dose. Exploratory objectives included assessment of cell-mediated immune (CMI) responses and assessment of the correlation between VNA titres and S-bAbs in a subset of participants at selected timepoints.

2.3. Study interventions and procedures

Blood samples were collected prior to, 14 and 28 days after each vaccination; prior to, 7 and 28 days after vaccine antigen exposure; and at 12 months post-vaccination. Wuhan SARS-CoV-2 S-specific antibody binding and neutralising activity were measured respectively by a Spike-specific enzyme-linked immunosorbent assay (S-ELISA) in all participants, and a wild-type virus-neutralisation assay in a randomly selected subset of participants as described previously [12]. VNAs were reported as 50 % inhibitory concentration (IC50) SARS-CoV-2 infection data (PCR test, N-ELISA) were collected up to the end of study. PCR tests were performed on nasal swabs collected from participants with COVID 19-like symptoms. N-ELISA was performed at regular intervals in all study groups.

T cell responses were assessed by intracellular cytokine staining after SARS-CoV-2 S peptide stimulation of peripheral blood mononuclear cells [12] at baseline, 28 days after each vaccination (Days 29 and 85), 28 days post vaccine antigen exposure (Day 197), and 6 months post-vaccine antigen exposure (Day 393) in a subset of 21 participants in Groups 1 to 5 and 7 participants in Group 6 (Table 1). A Th1 response was determined as the percentage of samples positive for CD4 + T cells

expressing IFN γ and/or IL-2. A Th2 response was determined as the percentage of CD4 + T cells expressing IL-4 and/or IL-5/IL-13 and CD40L. Detection of CD8 + T cell responses was characterised by the percentage of CD8 + T cells producing IFN γ and/or IL-2.

Solicited adverse events (AEs) within 7 days after vaccination were recorded in a diary and all other unsolicited AEs were recorded for 28 days after each vaccination. Serious adverse events (SAEs), AEs leading to study discontinuation were recorded for the entire study duration. While the study was ongoing, thrombosis with thrombocytopenia syndrome (TTS) was recognised as a rare AE following adenovirus-vectored COVID-19 vaccine administration in a different study. A series of protocol amendments was enacted to capture TTS as an AEs of special interest (AESI), and to include measurement of D-dimer, lupus anticoagulant, anti- β 2 glycoprotein and anti-cardiolipin in all participants. AESI were captured until study end.

2.4. Statistical analysis

The Full Analysis Set included all participants with at least one vaccine administration documented and the Per Protocol Immunogenicity population included all randomised and vaccinated participants for whom immunogenicity data were available. Participants with SARS CoV 2 infection defined as a positive PCR test or positive N-serology were excluded from the Per Protocol Immunogenicity analyses.

A study pause occurred in October 2020 as part of the voluntary pause of all Ad26.COVS.S vaccine studies during evaluation of an SAE in another study that met a study pausing rule [5]. As a result, the majority of participants in Groups 7 and 8 missed their visit window for Vaccination 2 at Day 28, rendering it infeasible to evaluate the intended 28-day vaccination interval. As their actual vaccination timing coincided with the scheduled intervals in Groups 1 and 6, reactogenicity and safety data were pooled for Ad26.COVS.S Groups 1 and 7, and placebo Groups 6 and 8 (Table 1). Immunogenicity data for Group 7 are not presented.

Descriptive statistics (geometric mean and confidence intervals [CIs], or median and interquartile range Q1-Q3, as appropriate) were calculated for continuous immunologic parameters. Responders were defined as participants with post-vaccination VNAs or S-bAbs greater than the lower limit of quantitation (LLOQ) in initially seronegative participants, or with a 4-fold increase in titre/concentration compared to baseline in participants who were initially seropositive. Correlations between VNAs and S-bAbs were calculated with CIs. Safety endpoints were analysed descriptively by vaccine group.

For the calculation of the geometric mean and its corresponding 95 % CI, the arithmetic mean and its corresponding 95 % CI were calculated on the log10 transformed values. These values were back transformed to provide the geometric mean and its corresponding 95 % CI. For a binomial proportion (such as the number of responders) the exact

Clopper Pearson method was used.

3. Results

3.1. Study participants

This study in adults was conducted between 31 August 2020 and 8 March 2022. Initial vaccination occurred from 14 Sept 2020 to 22 Sept 2020. Of 946 participants screened, 582 were randomised and vaccinated, and 515 (88.5 %) completed the study vaccinations (Fig. 1). The most common reasons for discontinuing vaccination were 34 Other (5.8 %, most commonly unblinding due to participant request to be informed of their COVID-19 vaccination status [$n = 22$]), withdrawal by participant (12, 2.1 %) and SARS-COV-2 infection (9, 1.5 %). Three participants discontinued vaccination due to AEs (lung adenocarcinoma [SAE], acute myeloid leukaemia [SAE], and paraesthesia [Grade 1 AE]). One participant discontinued the study due to death due to unknown causes. Only the case of paraesthesia was considered related to vaccination by the investigator.

Protocol deviations were recorded for 245 (42.1 %) participants across all study groups. Most protocol deviations (173, 29.7 %) were due to receipt of a disallowed concomitant treatment (usually another COVID-19 vaccine) during the study. A further 100 (17.2 %) protocol deviations were due to failure to comply with protocol-defined procedures; 28 (4.8 %) were due to COVID-19 illness; 5 (0.9 %) participants were enrolled into the study but did not meet enrolment criteria; and 1 participant (0.2 %) received the wrong vaccine.

Demographics characteristics were generally well balanced between the vaccination groups (Table 2).

3.2. Humoral immunogenicity

The number of participants included in the per protocol immunogenicity analysis in each group and in each age-group is provided in Table S1. There was no detectable immune response in the placebo groups. Results for placebo Group 6 are provided in Tables S2 and Table S3 as illustrative; immunogenicity results for placebo recipients are not discussed further. All participants were seronegative for S-bAbs at baseline, and all but two participants were also seronegative for VNAs. Geometric mean titres/concentrations (GMTs/GMCs) at each blood sampling timepoint for all Ad26.COVS.S groups are shown in Fig. 2.

3.2.1. Immunogenicity by dose level

3.2.1.1. Single dose regimen. The impact of dose level on the immunogenicity of a single dose of Ad26.COVS.S was assessed in Groups 1 to 5,

Table 1
Planned sample size and vaccination schedule in adults 18 to ≤ 55 years and ≥ 65 years.

Group	N	Day 1 (Vaccination 1)	Day 29 (Vaccination 2)	Day 57 (Vaccination 2)	Day 85 (Vaccination 2)	4 months post-vaccination 2 (Injection 3) ¹
1	75	5×10^{10} vp		5×10^{10} vp		1.25×10^{10} vp
2	75	2.5×10^{10} vp		2.5×10^{10} vp		1.25×10^{10} vp
3	75	1.25×10^{10} vp		1.25×10^{10} vp		1.25×10^{10} vp
4	75	1×10^{11} vp		Placebo		1.25×10^{10} vp ²
5	75	5×10^{10} vp		Placebo		1.25×10^{10} vp ²
6	25	Placebo		Placebo		Placebo
7	50	5×10^{10} vp	5×10^{10} vp	Approximate actual administration ³		1.25×10^{10} vp
8	25	Placebo	Placebo			Placebo
9	50	5×10^{10} vp			5×10^{10} vp	1.25×10^{10} vp
10	25	Placebo			Placebo	Placebo

N, number of participants; vp, virus particles.

