

ORIGINAL ARTICLE

Interim Results of a Phase 1–2a Trial of Ad26.COV2.S Covid-19 Vaccine

J. Sadoff, M. Le Gars, G. Shukarev, D. Heerwegh, C. Truyers, A.M. de Groot, J. Stoop, S. Tete, W. Van Damme, I. Leroux-Roels, P.-J. Berghmans, M. Kimmel, P. Van Damme, J. de Hoon, W. Smith, K.E. Stephenson, S.C. De Rosa, K.W. Cohen, M.J. McElrath, E. Cormier, G. Scheper, D.H. Barouch, J. Hendriks, F. Struyf, M. Dougouih, J. Van Hoof, and H. Schuitemaker

ABSTRACT

BACKGROUND

Efficacious vaccines are urgently needed to contain the ongoing coronavirus disease 2019 (Covid-19) pandemic of infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A candidate vaccine, Ad26.COV2.S, is a recombinant, replication-incompetent adenovirus serotype 26 (Ad26) vector encoding a full-length and stabilized SARS-CoV-2 spike protein.

METHODS

In this multicenter, placebo-controlled, phase 1–2a trial, we randomly assigned healthy adults between the ages of 18 and 55 years (cohort 1) and those 65 years of age or older (cohort 3) to receive the Ad26.COV2.S vaccine at a dose of 5×10^{10} viral particles (low dose) or 1×10^{11} viral particles (high dose) per milliliter or placebo in a single-dose or two-dose schedule. Longer-term data comparing a single-dose regimen with a two-dose regimen are being collected in cohort 2; those results are not reported here. The primary end points were the safety and reactogenicity of each dose schedule.

RESULTS

After the administration of the first vaccine dose in 805 participants in cohorts 1 and 3 and after the second dose in cohort 1, the most frequent solicited adverse events were fatigue, headache, myalgia, and injection-site pain. The most frequent systemic adverse event was fever. Systemic adverse events were less common in cohort 3 than in cohort 1 and in those who received the low vaccine dose than in those who received the high dose. Reactogenicity was lower after the second dose. Neutralizing-antibody titers against wild-type virus were detected in 90% or more of all participants on day 29 after the first vaccine dose (geometric mean titer [GMT], 224 to 354), regardless of vaccine dose or age group, and reached 100% by day 57 with a further increase in titers (GMT, 288 to 488) in cohort 1a. Titers remained stable until at least day 71. A second dose provided an increase in the titer by a factor of 2.6 to 2.9 (GMT, 827 to 1266). Spike-binding antibody responses were similar to neutralizing-antibody responses. On day 14, CD4+ T-cell responses were detected in 76 to 83% of the participants in cohort 1 and in 60 to 67% of those in cohort 3, with a clear skewing toward type 1 helper T cells. CD8+ T-cell responses were robust overall but lower in cohort 3.

CONCLUSIONS

The safety and immunogenicity profiles of Ad26.COV2.S support further development of this vaccine candidate. (Funded by Johnson & Johnson and the Biomedical Advanced Research and Development Authority of the Department of Health and Human Services; COV1001 ClinicalTrials.gov number, NCT04436276.)

The authors' full names, academic degrees, and affiliations are listed in the Appendix. Address reprint requests to Dr. Schuitemaker at Janssen Vaccines and Prevention, Newtonweg 1, Leiden 2301CA, the Netherlands, or at hschuite@its.jnj.com.

Drs. Sadoff and Le Gars contributed equally to this article.

This article was published on January 13, 2021, and last updated on January 21, 2021, at NEJM.org.

DOI: [10.1056/NEJMoa2034201](https://doi.org/10.1056/NEJMoa2034201)

Copyright © 2021 Massachusetts Medical Society.

THE ONGOING CORONAVIRUS DISEASE 2019 (Covid-19) pandemic that is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)^{1,2} has affected millions of people globally. To contribute to the containment of this pandemic — and to stop the pressure on health care systems and the negative effects on the global economy — efficacious Covid-19 vaccines are urgently needed.³⁻⁶

One of the candidate vaccines, Ad26.COV2.S, is a recombinant, replication-incompetent adenovirus serotype 26 (Ad26) vector encoding a full-length and stabilized SARS-CoV-2 spike (S) protein.^{7,8} The vaccine was derived from the first clinical isolate of the Wuhan strain (Wuhan 2019; whole genome sequence, NC_045512). The Ad26 vector is used in the Ebola vaccine that was approved by the European Medicines Agency and in vaccine candidates against respiratory syncytial virus, human immunodeficiency virus, and Zika virus.⁹⁻¹¹ Ad26-based vaccines are generally safe and highly immunogenic.⁹⁻¹¹ Here, we report the interim results of a multicenter, randomized, double-blind, placebo-controlled, phase 1–2a clinical trial (COV1001) involving healthy adults in two age cohorts to evaluate the safety, reactogenicity, and immunogenicity of Ad26.COV2.S.

METHODS

TRIAL DESIGN AND PARTICIPANTS

The trial was initiated on July 22, 2020, at 12 centers in Belgium and the United States. Trial participants included healthy adults between the ages of 18 and 55 years and those 65 years of age or older. The younger group was divided into cohort 1a (with a target enrollment of 375 participants) and cohort 1b (an exploratory cohort for in-depth analysis of immunogenicity, with a target enrollment of 25 participants). The older age group was included in cohort 3, with a target enrollment of 375 participants. In November 2020, enrollment was initiated in cohort 2 to collect longer-term data comparing a single-dose regimen with a two-dose regimen (as described in the Supplementary Appendix, available with the full text of this article at NEJM.org). Only the interim results in cohorts 1 and 3 are reported here. All the participants provided written informed consent before enrollment.

Participants in cohorts 1 and 3 received Ad26.COV2.S at a dose of either 5×10^{10} viral particles

(low dose) or 1×10^{11} viral particles (high dose) per milliliter, administered intramuscularly in a single-dose or two-dose schedule 56 days apart. The trial design called for an evaluation of the boosting effect of Ad26.COV2.S at 6 months and 1 year after vaccination with respect to safety, reactogenicity, and immunogenicity in each cohort. Additional details regarding the trial design are provided in Table S1 and Fig. S1 in the Supplementary Appendix and in the protocol, also available at NEJM.org.

