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MAJOR ARTICLE

Homologous and Heterologous Boosting of the Chadox1-S1-S COVID-19 Vaccine With the SCB-2019 Vaccine Candidate: A Randomized, Controlled, Phase 2 Study

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Background. Ongoing outbreaks of coronavirus disease 2019 (COVID-19) are driven by waning immunity following primary immunizations and emergence of new severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants that escape vaccine-induced neutralizing antibodies. It has been suggested that heterologous boosters could enhance and potentially maintain population immunity.

Methods. We assessed the immunogenicity and reactogenicity of booster doses of different formulations of aluminium hydroxide–adjuvanted SCB-2019 vaccine (9 μg of SCB-2019, with or without CpG-1018 adjuvant, or 30 μg of SCB-2019 with CpG-1018) in Brazilian adults primed with ChAdOx1-S vector vaccine. S-protein antibodies and ACE2-binding inhibition were measured by enzyme-linked immunosorbent assay (ELISA) on days 1, 15, and 29. Participants self-reported solicited adverse events and reactions.

Results. All SCB-2019 formulations increased S-protein ELISA antibodies and ACE2 binding inhibition to a greater extent than ChAdOx1-S. After 30 μg of SCB-2019 + CpG + aluminium hydroxide, titers against wild-type S-protein were significantly higher than after ChAdOx1-S on days 15 and 29, as were titers of neutralizing antibodies against the wild-type strain and Beta, Gamma, Delta, and Omicron variants. Boosting with SCB-2019 or ChAdOx1-S was well tolerated, with no vaccine-related serious or severe adverse events.

Conclusions. Boosting ChAdOx1-S-primed adults with SCB-2019 induced higher levels of antibodies against a wild-type strain and SARS-CoV-2 variants than a homologous ChAdOx1-S booster, with the highest responses being with the 30-μg SCB-2019 + CpG + aluminium hydroxide formulation.

Clinical Trials Registration. NCT05087368

Keywords. COVID-19; SCB-2019; booster; chAdOx1-S; heterologous; homologous; vaccine.

The coronavirus disease 2019 (COVID-19) pandemic has been ongoing for 2 years, during which time large proportions of high-income country populations have achieved vaccine-induced immunity following national immunization campaigns [1]. However, new variants of the wild-type severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) from Wuhan have continued to emerge, each displaying new mutations of the spike protein (S-protein) [2]. As the S-protein is the main antigenic target of most of the authorized vaccines, the accumulating mutations have resulted in these new variants becoming successively less susceptible to the neutralizing immunity induced by the first immunization campaigns [3–7]. This has resulted in new waves of pandemic COVID-19 outbreaks, most notably associated with the Beta (B.1.351), Gamma (P.1), Delta (B.1.617.2), and Omicron (B.1.1.529) variants [8]. The efficacy of authorized vaccines against infection has been seen to decrease with each new variant, both due to waning immunity following immunization and the changes in the antigenic target, while protection against severe disease is largely preserved.

This has led to the implementation of further immunization campaigns with booster doses of vaccines to broaden the immune response. Early indications are that heterologous boosters

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are mostly more effective than homologous boosters [9–11]. Most data on such immunity has been obtained with mRNA vaccines, which were the first to be authorized for use in immunization campaigns and have been widely used in high-income countries. However, many low- and middle-income countries are still in the phase of implementing full primary immunization of their populations, with widespread use of viral vector (eg, ChAdOx1-S1; AstraZeneca, United Kingdom) or inactivated (eg, CoronaVac; Sinovac Biotech, China) vaccines. Clover Biopharmaceuticals has developed a recombinant SARS-CoV-2 S-protein vaccine (S-Trimer), SCB-2019, that has been stabilized in the native prefusion trimeric conformation using the company's proprietary Trimer-Tag technology and adjuvanted with the Toll-like receptor 9 (TLR 9) agonist CpG-1018 and aluminum hydroxide ($AlOH_3$). The SPECTRA phase 2/3 efficacy trial demonstrated that 2 doses of 30 μg of SCB-2019 had 67.2% (95.72% CI, 54.3%–76.8%) efficacy against any COVID-19, and specific efficacies of 78.7% (95% CI, 57.3%–90.4%), 91.8% (95% CI, 44.9%–99.8%), and 58.6% (95% CI, 13.3%–81.5%) against the Delta, Gamma, and Mu variants, respectively [12]. The present study was conducted to investigate use of SCB-2019 in heterologous booster regimens compared with homologous boosters in individuals who have received a 2-dose primary vaccination series of the adenovirus vector vaccine ChAdOx1-S, which was authorized in Brazil. We assessed the safety and immunogenicity of 3 different formulations of SCB-2019: the standard 30-μg dose formulated with CpG-1018 and aluminum hydroxide used in the SPECTRA efficacy trial and 2 low-dose formulations containing 9 μg of SCB-2019 and aluminum hydroxide, 1 with and 1 without the CpG-1018 adjuvant, to investigate possible dose-sparing. These were given as a heterologous booster in persons primed with 2 doses of ChAdOx1-S, and responses were compared with a dose of ChAdOx1-S given as a homologous booster.

METHODS

This phase 2 randomized, controlled, multicenter study is ongoing at 3 sites in Brazil: Hospital de Clínicas de Porto Alegre, Hospital Gloria D'or, Rio de Janeiro and Centro de Estudos e Pesquisa em Moléstias Infecciosas (CEPCLIN), Natal. The objective reported here was to select the optimal SCB-2019 formulation to use in the heterologous boosting of individuals primed with 2 doses of the ChAdOx1-S1-S vaccine. Selection was to be based on the safety and immunogenicity and potential impact on supply of the chosen formulation, that is, dose-sparing.