¹ Vaccine antigen exposure.

² 6 months after vaccination 1 in the single-dose regimens (Groups 4 and 5).

³ Due to a study pause in October 2020 the majority of participants in Groups 7 and 8 missed their visit window for Vaccination 2 at Day 28. Their actual vaccination timing coincided with the scheduled intervals in Groups 1 and 6.

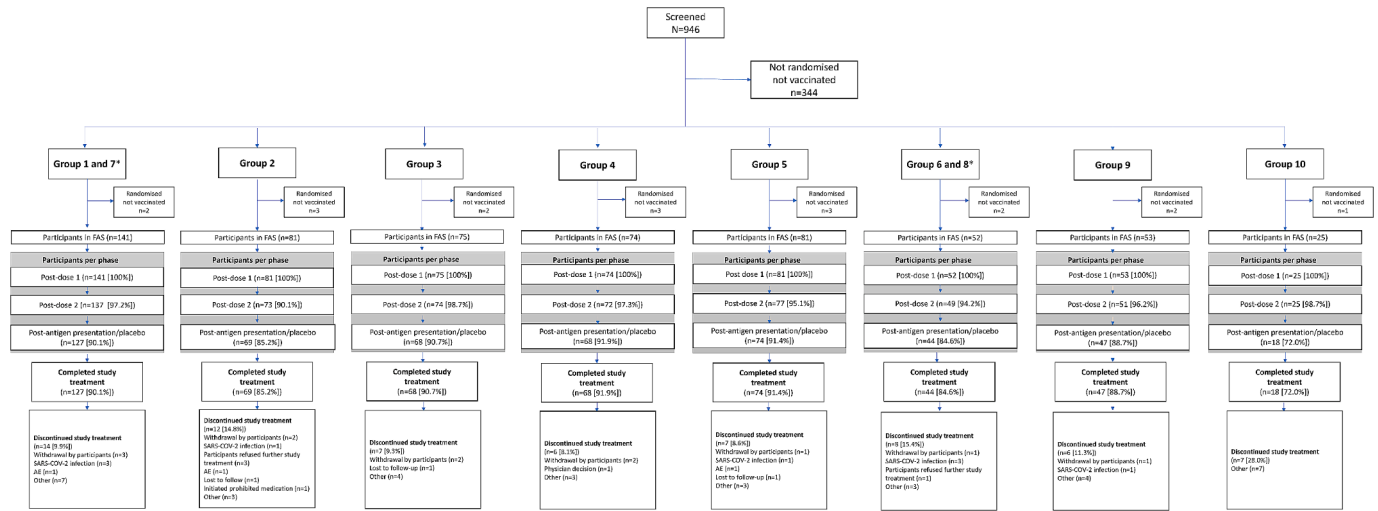


Fig. 1. Consort diagram. *Groups combined as the actual vaccination timing for Groups 7 and 8 coincided with the scheduled intervals in Groups 1 and 6.

Table 2

Demographic characteristics (Full analysis set).

	Group 3	Group 2	Groups 1&7*	Group 5	Group 4	Groups 6&8*	Group 9	Group 10	Total
Dose	Ad26 1.25 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Ad26 2.5 × 10 ¹⁰ , Ad26 2.5 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Ad26 5 × 10 ¹⁰ , Ad26 5 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Ad26 5 × 10 ¹⁰ , Placebo, Ad26 1.25 × 10 ¹⁰	Ad26 1 × 10 ¹¹ , Placebo, Ad26 1.25 × 10 ¹⁰	Placebo, Placebo, Placebo	Ad26 5 × 10 ¹⁰ , Ad26 5 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Placebo, Placebo, Placebo	
Number	75	81	141	81	74	52	53	25	582
Age, years									
Mean (SD)	49.2 (17.33)	49.1 (19.44)	49.1 (17.65)	48.6 (18.50)	50.3 (17.10)	49.1 (17.02)	48.1 (18.99)	52.4 (13.63)	49.2 (17.76)
Median	50.0	50.0	48.0	51.0	50.0	48.0	42.0	51.0	49.0
Range	(19; 84)	(19; 83)	(19; 81)	(18; 82)	(18; 78)	(19; 73)	(21; 80)	(32; 75)	(18; 84)
18–40	25 (33.3 %)	33 (40.7 %)	52 (36.9 %)	33 (40.7 %)	22 (29.7 %)	14 (26.9 %)	22 (41.5 %)	6 (24.0 %)	207 (35.6 %)
41–55	27 (36.0 %)	15 (18.5 %)	41 (29.1 %)	18 (22.2 %)	26 (35.1 %)	18 (34.6 %)	11 (20.8 %)	10 (40.0 %)	166 (28.5 %)
65–75	19 (25.3 %)	28 (34.6 %)	37 (26.2 %)	27 (33.3 %)	24 (32.4 %)	20 (38.5 %)	18 (34.0 %)	9 (36.0 %)	182 (31.3 %)
>75	4 (5.3 %)	5 (6.2 %)	11 (7.8 %)	3 (3.7 %)	2 (2.7 %)	0	2 (3.8 %)	0	27 (4.6 %)
Sex									
Female	22 (29.3 %)	30 (37.0 %)	40 (28.4 %)	35 (43.2 %)	32 (43.2 %)	23 (44.2 %)	19 (35.8 %)	13 (52.0 %)	214 (36.8 %)
Male	53 (70.7 %)	51 (63.0 %)	101 (71.6 %)	46 (56.8 %)	42 (56.8 %)	29 (55.8 %)	34 (64.2 %)	12 (48.0 %)	368 (63.2 %)
Race									
White	71 (94.7 %)	76 (93.8 %)	137 (97.2 %)	79 (97.5 %)	73 (98.6 %)	51 (98.1 %)	53 (100 %)	24 (96.0 %)	564 (96.9 %)
Asian	2 (2.7 %)	0	1 (0.7 %)	1 (1.2 %)	1 (1.4 %)	1 (1.9 %)	0	0	6 (1.0 %)
Other/not reported	2 (2.7 %)	5 (6.2 %)	3 (2.1 %)	1 (1.2 %)	0	0	0	1 (4.0 %)	12 (2.1 %)

SD, standard deviation*Groups combined as the actual vaccination timing for Groups 7 and 8 coincided with the scheduled intervals in Groups 1 and 6.

after the first or single dose of Ad26.COV2.S 1.25×10^{10} vp, 2.5×10^{10} vp, 5×10^{10} vp (2 groups) or 1×10^{11} vp. VNA GMTs increased after a single vaccination following each dose level of Ad26.COV2.S, with a dose–response observed (Fig. 2). GMTs were highest in the 1×10^{11} vp group and lowest in the 1.25×10^{10} vp group. At Day 29, the GMT (95 % CI) across the increasing dose levels was 148 (106–205) (1.25×10^{10} vp), 170 (126–227) (2.5×10^{10} vp), 243 (176–337) and 253 (207–308) (5×10^{10} vp), and 263 (201–345) (1×10^{11} vp) (Table S2).

In the single-dose groups, VNA GMTs persisted for 6 months after vaccination (Day 167). At Day 167 the GMT (95 % CI) was 154 (95–250) (5×10^{10} vp group), and 206 (148–288) (1×10^{11} vp group) (Table S2).

Levels of S-bAbs increased following a single vaccination of Ad26.COV2.S at each dose level and a similar dose–response trend was observed. At Day 29, the GMT (95 % CI) across the increasing dose levels

was 220 (168–288) (1.25×10^{10} vp), 299 (233–384) (2.5×10^{10} vp), 311 (241–400) and 360 (276–471) (5×10^{10} vp), and 505 (415–614) (1×10^{11} vp) (Table S3).

