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# Safety and immunogenicity of S-Trimer (SCB-2019), a protein subunit vaccine candidate for COVID-19 in healthy adults: a phase 1, randomised, double-blind, placebo-controlled trial

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## Summary

**Background** As part of the accelerated development of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), we report a dose-finding and adjuvant justification study of SCB-2019, a protein subunit vaccine candidate containing a stabilised trimeric form of the spike (S)-protein (S-Trimer) combined with two different adjuvants.

**Methods** Our study is a phase 1, randomised, double-blind placebo-controlled trial at a specialised clinical trials centre in Australia. We enrolled healthy adult volunteers in two age groups: younger adults (aged 18–54 years) and older adults (aged 55–75 years). Participants were randomly allocated either vaccine or placebo using a list prepared by the study funder. Participants were to receive two doses of SCB-2019 (either 3 µg, 9 µg, or 30 µg) or a placebo (0·9% NaCl) 21 days apart. SCB-2019 either had no adjuvant (S-Trimer protein alone) or was adjuvanted with AS03 or CpG/Alum. The assigned treatment was administered in opaque syringes to maintain masking of assignments. Reactogenicity was assessed for 7 days after each vaccination. Humoral responses were measured as SCB-2019 binding IgG antibodies and ACE2-competitive blocking IgG antibodies by ELISA and as neutralising antibodies by wild-type SARS-CoV-2 microneutralisation assay. Cellular responses to pooled S-protein peptides were measured by flow-cytometric intracellular cytokine staining. This trial is registered with ClinicalTrials.gov, NCT04405908; this is an interim analysis and the study is continuing.

**Findings** Between June 19 and Sept 23, 2020, 151 volunteers were enrolled; three people withdrew, two for personal reasons and one with an unrelated serious adverse event (pituitary adenoma). 148 participants had at least 4 weeks of follow-up after dose two and were included in this analysis (database lock, Oct 23, 2020). Vaccination was well tolerated, with two grade 3 solicited adverse events (pain in 9 µg AS03-adjuvanted and 9 µg CpG/Alum-adjuvanted groups). Most local adverse events were mild injection-site pain, and local events were more frequent with SCB-2019 formulations containing AS03 adjuvant (44–69%) than with those containing CpG/Alum adjuvant (6–44%) or no adjuvant (3–13%). Systemic adverse events were more frequent in younger adults (38%) than in older adults (17%) after the first dose but increased to similar levels in both age groups after the second dose (30% in older and 34% in younger adults). SCB-2019 with no adjuvant elicited minimal immune responses (three seroconversions by day 50), but SCB-2019 with fixed doses of either AS03 or CpG/Alum adjuvants induced high titres and seroconversion rates of binding and neutralising antibodies in both younger and older adults (anti-SCB-2019 IgG antibody geometric mean titres at day 36 were 1567–4452 with AS03 and 174–2440 with CpG/Alum). Titres in all AS03 dose groups and the CpG/Alum 30 µg group were higher than were those recorded in a panel of convalescent serum samples from patients with COVID-19. Both adjuvanted SCB-2019 formulations elicited T-helper-1-biased $CD4^+$ T-cell responses.

**Interpretation** The SCB-2019 vaccine, comprising S-Trimer protein formulated with either AS03 or CpG/Alum adjuvants, elicited robust humoral and cellular immune responses against SARS-CoV-2, with high viral neutralising activity. Both adjuvanted vaccine formulations were well tolerated and are suitable for further clinical development.

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## Introduction

As of Jan 23, 2021, the global COVID-19 pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has caused almost 100 million infections and 2·1 million deaths.[1] Infections are causing unprecedented numbers of cases of severe respiratory illness, with substantial proportions of patients requiring admission to intensive care units.[2] COVID-19 is associated with high transmission and, without adequately effective treatment, rising numbers of cases of respiratory distress are threatening to overwhelm global health-care capacity. Interventions are urgently required to reduce this disease