Patient Consent

The study protocol was approved by each hospital's ethical review committee and conducted according to the Declaration of Helsinki and Council for International Organizations of Medical Sciences International ethical guidelines and International Conference on Harmonization Good Clinical Practices guidelines. The protocol was registered on ClinicalTrials.gov, registration number NCT 05087368. Participants supplied written informed consent at enrollment.

Participants

Eligible participants were male or female adults, ≥18 years of age, who had previously received 2 doses of ChAdOx1-S1-S vaccine 6 months (±4 weeks) before enrollment and were willing and able to comply with the study requirements, including all scheduled visits, vaccinations, laboratory tests, and other study procedures. Inclusion criteria included being healthy or having a preexisting but stable medical condition at the screening examination, with the main exclusion criterion being any previous laboratory-confirmed SARS-CoV-2 infection.

Vaccine

The investigational SCB-2019 vaccine was supplied in a 1.0-mL prefilled syringe containing 720 μg of SCB-2019. Adjuvants were CpG-1018 (Dynavax Technologies) presented in a 2.0-mL vial containing 12 mg/mL of a 22-mer phosphorothioate oligodeoxynucleotide in Tris buffered saline (24 mg per vial) and aluminum hydroxide (Alhydrogel, Croda Health Care) supplied in vials of 10 mg/mL. The final vaccine formulations per dose contained either 30 μg of SCB-2019 with 1.5 mg of CpG-1018 and 0.75 mg of aluminum hydroxide in a 0.5-mL volume, as used in the reported efficacy trial [12], 9 μg of SCB-2019 with 0.225 mg of aluminum hydroxide in a 0.15-mL dose, or 9 μg of SCB-2019 with 0.45 mg of CpG-1018 and 0.225 mg of aluminum hydroxide in a 0.15-mL volume. The comparator vaccine (ChAdOx1-S1-S) was Fiocruz COVID-19 vaccine (Rio de Janeiro, Brazil), containing a chimpanzee adenovirus (ChadOx1) encoding the SARS-CoV-2 spike glycoprotein, with not less than 2.5×10^8 infectious units (Inf.U) in each 0.5-mL dose. These vaccine formulations were prepared on the day of use by trained unblinded vaccine administrators who administered them by intramuscular injection in the upper deltoid of the nondominant arm. For accurate administration of the 0.15-mL volume, 1-mL tuberculin syringes (Precisionglide, Becton Dickenson) were used. These vaccine administrators played no further part in the study, and all other study staff, investigators, and participants were masked to which vaccine had been given.

Procedures

At enrollment, participants were randomly allocated 1:1:1:1 using a block size of 8 to 4 equal groups to receive a third dose of ChAdOx1-S1 or a dose of 1 of the 3 formulations of SCB-2019. At their first study visit on day 1, after providing their baseline blood sample, participants received their assigned vaccination and were monitored for 30 minutes for any immediate reactions. Using diary cards, they then recorded for 7 days the