TRIAL OVERSIGHT

The trial was reviewed and approved by the local ethics committee or institutional review board at each site. Janssen Vaccines and Prevention, one of the Janssen Pharmaceuticals companies acquired by Johnson & Johnson, was the regulatory sponsor of the trial and holder of the Investigational New Drug application. The trial was funded by Johnson & Johnson and the Biomedical Advanced Research and Development Authority of the Department of Health and Human Services. Janssen representatives designed and manufactured the vaccine candidate, designed the trial, developed the statistical analysis plan, and performed the analyses. The decision to submit the manuscript for publication was made by all authors, who vouch for the accuracy and completeness of the reported data and for the fidelity of the trial to the protocol. No one who is not an author contributed to the writing of the manuscript.

PROCEDURES

In cohorts 1 and 3, we randomly assigned the participants in a 1:1:1:1:1 ratio to one of five vaccination groups: low dose followed by low dose, low dose followed by placebo, high dose followed by high dose, high dose followed by placebo, and placebo followed by placebo (Fig. S1). Data that are reported here were collected after the administration of the second dose (either vaccine or placebo) in cohort 1a and after the first dose in cohort 3. Randomization was performed by means of an interactive Web-response system and stratified according to site with the use of randomly permuted blocks. Participants and investigators remained unaware of trial-group assignments throughout the trial. To meet the criteria for blinding, the sponsor and statisticians were informed about group assignments for the primary analysis of results in cohorts 1 and 3 after 8 days

had elapsed since the administration of the second vaccine dose in all the participants.

PRIMARY AND SECONDARY END POINTS

The primary end points were the safety and reactogenicity of each dose schedule. Follow-up visits to evaluate reactogenicity, safety, and immunogenicity were scheduled on days 7, 28, and 71 after vaccination in each cohort. We collected data regarding solicited adverse events from patients' diary cards for 7 days after vaccination, data regarding unsolicited adverse events for 28 days after vaccination, and data regarding serious adverse events throughout the course of the trial after each vaccination. Adverse events were graded according to the guidance document Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials of the Food and Drug Administration. Safety data are included up to the cutoff date of October 30, 2020, in cohorts 1 and 3. The secondary end point was humoral and cellular immunity to the SARS-CoV-2 S protein.

LABORATORY ANALYSES

We used an enzyme-linked immunosorbent assay (ELISA) to measure SARS-CoV-2 S-specific binding antibodies at baseline and on days 15, 29, 57, and 71. Seropositivity was defined as a titer above the lower limit of quantitation of the assay (50.3 EU per milliliter). Clinicians at Public Health England measured SARS-CoV-2 serum neutralizing-antibody titers in a random subgroup of samples by means of a wild-type virus microneutralization assay using the Victoria/1/2020 SARS-CoV-2 strain, with seropositivity defined as a 50% maximal inhibitory concentration (IC_{50}) titer of more than 58 at the lower limit of quantitation. IC_{80} titers on wild-type virus microneutralization assay were also assessed at these time points. S-specific T-cell responses were measured at baseline and on day 15 by intracellular cytokine staining with the use of two pools of S-peptide pools of 15 mers overlapping by 11 amino acids. In CD4+ T cells, a response in type 1 helper T (Th1) cells was characterized by the expression of interferon- γ , interleukin-2, or both and not interleukin-4, interleukin-5, or interleukin-13; a response in type 2 helper T (Th2) cells was characterized by the expression of interleukin-4, interleukin-5, or interleukin-13 (or all three cytokines) plus CD40L. All assays were conducted in

a blinded fashion and are described in detail in the Supplementary Appendix.

STATISTICAL ANALYSIS

We determined that the enrollment of approximately 805 participants would provide a descriptive safety and immunogenicity assessment. We first enrolled 15 participants (3 per vaccination group) in cohort 1a; after the review of safety data for the sentinel enrollees, we proceeded to full enrollment of cohort 1a. The same process was used in the enrollment of participants in cohort 3. We used log-transformed data to calculate the confidence intervals of the geometric means. Details regarding the statistical analysis plan are provided in the Supplementary Appendix and in the protocol.

RESULTS

PARTICIPANTS

From July 22 to August 7, 2020, a total of 593 persons underwent screening for enrollment in cohort 1 (including 1a and 1b combined) (Fig. S1). Of these persons, 405 were enrolled and 402 received the first dose of Ad26.COV2.S; these participants had received the second dose by November 7, 2020. From August 3 to August 24, 2020, a total of 660 persons underwent screening for cohort 3. Of these participants, 405 were enrolled and 403 received the first dose of Ad26.COV2.S. (Details regarding age distribution are provided in Table S2.) Analyses of data obtained from participants in cohort 3 after the administration of the second dose, as well as durability and longer-term safety data, are ongoing.

At baseline, the percentage of participants who were seropositive for SARS-CoV-2 S-specific antibodies was 2% in cohort 1a and 1% in cohort 3. The baseline characteristics of the participants were broadly similar across the groups (Table 1).

VACCINE SAFETY AND REACTOGENICITY

Data regarding both solicited and unsolicited adverse events and serious adverse events were available for more than 99% of the participants who returned diary cards. The investigator's assessment of reactogenicity after the administration of the first dose of vaccine was available for 402 participants in cohort 1 and for 403 participants in cohort 3. In the two cohorts, solicited local adverse events were mostly of grade 1 or 2; the