In the single-dose groups, S-bAbs persisted for 6 months after vaccination (Day 167). At Day 167 the GMC (95 % CI) was 337 (242–468) (5×10^{10} vp group), and 472 (333–669) (1×10^{11} vp group).

3.2.1.2. 2-dose regimen. The effect of dose level in 2-dose regimens was assessed in Groups 1 to 3, in which participants who received a first dose of Ad26.COV2.S 1.25×10^{10} vp, 2.5×10^{10} vp, 5×10^{10} vp and a second dose of the same vaccine on Day 57 (Fig. 2).

Fourteen days after the second Ad26.COV2.S dose (Day 71), VNA GMTs (95 % CI) increased by 6.5 to 9.3-fold in each group. The GMT (95 % CI) was 346 (220–544) after the 1.25×10^{10} vp dose, 538 (403–718)

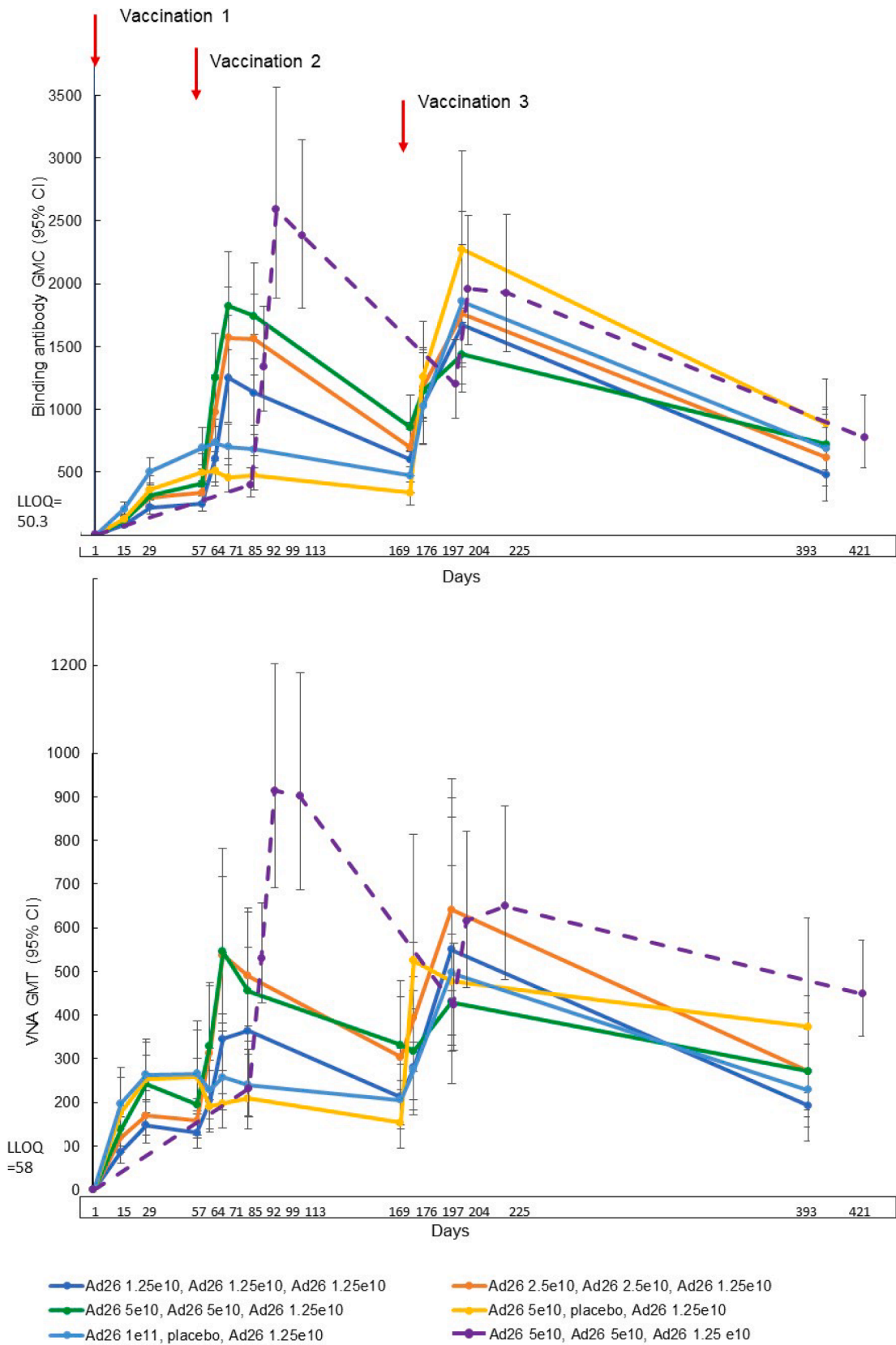


Fig. 2. Kinetics of SARS-CoV-2 S binding antibody (EU/ml) geometric mean concentrations (GMCs) and virus neutralising antibodies (VNA) geometric mean titres (GMTs) over the study (per protocol immunogenicity set). Purple dashed line shows results from Group 9. Participants received Ad26 5×10^{10} at Day 0 and 84 followed by vaccine antigen exposure and accordingly underwent blood samples at different time points. Red arrows show vaccination points for Groups 1–5. Error bars show 95 % confidence intervals. Data are tabulated in [Table S2](#), [Table S3](#), and [Table S4](#). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

after the 2.5×10^{10} vp dose, and 545 (381–781) after the 5×10^{10} vp dose. VNA GMTs were similar in the 2.5×10^{10} vp and 5×10^{10} vp dose groups from Day 71 until vaccine antigen exposure on Day 169, but tended to be higher than GMTs in the lower dose 1.25×10^{10} vp group (Table S2).

Four months after dose 2 (Day 169), the VNA GMT (95 % CI) was 213 (140–324) in the 1.25×10^{10} vp dose group, 304 (209–443) in the 2.5×10^{10} vp, and 332 (230–478) in the 5×10^{10} vp dose groups.

S-bAbs GMCs (95 % CI) increased by 4.3 to 4.9-fold in each group 14 days after the second Ad26.COV2.S dose. The S-bAbs GMC was 1254 (898–1750) after the second 1.25×10^{10} vp dose, 1569 (1250–1969) after the 2.5×10^{10} vp dose, and 1822 (1475–2251) after the 5×10^{10} vp dose. The apparent dose response continued to be observed across subsequent timepoints until vaccine antigen exposure (Table S2).

Four months after dose 2 (Day 169), the S-bAb GMC (95 % CI) was 600 (427–843) in the 1.25×10^{10} vp dose group, 698 (544–895) in the 2.5×10^{10} vp, and 859 (664–1112) in the 5×10^{10} vp dose groups.

3.2.2. Immunogenicity by dose number

The impact of dose number on immunogenicity was assessed in Groups 5 and 1 in which participants received a single dose of Ad26.COV2.S 5×10^{10} vp followed by placebo, or a second Ad26.COV2.S dose, respectively, on Day 57.

The VNA GMT increased steeply in participants who received a second dose Ad26.COV2.S vs placebo; VNA GMT of 545 (95 % CI 381–781) vs 198 (95 % CI 142–274), respectively 14 days post-second vaccination (Table S2). GMTs remained higher after the second Ad26.COV2.S dose at each subsequent timepoint until vaccine antigen exposure approximately 4 months later (Fig. 2).