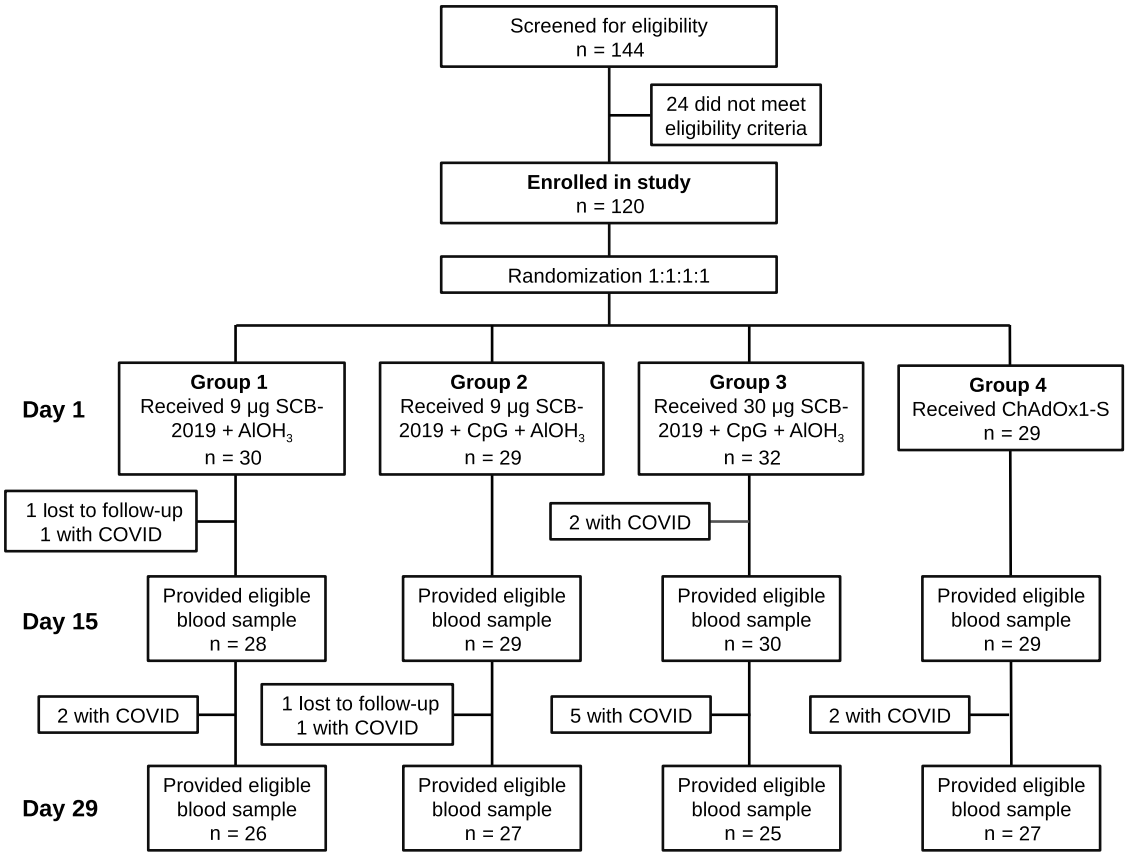

Figure 1. Study flowchart showing the disposition of the participants to each of the 4 groups. Abbreviation: COVID, coronavirus disease 2019.

occurrence of solicited local reactions (pain, erythema, and swelling at the injection site) and systemic adverse events (fatigue, headache, myalgia, arthralgia, loss of appetite, nausea, chills, fever [axillary temperature ≥38°C]) daily, together with any unsolicited adverse events, serious adverse events (SAEs), or medically attended adverse events (MAAEs) occurring up to the third study visit on day 29. At this study visit, the investigator assessed any reported adverse event (AE) as mild (no interference with daily activities), moderate (interferes with daily activities), or severe (prevents daily activity) and the relationship to the study procedures.

Immunogenicity

Serum samples obtained on day 1 (before vaccination) and at the second (day 15) and third (day 29) visits were used to assess immune responses. The primary end point was the response assessed by enzyme-linked immunosorbent assay (ELISA) as immunoglobulin G antibodies against SCB-2019 S-protein on day 15 [12]. Inhibition of binding of S-protein to the human angiotensin converting enzyme 2 (ACE2) was measured using a competitive ELISA with SCB-2019. Additional exploratory analyses included virus-neutralizing activity (VNA) titers measured on days 1, 15, and 29 in a microneutralization assay (MN_{50}) against the prototype Wuhan strain and the Beta (B.1.351), Delta (B.1.617.2), Gamma (P.1), and Omicron (B.1.1.529) variants of SARS-CoV-2.

Statistics

There was no formal hypothesis tested in this first stage of the study, all results being presented and analyzed descriptively. The sample size was considered adequate for the purposes of down-selection of formulation. The primary immunogenicity end point was ELISA antibody titers against SCB-2019 S-protein expressed as geometric mean titers (GMTs), geometric mean-fold rise in titers over baseline (GMFR), and seroconversion rates (SCRs) on days 15 and 29 in all participants who received the correct vaccination and had no major protocol deviation reported or suffered a COVID-19 infection before blood draw. Seroconversion was defined as a ≥4-fold increase in postvaccination titer in those with a baseline titer above the lower limit of quantitation (LLOQ) or a postvaccination titer ≥4-fold the LLOQ in those with no detectable activity at baseline. Although no formal analyses were planned, exploratory post hoc comparisons between groups were made by an analysis of covariance (ANCOVA) model with vaccine group as a fixed variable and baseline antibody result and site as covariates.

Table 1. Demographics of the Participants in the Full Analysis Set

Characteristics	Group 1: 9 μg SCB-2019 + $AlOH_3$	Group 2: 9 μg SCB-2019 + CpG + $AlOH_3$	Group 3: 30 μg SCB-2019 + CpG + $AlOH_3$	Group 4: ChAdOx1-S
	n = 30	n = 29	n = 32	n = 29
Sex, No. (%)				
Male	15 (50)	12 (41)	12 (38)	13 (45)
Female	15 (50)	17 (59)	20 (63)	16 (55)
Mean age ± SD, y	43.4 ± 14.4	40.0 ± 13.6	39.8 ± 12.1	36.8 ± 12.7
(Range)	(20–66)	(21–63)	(22–63)	(21–64)
Race, No. (%)				
American Indian/Alaskan native	0 (0)	0 (0)	0 (0)	1 (3)
Black or African American	1 (3)	4 (14)	2 (6)	4 (14)
White	25 (83)	22 (76)	25 (78)	21 (72)
Other	2 (7)	3 (10)	4 (13)	2 (7)
Unknown/not reported	2 (7)	0 (0)	1 (3)	1 (3)
Ethnic group, No. (%)				
Hispanic or Latino	21 (70)	26 (90)	20 (63)	19 (66)
Not Hispanic or Latino	2 (7)	1 (3)	9 (28)	4 (14)
Unknown/not reported	7 (23)	2 (7)	3 (9)	6 (21)
Mean body mass index ± SD, kg/m^2	28.5 (4.9)	28.9 (6.4)	27.5 (5.6)	27.8 (6.5)
(Range)	(19.8–37.9)	(17.3–42.3)	(18.3–46.3)	(20.0–44.5)
Risk of severe COVID-19,[a] No. (%)				
Low	18 (60)	18 (62)	24 (75)	21 (72)
High	12 (40)	11 (38)	8 (25)	8 (28)
COVID-19 infections during the study, No. (%)	8 (27)	7 (24)	9 (28)	6 (21)
Mean time from vaccination ± SD, d	32.4 ± 12.0	44.3 ± 14.6	24.0 ± 8.2	43.2 ± 23.2
(Range)	(8–47)	(32–67)	(13–37)	(8–47)

Abbreviation: COVID-19, coronavirus disease 2019.