## Research in context

### Evidence before this study

The COVID-19 pandemic has resulted in accelerated development of many vaccine candidates based on various immunological methods to target specific antigens of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). We did an unrestricted PubMed search on Dec 6, 2020, with the terms "COVID-19", "SARS-CoV-2", and "vaccine". We initially identified 268 references but this number decreased to 12 when we included the term "clinical trial". Of these references, nine were reports of human clinical trials of SARS-CoV-2 vaccines, and several were from the same studies of vaccines based on mRNA and human or chimpanzee adenovirus-vectored mRNA coding for SARS-CoV-2 spike (S)-protein, or SARS-CoV-2-inactivated vaccines. We separately identified a pre-publication paper on a clinical study of a nanoparticle vaccine containing recombinant SARS-CoV-2 S-protein in a saponin-based adjuvant.

### Added value of this study

Our study is, to the best of our knowledge, the first to assess the effect of two different adjuvants (AS03 and CPG/Alum) on an S-protein subunit vaccine against SARS-CoV-2 (SCB-2019), which uses Trimer-Tag technology (Clover Biopharmaceuticals, Chengdu, China) to keep the natural trimeric structure of the S-protein (S-Trimer). Immune responses to the S-Trimer protein alone (SCB-2019 with no adjuvant) were inadequate but, with both tested adjuvants (AS03 and CPG/Alum), immune responses of SCB-2019 were increased to achieve neutralising antibody titres after two vaccinations. Responses were consistent with those recorded in a panel of convalescent serum samples from patients with COVID-19. This neutralising activity directly correlates with the immune responses assessed as antibodies to the S-Trimer S-protein component and its receptor binding domain.

### Implications of all the available evidence

mRNA vaccines coding for S-protein of SARS-CoV-2 have been reported to elicit protective immune responses, based on results in clinical trials and efficacy assessments, while producing neutralising antibody titres. For stability of mRNA candidate vaccines, storage is required at less than –70°C, whereas another vaccine candidate can be stored at 2–8°C. Using proprietary technology, we created the vaccine candidate SCB-2019, which comprises S-Trimer, a trimeric form of S-protein in its natural configuration, in two adjuvanted formulations, which are stable when stored at 2–8°C, greatly facilitating distribution and use. Both SCB-2019 adjuvanted formulations were generally well tolerated and are highly immunogenic when administered as two doses 21 days apart. They elicited levels of neutralising titres comparable with those recorded in convalescent serum samples from patients with COVID-19. These findings are similar to those seen with two doses of an adjuvanted recombinant S-protein nanoparticle vaccine, supporting the rationale of this protein subunit vaccine approach. Our data support further clinical development of both SCB-2019 adjuvanted formulations.

burden, leading to accelerated clinical development of at least 64 vaccine candidates.[3]

The main viral antigenic target is the glycosylated spike (S) protein, a trimeric protein consisting of two subunits (S1 and S2)[4] that is essential for viral binding, fusion, and uptake into mammalian cells.[5] The S1 receptor binding domain (RBD) interacts with human cell-surface human ACE2 and, after proteolytic cleavage, the S2 subunit undergoes a major conformational change leading to fusion and intracellular uptake of viral mRNA for replication.[6,7] Interference with this process is the basis of most immunological approaches to prevent SARS-CoV-2 infections including vaccines.[8]

Trimer-Tag (Clover Biopharmaceuticals, Chengdu, China) is derived from the C-terminal region of human type I procollagen and is capable of self-trimerisation.[9] When soluble receptors or biologically active proteins are fused in-frame to Trimer-Tag, the resulting fusion proteins expressed in mammalian cells are secreted as disulphide bond-linked homotrimers. S-Trimer is a recombinant SARS-CoV-2 fusion protein produced using Trimer-Tag technology in Chinese hamster ovary cells. It preserves the native trimeric structure of S-protein in the prefusion form of the antigenic epitope, which is necessary for viral neutralisation, and it binds with high affinity to human ACE2.[10] S-Trimer was highly purified via multiple steps, including removal of host cell proteins, residual DNA, and preventative viral inactivation agents, to meet International Council for Harmonisation (ICH) guidelines. The resulting vaccine candidate, SCB-2019, is stable in liquid solution formulations at 2–8°C for at least 6 months, with longer term stability studies ongoing.[10] When formulated with the oil-in-water emulsion adjuvant AS03, or the TLR9 agonist CpG combined with Alum (CpG/Alum),[11] SCB-2019 induced protective immunity to SARS-CoV-2 in animal challenge studies.[10] We report an interim analysis of the first stage of a first-in-human phase 1 dose-finding and adjuvant justification study done to assess the safety, tolerability, and immunogenicity of three dose levels of SCB-2019 administered to healthy adults as two doses 21 days apart non-adjuvanted or formulated with either AS03 or CpG/Alum.