Table 1. Characteristics of the Participants at Baseline.*				
Characteristic	Low-Dose Vaccine Group	High-Dose Vaccine Group	Placebo Group	All Participants
Cohort 1 (ages 18–55 yr)†				
No. of participants	162	158	82	402
Sex — no. (%)				
Male	78 (48)	72 (46)	40 (49)	190 (47)
Female	84 (52)	85 (54)	42 (51)	211 (52)
Nonbinary	0	1 (1)	0	1 (<1)
Age — yr				
Mean	36.1±10.1	34.8±10.3	35.4±10.0	35.4±10.2
Range	18–55	19–55	19–55	18–55
Race or ethnic group — no. (%)‡				
White	149 (92)	145 (92)	70 (85)	364 (91)
Black	4 (2)	7 (4)	9 (11)	20 (5)
Asian	5 (3)	5 (3)	0	10 (2)
Native Hawaiian or other Pacific Islander	1 (1)	0	0	1 (<1)
American Indian or Alaska Native	3 (2)	0	0	3 (1)
Hispanic or Latino	8 (5)	5 (3)	4 (5)	17 (4)
Multiple	0	1 (1)	0	1 (<1)
Unknown	0	0	3 (4)	3 (1)
Body-mass index§	24.5±3.3	24.6±3.1	24.5±3.0	24.6±3.2
SARS-CoV-2 seropositive — no. (%)¶	3 (2)	2 (1)	2 (2)	7 (2)
Cohort 3 (age ≥65 yr)				
No. of participants	161	161	81	403
Sex — no. (%)				
Male	84 (52)	79 (49)	38 (47)	201 (50)
Female	77 (48)	82 (51)	43 (53)	202 (50)
Age — yr				
Mean	69.6±4.0	70.0±4.2	69.9±3.7	69.8±4.0
Range	65–83	65–88	65–79	65–88
Race or ethnic group — no. (%)				
White	158 (98)	158 (98)	81 (100)	397 (99)
Black	1 (1)	2 (1)	0	3 (1)
American Indian or Alaska Native	1 (1)	0	0	1 (<1)
Hispanic or Latino	1 (1)	2 (1)	3 (4)	6 (1)
Unknown	1 (1)	0	0	1 (<1)
Not reported	0	1 (1)	0	1 (<1)
Body-mass index§	25.3±2.8	25.5±2.7	25.2±3.1	25.4±2.8
SARS-CoV-2 seropositive — no. (%)	1 (1)	2 (1)	1 (1)	4 (1)

* Plus–minus values are means ±SD. The participants in cohorts 1 and 3 received Ad26.COV2.S at a dose of either 5×10^{10} viral particles (low-dose group) or 1×10^{11} viral particles (high-dose group) in a 1-ml volume. The participants were grouped according to pooled groups (low dose followed by low dose together with low dose followed by placebo, high dose followed by high dose together with high dose followed by placebo, and placebo followed by placebo).

† Cohort 1 includes both cohorts 1a and 1b.

‡ Race or ethnic group was reported by the participants, who could report more than one category.

§ The body-mass index is the weight in kilograms divided by the square of the height in meters. This calculation was based on the weight and height measured at the time of screening.

¶ Only seronegative participants were enrolled in cohort 1b, according to the protocol.

most frequent event was injection-site pain. In cohort 1, solicited local adverse events were reported in 103 of 162 low-dose recipients (64%), in 123 of 158 high-dose recipients (78%), and in 7 of 82 placebo recipients (9%) (Fig. 1A and Table S3). In cohort 3, solicited local adverse events were reported in 66 of 161 low-dose recipients (41%), in 68 of 161 high-dose recipients (42%), and in 11 of 81 placebo recipients (14%) (Fig. 1B).

In the two cohorts, most solicited systemic adverse events were of grade 1 or 2; the most frequent events were fatigue, headache, and myalgia. In cohort 1, solicited systemic adverse events were reported in 105 low-dose recipients (65%), in 133 high-dose recipients (84%), and in 21 placebo recipients (26%). In cohort 3, solicited systemic adverse events were reported in 74 low-dose recipients (46%), in 88 high-dose recipients (55%), and in 19 placebo recipients (23%).

In cohort 1, solicited grade 3 systemic adverse events were reported in 15 low-dose recipients (9%) and in 32 high-dose recipients (20%); no placebo recipients reported such events. In cohort 1a, among the participants between the ages of 18 and 30 years who had one or more solicited grade 3 adverse events, 24% had received the low dose and 26% had received the high dose; in those between the ages of 31 and 45 years, the corresponding percentages were 43% and 14%; and in those between the ages of 46 and 55 years, the corresponding percentages were 3% and 11%. In cohort 3, grade 3 solicited systemic adverse events were reported in 1 low-dose recipient (1%) and in 4 high-dose recipients (2%); no placebo recipients reported having such events.

In cohort 1, fever was reported in 25 low-dose recipients (15%) and in 62 high-dose recipients (39%); grade 3 fever (temperature range, 39.0 to 40.0°C) was reported in 8 low-dose recipients (5%) and in 15 high-dose recipients (9%). In cohort 3, fever was reported in 7 low-dose recipients (4%) and in 14 high-dose recipients (9%); grade 3 fever was reported in no low-dose recipients and in 2 high-dose recipients (1%). No participants in the placebo group in either cohort reported having fever. All cases of fever occurred within 2 days after immunization and resolved within 1 or 2 days; more than 80% of the participants with fever received an antipyretic drug at the onset of symptoms.

In cohort 1, unsolicited adverse events were

reported in 34 low-dose recipients (21%), in 56 high-dose recipients (35%), and in 14 placebo recipients (17%). In cohort 3, unsolicited adverse events were reported in 27 low-dose recipients (17%), in 38 high-dose recipients (24%), and in 13 placebo recipients (16%) (Table S4). No grade 4 adverse events (solicited or unsolicited) were reported in any cohort.

In cohort 1a, safety data after the administration of the second dose of vaccine were available for 363 participants (Fig. S2). One or more solicited adverse events were noted in 77% and 80% of the participants in the low-dose and high-dose groups, respectively, as compared with 34% and 31% of those who received placebo as a second dose after a first dose of vaccine and in 22% of those who received placebo for both doses. Solicited adverse events of grade 3 or higher were noted in 1% of low-dose recipients and in 7% of high-dose recipients; the corresponding percentages were 1% and 2% among participants in the placebo group who received a first dose of vaccine and in no participants who received placebo for both doses. No grade 3 fevers were reported in any group after a second dose of vaccine.

No participant discontinued the trial because of an adverse event. Five serious adverse events occurred: one case of hypotension that was deemed by the investigator to be unrelated to the vaccine because of a history of recurrent hypotension; one case of bilateral nephrolithiasis in a participant with a history of kidney stones (not related); one case of legionella pneumonia (not related); one worsening of multiple sclerosis, which had remained undiagnosed for approximately 8 to 10 years on the basis of findings on magnetic resonance imaging (not related); and one case of fever that resulted in hospitalization because of suspicion of Covid-19. In the last case, the participant recovered within 12 hours, and the fever was subsequently deemed by the investigator to be related to the vaccine. Details regarding all safety data are provided in the Supplementary Appendix.