[a]Risk due to presence of known comorbidities.

Immunogenicity results against the different SARS-CoV-2 variants are also expressed as GMTs, GMFR, and SCRs for group and variant. ELISA GMTs of neutralizing antibodies against SCB-2019 S-protein prototype strain are presented in IU/mL; GMTs of neutralizing antibodies against variants and ACE2 are expressed in reciprocal units. Safety data are presented descriptively as proportions of groups (percentages) reporting any solicited reaction or adverse event or unsolicited adverse event.

RESULTS

Recruitment began on November 26, 2021, and the last volunteer was enrolled on March 7, 2022. During this period, there was a major outbreak of Omicron infections. Of 144 volunteers screened, a total of 120 volunteers were enrolled and randomized to the 4 study groups (Figure 1). Only 2 enrolled participants did not attend through visit 3; 1 was lost to follow-up from Group 1 after visit 1, and the second was lost to follow-up from Group 2 after visit 2. All of the remaining 118 randomized participants completed through visit 3, except for 1 from Group 1 who did not provide blood for immunology assessment. Demographics in the 4 study groups were comparable (Table 1). Over the course of the study, there were 30 suspected cases of COVID-19 in the study population, 26 of which were confirmed by RT-PCR and 2 by rapid antigen test (RAT). These occurred in all 4 study groups, with onset from 8 to 77 days after vaccination (Table 1). Three cases had onset before day 15, and a further 10 before day 29, with the remaining 17 having onset after visit 3. The participants with confirmed COVID-19 infection before the second blood sample were excluded from the relevant immunogenicity analyses.

Immunogenicity

Vaccination in any of the 4 study groups resulted in increases in titers of binding antibodies against SCB-2019 and inhibition of S-protein binding to ACE2, which were notably higher in Groups 1–3, which received the heterologous SCB-2019 formulation, than Group 4 after a homologous ChAdOx1-S booster (Figure 2). Following the SCB-2019 boosters, these increases were apparently dose dependent; in Groups 1 and 2, which received 9 μg of SCB-2019, the respective GMFR from day 1 were 9 and 11 at day 15 and 8 and 9 at day 29, while in Group 3 the GMFR were 18 and 16 at days 15 and 29, suggesting the 30-μg SCB-2019 dose was more effective in inducing a booster response (Figure 2*A*). Notably, all 3 SCB-2019 groups elicited significantly higher GMFR than the 4-fold increase elicited by the homologous ChAdOx1-S booster vaccination at day 15. The