## Methods

### Study design and participants

We did a phase 1, randomised, double-blind placebo-controlled trial at Linear Clinical Research, a specialised clinical trials centre with its own bed facility and pharmacy operating out of the QEII Medical Centre (Perth, WA, Australia). The first stage of the trial (reported here) was a placebo-controlled dose-escalation study done in two parts, with the first part done in younger adults (aged

18–54 years) and the second part done in older adults (aged 55–75 years). Ten participants were each included in dose (3 µg, 9 µg, or 30 µg) and formulation (no adjuvant or AS03 or CpG/Alum adjuvant) groups.

The first part of the study (done in younger adults) used a sentinel strategy in which the first two participants in each dose and formulation group were randomly assigned either vaccine or placebo and had study injections. These two sentinels were monitored for 48 h and safety data were reviewed by a safety monitoring committee (SMC) to assess any clinically significant adverse events that occurred. The remaining eight participants in each of the nine younger adult groups were then randomised to either vaccine or placebo (seven vaccine, one placebo).

No sentinel strategy was applied to the second part of the study done in older adults who were only recruited after safety data from the equivalent younger adult group (same dose and formulation) had been evaluated by the SMC. Older adults only received adjuvanted vaccine.

Eligible participants were male or female adults aged 18–75 years who were healthy at enrolment based on medical history and medical assessment. Participants were recruited from an existing database maintained by Linear Clinical Research and through ethics-approved advertising on the study site's website and social media as well as posters in the QEII Medical Centre. All volunteers were screened for serum antibodies against SARS-CoV-2, as evidence of previous infection, and for acute exposure using RT-PCR; screening was repeated at study visits on days 22, 36, and 50. Inclusion criteria included the ability to understand and sign the informed consent, having a body-mass index of 18·5–35·0 kg/m², and availability for the duration of the study (6 months). Female participants of childbearing potential were not to be pregnant or breastfeeding and had to agree to use protocol-approved forms of contraception until 6 months after the first vaccination. Men were to use a protocol-approved form of contraception for 6 months from the day of first vaccination and to refrain from donating sperm over the same period.

Main exclusion criteria included positive serology for SARS-CoV-2; any uncontrolled chronic medical disorders; any known or suspected impairment of the immune system due to known immunosuppressive conditions, or any treatment with immunosuppressants or immunostimulants; known allergy to any vaccine components; malignant diseases; positive serology for HIV, hepatitis B, or hepatitis C; or previous receipt of any other SARS-CoV-2 vaccine. All volunteers were asked to avoid strenuous exercise from screening to day 50.

See Online for appendix

The study protocol was approved by the study centre institutional review board and done according to ICH and Good Clinical Practice guidelines. All participants provided signed informed consent.

### Randomisation and masking

Participants were randomly assigned to either non-adjuvanted, AS03-adjuvanted, or CpG/Alum-adjuvanted groups using randomisation lists prepared by the study funder and formatted in accordance with Medidata standards (Medidata Solutions, New York, NY, USA), with randomisation codes uploaded through the Medidata randomisation and trial management system (RTMS). Only unblinded personnel had access to this list through Medidata RTMS. Participants were assigned a study number at enrolment and were vaccinated according to the randomisation list. All participants and personnel involved in safety data collection and immunogenicity assessments were unaware of the study treatment. Vaccine preparation and administration was done by different unblinded study personnel, using opaque syringes to maintain masking of the participants since the vaccine and placebo are visually different.

