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RESEARCH ARTICLE

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# Safety and immunogenicity of Ad26.COV2.S in adolescents: Phase 2 randomized clinical trial

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**ABSTRACT**

We conducted a randomized, Phase 2 trial to assess the safety and humoral immunogenicity of reduced doses/dose volume of the standard dose of Ad26.COV2.S COVID-19 vaccine ($5 \times 10^{10}$ viral particles [vp]) in healthy adolescents aged 12–17 years. Participants were randomly assigned to receive Ad26.COV2.S at reduced dose levels of $0.625 \times 10^{10}$ (0.5 mL), $1.25 \times 10^{10}$ (0.5 mL) or $2.5 \times 10^{10}$ (0.5 mL or low volume 0.25 mL) vp in a 1- or 2-dose (56-day interval) primary schedule. Adolescents who received a 1-dose primary schedule received a $2.5 \times 10^{10}$ vp booster dose 6 months later. Safety and humoral immunogenicity were assessed up to 6 months post-last vaccination. All regimens were well tolerated, with no safety concerns identified. Local and systemic solicited AEs in adolescents were consistent with the known safety profile in adults. All 1- and 2-dose Ad26.COV2.S primary schedules elicited robust peak Spike-binding antibody responses and virus neutralizing titers against the reference strain, in participants with and without preexisting SARS-CoV-2 immunity. Immune responses were durable for at least 6 months. Spike-binding antibody responses were comparable to those elicited in young adults aged 18–25 years who received a standard dose of Ad26.COV2.S in Phase 3 efficacy studies Reduced doses/dose volume of Ad26.COV2.S had an acceptable safety profile and elicited robust humoral immune responses in adolescents aged 12–17 years. All 1- and 2-dose schedules elicited Spike-binding antibody responses that were comparable to an adult population in whom efficacy has been demonstrated using a higher vaccine dose. (clinicaltrials.gov NCT05007080).

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Ad26.COV2.S; COVID-19 vaccine; adolescent; immunogenicity; safety; virus vector vaccine; dose-range study; shelf life

## Introduction

The rapid global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) led to substantial morbidity, mortality, and economic disruption after its emergence in December 2019. The disease caused by SARS-CoV-2 is referred to as coronavirus disease 2019 (COVID-19). Symptoms range from mild to severe, and can lead to death.[1–3] Clinical manifestations of COVID-19 are typically less severe in children than in adults[4]; however, the potential exists for serious complications such as multisystem inflammatory syndrome. This potential underscores the importance of thorough evaluation of COVID-19 vaccines for safety and efficacy in the pediatric and adolescent population.[5–7]

Ad26.COV2.S is a monovalent COVID-19 vaccine composed of a recombinant, replication-incompetent human adenovirus type 26 (Ad26) vector, constructed to encode a stabilized Spike protein derived from a SARS-CoV-2 clinical isolate (Wuhan, 2019, or 'reference strain'), in the pre-fusion conformation.[8,9] In adults, a single immunization with $5 \times 10^{10}$ viral particles (vp) of Ad26.COV2.S was effective in preventing COVID-19, especially moderate-to-severe/critical COVID-19, with increased efficacy observed after a second dose administered after 2 months.[10,11]

One of the key challenges of the COVID-19 pandemic was managing limited vaccine supply in the face of unprecedented global demand.[12] Dose-sparing strategies can expand vaccine availability and increase coverage, optimizing use of limited antigen supply.[13] The adenovirus vector platform continues to have an important role in vaccine development,[14] and could play an additional role during epidemics or pandemics when vaccines need to be rapidly manufactured and deployed. Other Ad26-vectored vaccines, such as the first component of the Ad26- and Modified Vaccinia Ankara-based Ebola vaccine, are highly immunogenic in adolescents and children.[15,16] It is expected that lower doses and/or lower dose volumes of Ad26.COV2.S could provide satisfactory immunogenicity in adolescents, facilitating dose-sparing strategies which could be important in emergency settings. Evaluation of very low dose levels could also support future studies of other Ad26-vectored vaccines in younger pediatric age groups.

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We evaluated the safety and immunogenicity of three reduced doses of Ad26.COV2.S as 1- or 2-dose primary regimens in healthy adolescents aged 12–17 years. In one group, a $2.5 \times 10^{10}$ vp dose was obtained by administering half of the volume of the full 0.5 mL standard dose. Here we report the safety and humoral immunogenicity results in terms of Spike-binding and neutralizing antibody titers through 6 months after the last vaccination. Levels of Spike-binding antibodies were compared with young adults in whom efficacy was demonstrated in Phase 3 trials.

## Patients and methods

### Study design and participants

This randomized, observer-blind Phase 2 study was conducted in 15 centers in Brazil (4 centers), Argentina (2), Mexico (2), India (3) and South Africa (4) (clinicaltrials.gov NCT05007080). The coprimary objectives were to assess the reactogenicity, safety, and humoral immune response to three reduced doses of Ad26.COV2.S when administered as 1-dose or 2-dose primary regimens. Secondary objectives included safety and immunogenicity up to 6 months post-last dose.

The primary safety objective included assessment of solicited local and systemic adverse events (AEs), unsolicited AEs, medically-attended AEs, serious AEs (SAEs) and adverse events of special interest (AESI). The primary immunogenicity objective was Spike-binding and SARS-CoV-2 neutralizing antibody responses 28 days after the first administration (dose 1) and 14 days after the second administration (dose 2).

Eligible participants were healthy adolescents aged 12–17 years who had no history of COVID-19 vaccination. Participants were screened for previous SARS-CoV-2 infection using local rapid finger-prick serology tests and only those participants testing negative were eligible for enrollment. Individuals who had previously received a coronavirus vaccine were excluded. Full inclusion and exclusion criteria are listed in Supplementary information.

Study oversight was provided by an Independent Data Monitoring Committee which reviewed safety and reactogenicity data on an ongoing basis. The study was reviewed and approved by appropriate national, regional, or institutional review boards or independent ethics committees. All participants provided written informed consent before enrollment.

### Interventions and procedures

Participants were randomly assigned in a 1:1:1:1:1:1 ratio to one of six study groups (Figure 1); three 1-dose groups – Ad26.COV2.S at $2.5 \times 10^{10}$ vp (0.25 mL, Low Volume [LV]), $1.25 \times 10^{10}$ vp, or $0.625 \times 10^{10}$ vp (both 0.5 mL) (one-half, one-quarter, and one-eighth of the approved dose in adults, respectively) on Day 1, and placebo (0.5 mL 0.9% sodium chloride solution) on Day 57 in the primary regimen. Three 2-dose groups received two doses of Ad26.COV2.S at each dose level (all 0.5 mL) on Day 1 and Day 57. Participants in the 1-dose groups received an Ad26.COV2.S $2.5 \times 10^{10}$ vp booster dose 6 months after