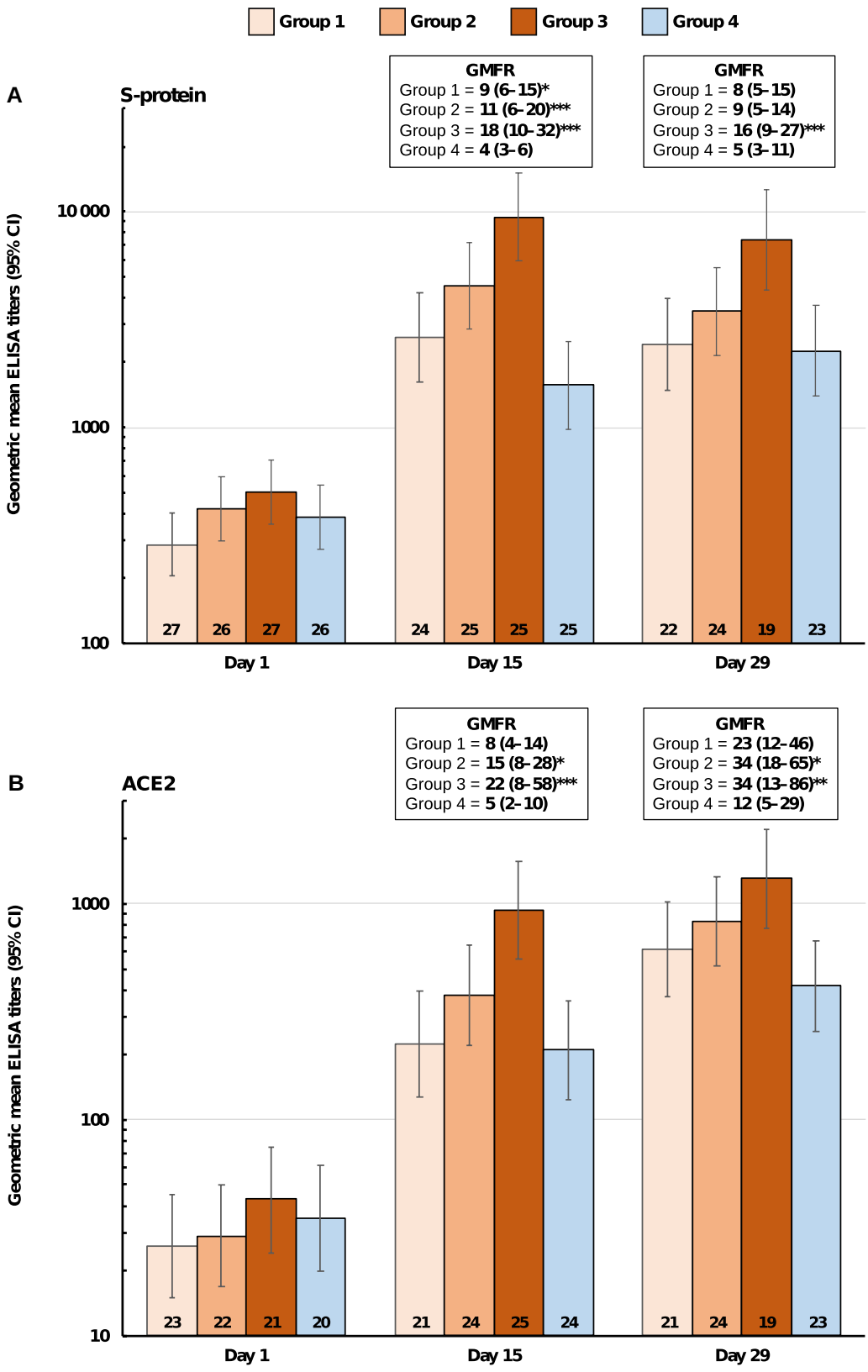

Figure 2. Booster vaccination responses shown as geometric mean titers (with 95% CIs) of ELISA antibodies against SCB-2019 (A) and ACE2 (B) at days 15 and 29 after vaccination. Geometric mean-fold rises from day 1 (95% CI) are shown with ANCOVA *P* values of intergroup differences between individual Groups 1, 2, and 3 (SCB-2019) and Group 4 (ChAdOx1-*S*): **P* < .05; ***P* < .01; ****P* < .001. Numbers in columns are *n* values per group. Abbreviations: ANCOVA, analysis of covariance; ELISA, enzyme-linked immunosorbent assay.

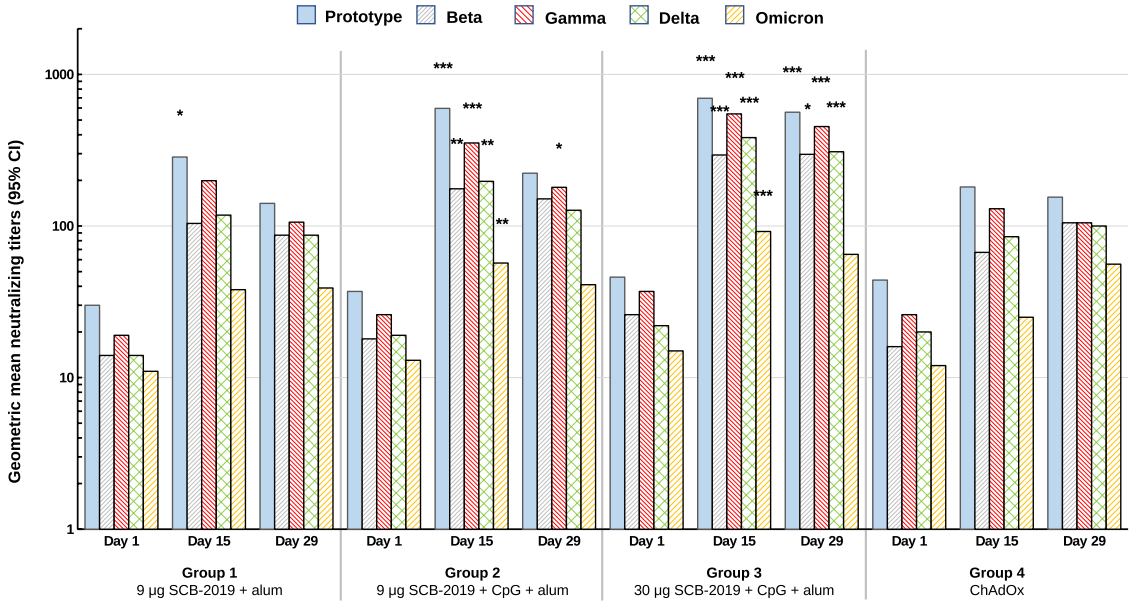

Figure 3. Booster vaccination responses shown as geometric mean neutralizing titers (with 95% CIs) against the indicated SARS-CoV-2 variants 15 days after vaccination. Differences in GMTs of Groups 1–3 vs Group 4 at day 15 were tested by ANCOVA: *$P < .05$; **$P < .01$; ***$P < .001$. Abbreviations: ANCOVA, analysis of covariance; GMTs, geometric mean titers; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

response increased further in Group 4 at day 29, but a significant difference persisted between Groups 3 and 4.

A similar profile was observed when measuring ACE2 binding (Figure 2*B*), with higher fold increases in all groups at days 15 and 29 than with the S-protein assay, which were still dose dependent in the SCB-2019 groups. This immune response was significantly higher in Groups 2 and 3 than in Group 4, the ChAdOx1-S-boosted group, and this significant difference persisted at day 29, when all 4 groups displayed higher titers, with GMFR ranging from 23 to 34 in the 3 SCB-2019 groups compared with a GMFR of 12 in the ChAdOx1-S group.