IMMUNOGENICITY AND SEROCONVERSION

Immunogenicity data for this interim analysis were unblinded according to dose level. In all five groups in cohort 1a, the binding-antibody geometric mean concentration (GMC), as reported in ELISA units per milliliter, was measured against

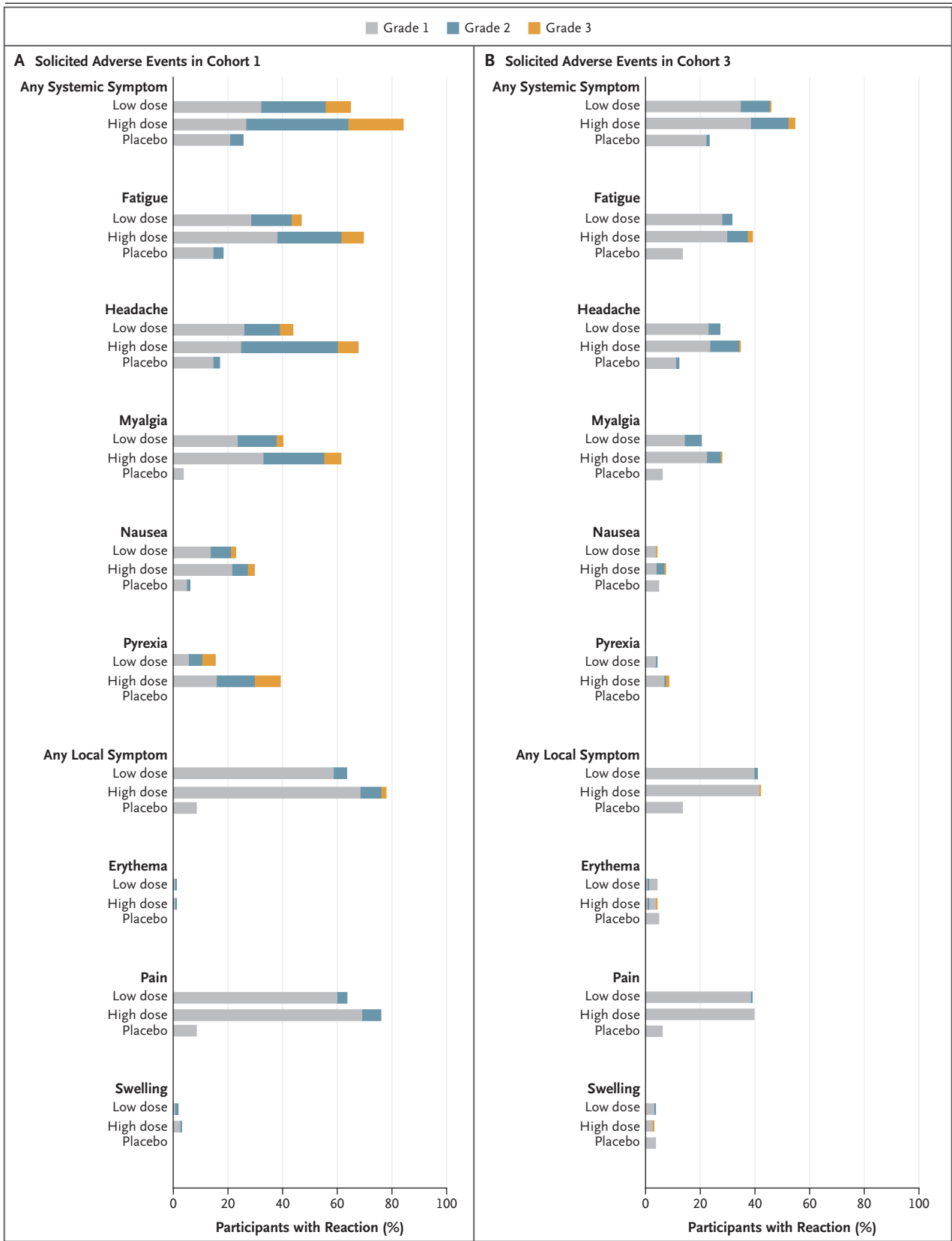


Figure 1 (facing page). Solicited Adverse Events in Cohorts 1 and 3 after the First Vaccine Dose.

Shown are solicited adverse events in participants who received the Ad26.COVID-19 vaccine at a dose of 5×10^{10} viral particles (low dose) or 1×10^{11} viral particles (high dose) per milliliter or placebo. Healthy adults between the ages of 18 and 55 years were included in cohort 1 (Panel A), and those 65 years of age or older were included in cohort 3 (Panel B). The younger group was divided into cohorts 1a and 1b, with the latter designated as an exploratory cohort for in-depth analysis of immunogenicity. As shown here, data for cohorts 1a and 1b have been pooled. Data for patients in cohort 1a who received a second dose of vaccine are provided in Figure S2 in the Supplementary Appendix.

a stabilized SARS-CoV-2 full-length spike protein. At baseline, the GMC values in all the participants were lower than the lower limit of quantitation. By day 29 after vaccination, the values had increased to 478 (95% confidence interval [CI], 379 to 603) in the low-dose/placebo group, 586 (95% CI, 445 to 771) in the low-dose/low-dose group, 625 (95% CI, 505 to 773) in the high-dose/placebo group, and 788 (95% CI, 628 to 988) in the high-dose/high-dose group, with an incidence of seroconversion of 99% or more in all the groups (Fig. 2A and Fig. S3A). By day 57, the corresponding GMC values had further increased to 660 (95% CI, 513 to 849), 754 (95% CI, 592 to 961), 873 (95% CI, 701 to 1087), and 1100 (95% CI, 908 to 1332). After the first dose, the incidence of seroconversion was 100% in all but the high-dose/placebo group (97%). Fourteen days after the second dose, the GMC was 1677 (95% CI, 1334 to 2109) in the low-dose/low-dose group and 2292 (95% CI, 1846 to 2845) in the high-dose/high-dose group, with 100% seroconversion in each group. On day 71, in the low-dose/placebo and high-dose/placebo groups, the GMC was 600 (95% CI, 443 to 814) and 951 (95% CI, 696 to 1,300), respectively, values that were similar to those on day 57.

In cohort 3, the GMCs in all the participants were also below the lower limit of quantitation at baseline. By day 15 after vaccination, the GMC had increased to 122 (95% CI, 97 to 152) in the low-dose group and to 141 (95% CI, 114 to 175) in the high-dose group, with a seroconversion incidence of 75% and 77%, respectively. By day 29, the GMC was 312 (95% CI, 246 to 396) in the low-dose group and 350 (95% CI, 281 to 429) in the high-dose group, with 96% seroconversion.