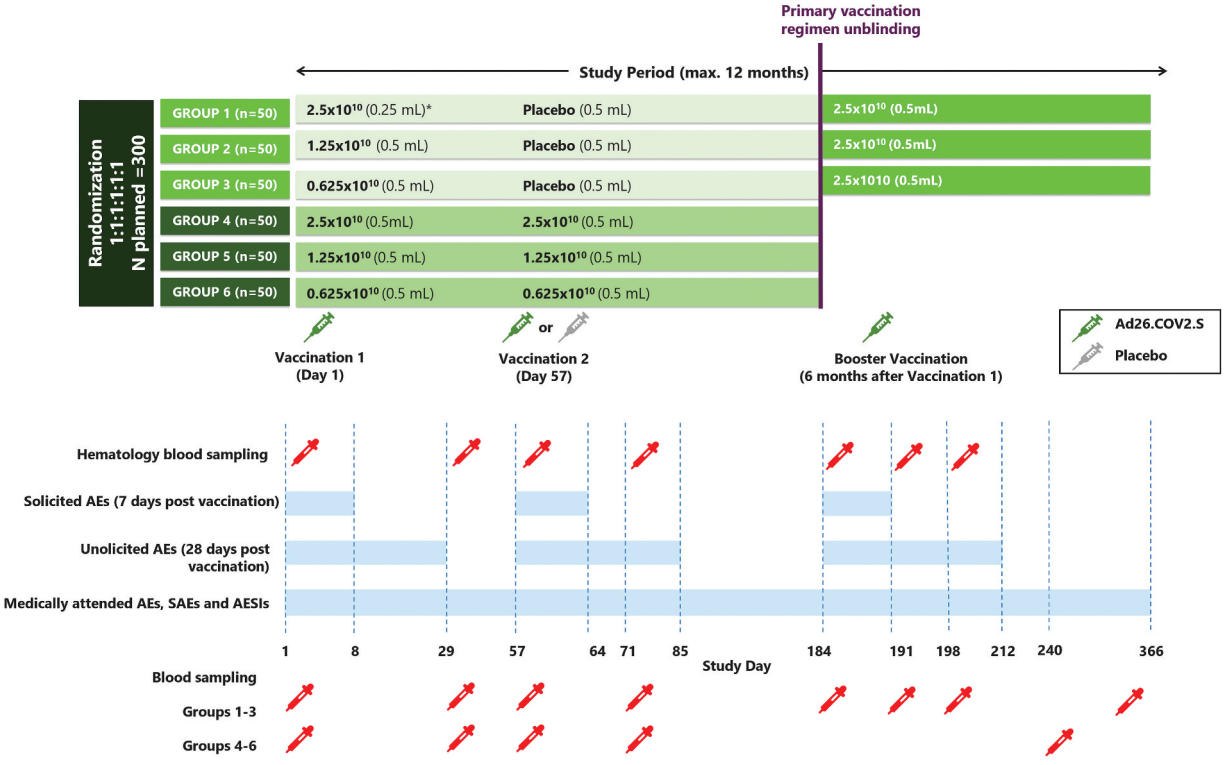


**Figure 1.** Study design and procedures. AE, adverse event; AESI, adverse event of special interest; SAE, serious adverse event *The $2.5 \times 10^{10}$ vp per 0.25 mL group was included to assess safety and immunogenicity of the lower volume for vaccine administration in potential future pediatric studies.

dose 1. Participants were followed up until 6 months after the last active vaccination.

Blood samples were collected from all participants for serum chemistry, hematology, and immunogenicity assessment at the time points specified in Figure 1.

### Randomization and blinding

Participants were randomized using an interactive web response system balanced with randomly permuted blocks and were stratified by age group (16–17 years, 12–15 years), and sex (male/female). Participants and investigators were blinded to dose levels throughout the study but were unblinded to the primary regimen (1- or 2-dose) at approximately 6 months post-dose 1. Blinding was guaranteed by the separation between preparation and administration of vaccine and placebo, with administration performed by an unblinded study vaccine administrator using masked syringes. The sponsor and statisticians were unblinded at an interim analysis when all participants had completed the Day 85 visit. Complete unblinding occurred when all participants completed the last visit.

### Evaluation of safety and reactogenicity

Solicited AEs were recorded by the participant in an electronic diary for 7 days post-vaccination and were subsequently evaluated by the investigator. AEs were graded according to FDA guidance criteria.[17]

All other (unsolicited) AEs were recorded for 28 days after each vaccination. Medically attended AEs were collected for 6 months after each vaccination. SAEs, AEs leading to discontinuation from the study/vaccination regimen and AESI were collected up to study end. AESIs were thrombosis with thrombocytopenia syndrome (TTS) at any age, and multisystem inflammatory syndrome in children. Suspected cases of TTS, defined as a thrombotic event with concurrent thrombocytopenia (platelet count < 150,000/μL), were to be assessed by an TTS expert Adjudication Committee.

### Evaluation of immunogenicity

SARS-CoV-2 Spike-binding antibody concentrations were assessed by a previously described human SARS-CoV-2 pre-Spike-specific IgG enzyme-linked immunosorbent assay (S-ELISA).[18] Neutralizing titers were measured by a pseudotyped virus neutralization assay based on pseudovirions expressing the SARS-CoV-2 S protein from the reference strain (D614G mutation), Delta variant (B.1.617.2), or Omicron BA.1 subvariant (B.1.1.529.1) (Supplementary information).

After screening with the finger prick test, baseline SARS-CoV-2 serostatus was determined for the purpose of immunogenicity analyses according to Day 1 S-ELISA and nucleocapsid (N)-ELISA assays, with a participant declared seropositive if at least one of those results was positive (>lower limit of quantitation [LLOQ]), and seronegative if both results were negative. Asymptomatic COVID-19 cases during the study were identified by polymerase chain reaction (PCR) or N-ELISA seroconversion.

### Statistical analysis

The statistical analysis was descriptive. Approximately 300 adolescents were to be enrolled.

Safety results are presented for the full analysis set, which included all participants with at least one documented vaccine administration. Immunogenicity results are presented for the per-protocol immunogenicity population, which included all randomized and vaccinated participants for whom immunogenicity data were available, excluding data from participants with protocol deviations expected to impact immunogenicity outcomes. Immunogenicity results obtained from participants after SARS-CoV-2 infection were censored.

Descriptive statistics (geometric mean concentrations/titers [GMC/GMT] and confidence intervals [CIs]) were calculated for continuous immunologic parameters. GMTs/GMCs were calculated using the $\log_{10}$ transformed antibody concentrations/titers 28 days post-dose 1- and 14-day post-dose 2. Geometric mean fold increases in antibody concentrations/titers over baseline and the corresponding 95% CI were calculated for each timepoint. For the calculation of geometric mean and median, values <LLOQ were imputed with LLOQ/2 and values above the upper limit of quantification (ULOQ) were imputed with the ULOQ. For the calculation of fold increases from baseline, values <LLOQ were imputed with the LLOQ and values >ULOQ were imputed with the ULOQ.

The percentage of participants with a response was estimated. A responder was a participant with a baseline value ≤LLOQ and a post-baseline value >LLOQ, or a baseline value >LLOQ and a post-baseline value 4-fold higher than baseline.

A *post hoc* analysis of Spike-binding antibodies excluded results from participants with a potential SARS-CoV-2 infection defined as a twofold increase in N-ELISA titer between any two consecutive time points. In view of the potential impact of COVID-19 infections on immunogenicity results, the *post hoc* analysis results are given below. Results of the main analysis that includes all subjects are provided in the Supplement Tables S1 to S4.

Humoral immune responses at lower dose levels/dose volume in adolescents were compared to those elicited in a population in whom vaccine efficacy was demonstrated. Peak post-dose 1 and post-dose 2 Spike-binding antibody responses were descriptively compared to those observed in young adults aged 18–25 years who received one or two standard Ad26.COV2.S doses ($5\times10^{10}$ vp) in Phase 3 studies (NCT04535453, NCT04614948[11] for SARS-CoV-2-naive participants, NCT05091307, NCT04908722 for SARS-CoV-2- seropositive participants).