When assessed for neutralizing activity against the protype strain and the different SARS-CoV-2 variants at day 15, these differences between different doses of SCB-2019 and between SCB-2019 and ChAdOx1-S were still evident (Figure 3). All groups displayed neutralizing activity against all 5 variants at baseline, with the highest responses being against the prototype Wuhan virus and the lowest against the most recent variant, Omicron (B.1.1529). Two weeks after vaccination, there were marked increases against all 5 variants in all 4 groups, with significantly higher increases after heterologous SCB-2019 doses in Groups 2 and 3 than the homologous ChAdOx1-S dose in Group 4. Overall, the biggest increases in SCB-2019 groups were against the prototype virus, with GMFR ranging from 10 to 15 (Table 2), and Delta variant (GMFR 9 to 17), and the lowest increases against Omicron, with GMFR ranging from 4 to 6. The GMFR in Group 4, after a booster ChAdOx1-S vaccination, ranged from 2 to 5 for the different variants. When the GMTs of each group vs Group 4 were compared, the responses in Groups 2 and 3 were significantly higher than Group 4 against all variants (Figure 3). Similarly, seroconversion rates for each variant were highest with the 30-μg SCB-2019 formulation, with a notably higher response against Omicron than the homologous ChAdOx1-S booster (Table 2). Interestingly, the significant differences between Groups 2 and 4, the low-dose fully adjuvanted SCB-2019 and ChAdOx1-S, did into persist to day 29 except for the Gamma variant. This was due in equal parts to waning titers in Group 2 and increasing titers in Group 4. While significant differences did persist between Groups 3 and 4 for the protype virus and the Beta, Gamma, and Delta variants, titers against Omicron were not different in these 2 groups.

Safety

Overall, all 4 vaccine formulations were well tolerated, with no vaccine-related adverse events or severe adverse events, no withdrawals due to an AE, and no deaths (Table 3). The only reported SAE was a leg fracture, which was not related to the study procedures. One female participant with a history of hypertension and prior COVID-19 infection reported a mild allergic reaction 13 days after vaccination that resolved but was repeated 5 days later. The investigator considered the first occurrence to be related to the study vaccine, but not the second.

There were no clinically meaningful differences in rates of solicited local reactions between the groups. Rates were highest in Group 2 (41%) and Group 3 (47%), after 9 μg and 30 μg of SCB-2019 with CpG and aluminum hydroxide, respectively. The rate was lower (29%) in Group 1, which received 9 μg of

Table 2. Geometric Mean-Fold Rises at Days 15 and Day 29 From Day 0 and Seroconversion Rates on Days 15 and 29 for Antibodies Against the Prototype SARS-CoV-2 and 4 Variants Measured by Microneutralization Test

Day	Booster Vaccine	Group 1: 9 μg SCIB-2019 + $AlOH_3$	Group 2: 9 μg SCIB-2019 + CpG + $AlOH_3$	Group 3: 30 μg SCIB-2019 + CpG + $AlOH_3$	Group 4: ChAdOx1-S
Prototype SARS-CoV-2					
15	No.	24	25	25	25
	GMFR (95% CI)	10 (6–17)	17 (10–30)	15 (9–27)	4 (3–7)
	SCR (95% CI), %	79 (58–93)	84 (64–96)	84 (64–96)	48 (28–69)
29	No.	22	24	19	23
	GMFR (95% CI)	5 (3–7)	6 (4–11)	13 (7–23)	3 (2–6)
	SCR (95% CI), %	64 (41–83)	71 (49–87)	84 (60–97)	39 (20–62)
Beta variant					
15	No.	24	25	25	25
	GMFR (95% CI)	7 (5–12)	10 (6–18)	11 (7–20)	5 (3–7)
	SCR (95% CI), %	75 (53–90)	76 (55–91)	80 (59–93)	52 (31–72)
29	No.	22	24	19	23
	GMFR (95% CI)	6 (4–9)	10 (6–17)	12 (7–20)	6 (3–12)
	SCR (95% CI), %	68 (45–86)	83 (63–95)	90 (67–99)	61 (39–80)
Gamma variant					
15	No.	24	25	25	25
	GMFR (95% CI)	11 (7–18)	14 (8–25)	15 (8–28)	5 (3–9)
	SCR (95% CI), %	79 (58–93)	84 (64–96)	80 (59–93)	56 (35–76)
29	No.	22	24	19	23
	GMFR (95% CI)	6 (4–9)	8 (5–13)	13 (7–25)	4 (2–7)
	SCR (95% CI), %	68 (45–86)	75 (53–90)	79 (54–94)	44 (23–66)
Delta variant					
15	No.	24	25	25	25
	GMFR (95% CI)	9 (5–14)	11 (6–19)	17 (10–27)	5 (3–8)
	SCR (95% CI), %	75 (53–90)	76 (55–91)	88 (69–98)	56 (35–76)
29	No.	22	24	19	23
	GMFR (95% CI)	6 (4–11)	7 (4–13)	16 (10–27)	5 (2–9)
	SCR (95% CI), %	68 (45–86)	75 (53–90)	90 (67–99)	48 (27–69)
Omicron variant					
15	No.	24	25	25	25
	GMFR (95% CI)	4 (2–6)	4 (3–7)	6 (4–10)	2 (1–4)
	SCR (95% CI), %	50 (29–71)	64 (43–82)	68 (47–85)	20 (6.8–41)
29	No.	22	24	19	23
	GMFR (95% CI)	4 (2–6)	4 (2–6)	5 (3–8)	5 (2–9)
	SCR (95% CI), %	55 (32–76)	58 (37–78)	58 (34–80)	52 (31–73)

Seroconversion was defined as a 4-fold increase in titer over baseline at day 1 or from LLOQ if day 1 titer was < LLOQ.

Abbreviations: GMFR, geometric mean fold rise from day 1 at day 15 or day 29; LLOQ, lower limit of quantification; SCR, seroconversion rate; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

SCB-2019 with aluminum hydroxide alone. After the homologous ChAdOx1-S vaccination, 33% of Group 4 reported a local reaction. Local reactions in all 4 groups mainly consisted of mild pain at the injection site, with a few cases described as moderate but none as severe (Figure 4). Solicited systemic AEs were most frequently reported after the ChAdOx1-S vaccine in Group 4 (63%), with lower rates (39%–43%) in the SCB-2019 groups. The most frequent solicited systemic AEs were headache, fatigue, and myalgia (Figure 4), which were mainly mild and transient, with no severe cases reported. As with local reactions, there were no clinically meaningful differences in systemic AEs between groups. There were no adverse events of special interest or severe unsolicited AEs reported over the course of the study.