### Procedures

SCB-2019 (Clover Biopharmaceuticals, Chengdu, China) is supplied in single-use vials as a sterile, clear-to-slightly opalescent, colourless solution for injection, stored at 2–8°C. For use, the appropriate dose of SCB-2019 was diluted in a vial with sodium chloride (NaCl 0·9%). For adjuvant mixing and administration, 0·25 mL of AS03 (GSK Vaccines, Wavre, Belgium) or 1·5 mg CpG 1018 (Dynavax Technologies, Emeryville, CA, USA) plus 0·75 mg Alum (Alhydrogel, Croda, Goole, UK) per dose were added to the vial and mixed by gentle inversion at room temperature a maximum of 1 h before administration. Each 0·5 mL dose contained 3 µg, 9 µg, or 30 µg SCB-2019 and adjuvants as appropriate, with sodium phosphate buffer and 0·05 mg polysorbate 80 in 0·9% NaCl. Doses were withdrawn into a syringe and administered by intramuscular injection in the deltoid region. Placebo was 0·5 mL 0·9% NaCl for injection.

On day 1, before vaccination, each adult received a full physical examination, at which vital signs were recorded and blood was collected for baseline safety laboratory variables. Further blood samples for safety analyses were obtained on days 8, 22, 36, and 50 for haematology, coagulation panel, serum chemistry, and urinalysis.

After vaccination, sentinel participants were monitored in the study centre for 6 h, whereas all other participants remained under observation for 60 min for potential immediate post-vaccination reactions. Participants then recorded solicited local reactions (pain, redness, and swelling at the injection site), systemic adverse events (headache, fatigue, myalgia, nausea or vomiting, and diarrhoea and vomiting), and body temperature for 7 days on electronic diary cards. Solicited reactions and adverse events were graded for severity (appendix pp 2–3) by the participants and assessed for causality by the investigator during interview at the following study visit. All unsolicited adverse events were recorded from day 1 to day 50. Serious adverse events and adverse events of special interest (appendix p 2) occurring before database lock (on Oct 20, 2020) were to be reported immediately to the investigator and then to the study funder within

24 h. Any medication used during the study, including paracetamol or non-steroidal anti-inflammatory drugs for prophylaxis, was recorded. During the study, the SMC continuously assessed safety data with the option to authorise use of stopping or pausing rules predefined in the protocol (appendix pp 14–122). During trial procedures, personal protection and safety measures for study staff and volunteers were maintained at a high level to avoid SARS-CoV-2 infection.

Serum samples were prepared for immunogenicity assessments before vaccinations on days 1 and 22, then on days 36 and 50. Serum samples were stored at –80°C or lower until shipment to the immunological laboratory (360biolabs, Melbourne, VIC, Australia) for analysis. Three immunological assays were done to evaluate humoral immune responses to vaccination. The primary immunogenicity endpoint was based on the anti-SCB-2019 IgG antibody titre at each blood sampling timepoint, measured by ELISA. Secondary immunogenicity assessments included an ACE2-competitive ELISA measuring the inhibition of SCB-2019 binding to human ACE2 receptor by serum IgG antibodies, and anti-wild-type SARS-CoV-2 neutralising activity measured by wild-type microneutralisation assay (WT-$MN_{50}$). A panel of 20 human convalescent serum samples from three hospitalised adults and 17 non-hospitalised adults with COVID-19 (mean age 37 [SD 11; range 18–54] years; serum samples collected 20–57 [mean 39] days after symptom onset), and the National Institute for Biological Standards and Control (NIBSC; Potters Bar, UK) reference serum sample 20/130, were analysed using the same validated assays as comparators for the post-vaccination serum samples. Details of immunological assay methods are provided in the appendix (pp 4–5).