## Results

### Study participants

The study was initiated in September 2021 and ended August 2023. A total of 300 adolescents aged 12–17 years were randomized and 299 were vaccinated (Figure 2), 55

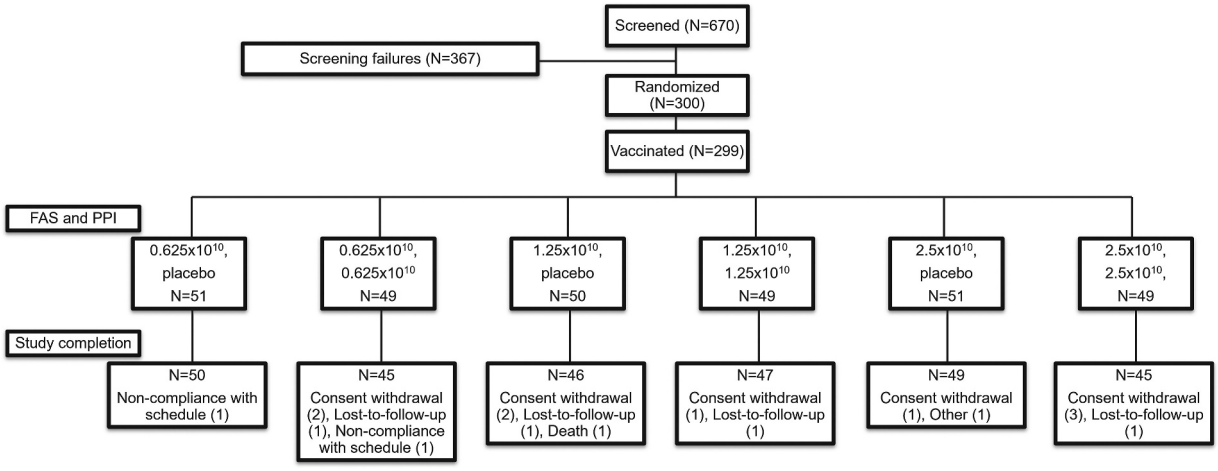


**Figure 2.** Participant flow.

(18.4%) from Brazil, 35 (11.7%) from Argentina, 37 (12.4%) from Mexico, 108 (36.1%) from India, and 64 (21.4%) from South Africa. One additional participant did not have a valid informed consent form but was vaccinated. This participant was therefore not included in any analysis. Seventeen participants discontinued the study prematurely, most frequently due to consent withdrawal by the participant or parent/guardian ($n = 9$). One participant ($1.25 \times 10^{10}$ vp/placebo group) died due to recreational drug overdose 125 days post-booster. This event was considered by the investigator to be unrelated to vaccination. No other study discontinuations were due to AEs.

Demographic and other baseline characteristics were balanced between groups (Table 1). The mean age of all participants was 14.2 years, 57.9% were male, and approximately equal proportions were Asian, Black or African American, or White. Most (69.2%) participants were aged 12–15 years due to pediatric COVID-19 vaccination programs targeting adolescents that commenced during the study.

All participants were seronegative based on rapid finger prick local serology tests at screening. However, on analysis of baseline serum samples with S- and N-ELISA, the majority of participants (220/299, 73.6%) were SARS-COV-2 seropositive at baseline.

## Reactogenicity and safety

All vaccine doses were well tolerated. Pain was the most frequently reported AE after active immunization (Figure 3). There was no apparent effect of dose or dose volume on the incidence or severity of solicited local AEs after primary vaccination with one or two doses. Pain and swelling appeared to be higher after the $2.5 \times 10^{10}$ vp booster dose than after primary vaccination following a 1-dose primary schedule

**Table 1.** Demographic characteristics of the study population.

| | $0.625 \times 10^{10}$, placebo | $0.625 \times 10^{10}$, $0.625 \times 10^{10}$ | $1.25 \times 10^{10}$, placebo | $1.25 \times 10^{10}$, $1.25 \times 10^{10}$ | $2.5 \times 10^{10}$, placebo | $2.5 \times 10^{10}$, $2.5 \times 10^{10}$ |
|---|---|---|---|---|---|---|
| Full Analysis Set | 51 | 49 | 50 | 49 | 51 | 49 |
| **Age (years)** | | | | | | |
| Mean (SD) | 14.3 (1.76) | 14.1 (1.84) | 14.0 (1.82) | 14.2 (1.80) | 14.1 (1.87) | 14.4 (1.75) |
| Range | (12; 17) | (12; 17) | (12; 17) | (12; 17) | (12; 17) | (12; 17) |
| 12–15 n (%) | 36 (70.6) | 34 (69.4) | 35 (70.0) | 34 (69.4) | 35 (68.6) | 33 (67.3) |
| 16–17 n (%) | 15 (29.4) | 15 (30.6) | 15 (30.0) | 15 (30.6) | 16 (31.4) | 16 (32.7) |
| **Sex** n (%) | | | | | | |
| Female | 21 (41.2) | 20 (40.8) | 22 (44.0) | 21 (42.9) | 22 (43.1) | 20 (40.8) |
| Male | 30 (58.8) | 29 (59.2) | 28 (56.0) | 28 (57.1) | 29 (56.9) | 29 (59.2) |
| **Race** n (%) | | | | | | |
| Asian | 17 (33.3) | 14 (28.6) | 19 (38.0) | 19 (38.8) | 17 (33.3) | 22 (44.9) |
| Black/African American | 16 (31.4) | 16 (32.7) | 13 (26.0) | 14 (28.6) | 17 (33.3) | 12 (24.5) |
| White | 14 (27.5) | 17 (34.7) | 13 (26.0) | 13 (26.5) | 13 (25.5) | 12 (24.5) |
| Other | 1 (2.0) | 2 (4.1) | 5 (10.0) | 2 (4.1) | 3 (5.9) | 1 (2.0) |
| Not reported/unknown | 3 (5.9) | 0 | 0 | 1 (2.0) | 1 (2.0) | 2 (4.1) |
| **Ethnicity** n (%) | | | | | | |
| Hispanic or Latino | 24 (47.1) | 26 (53.1) | 19 (38.0) | 16 (32.7) | 22 (43.1) | 17 (34.7) |
| Not Hispanic or Latino | 27 (52.9) | 23 (46.9) | 30 (60.0) | 31 (63.3) | 29 (56.9) | 32 (65.3) |
| Not reported/unknown | 0 | 0 | 1 (2.0) | 2 (4.1) | 0 | 0 |
| **SARS-CoV-2 serostatus at baseline*** n (%) | | | | | | |
| Positive | 41 (80.4) | 36 (73.5) | 34 (68.0) | 34 (69.4) | 38 (74.5) | 37 (75.5) |
| Negative | 10 (19.6) | 13 (26.5) | 16 (32.0) | 15 (30.6) | 13 (25.5) | 12 (24.5) |

SD, standard deviation.

*All participants tested negative using local rapid finger-prick serology tests. A majority of participants subsequently had positive baseline SARS-CoV-2 serostatus based on Day 1 S or N serology (ELISA), with a subject being declared seropositive if either one of these tests was positive.