Similar trends were observed for S-bAbs. S-bAbs increased steeply in participants who received a second Ad26.COV2.S dose vs placebo; GMC 1822 (95 % CI 1475–2251) vs 459 (95 % CI 345–609) respectively, 14 days post-second vaccination (Table S3). GMCs remained higher after the second Ad26.COV2.S dose at each subsequent timepoint until vaccine antigen exposure (Fig. 2).

3.2.3. Immunogenicity by dose interval

The impact of dose interval on immunogenicity was assessed in Groups 1 and 9 in which participants received two doses of Ad26.COV2.S 5×10^{10} vp given at either 56 or 84-day intervals. At the post-dose 2 timepoints, VNA GMTs were numerically higher in participants after an 84-day interval between doses (Fig. 2). At 14 days post-dose 2, the VNA GMT was 545 (95 % CI 381–781) in a 56-day interval vs 914 (95 % CI 692–1205) after an 84-day interval and the GMCs compared to baseline were 9.2 (95 % CI 6.4–13.3) and 15.8 (11.9–20.8), respectively (Table S2, Table S4). GMTs remained higher in the 84-day interval group at each subsequent timepoint until vaccine antigen exposure at 4 months post dose 2.

The same trend was observed for S-bAbs. At 14 days post-dose 2 the antibody GMC was 1822 (95 % CI 1475–2251) after a 56-day interval, and 2597 (95 % CI 1886–3575) after an 84-day interval (Table S3, Table S4). GMCs compared to baseline were 36.4 (95 % CI 29.3–45.2) and 51.6 (95 % CI 37.5–71.1) in the respective groups. GMCs remained higher in the 84-day interval group at each subsequent timepoint until vaccine antigen exposure (Fig. 2).

3.2.4. Anamnestic response and antibody persistence

Memory responses were assessed in all study groups by administration of Ad26.COV2.S 1.25×10^{10} vp approximately 4 months after the second vaccination with Ad26.COV2.S or placebo. Vaccine antigen exposure induced a rapid anamnestic response with marked increases in VNA titres and S-bAb concentrations after all Ad26.COV2.S doses and regimens tested, indicating booster effects and the presence of immune memory (Fig. 2, Table S2, Table S3). The magnitude of the S-bAb anamnestic response was highest in participants who received a single dose of Ad26.COV2.S 5×10^{10} vp.

Six months after vaccine antigen exposure, the VNA GMT (95 % CI) was 193 (112–334) in the 1.25×10^{10} vp dose group, 272 (166–445) in the 2.5×10^{10} vp dose group, 271 (182–404) in the 5×10^{10} vp 2-dose group, 373 (223–623) in the 5×10^{10} vp 1-dose group, and 229 (143–367) in the 1×10^{11} vp group (Table S2). GMCs for S-bAbs were 482 (271–858), 617 (395–964), 721 (520–1001), 888 (636–1241), and 692 (469–1020) in the respective groups (Table S3). Both VNA GMTs and S-bAb GMCs were higher 6 months post-vaccine antigen exposure than at baseline.

3.2.5. Humoral immunogenicity by age group 18–55 and ≥ 65 years

There was an overall trend for numerically lower VNA GMTs and S-bAb GMCs in participants aged ≥ 65 years vs participants aged 18–55 years, although the 95 % CIs were wide due to the low number of subjects in each age-category (Table S1, Table S5, Table S6, Table S7). A dose–response was observed, with similar kinetics of the VNA and S-bAb responses in each age-group.

3.3. Correlation between S-ELISA and VNA

Wild-type VNA titres (IC50) correlated highly with S-bAb concentrations (EU/mL) at all blood sampling time points evaluated in all Ad26.COV2.S vaccine groups from Day 29 (Fig. S3 and S4). The Spearman correlation was ≥ 0.70 at each timepoint, indicating high correlation between the two assays.

3.4. Cell-mediated immunity

At Day 29 the percentage of samples positive for CD4 + T cells expressing IFN γ and/or IL-2 (Th1), was 39 % (1.25×10^{10} vp), 53 % (2.5×10^{10} vp), 54 % and 56 % (5×10^{10} vp), and 38 % (1×10^{11} vp) (Table S8). SARS-CoV-2 S-specific CD4 + T cells producing Th1 cytokines increased in all groups compared to Day 85 after vaccine antigen exposure at 4 months post-dose 2 (Fig. S1). At Day 197, the percentage of samples positive for CD4 + Th1 cells in groups that received two priming doses was 50 % (1.25×10^{10} vp), 75 % (2.5×10^{10} vp), and 64 % (5×10^{10} vp). The percentage of positive participants in groups that received a single priming dose was 56 % (5×10^{10} vp), and 36 % (1×10^{11} vp). Six months post-vaccine antigen exposure, positive CD4 + Th1 cells were observed in 31 % (1.25×10^{10} vp), 75 % (2.5×10^{10} vp), and 33 % (5×10^{10} vp) of samples from groups that received 2-dose priming. The percentage in groups that received a single priming dose was 55 % (5×10^{10} vp), and 67 % (1×10^{11} vp). No Th2 responses were detected at any timepoint post-vaccination.

The percentage of samples positive for CD8 + T cells expressing IFN γ and/or IL-2 at Day 29 was 31 % (1.25×10^{10} vp), 40 % (2.5×10^{10} vp), 56 % and 36 % (5×10^{10} vp), and 11 % (1×10^{11} vp) (Table S9). After vaccine antigen exposure, SARS-CoV-2 S-specific CD8 + T cell responses in groups that received two priming doses were observed in 67 % (1.25×10^{10} vp), 67 % (2.5×10^{10} vp), and 50 % (5×10^{10} vp). The percentage in groups that received a single priming dose was 56 % (5×10^{10} vp), and 46 % (1×10^{11} vp). Six months post-vaccine antigen exposure, positive CD8 + cells responses were observed in 46 % (1.25×10^{10} vp), 58 % (2.5×10^{10} vp), and 23 % (5×10^{10} vp) of samples from groups that received 2-dose priming. The percentage in groups that received a single priming dose was 46 % (5×10^{10} vp), and 46 % (1×10^{11} vp). Overall, there was a trend for higher CD8 + T cell responses post-dose 2, post-vaccine antigen exposure, and 6 months post-vaccine antigen exposure in Group 5 (5×10^{10} vp, placebo) and Group 2 (2.5×10^{10} , 2.5×10^{10} vp) vs other Ad26.COV2.S groups.

3.5. Reactogenicity and safety

3.5.1. Post-dose 1

Solicited AEs were reported by 53.1–77.0 % of participants across the Ad26.COV2.S groups and by 40 % in the placebo groups (Table 3).

Pain at the injection site was the most frequently reported local solicited AE in all groups, ranging from 32.0–53.2 % in the Ad26.COV2.S groups and 8.0–13.5 % in the placebo groups. There was a trend for a dose relationship, with fewer AEs reported at the lower Ad26.COV2.S dose levels (1.25×10^{10} vp, and 2.5×10^{10} vp) than at the higher dose level of 1×10^{11} vp, with the 5×10^{10} vp groups falling in between.

The most frequently reported systemic solicited AEs in all groups were fatigue (reported for 28.0–51.4 % of participants in the Ad26.COV2.S groups vs 16.0–17.3 % in the placebo groups), headache (25.3–60.8 % vs 20.0–21.2 %, respectively), and myalgia (9.3–38.3 % vs 7.7–16.0 %), with the lowest incidences consistently reported for the 1.25×10^{10} vp and 2.5×10^{10} vp groups (Table 4).