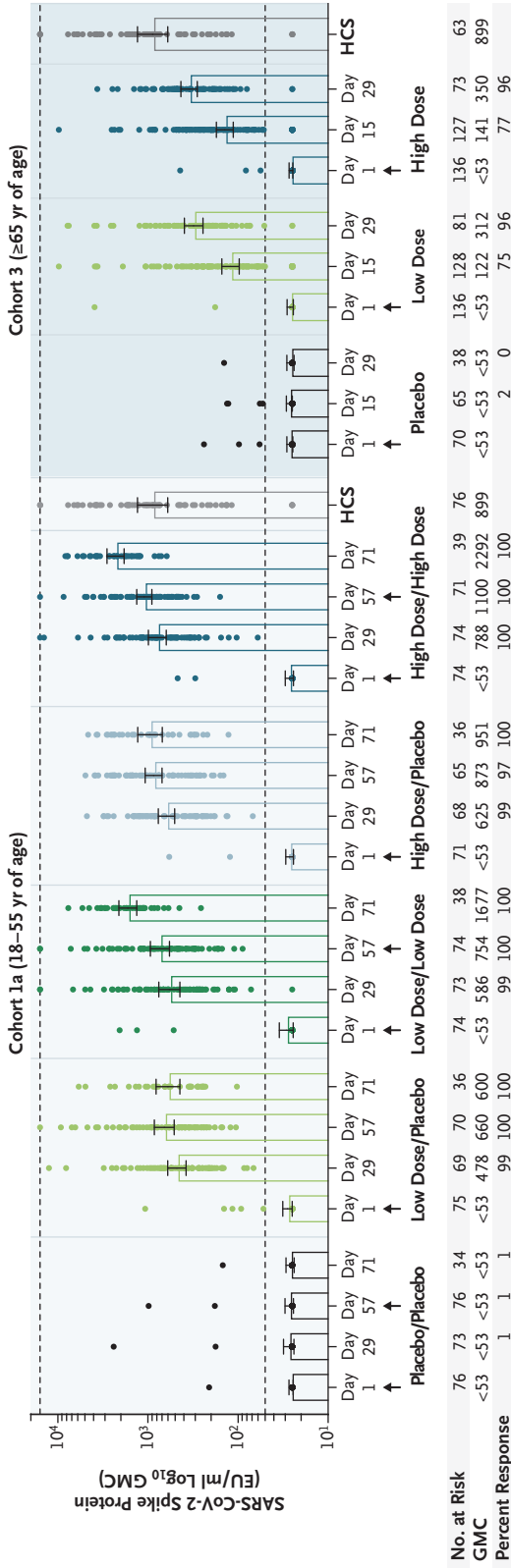
The SARS-CoV-2 neutralizing-antibody titer (IC_{50}) was measured in a random subgroup of participants in cohorts 1a and 3. In cohort 1a, the geometric mean titer (GMT) was below the lower limit of quantitation at baseline and by day 29 after vaccination had increased to 224 (95% CI, 158 to 318) in the low-dose/placebo group, 224 (95% CI, 168 to 298) in the low-dose/low-dose group, 215 (95% CI, 169 to 273) in the high-dose/placebo group, and 354 (95% CI, 220 to 571) in the high-dose/high-dose group, with an incidence of seroconversion of 96%, 88%, 96%, and 92%, respectively (Fig. 2B and Fig. S3B). By day 57, the GMT had further increased to 310 (95% CI, 228 to 422), 288 (95% CI, 221 to 376), 370 (95% CI, 268 to 511), and 488 (95% CI, 334 to 714), respectively, with a 100% incidence of seroconversion in the low-dose/placebo group and 96% seroconversion in the other groups.

In cohort 1a, 14 days after the second dose, the GMT was 827 (95% CI, 508 to 1183) in the low-dose/low-dose group and 1266 (95% CI, 746 to 2169) in the high-dose/high-dose group, with 100% seroconversion in the two dose groups. On day 71, the GMT was 321 (95% CI, 227 to 438) in the low-dose/placebo group and 388 (95% CI, 290 to 509) in the high-dose/placebo group, values that were similar to those on day 57; the incidence of seroconversion was 100% in both groups.

In cohort 3, the GMTs in all the participants were below the lower limit of quantitation at baseline and had increased to 212 (95% CI, 137 to 284) in the low-dose group and 172 (95% CI, 119 to 269) in the high-dose group on day 15 and to 277 (95% CI, 193 to 307) and 212 (95% CI, 163 to 266), respectively, on day 29. The incidence of seroconversion was 91% and 84%, respectively, on day 15 and 96% and 88%, respectively, on day 29. These data were confirmed on IC_{80} analysis (Fig. S4).

Antibody levels as measured on wild-type virus neutralization assay and ELISA were strongly correlated in the two cohorts (Fig. S5). However, the correlation had a wider elliptical shape in cohort 3, which suggested more variability in the relationship between the neutralizing-antibody titer and the binding-antibody titer in the older adults. Antibody levels in the different human convalescent serum panels that were included in assays for humoral-immunity assessment that were performed in different laboratories and in serum samples that were obtained from vaccine recipi-