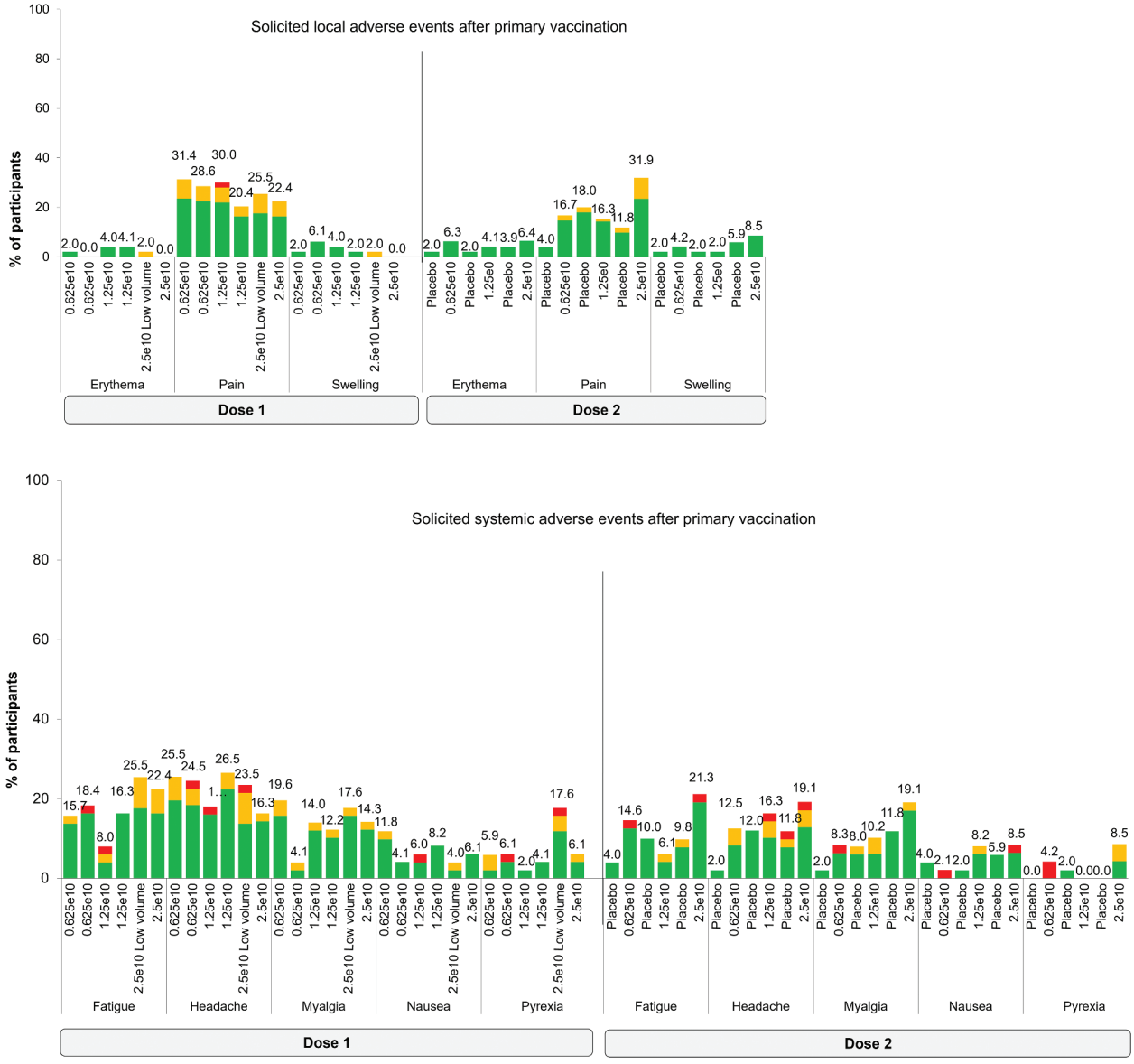


**Figure 3.** Percentage of participants with solicited local and solicited systemic adverse events after primary vaccination – full analysis set. Green = Grade 1; Orange = Grade 2; Red = Grade 3. Pain: Grade 1 = Aware of symptoms but easily tolerated, does not interfere with activity, discomfort only 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. Nausea: Grade 1 = minimal symptoms, causes minimal or no interference with work, school, or self-care 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: Hospitalization, inability to perform basic self-care functions.

(Figure 4). Across all doses, the medians for the duration of solicited local AEs ranged from 1–4.5 days.

The most frequently reported solicited systemic AEs after each dose were fatigue, headache, and myalgia (Figures 3, 4). There was no apparent effect of dose or dose volume on solicited systemic AEs after any dose. There was no increase in the incidence or severity of systemic AEs after dose 2 of the primary schedule, or after the booster dose following a 1-dose primary schedule. Across all doses, the median for the duration of solicited systemic AEs ranged between 1–3 days. The majority of local and systemic AEs were Grade 1. Grade 3 AEs were reported by no more than three participants in any dose level or group and did not increase in frequency after consecutive doses.

Unsolicited symptoms were reported by 6.1–20.4% of participants across study groups within 28 days after dose 1, 13.7–18.8% after dose 2, and 11.8–20.4% after the booster dose (Table S5). Grade 3 unsolicited AEs or any unsolicited AEs considered by the investigator to be vaccine-related were reported by no more than two participants in any group after any dose. There was no change in the number

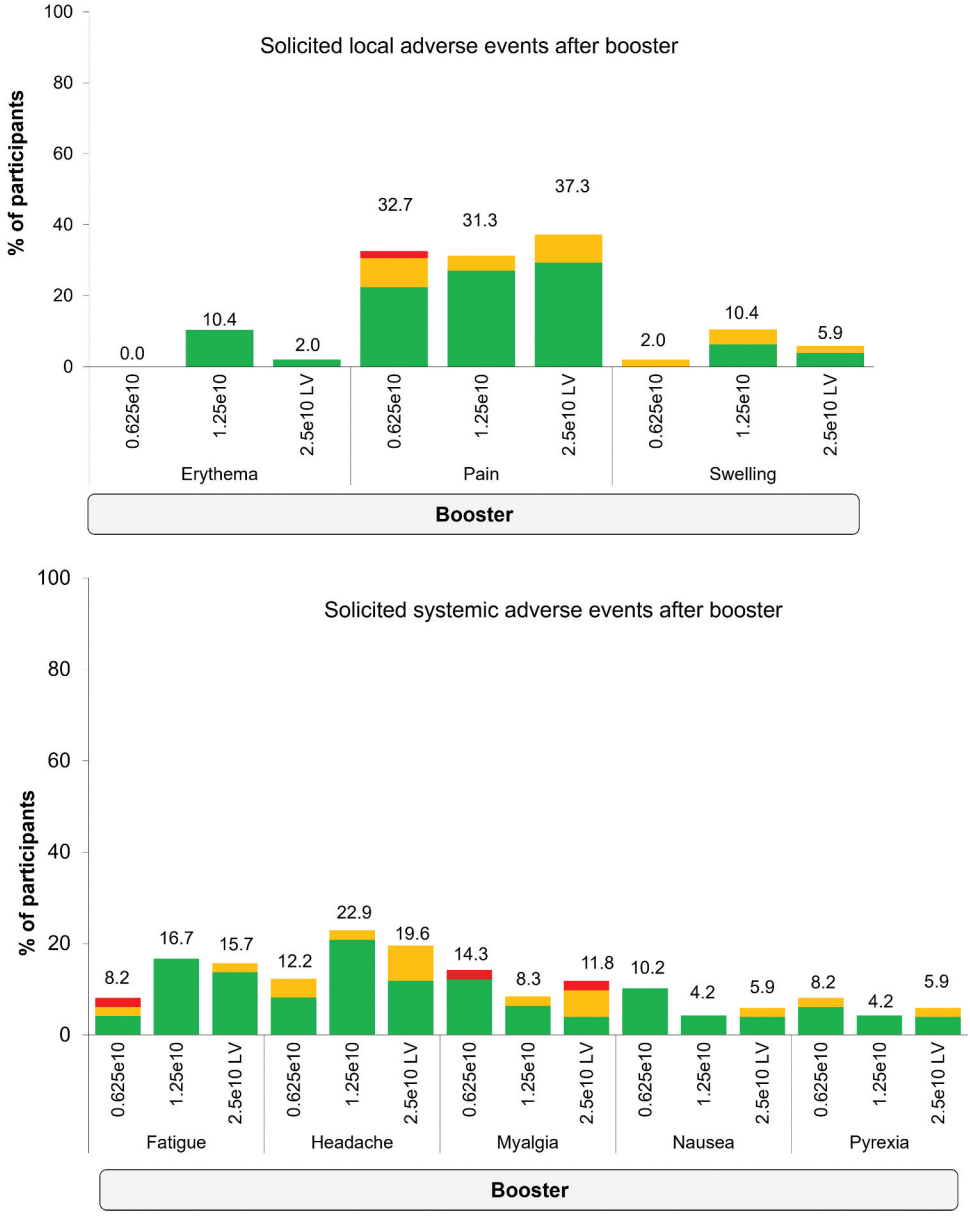


**Figure 4.** Percentage of participants with solicited local and solicited systemic adverse events after booster following a 1-dose primary schedule– full analysis set. Green = Grade 1; Orange = Grade 2; Red = Grade 3. Pain: Grade 1 = Aware of symptoms but easily tolerated, does not interfere with activity, 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. Nausea: Grade 1 = minimal symptoms, causes minimal or no interference with work, school, or self-care 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: Hospitalization, inability to perform basic self-care functions.