No Grade 3 local AEs were reported after dose 1. Grade 3 Systemic AEs were uncommon and were more frequently reported in the highest dose group (1×10^{11} vp): up to 8.1 % for individual AEs vs up to 3.8 % for other groups.

Unsolicited AEs were reported by 15.1–44.6 % of participants in the Ad26.COV2.S groups vs 17.3–20.0 % in the placebo groups. The most frequently reported unsolicited AE was headache, reported by 4.0–12.3 % in Ad26.COV2.S groups and 5.8–8.0 % in placebo groups.

3.5.2. Post-dose 2

The frequencies of solicited local and systemic AEs after the second dose of Ad26.COV2.S were generally similar to post-dose 1, with no evidence of any increase in the type or intensity of AEs (Tables 3 and 4). Unsolicited AEs were reported by 19.6–28.6 % of participants after a second dose of Ad26.COV2.S vs 16.0–18.4 % after placebo.

3.5.3. Post-vaccine antigen exposure

The incidence and intensity of local and systemic symptoms after vaccine antigen exposure was similar or less than previous doses. Unsolicited AEs were reported by 8.8–16.2 % of participants after vaccine antigen exposure vs 9.1–22.2 % after placebo.

The reactogenicity profile reported after all study doses is provided in Fig. 3.

3.5.4. Reactogenicity by age group category

There was a trend towards a decrease in the frequency and severity of solicited local and systemic AEs with increasing age of participants in the Ad26.COV2.S vaccine groups (Table S10, Table S11).

Table 3

Percentage of participants with solicited local adverse events after each dose and after vaccine antigen exposure.

	Group 3	Group 2	Groups 1/7	Group 5	Group 4	Groups 6/8	Group 9	Group 10
	Ad26 1.25×10^{10} , Ad26 1.25×10^{10} , Ad26 1.25×10^{10}	Ad26 2.5×10^{10} , Ad26 2.5×10^{10} , Ad26 1.25×10^{10}	Ad26 5×10^{10} , Ad26 5×10^{10} , Ad26 1.25×10^{10}	Ad26 5×10^{10} , Placebo, Ad26 1.25×10^{10}	Ad26 1×10^{11} , Placebo, Ad26 1.25×10^{10}	Placebo,Placebo, Placebo	Ad26 5×10^{10} , Ad26 5×10^{10} , Ad26 1.25×10^{10}	Placebo,Placebo, Placebo
Post-dose 1	75	81	141	81	74	52	53	25
Vaccination site erythema								
Any	0	2 (2.5 %)	1 (0.7 %)	1 (1.2 %)	2 (2.7 %)	0	0	0
Grade 1	0	1 (1.2 %)	1 (0.7 %)	1 (1.2 %)	1 (1.4 %)	0	0	0
Grade 2	0	1 (1.2 %)	0	0	1 (1.4 %)	0	0	0
Vaccination site pain								
Any	24 (32.0 %)	27 (33.3 %)	75 (53.2 %)	42 (51.9 %)	39 (52.7 %)	7 (13.5 %)	21 (39.6 %)	2 (8.0 %)
Grade 1	24 (32.0 %)	27 (33.3 %)	68 (48.2 %)	40 (49.4 %)	35 (47.3 %)	7 (13.5 %)	20 (37.7 %)	2 (8.0 %)
Grade 2	0	0	7 (5.0 %)	2 (2.5 %)	4 (5.4 %)	0	1 (1.9 %)	0
Vaccination site swelling								
Any	1 (1.3 %)	1 (1.2 %)	1 (0.7 %)	2 (2.5 %)	3 (4.1 %)	0	1 (1.9 %)	0
Grade 1	0	1 (1.2 %)	1 (0.7 %)	0	3 (4.1 %)	0	0	0
Grade 2	1 (1.3 %)	0	0	2 (2.5 %)	0	0	1 (1.9 %)	0
Post-dose 2	74	73	137	77	72	49	51	25
Vaccination site erythema								
Any	0	2 (2.7 %)	1 (0.7 %)	0	0	0	0	0
Grade 1	0	2 (2.7 %)	0	0	0	0	0	0
Grade 2	0	0	1 (0.7 %)	0	0	0	0	0
Vaccination site pain								
Any	29 (39.2 %)	32 (43.8 %)	63 (46.0 %)	4 (5.2 %)	5 (6.9 %)	3 (6.1 %)	20 (39.2 %)	2 (8.0 %)
Grade 1	29 (39.2 %)	30 (41.1 %)	54 (39.4 %)	4 (5.2 %)	5 (6.9 %)	2 (4.1 %)	18 (35.3 %)	2 (8.0 %)
Grade 2	0	2 (2.7 %)	8 (5.8 %)	0	0	1 (2.0 %)	2 (3.9 %)	0
Grade 3	0	0	1 (0.7 %)	0	0	0	0	0
Vaccination site swelling								
Any	0	1 (1.4 %)	0	0	0	0	0	0
Grade 1	0	1 (1.4 %)	0	0	0	0	0	0
Post-vaccine antigen exposure or placebo	68	69	127	74	68	44	47	18
Vaccination site erythema								
Any	0	0	0	0	0	0	0	0
Vaccination site pain								
Any	29 (42.6 %)	28 (40.6 %)	50 (39.4 %)	35 (47.3 %)	31 (45.6 %)	1 (2.3 %)	16 (34.0 %)	2 (11.1 %)
Grade 1	29 (42.6 %)	28 (40.6 %)	45 (35.4 %)	30 (40.5 %)	30 (44.1 %)	1 (2.3 %)	14 (29.8 %)	2 (11.1 %)
Grade 2	0	0	5 (3.9 %)	5 (6.8 %)	1 (1.5 %)	0	1 (2.1 %)	0
Grade 3	0	0	0	0	0	0	1 (2.1 %)	0
Vaccination site swelling								
Any	1 (1.5 %)	0	1 (0.8 %)	3 (4.1 %)	2 (2.9 %)	0	0	0
Grade 1	0	0	0	3 (4.1 %)	0	0	0	0
Grade 2	1 (1.5 %)	0	1 (0.8 %)	0	2 (2.9 %)	0	0	0

Pain: Grade 1 = Aware of symptoms but easily tolerated, does not interfere with activity, discomfort only to touch; Grade 2 = Notable symptoms, requires modification in activity or use of medications, discomfort with movement; Grade 3 = Incapacitating symptoms, inability to do work, school, or usual activities, use of narcotic pain reliever.

Erythema and swelling: Grade 1 = 25–50 mm; Grade 2 = 51–100 mm; Grade 3 = >100 mm.

Table 4

Percentage of participants with solicited systemic adverse events after each dose and after vaccine antigen exposure.