A ELISA Analysis



B Virus Neutralization Assay

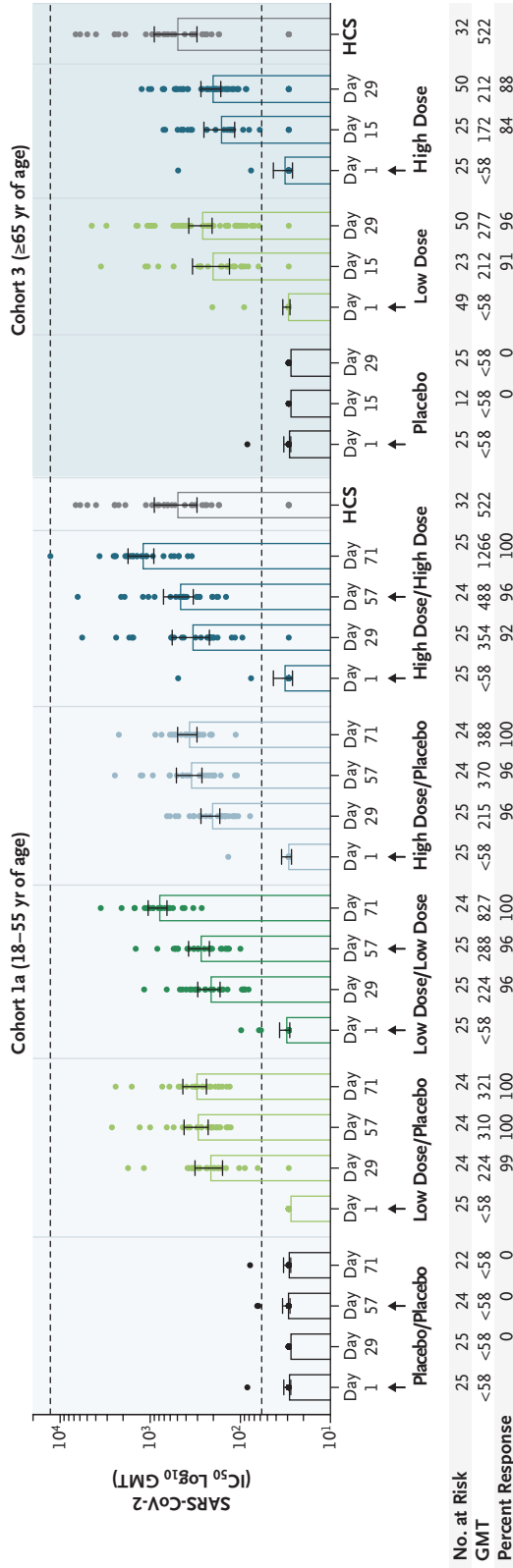


Figure 2 (facing page). Humoral Immunogenicity.

Shown are measures of humoral immunogenicity in serum samples obtained from the participants in cohort 1a (left side) and cohort 3 (right side), according to the receipt of the low or high dose of Ad26.COV2.S or placebo. In cohort 1a, the participants received two injections of high-dose or low-dose vaccine or placebo, as indicated with slashes (e.g., placebo/placebo if they received two injections of placebo). The samples were measured on enzyme-linked immunosorbent assay (ELISA) in ELISA units (EU) per milliliter (Panel A) and on wild-type virus neutralization assay, with seropositivity defined as a half maximal inhibitory concentration (IC₅₀) titer of more than 58 at the lower limit of quantitation (Panel B). Logarithmic values are reported as the geometric mean concentration (GMC) in the ELISA analyses and as the geometric mean titer (GMT) in the neutralizing-antibody analyses. The values were measured at baseline and at day 29 after vaccination in all the participants and on days 57 and 71 in those in cohort 1a. The two horizontal dotted lines in each panel indicate the lower and upper limits of quantitation of the respective assay; values below the lower line have been imputed to half the lower limit of quantitation. I bars indicate 95% confidence intervals. HCS denotes human convalescent serum.

dose recipient in cohort 3 had a measurable Th2 response (Fig. 3B). However, all the participants who had a measurable Th1 or Th2 response had a Th1:Th2 ratio that was well above 1, which indicated a vaccine-induced Th1-skewed response.

S-specific CD8+ T-cell responses, as identified by the expression of interferon- γ or interleukin-2 cytokines on S-peptide stimulation, were absent at baseline in the two cohorts (Fig. 3C). On day 15 in cohort 1a, a CD8+ T-cell response was detected in 51% of participants (95% CI, 39 to 63) in the low-dose group and in 64% (95% CI, 52 to 75) in the high-dose group, with a median S-specific CD8+ T-cell response of 0.07% (IQR, 0.03 to 0.19) and 0.09% (IQR, 0.05 to 0.19), respectively. In cohort 3, CD8+ T-cell responses were lower, with an incidence of 36% (95% CI, 23 to 51) in the low-dose group and 24% (95% CI, 13 to 37) in the high-dose group, with a median response of 0.06% (IQR, 0.02 to 0.12) and 0.02% (IQR, 0.01 to 0.08), respectively. The correlation between CD4+ Th1 and CD8+ T-cell response was poor in the two cohorts (Fig. S7).

ents were in the same range. Details regarding differences in values according to demographic characteristics are provided in Tables S5 and S6 in the Supplementary Appendix. Levels of Ad26 neutralizing antibodies at baseline or after the first dose of vaccine did not correlate with the levels of SARS-CoV-2 neutralizing antibodies on either day 29 or day 71 (Fig. S6).

S-SPECIFIC T-CELL RESPONSES

The vaccine-elicited responses in S-specific CD4+ Th1 and Th2 cells and in CD8+ T cells were assessed in a subgroup of participants at baseline and 15 days after the first dose. In cohort 1a, a Th1 response to S peptides was detected in 76% (95% CI, 65 to 86) of low-dose recipients and in 83% (95% CI, 73 to 91) of high-dose recipients; the corresponding values in cohort 3 were 60% (95% CI, 46 to 74) and 67% (95% CI, 53 to 79), respectively (Fig. 3A). In cohort 1a, the median CD4+ Th1 response to S peptides increased from an undetectable level at baseline to a median of 0.08% (interquartile range [IQR], 0.05 to 0.16) in low-dose recipients and 0.11% (IQR, 0.07 to 0.16) in high-dose recipients on day 15; in cohort 3, the corresponding values were 0.09% (IQR, 0.04 to 0.17) and 0.11% (IQR, 0.04 to 0.15), respectively. A low-dose recipient in cohort 1a and a high-

DISCUSSION

The interim analysis of our phase 1–2a trial showed that the Ad26.COV2.S vaccine had an acceptable safety and reactogenicity profile and was immunogenic after a single vaccination with either the low or high dose. After the administration of the first dose, a trend toward a higher incidence of solicited systemic adverse events was noted with the higher vaccine dose, and a clear trend for decreasing grade 3 adverse events with increasing age was observed. The local and systemic reactions occurred on the day of immunization or the next day and generally resolved within 24 hours. The systemic reactions were very responsive to antipyretic drugs, and no need for the prophylactic use of such drugs was identified. After the second dose among participants between the ages of 18 and 55 years, the incidence of grade 3 solicited systemic adverse events was much lower than that after the first immunization in both the low-dose and high-dose groups, a finding that contrasts with observations with respect to messenger RNA–based vaccines, for which the second dose has been associated with increased reactogenicity.^{4,6}

Although all ongoing phase 3 studies of other Covid-19 vaccines have assessed two-dose sched-