or intensity of AEs after each dose. In each group, most AEs were reported once. AEs occurring in at least 10% of participants in any study group over the entire study period were COVID-19 (reported by 7.8–13.7% of participants in each study group), influenza (4.1–11.8%), and upper respiratory tract infection (0–10.0%).

Over the entire study period MAAEs were reported by 4.1–18.4% of participants in each study group (Table S5). No MAAEs led to vaccine discontinuation.

In addition to the fatal recreational drug overdose mentioned above, two SAEs were reported in two participants; one femur fracture ($2.5 \times 10^{10}$ vp/placebo group) and one vaginal cyst excision ($1.25 \times 10^{10}$ vp/$1.25 \times 10^{10}$ vp group), both of which resolved and were considered by the Investigator to be unrelated to vaccination.

One participant in the $0.625 \times 10^{10}$ vp/$0.625 \times 10^{10}$ vp primary vaccination group reported asymptomatic Grade 2 "platelet count decreased" 1 day after the first vaccination that

remained stable throughout the study (nadir value 102,000/μl). As there was no concurrent thrombotic event, the suspected AESI event did not qualify for TTS assessment. No cases of multisystem inflammatory syndrome were reported.

### *Immunogenicity*

Spike-binding antibodies after 1- or 2-dose Ad26.COV2.S priming regimens in adolescents. All doses of Ad26.COV2.S elicited Spike-binding antibodies 28 days after a single dose administered to participants with preexisting SARS-CoV-2 immunity (Figure 5a). Fold increases from baseline at Day 29 ranged from 8.7 to 19.1 across all groups. The Spike-binding antibody GMT was 9441 (95% CI 6387–13,953) and 8741 (5860–13,038) in the $0.625 \times 10^{10}$ groups 15,918 (11,803–-21,467) and 11,096 (7975–15,438) in the $1.25 \times 10^{10}$ groups 10,887 (7365–16,093) in the $2.5 \times 10^{10}$ LV group, and 17,520 (11,993–25,594) in the $2.5 \times 10^{10}$ group (Table S6).

In 1-dose primary regimens, responses were durable up to at least 6 months post-dose 1 (Day 184). A boost with Ad26.COV2.S $2.5 \times 10^{10}$ vp at Day 184 induced 1.8- to 2.2-fold increases by 7 days post-boost compared to pre-booster levels. The post-booster GMT was 6749 (95% CI 4809–9470) in the $0.625 \times 10^{10}$ group, 8169 (5114–13,047) in the $1.25 \times 10^{10}$ group, and 6288 (4624–8552) in the $2.5 \times 10^{10}$ LV group. Spike-binding antibody GMTs declined by Day 366 but remained well above baseline levels (Figure 5a).

In 2-dose primary regimens, there was a minimal appreciable increase in Spike-binding antibody GMTs after the second Ad26.COV2.S vaccination. Antibody levels declined by 6 months post-dose 2 (Day 240) but remained well above baseline levels, and were similar to those observed at 6 months post-dose 1 in the groups that received a 1-dose primary schedule (Figure 5a).

The number of participants without preexisting SARS-CoV-2 immunity was low (10–16 participants per group at baseline). In this seronegative population, all doses of Ad26.COV2.S elicited Spike-binding antibodies 28 days after a single dose (Figure 5b). Fold increases from baseline at Day 29 ranged between 9.4 and 29.8 across groups. Spike-binding antibody GMTs were 535 (95% CI 338–847) and 475 (235–959) in the $0.625 \times 10^{10}$ groups, 1501 (703–3205) and 969 (554–1697) in the $1.25 \times 10^{10}$ groups, 1001 (480–2087) in the $2.5 \times 10^{10}$ LV group and 867 (404–1864) in the $2.5 \times 10^{10}$ group (Table S7).

In initially seronegative individuals in 2-dose priming groups, a second Ad26.COV2.S vaccination induced further increases in Spike-binding antibodies of 3.6-, 5.2- and 3.9-fold across increasing dose levels (Figure 5b). The number of results from initially seronegative individuals at subsequent timepoints was low, therefore no conclusions can be drawn and results are provided in Table S7.