As noted, there were 30 cases of COVID-19 reported, including 13 from day 1 to day 29, when immunogenicity was assessed. Of these, 25 were considered to be mild and 5 to be moderate in severity; no cases were described as severe or had associated pneumonia or required hospitalization.

DISCUSSION

This study is the first investigation of boosting immune responses with Clover's recombinant SARS-CoV-2 S-trimer fusion protein vaccine (SCB-2019) as a heterologous vaccine to the primary vaccine. This study also investigated the use of a lower dose of SCB-2019, with and without the CpG-1018 adjuvant, to allow for dose-sparing. The data show that the best

Table 3. Reactogenicity in the 29 Days After the Booster Doses of Vaccines as Indicated in the Safety Population

Vaccine	Group 1 9 μg SCB-2019 + $AlOH_3$ (n = 30), No. (%)	Group 2 9 μg SCB-2019 + CpG + $AlOH_3$ (n = 29), No. (%)	Group 3 30 μg SCB-2019 + CpG + $AlOH_3$ (n = 32), No. (%)	Group 4 ChAdOx1-S (n = 29), No. (%)
Any solicited local AE	8/28 (29)	11/27 (41)	14/30 (47)	9/27 (33)
Mild	7/28 (25)	11/27 (41)	13/30 (43)	8/27 (30)
Moderate	1/28 (4)	0/27 (0)	1/30 (3)	1/27 (4)
Any solicited systemic AE	11/28 (39)	14/27 (52)	13/30 (43)	17/27 (63)
Mild	9/28 (32)	10/27 (37)	7/30 (23)	13/27 (48)
Moderate	2/28 (7)	4/27 (15)	6/30 (20)	4/27 (15)
Any unsolicited AE				
Any	10 (33)	19 (66)	16 (50)	11 (38)
Grade 3 related	0	0	0	0
Grade 3 not related	0	1	0	0
Serious adverse events				
Any	0	1 (3)[a]	0	0
Related	0	0	0	0
Medically attended AEs	5 (17)	7 (24)	9 (28)	6 (21)
AEs of special interest, AEs leading to early withdrawal or death	0	0	0	0

Abbreviations: AE, adverse event.

[a]One participant suffered a leg fracture, which was considered to be unrelated to the study.

booster response in participants primed with 2 doses of the adenoviral vector vaccine, ChAdOx1-S, was provided by the standard formulation containing 30 μg of SCB-2019 with the Toll-like receptor 9 agonist CpG-1018 and aluminum hydroxide. This formulation demonstrated 67.2% efficacy against COVID-19 of any severity in the SPECTRA study and 100% efficacy against severe disease [12]. As a heterologous booster, it was not associated with any safety concerns and had acceptable reactogenicity, comparable to that observed in the SPECTRA study following primary immunizations [12]. The 30-μg SCB-2019 dose elicited significantly higher immunity against the 2 key antigenic targets, measured as antibodies against SARS-CoV-2 S-protein and inhibition of the binding of S-protein to the ACE2 receptor, than the homologous ChAdOx1-S vaccine. Further, neutralizing antibody responses against 4 of the major SARS-CoV-2 variants were significantly higher than with the homologous ChAdOx1-S vaccine. These observations are important as ChAdOx1-S has been shown to be highly protective against severe disease and death since its global rollout.

One exception was the level of neutralizing activity against the Omicron variant; although significantly lower 15 days after homologous boosting than with heterologous with SCB-2019 + CpG + aluminum hydroxide formulations, titers against Omicron were comparable at day 29. This was partly due to a continuing increase in these titers in the ChAdOx1-S group, while they waned slightly in the SCB-2019 groups. The question of whether this indicates a difference in the kinetics of the response to the homologous booster requires further investigation. It has previously been observed that the immune response to a heterologous second vaccination using mRNA vaccines after a primary dose of ChAdOx1-S is more rapid than homologous vaccination, but we are unaware of similar observations with a protein or inactivated vaccine [13].

The COVID-19 pandemic has decreased in severity but has endured in numbers of infections with the appearance of new variants, which, despite appearing to be less sensitive to vaccine-induced immunity, are also leading to less severe forms of disease with fewer hospitalizations and deaths [14]. However, SARS-CoV-2 remains a threat to global health, and the experience of a series of novel variants emerging and rapidly predominating in circulation highlights the potential for future outbreaks. In a situation analogous to influenza, in a population that now has immune experience due to infection or vaccination, future variants may lead to seasonal outbreaks. For that reason, it is essential that high levels of immunity be maintained in global populations to ensure that there are no more explosive outbreaks of serious illness such as those the world has recently experienced [15].

Many countries have already achieved high levels of immunity, notably those high-income countries that were able to initiate mass immunization campaigns with the first vaccines to be authorized, mainly the mRNA and vector vaccines targeting the S-protein of the prototype virus [1]. Middle- and low-income countries are now playing catch-up, typically using less expensive and more easily managed inactivated vaccines. However, the steady emergence of a series of novel variants, the majority of which have changes in the main antigenic target, the S-protein, has seen a decline in the extent of protective immunity afforded by the initial vaccines [3–8]. The combination of waning antibodies and lower immunity against the novel variant has caused resurgence of COVID-19 outbreaks around the world, which may be countered by use of booster vaccinations [16]. Unfortunately, boosters only provide a temporary solution as waning immunity and emergence of new escape variants mean that the added protection will be short lived.