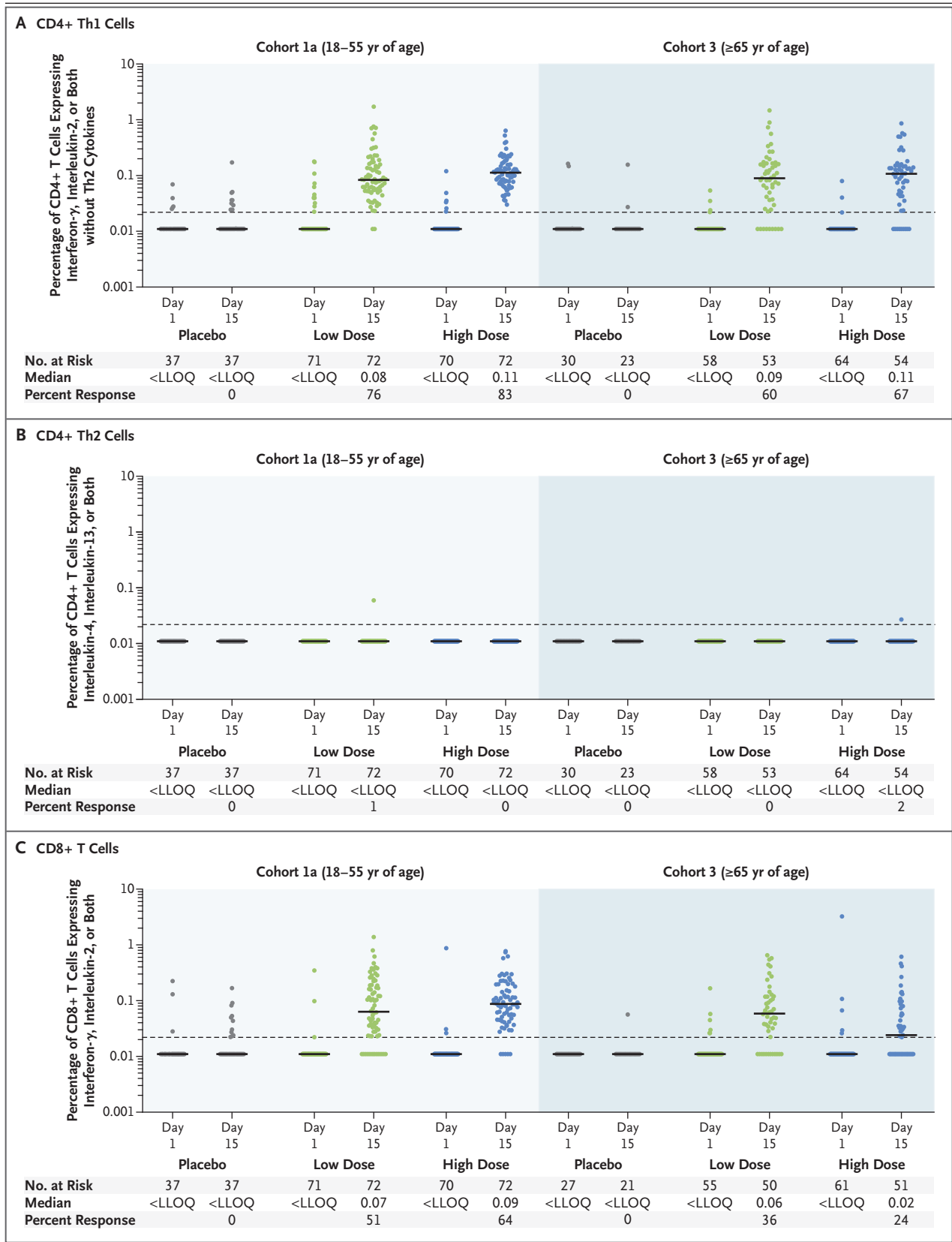


Figure 3 (facing page). Cellular Immunogenicity of Ad26.COVS.

In CD4+ T cells, the response to low-dose or high-dose vaccine or placebo in type 1 helper T (Th1) cells was characterized by the expression of interferon- γ , interleukin-2, or both, without cytokines expressed by type 2 helper T (Th2) cells (Panel A). The response in CD4+ Th2 cells was characterized by the expression of interleukin-4, interleukin-5, or interleukin-13 (or all three cytokines) plus CD40L (Panel B). In CD8+ T cells, the response was measured by the expression of interferon- γ , interleukin-2, or both (Panel C). In all three panels, the horizontal bars indicate median values on intracellular cytokine staining for individual responses to a SARS-CoV-2 S protein peptide pool in peripheral-blood mononuclear cells at baseline and 15 days after vaccination in a subgroup of participants in cohort 1a (left side) and cohort 3 (right side), according to the receipt of the low or high dose of Ad26.COVS or placebo. The horizontal dotted line in each panel indicates the lower limit of quantitation (LLOQ); values below the line have been imputed to half the LLOQ.

ules, a single dose of Ad26.COVS elicited a strong humoral response in a majority of vaccine recipients, with the presence of S-binding and neutralizing antibodies in more than 90% of the participants, regardless of either age group or vaccine dose. In addition, during 71 days of follow-up after the first dose, antibody titers further increased and stabilized, which suggests durability of the Ad26.COVS-elicited immune response.

The potency of Ad26.COVS is supported by the results of our study involving nonhuman primates, in which a single dose provided complete protection against SARS-CoV-2 replication in the lung and near complete protection in the nose.¹² An efficacious single-dose Covid-19 vaccine has obvious logistic advantages over a two-dose vaccine, especially during a pandemic. We observed that among participants between the ages of 18 and 55 years, a second vaccine dose at day 57 further increased the antibody titer, a finding that was also in line with our recent observations in nonhuman primates.¹⁴ Neutralizing-antibody titers against the Ad26 vector that were elicited by the first vaccination did not correlate with the magnitude of response after the second dose. Whether a second dose will provide additional benefit for either improved efficacy or durability in humans, especially in elderly persons in whom the immune response after the first dose tended to be modestly lower than that in younger participants, is

currently being studied in a phase 3 clinical trial (ClinicalTrials.gov number, NCT04614948).

The lack of standards and use of different assays complicate the comparison of performance of the various Covid-19 vaccines that are currently in development.³⁻⁶ In addition, comparisons of convalescent serum panels are rather arbitrary, since the reported GMTs have varied according to the composition of the panels (i.e., Covid-19 severity of the donors, time of sampling since disease onset, and other factors).