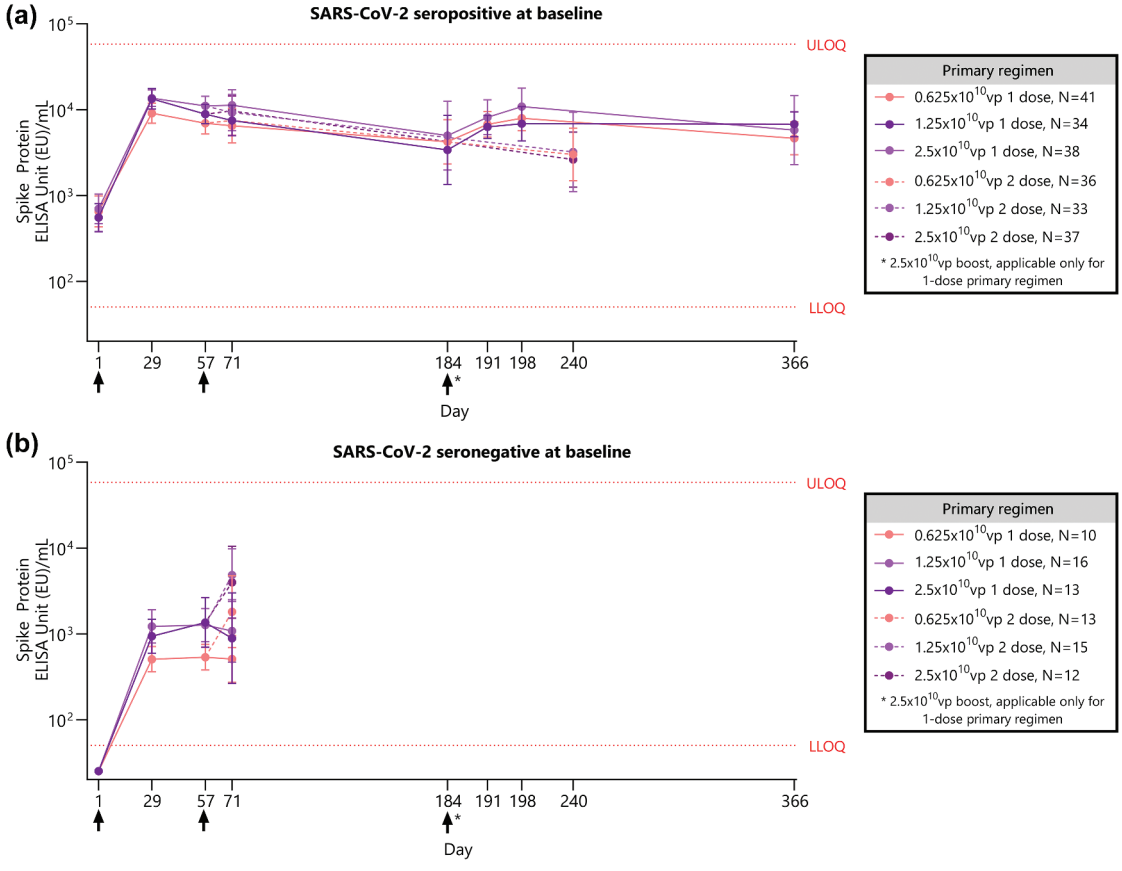


**Figure 5.** SARS-CoV-2 spike-binding antibody levels in adolescents with (a) and without (b) preexisting SARS-CoV-2 immunity. N, number of participants with data at baseline; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification. Solid lines = 1-dose regimens. Dotted lines = 2-dose regimens. Arrows indicate vaccination with Ad26.COV2.S at Day 1, Ad26.COV2.S or placebo at Day 57, and a boost with Ad26.COV2.S at Day 184 for groups receiving a 1-dose primary regimen. Up to Day 57, data from participants in the 1- and 2-dose primary regimens receiving the same dose level at Day 1 are combined. Data at later time points in participants without preexisting immunity is not shown due to the small sample size at these time points ($N < 2$). Geometric mean concentrations are shown as dots, with the corresponding 95% CIs.

### Comparison with responses in adults

All reduced Ad26.COV2.S doses administered to adolescents aged 12–17 years induced similar or higher Spike-binding antibody responses compared to the standard dose administered to young adults aged 18–25 years in Phase 3 studies (Figure 6). In seropositive participants, geometric mean increases from baseline up to Day 29 ranged between 12.3 and 18.8 in 12–17-year-olds, versus 9.5 in 18–25-year-olds. In seronegative participants, Spike-binding antibody GMTs were similar or higher in 12–17-year-olds (range 506–1219 EU/mL) versus 18–25-year-olds (480, 95% CI 391–589 EU/mL) (Table S8).

### Impact of pre-existing immunity to SARS-CoV-2 on spike-binding antibody responses

Spike-binding antibody responses elicited by Ad26.COV2.S were modulated by preexisting SARS-CoV-2 immunity. There was a high positive correlation between Spike-binding antibody levels prior to dose 1 or 2 in the primary regimen and those at peak post-vaccination time points (Figure 7a,b). In addition, increasing preexisting Spike binding antibody levels were correlated with lower fold increases after vaccination, with negative correlations observed post-dose 1 (Figure 7c) of the primary regimen. Post-dose 2, a weak negative correlation was observed as most individuals showed no fold increase in binding antibody responses, i.e., fold increases close to 1 (Figure 7d).

### Neutralizing antibody responses

In participants with preexisting SARS-CoV-2 immunity, all Ad26.COV2.S dose levels induced neutralizing titers against the reference strain (D614G) and SARS-CoV-2 variants of concern (Delta and Omicron BA.1), which are heterologous to the antigen in Ad26.COV2.S (Figure 8a–c) (Tables S9 to S14). In 1-dose groups, neutralizing titers against all three variants peaked on Day 29, declined by 6 months, but remained well above baseline levels. A booster dose at 6 months post-dose 1 elicited further increases in neutralizing responses against all three variants which were durable through 6 months post-boost.

In 2-dose groups there was little change in neutralizing titers after the second Ad26.COV2.S vaccination for all dose levels evaluated. Antibody titers declined by 6 months but remained well above baseline levels.

Among the limited number of seronegative participants in 1-dose groups, neutralizing titers against the reference strain were detectable at 28 days, whereas neutralizing titers against Delta and Omicron BA.1 were low or undetectable (Figure 8d–

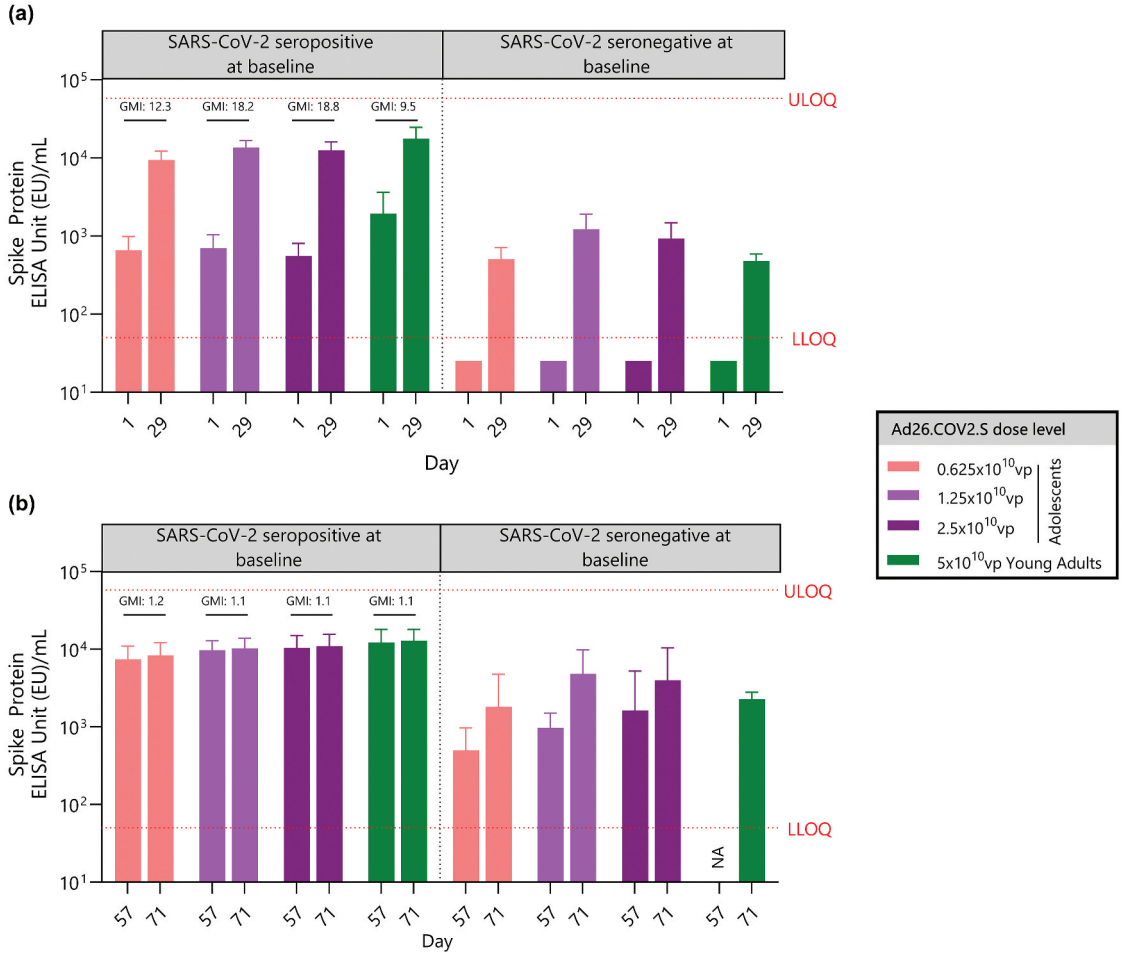


**Figure 6.** Peak SARS-CoV-2 spike-binding antibody levels (a) post-dose 1 and (b) post-dose 2 in adolescents 12–17 years (pink, light and dark purple) and young adults 18–25 years (green) with and without preexisting SARS-CoV-2 immunity.