	Group 3	Group 2	Groups 1/7	Group 5	Group 4	Groups 6/8	Group 9	Group 10
	Ad26 1.25 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Ad26 2.5 × 10 ¹⁰ , Ad26 2.5 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Ad26 5 × 10 ¹⁰ , Ad26 5 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Ad26 5 × 10 ¹⁰ , Placebo, Ad26 1.25 × 10 ¹⁰	Ad26 1 × 10 ¹¹ , Placebo, Ad26 1.25 × 10 ¹⁰	Placebo,Placebo, Placebo	Ad26 5 × 10 ¹⁰ , Ad26 5 × 10 ¹⁰ , Ad26 1.25 × 10 ¹⁰	Placebo,Placebo, Placebo
Post-dose 1	75	81	141	81	74	52	53	25
Fatigue								
Any	21 (28.0 %)	23 (28.4 %)	64 (45.4 %)	37 (45.7 %)	38 (51.4 %)	9 (17.3 %)	24 (45.3 %)	4 (16.0 %)
Grade 1	21 (28.0 %)	17 (21.0 %)	39 (27.7 %)	27 (33.3 %)	25 (33.8 %)	9 (17.3 %)	16 (30.2 %)	4 (16.0 %)
Grade 2	0	5 (6.2 %)	24 (17.0 %)	10 (12.3 %)	7 (9.5 %)	0	7 (13.2 %)	0
Grade 3	0	1 (1.2 %)	1 (0.7 %)	0	6 (8.1 %)	0	1 (1.9 %)	0
Headache								
Any	19 (25.3 %)	22 (27.2 %)	58 (41.1 %)	33 (40.7 %)	45 (60.8 %)	11 (21.2 %)	21 (39.6 %)	5 (20.0 %)
Grade 1	16 (21.3 %)	16 (19.8 %)	40 (28.4 %)	22 (27.2 %)	23 (31.1 %)	11 (21.2 %)	15 (28.3 %)	5 (20.0 %)
Grade 2	3 (4.0 %)	6 (7.4 %)	16 (11.3 %)	11 (13.6 %)	18 (24.3 %)	0	5 (9.4 %)	0
Grade 3	0	0	2 (1.4 %)	0	4 (5.4 %)	0	1 (1.9 %)	0
Myalgia								
Any	7 (9.3 %)	9 (11.1 %)	48 (34.0 %)	31 (38.3 %)	28 (37.8 %)	4 (7.7 %)	19 (35.8 %)	4 (16.0 %)
Grade 1	7 (9.3 %)	7 (8.6 %)	26 (18.4 %)	23 (28.4 %)	18 (24.3 %)	4 (7.7 %)	15 (28.3 %)	2 (8.0 %)
Grade 2	0	2 (2.5 %)	19 (13.5 %)	8 (9.9 %)	6 (8.1 %)	0	2 (3.8 %)	2 (8.0 %)
Grade 3	0	0	3 (2.1 %)	0	4 (5.4 %)	0	2 (3.8 %)	0
Nausea								
Any	3 (4.0 %)	4 (4.9 %)	13 (9.2 %)	9 (11.1 %)	15 (20.3 %)	0	5 (9.4 %)	0
Grade 1	2 (2.7 %)	4 (4.9 %)	8 (5.7 %)	8 (9.9 %)	10 (13.5 %)	0	5 (9.4 %)	0
Grade 2	1 (1.3 %)	0	4 (2.8 %)	1 (1.2 %)	2 (2.7 %)	0	0	0
Grade 3	0	0	1 (0.7 %)	0	3 (4.1 %)	0	0	0
Pyrexia								
Any	0	5 (6.2 %)	14 (9.9 %)	14 (17.3 %)	15 (20.3 %)	0	4 (7.5 %)	0
Grade 1	0	4 (4.9 %)	8 (5.7 %)	12 (14.8 %)	1 (1.4 %)	0	2 (3.8 %)	0
Grade 2	0	0	5 (3.5 %)	1 (1.2 %)	8 (10.8 %)	0	0	0
Grade 3	0	1 (1.2 %)	1 (0.7 %)	1 (1.2 %)	6 (8.1 %)	0	2 (3.8 %)	0
Post-Dose 2	74	73	137	77	72	49	51	25
Fatigue								
Any	16 (21.6 %)	15 (20.5 %)	62 (45.3 %)	11 (14.3 %)	14 (19.4 %)	8 (16.3 %)	23 (45.1 %)	3 (12.0 %)
Grade 1	13 (17.6 %)	10 (13.7 %)	48 (35.0 %)	10 (13.0 %)	13 (18.1 %)	7 (14.3 %)	18 (35.3 %)	2 (8.0 %)
Grade 2	3 (4.1 %)	5 (6.8 %)	14 (10.2 %)	1 (1.3 %)	1 (1.4 %)	1 (2.0 %)	3 (5.9 %)	1 (4.0 %)
Grade 3	0	0	0	0	0	0	2 (3.9 %)	0
Headache								
Any	15 (20.3 %)	24 (32.9 %)	50 (36.5 %)	12 (15.6 %)	16 (22.2 %)	9 (18.4 %)	14 (27.5 %)	4 (16.0 %)
Grade 1	13 (17.6 %)	16 (21.9 %)	32 (23.4 %)	9 (11.7 %)	15 (20.8 %)	7 (14.3 %)	10 (19.6 %)	3 (12.0 %)
Grade 2	2 (2.7 %)	8 (11.0 %)	17 (12.4 %)	3 (3.9 %)	1 (1.4 %)	2 (4.1 %)	3 (5.9 %)	1 (4.0 %)
Grade 3	0	0	1 (0.7 %)	0	0	0	1 (2.0 %)	0
Myalgia								
Any	5 (6.8 %)	11 (15.1 %)	37 (27.0 %)	3 (3.9 %)	7 (9.7 %)	1 (2.0 %)	11 (21.6 %)	2 (8.0 %)
Grade 1	5 (6.8 %)	9 (12.3 %)	24 (17.5 %)	1 (1.3 %)	7 (9.7 %)	0	5 (9.8 %)	2 (8.0 %)
Grade 2	0	2 (2.7 %)	11 (8.0 %)	2 (2.6 %)	0	1 (2.0 %)	6 (11.8 %)	0
Grade 3	0	0	2 (1.5 %)	0	0	0	0	0
Nausea								
Any	2 (2.7 %)	7 (9.6 %)	10 (7.3 %)	1 (1.3 %)	4 (5.6 %)	1 (2.0 %)	3 (5.9 %)	2 (8.0 %)
Grade 1	2 (2.7 %)	7 (9.6 %)	4 (2.9 %)	1 (1.3 %)	4 (5.6 %)	1 (2.0 %)	1 (2.0 %)	1 (4.0 %)
Grade 2	0	0	6 (4.4 %)	0	0	0	2 (3.9 %)	1 (4.0 %)
Pyrexia								
Any	0	0	5 (3.6 %)	0	0	1 (2.0 %)	3 (5.9 %)	0
Grade 1	0	0	3 (2.2 %)	0	0	1 (2.0 %)	3 (5.9 %)	0
Grade 2	0	0	2 (1.5 %)	0	0	0	0	0
Post-vaccine antigen exposure or placebo	68	69	127	74	68	44	47	18
Fatigue								
Any	17 (25.0 %)	22 (31.9 %)	37 (29.1 %)	21 (28.4 %)	15 (22.1 %)	10 (22.7 %)	10 (21.3 %)	4 (22.2 %)
Grade 1	15 (22.1 %)	18 (26.1 %)	31 (24.4 %)	15 (20.3 %)	13 (19.1 %)	8 (18.2 %)	7 (14.9 %)	4 (22.2 %)
Grade 2	2 (2.9 %)	3 (4.3 %)	6 (4.7 %)	6 (8.1 %)	2 (2.9 %)	1 (2.3 %)	3 (6.4 %)	0
Grade 3	0	1 (1.4 %)	0	0	0	1 (2.3 %)	0	0
Headache								
Any	8 (11.8 %)	14 (20.3 %)	25 (19.7 %)	18 (24.3 %)	12 (17.6 %)	7 (15.9 %)	5 (10.6 %)	2 (11.1 %)
Grade 1	7 (10.3 %)	12 (17.4 %)	18 (14.2 %)	10 (13.5 %)	9 (13.2 %)	5 (11.4 %)	3 (6.4 %)	1 (5.6 %)
Grade 2	1 (1.5 %)	2 (2.9 %)	7 (5.5 %)	8 (10.8 %)	3 (4.4 %)	2 (4.5 %)	2 (4.3 %)	1 (5.6 %)
Myalgia								
Any	10 (14.7 %)	11 (15.9 %)	18 (14.2 %)	11 (14.9 %)	9 (13.2 %)	4 (9.1 %)	7 (14.9 %)	3 (16.7 %)
Grade 1	10 (14.7 %)	9 (13.0 %)	14 (11.0 %)	10 (13.5 %)	8 (11.8 %)	2 (4.5 %)	6 (12.8 %)	3 (16.7 %)
Grade 2	0	2 (2.9 %)	4 (3.1 %)	1 (1.4 %)	1 (1.5 %)	1 (2.3 %)	1 (2.1 %)	0
Grade 3	0	0	0	0	0	1 (2.3 %)	0	0
Nausea								
Any	1 (1.5 %)	4 (5.8 %)	7 (5.5 %)	7 (9.5 %)	3 (4.4 %)	1 (2.3 %)	0	1 (5.6 %)