A theoretical risk of vaccine-associated enhanced respiratory disease (VAERD)¹⁵⁻¹⁷ has been associated with poorly neutralizing humoral immunity and Th2-skewed cellular immune responses. In this trial, all elicited CD4+ T-cell responses to Ad26.COVS were Th1-skewed, in line with previous experience with the Ad26-based vaccine platform.⁹⁻¹¹ Data that further minimize the theoretical risk of VAERD are the accompanying consistent CD8+ T-cell responses (albeit occurring at lower levels in older adults than in younger adults) and strong humoral responses.

The demographic characteristics of the participants in our trial confirm the lack of representation of minority groups. This finding is a point of focus in our clinical-development program to ensure the availability of data with respect to groups that seem to be affected most by the Covid-19 pandemic.

Our interim analysis indicates that vaccine candidate Ad26.COVS is safe and immunogenic in both younger and older adults. This finding, in combination with the results in preclinical challenge studies,^{12,13} has supported our decision to proceed with two phase 3 trials (NCT04505722 and NCT04614948) to evaluate the efficacy of either a single-dose or two-dose regimen of the lower dose (5×10^{10} viral particles) of Ad26.COVS.

Supported by Johnson & Johnson and by a contract (HH-S0100201700018C) with the Biomedical Advanced Research and Development Authority of the Department of Health and Human Services.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

We thank all the participants in this clinical trial; the COV1001 study team, including all the investigators at the clinical sites; the members of the microneutralization assay testing team of Public Health England; and the Nexelis team, including Helen Diamantakis, Steven Phay Tran, and Hossein Koohsari, for providing laboratory testing services.

APPENDIX

The authors' full names and academic degrees are as follows: Jerald Sadoff, M.D., Mathieu Le Gars, Ph.D., Georgi Shukarev, M.D., Dirk Heerwegh, Ph.D., Carla Truyers, Ph.D., Anne M. de Groot, Ph.D., Jeroen Stoop, Ph.D., Sarah Tete, Ph.D., Wim Van Damme, M.D., Isabel Leroux-Roels, M.D., Pieter-Jan Berghmans, M.D., Murray Kimmel, D.O., Pierre Van Damme, M.D., Jan de Hoon, M.D., William Smith, M.D., Kathryn E. Stephenson, M.D., Stephen C. De Rosa, M.D., Kristen W. Cohen, Ph.D., M. Juliana McElrath, M.D., Emmanuel Cormier, Ph.D., Gert Scheper, Ph.D., Dan H. Barouch, M.D., Jenny Hendriks, Ph.D., Frank Struyf, M.D., Macaya Dououguih, M.D., Johan Van Hoof, M.D., and Hanneke Schuitemaker, Ph.D.

The authors' affiliations are as follows: Janssen Vaccines and Prevention, Leiden, the Netherlands (J. Sadoff, M.L.G., G. Shukarev, A.M.G., J. Stoop, S.T., E.C., G. Scheper, J. Hendriks, M.D., J.V.H., H.S.); Janssen Research and Development, Beerse (D.H., C.T., F.S.), Janssen Clinical Pharmacology Unit, Merkssem (W.V.D.), the Center for Vaccinology, Ghent University, Ghent (I.L.-R.), SGS Life Sciences (P.-J.B.) and the Center for the Evaluation of Vaccination, University of Antwerp (P.V.D.), Antwerp, and the Center for Clinical Pharmacology, University Hospitals Leuven, Leuven (J. de Hoon) — all in Belgium; Optimal Research, Melbourne, FL (M.K.); the Alliance for Multispecialty Research, Knoxville, TN (W.S.); the Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston (K.E.S., D.H.B.); and the Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle (S.C.D.R., K.W.C., M.J.M.).

REFERENCES

- World Health Organization. Pneumonia of unknown cause — China. January 5, 2020 (<https://www.who.int/csr/don/05-january-2020-pneumonia-of-unknown-cause-china/en/>).
- Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727-33.
- Folegatti PM, Ewer KJ, Aley PK, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. *Lancet* 2020;396:467-78.
- Jackson LA, Anderson EJ, Roupheal NG, et al. An mRNA vaccine against SARS-CoV-2 — preliminary report. *N Engl J Med* 2020;383:1920-31.
- Zhu F-C, Guan X-H, Li Y-H, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2020;396:479-88.
- Mulligan MJ, Lyke KE, Kitchin N, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. *Nature* 2020;586:589-93.
- Bos R, Rutten L, van der Lubbe JEM, et al. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses. *NPJ Vaccines* 2020;5:91.
- Custers J, Kim D, Leyssen M, et al. Vaccines based on replication incompetent Ad26 viral vectors: standardized template with key considerations for a risk/benefit assessment. *Vaccine* 2020 October 2 (Epub ahead of print).
- Anywine Z, Whitworth H, Kaleebu P, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. *J Infect Dis* 2019;220:46-56.
- Williams K, Bastian AR, Feldman RA, et al. Phase 1 safety and immunogenicity study of a respiratory syncytial virus vaccine with an adenovirus 26 vector encoding prefusion F (Ad26.RSV.preF) in adults aged ≥60 years. *J Infect Dis* 2020;222:979-88.
- Barouch DH, Tomaka FL, Wegmann F, et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blind, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys (NHP 13-19). *Lancet* 2018;392:232-43.
- Mercado NB, Zahn R, Wegmann F, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* 2020;586:583-8.
- Tostanoski LH, Wegmann F, Martinot AJ, et al. Ad26 vaccine protects against SARS-CoV-2 severe clinical disease in hamsters. *Nat Med* 2020;26:1694-700.
- Solforsoli L, Kuipers H, Rosendal Huber SK, et al. Immunogenicity of one- and two-dose regimens of the Ad26.COV2.S COVID-19 vaccine candidate in adult and aged rhesus macaques. November 17, 2020 (<https://www.biorxiv.org/content/10.1101/2020.11.17.368258v1>). preprint.
- Deming D, Sheahan T, Heise M, et al. Vaccine efficacy in senescent mice challenged with recombinant SARS-CoV bearing epidemic and zoonotic spike variants. *PLoS Med* 2006;3(12):e525.
- Iwata-Yoshikawa N, Uda A, Suzuki T, et al. Effects of Toll-like receptor stimulation on eosinophilic infiltration in lungs of BALB/c mice immunized with UV-inactivated severe acute respiratory syndrome-related coronavirus vaccine. *J Virol* 2014;88:8597-614.
- Yasui F, Kai C, Kitabatake M, et al. Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV. *J Immunol* 2008;181:6337-48.

Copyright © 2021 Massachusetts Medical Society.