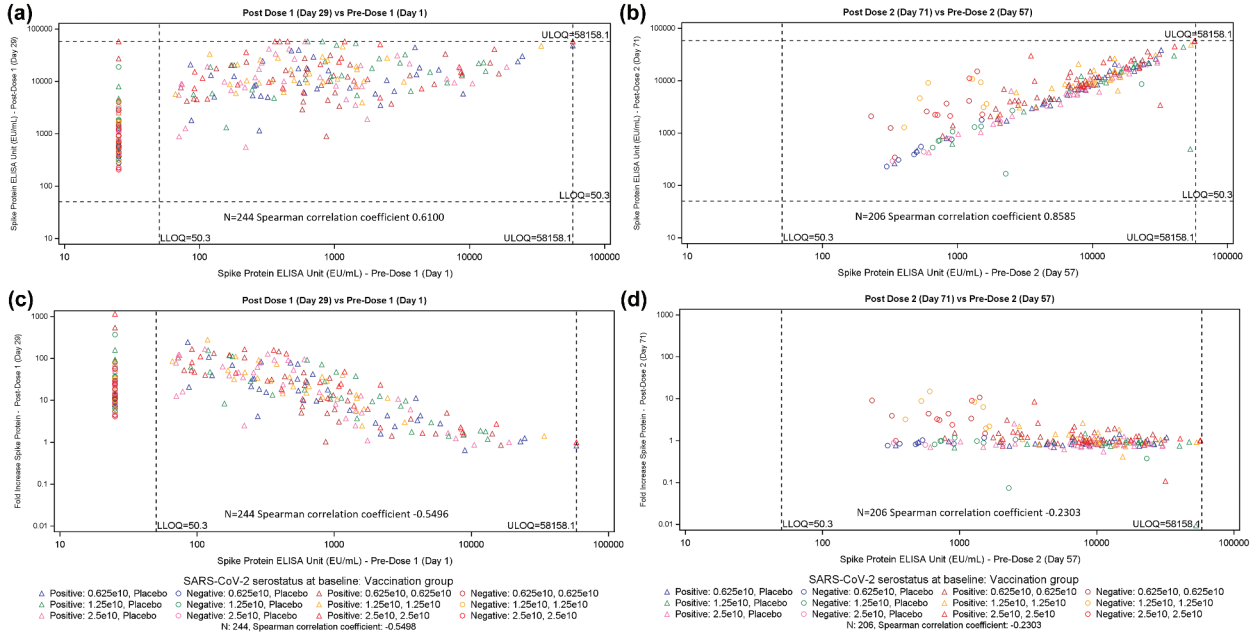


**Figure 7.** SARS-CoV-2 binding antibody levels prior to vaccination negatively correlate with fold increases post-vaccination. LLOQ, lower limit of quantification; ULOQ, upper limit of quantification. Correlations between Spike-binding antibody concentrations, as measured by S-ELISA, pre- and post-dose 1 (a) and pre- and post- dose 2 (b) are shown. Correlations between Spike binding antibody concentrations pre-dose 1 or 2 and fold increases 28 days post-dose 1 or 14 days post-dose 2 are shown in (c) and (d), respectively. Spearman correlation coefficients are shown for each correlation. Dotted lines indicate LLOQ and ULOQ for each assay.

f). A second Ad26.COV2.S vaccination at Day 57 elicited increases in neutralizing titers against the reference and Delta strains, while neutralizing titers against Omicron BA.1 were undetectable in most participants (Figure 8g).

## Discussion

In this study, we demonstrated that different dose levels, dose volumes, and dose regimens of Ad26.COV2.S were well tolerated in adolescents, with no safety concerns identified. The pattern of local and systemic solicited AEs was aligned with the known safety profile of Ad26.COV2.S in adults.[19] Grade 3 AEs were uncommon and no cases of TTS or multisystem inflammatory syndrome were observed, although this is not unexpected given the rarity of these AEs in the general population.[20]

All vaccine dose levels, dose volumes, and regimens elicited robust Spike-binding antibodies and neutralizing titers against the SARS-CoV-2 reference strain in adolescents. Previous studies have shown that the magnitude of antibody responses following COVID-19 is higher in children compared to adults.[21–23] In our study, reduced dose/dose volume of Ad26.COV2.S vaccine elicited strong immune responses in seropositive and seronegative adolescents that were comparable or higher to those observed in young adults in whom vaccine efficacy against moderate-to-severe COVID-19 was demonstrated after standard adult Ad26.COV2.S vaccination, suggesting similar efficacy in adolescents.[11] Humoral immune responses induced by Ad26.COV2.S persisted for at least 6 months post-vaccination, consistent with previous findings in adults.[24]

In agreement with studies using other COVID-19 vaccines,[25,26] circulating pre-vaccination Spike-binding antibodies negatively correlated with post-vaccination fold increases. Nevertheless, post-vaccination antibody levels were high in this population.

Hybrid immunity, i.e., immunity in individuals who received BNT162b2 COVID-19 vaccine or experienced COVID-19 prior to or after vaccination, has previously been associated with reduced SARS-CoV-2 infections.[27] Here we show broad cross-neutralizing antibody responses against the reference strain, Delta and Omicron BA.1 variants in adolescents with preexisting SARS-CoV-2 immunity. By contrast, limited-to-undetectable antibody responses against Delta and Omicron BA.1 were observed in SARS-CoV-2-naive participants. Our results underscore the benefits of hybrid immunity both in terms of the magnitude and breadth of antibody responses to SARS-CoV-2 and its variants in adolescents.

Ad26.COV2.S was voluntarily withdrawn by the sponsor in 2023. However, the adenovirus-vector vaccine platform remains a potent tool in vaccine development. Replication-deficient adenovirus vectors generally induce strong humoral and cell-mediated immune responses, have an acceptable safety profile, and are relatively easy to manufacture.[14] A major potential limitation is preexisting immunity against the adenovirus vector vaccine, which has been well documented for prevalent serotypes such as serotype 5, and which can attenuate antibody responses to the antigen inserts.[28,29] Ad26 is a low prevalence serotype and preexisting immunity is uncommon. Nevertheless, repeated exposure to Ad26 vaccines could potentially lead to anti-vector immunity with impacts on antigen responses. Studies in non-human primates and humans have shown no clear or consistent impact of anti-vector immunity on antibody responses to different target antigens.[30–33] These data support an ongoing future for Ad26 as vector in prophylactic and therapeutic vaccines.