Peripheral blood mononuclear cells were collected from all participants at days 1, 22, 36, and 50 to assess T-cell mediated immune responses to vaccination using intracellular cytokine staining flow cytometry to measure $CD4^+$ T cells expressing markers including interferon (IFN)-γ and interleukin (IL)-2, IL-4, IL-5, and

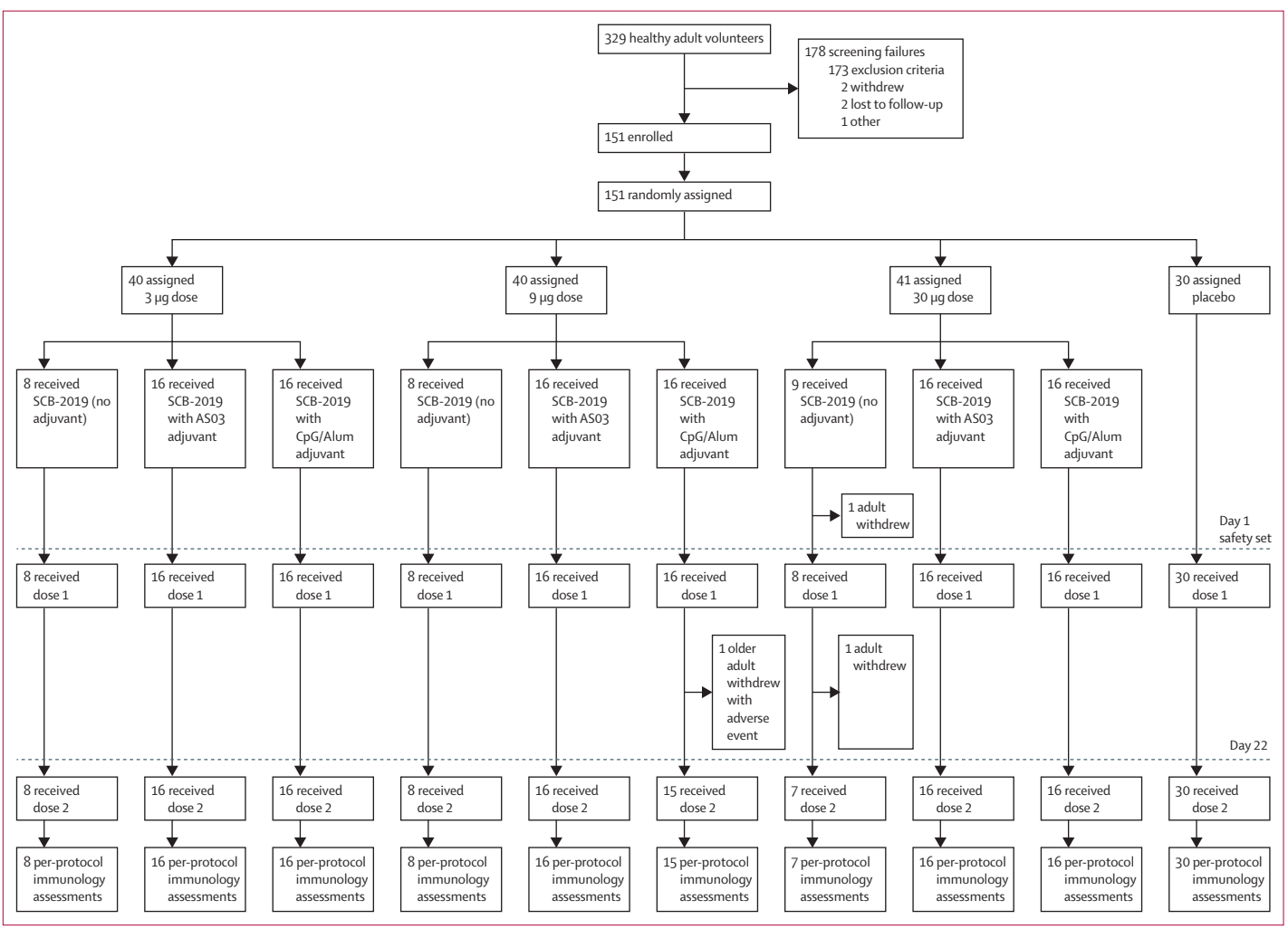


***Figure 1:*** **Trial profile (all ages combined)**

IL-17 after stimulation with SARS-CoV-2 S-protein peptide pools.