(continued on next page)

Table 4 (continued)

	Group 3	Group 2	Groups 1/7	Group 5	Group 4	Groups 6/8	Group 9	Group 10
Grade 1	1 (1.5 %)	3 (4.3 %)	7 (5.5 %)	7 (9.5 %)	1 (1.5 %)	1 (2.3 %)	0	1 (5.6 %)
Grade 2	0	1 (1.4 %)	0	0	2 (2.9 %)	0	0	0
Pyrexia								
Any	0	0	1 (0.8 %)	0	0	0	0	0
Grade 1	0	0	1 (0.8 %)	0	0	0	0	0

Nausea: Grade 1 = Minimal symptoms, causes minimal or no interference with work, school, or selfcare activities; Grade 2 = Notable symptoms, requires modification in activity or use of medications, does not result in loss of work, school, or cancellation of social activities; Grade 3 = Incapacitating symptoms, requires bed rest and/or results in loss of work, school, or cancellation of social activities; Grade 4 = Hospitalisation, inability to perform basic self-care functions.

Fever: Grade 1 = 38.0–38.4 °C; Grade 2 = 38.5–38.9 °C; Grade 3 = 39.0– 40.0 °C; Grade 4 = >40.0 °C.

Other symptoms: Grade 1 = Minimal symptoms causing no or minimal interference with usual social and functional activities; Grade 2 = Notable symptoms causing greater than minimal interference with usual social and functional activities (may require use of medications); Grade 3 = Severe symptoms causing inability to perform usual social and functional activities and requires medical intervention (may require use of narcotic pain reliever); Grade 4: Hospitalisation, inability to perform basic self-care functions.

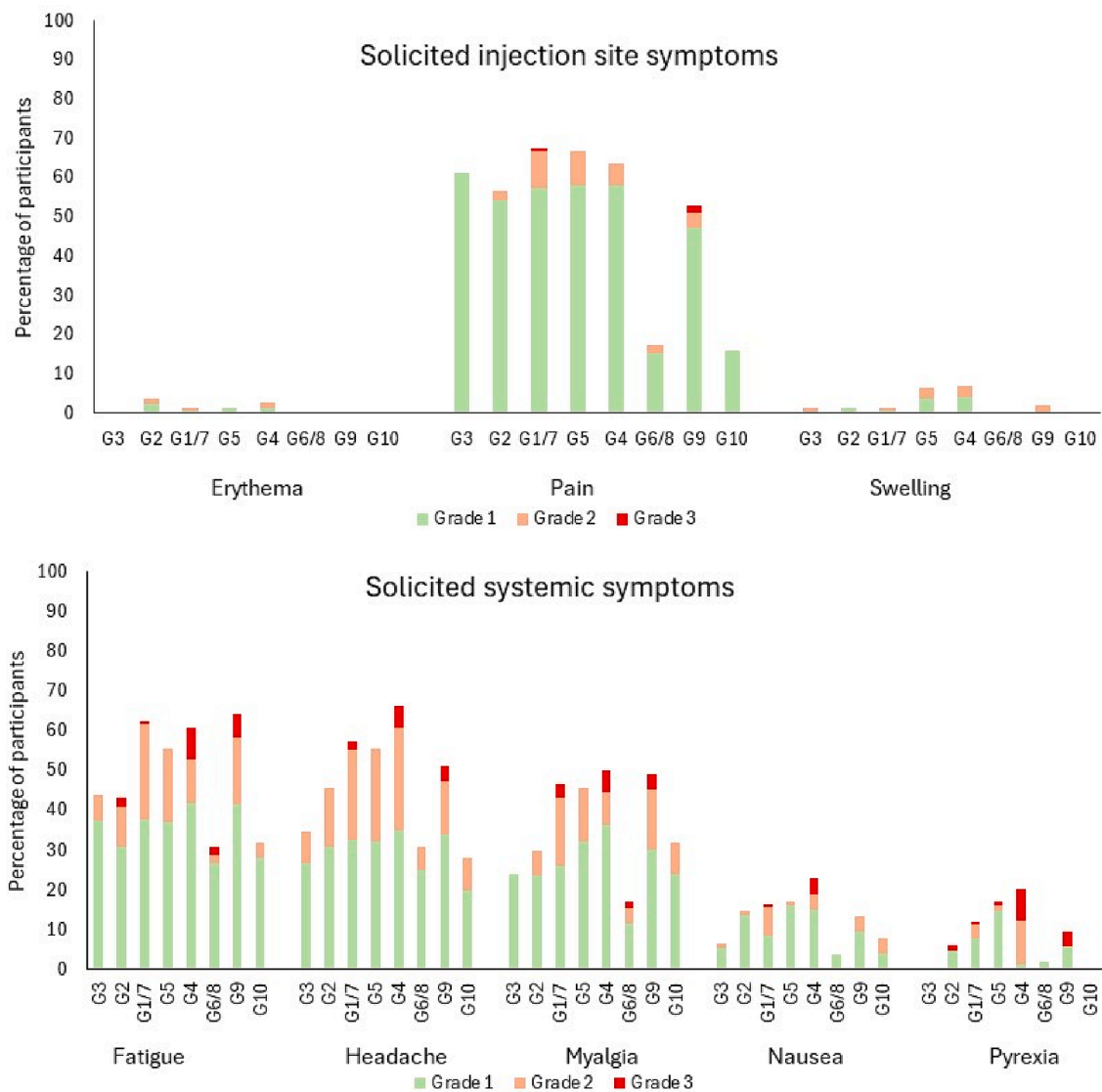


Fig. 3. Solicited symptoms reported post-dose 1, post-dose 2 and post-antigen presentation or placebo doses Definitions of severity are provided in the footnotes to Tables 2 and 3 Group definitions are provided in Table 1.

3.5.5. Saes and AESI

Nine participants had SAEs during the study, all were in an Ad26.COV2.S group. None were considered related to study vaccination by the investigator. There was 1 death reported due to unknown causes that occurred 188 days after the first dose of Ad26.COV2.S at 2.5×10^{10} vp.