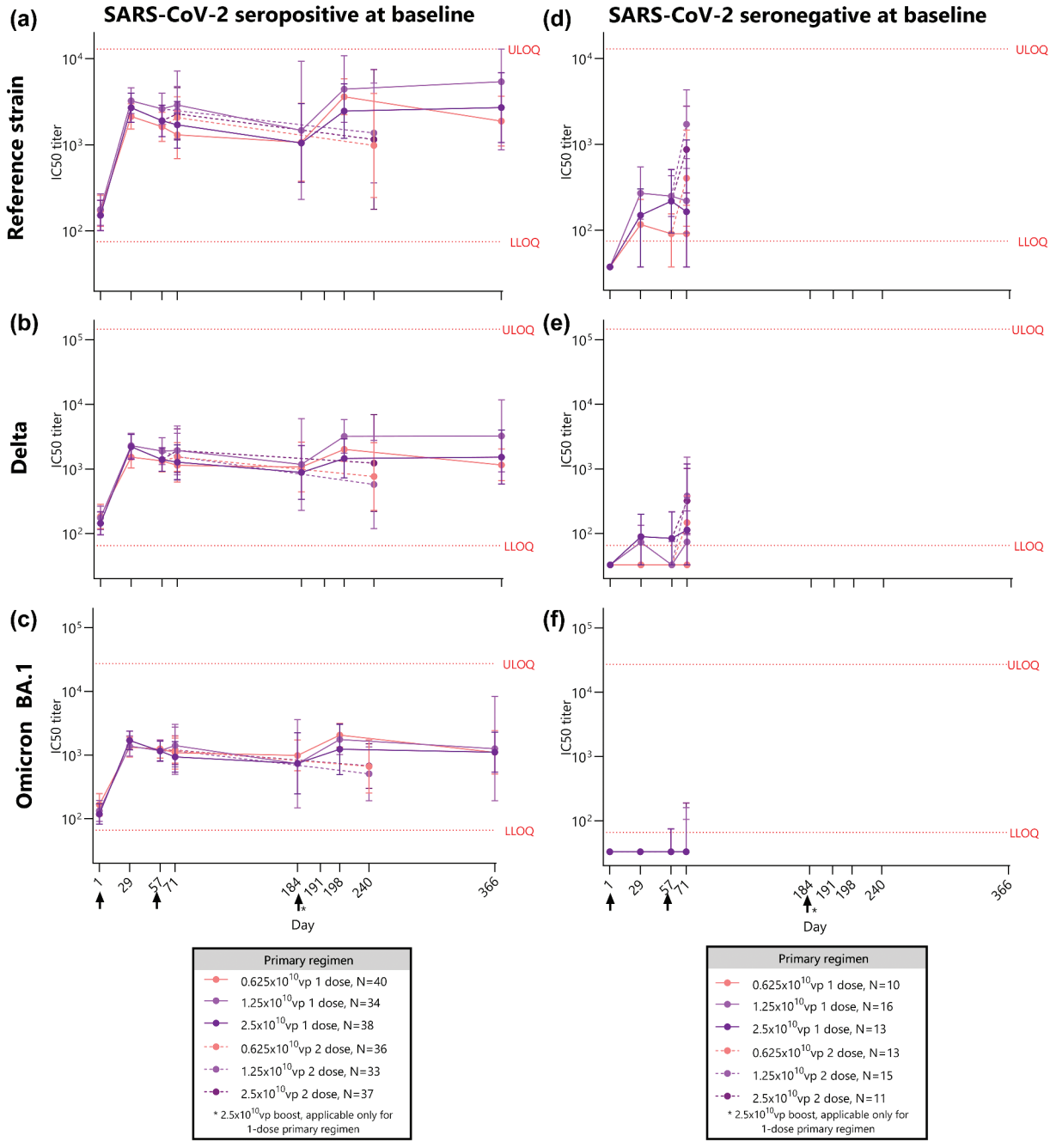


**Figure 8.** Neutralizing titers against the SARS-CoV-2 reference strain (D614G), delta and Omicron BA.1 in adolescents with and without preexisting SARS-CoV-2 immunity. LLOQ, lower limit of quantification; N. number of participants with data at baseline; ULOQ: upper limit of quantification. Neutralizing titers against the SARS-CoV-2 reference strain with a D614G mutation (a, d), Delta (b, e) and Omicron BA.1 (c, f), were measured at the indicated time points and are shown separately for participants with (a-c) and without (d-f) preexisting SARS-CoV-2 immunity. 1-dose regimens are shown with solid lines, while 2-dose regimens are shown with dotted lines. Arrows indicate vaccination with Ad26.COV2.S at Day 1, Ad26.COV2.S or placebo at Day 57, and a boost with Ad26.COV2.S at Day 184 for groups receiving a 1-dose primary regimen. Up to Day 57, data from participants in the 1- and 2-dose primary regimens receiving the same dose level at Day 1 is combined. Data at later time points in participants without preexisting immunity are not shown due to the small sample size at these time points ($N < 2$). Geometric mean concentrations are shown as dots, with the corresponding 95% CIs.

Potential study limitations are the low number of participants who were seronegative at baseline due to poor sensitivity of rapid finger-prick serology tests. In addition, antibody responses against more recent SARS-CoV-2 variants such as BA.2.75 or XBB.1.5, were not evaluated.

In conclusion, our findings add to insights into the safety and immunogenicity of Ad26.COV2.S in adolescents aged 12–17 years with and without preexisting SARS-CoV -2 immunity. Low vaccine doses of this adenovirus-vector vaccine induced robust immune responses in adolescents that were comparable to young adults vaccinated with a standard dose. The findings also shed light on the durability of immune responses, the influence of preexisting immunity on vaccine immunogenicity, and the potential benefits of hybrid immunity in the adolescent population. In more general terms, lower dose levels of this adenovirus-based vaccine elicited strong immune responses in adolescents that were comparable to young adults

vaccinated with a standard dose, which could be relevant for potential future use of vaccines that use this platform.

## COV3006 Study Group Collaborators

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## Disclosure statement

Jelena Tica was an employee of Johnson … Johnson at the time of this study.

Veronica V. Rezelj was an employee of Johnson & Johnson at the time of this study.

Benoit Baron is an employee of Johnson & Johnson.

Vitalija van Paassen is an employee of Johnson & Johnson and holds stock or shares in Johnson & Johnson, LLC.

Gert Scheper is an employee of Johnson & Johnson and holds stock or shares in Johnson & Johnson, LLC.

Mathieu Le Gars was an employee of Johnson & Johnson at the time of this study and holds stock or shares in Johnson & Johnson, LLC.

Frank Struyf was an employee of Johnson & Johnson at the time of this study and holds stock or shares in Johnson & Johnson, LLC. He holds shares in GSK as remuneration for past employment.

Macaya Douoguih was an employee of Johnson & Johnson at the time of this study and holds stock or shares in Johnson & Johnson, LLC.

Javier Ruiz-Guiñazú is an employee of Johnson & Johnson and holds stock or shares in Johnson & Johnson, LLC.

Javier Zaidman reports no conflict of interest.

Lee Fairlie reports receiving grant funding from Johnson & Johnson, LLC for study conduct.

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## Notes on contributor

*Javier Ruiz-Guiñazú*, MD, MSc, was born in 1969 in Argentina. He is a seasoned pediatrician and pediatric infectious diseases specialist with over 12 years of clinical practice. Dr. Ruiz-Guiñazú has extensive experience in vaccine clinical research, spanning more than two decades in both investigational site and industry roles. His expertise includes leading Phase I-III trials and engaging in regulatory interactions for approved vaccines. Currently, he is a Senior Director at Janssen Research & Development in Belgium, where he serves as the Medical Leader for the Extraintestinal pathogenic E. coli (ExPEC) vaccine program. Additionally, he played a pivotal role in the COVID-19 vaccine program. Dr. Ruiz-Guiñazú holds a Master's degree in Clinical Trials from the London School of Hygiene and Tropical Medicine and has contributed to numerous scientific publications in the field of infectious diseases and vaccine development.

## Authors contribution

Dr Jelena Tica: drafted the initial manuscript, design, data collection, performed analysis, writing – review and editing. Dr Veronica V. Rezelj: drafted the initial manuscript, design, data collection, performed analysis, writing – review and editing. Benoit Baron: design, data collection, performed analysis, writing – review and editing. Dr Vitalija van Paassen: design, performed analysis, writing – review and editing. Dr Javier Zaidman: data collection, writing – review and editing. Dr Lee Fairlie: data collection, writing – review and editing. Dr Gert Scheper: conceptualization, project administration, writing – review and editing. Dr Mathieu Le Gars: design, supervision, writing – review and editing. Dr Frank Struyf: writing – review and editing. Dr Macaya Douoguih: writing – review and editing. Dr Javier Ruiz-Guiñazú: conceptualization, design, performed analysis, writing – review and editing. All authors critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

## 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.

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