### Outcomes

Overall objectives were to assess the safety, tolerability, and immunogenicity of three increasing doses of SCB-2019, non-adjuvanted or adjuvanted with AS03 or CpG/Alum, in younger (aged 18–54 years) and older (aged 55–75 years) adults when administered as two intramuscular doses 21 days apart. Long-term follow-up to 24 months is planned, to generate data on safety and antibody persistence and to include SARS-CoV-2-seropositive participants.

### Statistical analysis

There was no formal statistical hypothesis in this phase 1 study and all data summaries are presented descriptively by group. The study sample size was not based on any statistical hypothesis but is typical of such phase 1 studies and was deemed adequate to provide a preliminary assessment of vaccine safety and reactogenicity in each cohort.

The safety analysis set consisted of all randomised participants who received at least one dose of study vaccine or placebo, analysed according to treatment received (per-protocol). Reported summary statistics include counts and percentages of participants who reported at least one solicited local reaction or systemic adverse event, unsolicited adverse events (with severity and causality), and serious adverse events and adverse events of special interest, after the first and second doses. For this report, safety data for all participants with at least 21-day safety follow-up after dose two are included.

The immunogenicity full analysis set consisted of all participants in the safety analysis set who had at least one post-vaccination blood sample collected and analysed for

| | Placebo | 3 µg SCB-2019 | | | 9 µg SCB-2019 | | | 30 µg SCB-2019 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant |
| **Younger adults (aged 18–54 years)** | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 9 | 8 | 8 |
| Age, years | 32·6 (10·7) | 37·6 (11·9) | 35·8 (9·3) | 41·9 (11·1) | 36·5 (12·4) | 37·0 (11·6) | 36·1 (15·0) | 30·9 (11·4) | 31·3 (11·3) | 39·1 (9·9) |
| Range | 18–52 | 20–50 | 24–53 | 20–53 | 20–54 | 21–53 | 19–55 | 18–49 | 19–47 | 21–50 |
| Sex | | | | | | | | | | |
| Male | 7 (39%) | 5 (63%) | 5 (63%) | 0 | 4 (50%) | 4 (50%) | 4 (50%) | 3 (33%) | 2 (25%) | 2 (25%) |
| Female | 11 (61%) | 3 (38%) | 3 (38%) | 8 (100%) | 4 (50%) | 4 (50%) | 4 (50%) | 6 (67%) | 6 (75%) | 6 (75%) |
| Race | | | | | | | | | | |
| Asian | 4 (22%) | 2 (25%) | 1 (13%) | 1 (13%) | 1 (13%) | 1 (13%) | 1 (13%) | 1 (11%) | 2 (25%) | 2 (25%) |
| Black | 0 | 0 | 0 | 0 | 0 | 0 | 1 (13%) | 0 | 0 | 0 |
| White | 14 (78%) | 6 (75%) | 6 (75%) | 7 (88%) | 6 (75%) | 7 (88%) | 6 (75%) | 8 (89%) | 6 (75%) | 6 (75%) |
| Other | 0 | 0 | 1 (13%) | 0 | 1 (13%) | 0 | 0 | 0 | 0 | 0 |
| Ethnicity | | | | | | | | | | |
| Hispanic or Latino | 1 (6%) | 0 | 0 | 2 (25%) | 2 (25%) | 1 (13%) | 0 | 0 | 1 (13%) | 0 |
| Not Hispanic or Latino | 17 (94%) | 8 (100%) | 8 (100%) | 6 (75%) | 6 (75%) | 7 (88%) | 8 (100%) | 9 (100%) | 7 (88%) | 8 (100%) |
| **Older adults (aged 55–75 years)** | | | | | | | | | | |
| Number of participants | 12 | 0 | 8 | 8 | 0 | 8 | 8 | 0 | 8 | 8 |
| Age, years | 62·3 (5·9) | .. | 62·8 (5·6) | 63·1 (5·7) | .. | 59·1 (3·4) | 60·3 (4·0) | .. | 59·8 (3·2) | 61·5 (6·4) |
| Range | .. | .. | 55–70 | 55–71 | .. | 55–64 | 55–67 | .. | 55–63 | 55–74 |
| Sex | | | | | | | | | | |
| Male | 3 (25%) | .. | 5 (63%) | 4 (50%) | .. | 5 (63%) | 5 (63%) | .. | 2 (25%) | 4 (50%) |
| Female | 9 (75%) | .. | 3 (38%) | 4 (50%) | .. | 3 (38%) | 3 (38%) | .. | 6 (75%) | 4 (50%) |
| Race | | | | | | | | | | |
| Asian | 0 | .. | 0 | 0 | .. | 0 | 0 | .. | 0 | 0 |
| Black | 0 | .. | 0 | 0 | .. | 0 | 0 | .. | 0 | 0 |
| White | 12 (100%) | .. | 8 (100%) | 8 (100%) | .. | 8 (100%) | 8 (100%) | .. | 8 (100%) | 8 (100%) |
| Other | 0 | .. | 0 | 0 | .. | 0 | 0 | .. | 0 | 0 |
| Ethnicity | | | | | | | | | | |
| Hispanic or Latino | 1 (8%) | .. | 0 | 1 (13%) | .. | 0 | 0 | .. | 0 | 1 (13%) |
| Not Hispanic or Latino | 11 (92%) | .. | 8 (100%) | 7 (88%) | .. | 8 (100%) | 8 (100%) | .. | 8 (100%) | 7 (88%) |