The event was considered unrelated to study vaccine by the investigator. There were two potential AESIs (defined as a thrombotic event or thrombocytopenia) during the study. One 55-year-old male participant in Group 5 had Grade 2 thrombophlebitis at Day 2 which resolved after 28 days. One 77-year-old female participant in Group 1 had a Grade 3

ischemic stroke 8 days post-vaccine antigen exposure (no platelet count data available) which resolved after 8 days. Neither event was considered related to study vaccine by the investigator. Neither event qualified for assessment as a potential case of TTS.

4. Discussion

This study set out to address important questions relating to the optimal dose regimen for the Ad26.COV2.S vaccine. Its results contributed to the approval for use of Ad26.COV2.S as a homologous booster dose in the US and Europe. Key findings include the observation of a dose response after a single dose or 2-dose primary regimen, with higher VNA and S-bAb responses following increasing dose levels; durable immune responses after a single Ad26.COV2.S dose (5×10^{10} vp) that persist at least 6 months; and increased magnitude of the immune response when the interval between dose 1 and dose 2 was increased. A second Ad26.COV2.S dose resulted in strong dose-related responses but did not lead to increased long term antibody persistence after antigen exposure when compared to a single Ad26.COV2.S dose. Additionally, rapid and robust booster responses observed in all groups after administration of 1.25×10^{10} vp to mimic virus exposure indicate effective priming and establishment of immune memory after one or two previous Ad26.COV2.S doses. Durable immune responses were observed in all groups up to 6 months after primary vaccination (time of antigen exposure), and for 6 months post-vaccine antigen exposure (last time-point assessed).

Higher dose levels were associated with increased reactogenicity versus lower dose levels. However, the incidence and severity of SAEs was similar across all Ad26.COV2.S groups. No cases of TTS were observed and there were no safety concerns identified.

Immunogenicity and reactogenicity tended to be higher in younger than older adults, consistent with the concept of immunosenescence and with results from other studies using Ad26.COV2.S [13].

Marked humoral and CMI-mediated booster responses were elicited with the low Ad26.COV2.S dose (1.25×10^{10}) regardless of the priming schedule, indicating that all priming doses and schedules were effective in inducing immune memory needed for an anamnestic response upon antigen exposure. Anamnestic responses are important in providing long-term protection against COVID-19 [14], which suggests that primary vaccination with Ad26.COV2.S followed by a SARS-CoV-2 infection confers an advantage in protecting against COVID-19. High quality polyfunctional Th1 responses were elicited by Ad26.COV2.S that were boostable and durable. This is consistent with previous studies using Ad26.COV2.S with persistence of CD4 + and CD8 + T cells for up to 8 months after a single vaccination, associated with expansion of the neutralising antibody breadth against SARS-CoV-2 variants over time [10].

These data indicate that booster vaccination using Ad26.COV2.S could be conducted using low dose vaccines, potentially expanding availability in epidemic/pandemic settings where very large numbers of doses may be required.

Strengths of the study include its double-blind, placebo-controlled design, 12-month follow-up after primary vaccination, and parallel investigation of multiple vaccination regimens allowing their direct comparison. Most individuals were still naïve to SARS-CoV-2 when the study started. A similar study would be difficult to conduct today. Potential limitations include the infeasibility of evaluating a 28-day interval between Ad26.COV2.S doses due to a study pause, and a high number of participants who were excluded from the per protocol analysis of immunogenicity due to the emergent availability of approved COVID-19 vaccines during the study. Study staff conducting the safety reporting and participants were unblinded more than 3 months and up to 10 months, after receipt of initial study vaccination. Safety and COVID-19 case analyses were censored at the date of unblinding or upon receipt of an unscheduled vaccine, whichever event occurred first, to avoid any effect on safety reporting. In view of the interval between

vaccination and unblinding, and censoring of these subjects in the analysis, it is unlikely that unblinding had any impact on the interpretation of safety data.

In conclusion, the study supports the use of the approved primary, single dose schedule with Ad26.COV2.S 5×10^{10} , and shows that a longer interval (84 vs 56 days) between the primary vaccination and a homologous booster dose resulted in a larger increase in humoral immune responses vs the 1-dose regimen in participants aged 18–55 and those 65 years or older. This study also provides a wealth of immunogenicity and safety data that can contribute to development of new vaccines using adenovirus platforms.

Author contributions

All authors confirm that they had full access to all the data in the study and accept responsibility to submit for publication.

Funding

This study was supported by Janssen Vaccines & Prevention BV in collaboration with the United States government under the Biomedical Advanced Research and Development Authority, the National Institutes of Health, the Department of Defense, and the COVID-19 Prevention Network. This project was funded in whole or in part with federal funds from the Administration for Strategic Preparedness and Response, Biomedical Advanced Research and Development Authority, under contract number HHSO100201700018C. The findings and conclusions herein are those of the authors and do not necessarily represent the views of the Department of Health and Human Services or its components.

CRediT authorship contribution statement

Vicky Cárdenas: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Conceptualization. **Mathieu Le Gars:** Writing – review & editing, Validation, Methodology, Investigation. **Carla Truysers:** Writing – review & editing, Validation, Formal analysis. **Javier Ruiz-Guínazú:** Writing – review & editing, Supervision, Investigation. **Frank Struyf:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Alicia Colfer:** Writing – review & editing, Project administration, Investigation. **Marc Bonten:** Writing – review & editing, Investigation. **Alberto Borobia:** Writing – review & editing, Investigation. **Emil C. Reisinger:** Writing – review & editing, Investigation. **Ingrid M.C. Kamerling:** Investigation, Writing – review & editing. **Macaya Douoguih:** Writing – review & editing, Methodology, Conceptualization. **Jerald Sadoff:** Writing – review & editing, Validation, Methodology, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Vicky Cardenas reports financial support was provided by Johnson & Johnson. Mathieu Le Gars reports financial support was provided by Johnson & Johnson. Carla Truysers reports financial support was provided by Johnson & Johnson. Javier Ruiz-Guinazu reports financial support was provided by Johnson & Johnson. Frank Struyf reports financial support was provided by Johnson & Johnson. Alicia Colfer reports financial support was provided by Johnson & Johnson. Macaya Douoguih reports financial support was provided by Johnson & Johnson. Jerald Sadoff reports financial support was provided by Johnson & Johnson. Marc Bonten reports a relationship with Shionogi, Merck, GSK and Janssen Vaccines that includes: consulting or advisory. Marc Bonten reports a relationship with Sanofi that includes: board membership. Frank Struyf reports a relationship with GSK that includes: equity or stocks. Marc Bonten reports a relationship with Johnson & Johnson,

Merck, Sanofi that includes: funding grants. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Data availability

Data will be made available on request.

Acknowledgements

The authors thank the study participants, investigators and personnel involved in study COV2001, as well as all representatives of Janssen who were involved in analyses of the data. The authors also thank Dr Blanca Sánchez Santiago, Dr Dolores Ochoa, Dr Stanislav Ignatenko, and Dr Jeroen van de Wetering de Rooij for their role in study conduct.

The authors thank Joanne Wolter (independent on behalf of Johnson & Johnson) for medical writing support and production assistance.

Data Sharing Statement

Janssen has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for the evaluation of requests for clinical study reports and participant-level data from investigators and physicians for scientific research that will advance medical knowledge and public health. Data will be made available following publication and approval by YODA of any formal requests with a defined analysis plan. For more information on this process or to make a request, please visit the YODA Project site at <http://yoda.yale.edu>. The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at <https://www.janssen.com/clinical-trials/transparency>.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.vaccine.2024.04.059>.

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