Evidence so far suggests that in most cases boosting with a heterologous vaccine is more effective than homologous boosters [17–19], which this report appears to confirm, with the heterologous SCB-2019 booster eliciting higher immunity than a dose of the heterologous ChAdOx1-S vaccine. In view of the global need for more COVID-19 vaccines, we also assessed the effect of a reduced dose of SCB-2019 to allow dose-sparing as well as omitting the CpG-1018 adjuvant, which may also be

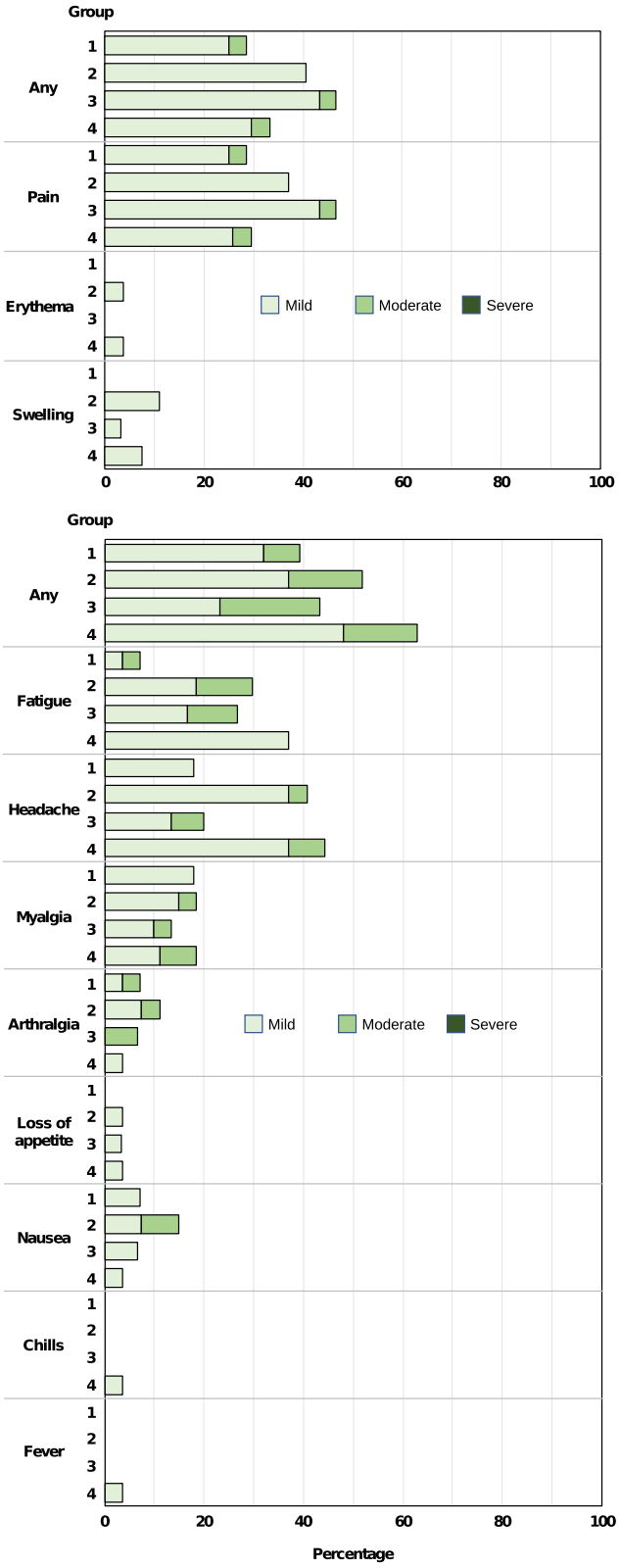

Figure 4. Solicited local reactions and systemic adverse events occurring within 7 days of vaccination by severity, reported as percentages of each group.

dose-limiting. Our results suggest that both formulations of SCB-2019 with CpG and aluminum hydroxide containing 9-μg or 30-μg doses provide an important boost in immunity with no evidence of increased reactogenicity.

This is a small study with several limitations, but the trends are confirmation of other observations. Several studies have shown that heterologous booster vaccination can heighten and broaden the immune response compared with homologous booster doses [17–19]. We restricted this study to 1 priming vaccine, ChAdOx1-S, but our results need to be confirmed with other vaccines, particularly mRNA and inactivated vaccines. We only assessed the immune responses out to 4 weeks after the booster vaccination, and persistence of any improved immune responses following the heterologous and homologous boosters will have to be assessed. Finally, we did not assess the efficacy of the booster immunization; although there were several cases of COVID-19 reported in this small study population, the study was not designed to include an efficacy assessment, which would also require a placebo group. Notably, none of these cases were severe, and there were no hospitalizations due to COVID-19.

In conclusion, the formulation of 30-μg SCB-2019 adjuvanted with CpG-1018 and aluminum hydroxide is safe and well tolerated as a heterologous booster vaccine in those previously primed with ChAdOx1-S and is immunologically more effective than that same vaccine given as a homologous booster.

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Potential conflicts of interest. F.P., P.L., I.E., H.-L.C., and I.S. are all full-time employees of the study sponsor. S.A.C.C. and R.C. are scientific advisors to the study sponsor. All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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