Data are mean (SD) or n (%), unless otherwise indicated.

***Table 1:*** **Demographics of safety population, by age and vaccine group**

immunogenicity. Data are summarised according to treatment received. Antibody responses are presented as geometric mean titres (GMTs) with 95% CIs at each blood sampling timepoint for each vaccine group. Geometric mean values are calculated on $\log_{10}$ (titres or data) values, with subsequent antilog transformations applied, the 95% CI being calculated using Student's *t* distribution. Seroconversion rates, defined as the percentage of participants with at least a four-fold increase in antibody titre over baseline within each study group, were calculated at days 22, 36, and 50. For between-group comparisons in geometric means, an ANOVA model was fitted to log-transformed assessment values (such as titre), based on participants with available data at each timepoint, then the geometric mean ratio and the 95% CI were calculated. Two-sided 95% CIs for the geometric mean ratio were obtained by calculating CIs using Student's *t* distribution for the mean difference of the logarithmically transformed results and antilog transformation of the confidence limits. In a post-hoc analysis, correlations between different antibody response measurements were evaluated with Pearson correlation coefficients computed for these analyses. All analyses, and summaries, were on group unblinded data and were done using SAS software (version 9.4 or higher) or GraphPad Prism (version 6.0c).

This trial is registered with ClinicalTrials.gov, NCT04405908.

### Role of the funding source

MD, BM, BH, IS, PiL, PeL, HHH, and JL are employed by the funder and participated in study design and development of the protocol, and in data analysis and data interpretation. RC and PR are scientific advisers for the study funder. The funder reviewed the protocol. PR worked with a medical writer (funded by Clover Biopharmaceuticals) to prepare a first draft report, which was reviewed and revised by all authors.

## Results

Between June 19 and Sept 23, 2020, 329 healthy adult volunteers were screened, of whom 151 (91 younger adults and 60 older adults) were enrolled after testing negative for SARS-CoV-2 (figure 1). Most screen failures (173 [97%] of 178) were due to exclusion criteria. One adult assigned 30 µg SCB-2019 withdrew before receiving any vaccination and was replaced with another volunteer. Demographics were similar across groups (table 1). In the younger adult group, the mean age of participants was 36·2 (SD 11·5) years for SCB-2019 recipients (combined doses) and 32·6 (10·7) years for placebo recipients; 36 (40%) of 91 younger volunteers were male and most described themselves as white (72 [79%]) and neither Hispanic nor Latino (84 [92%]). In the older adult group, the mean age of participants was 61·1 (SD 4·9) years for SCB-2019 recipients and 62·3 (5·9) years for placebo recipients; 28 (47%) of 60 older adults were male and all (100%) were white.

At database lock (Oct 23, 2020), no deaths or hospitalisations had been reported, and only two serious adverse events had been recorded, both in older adults. One older adult was diagnosed with cellulitis after a cat bite but completed the study, whereas another had hyponatraemia after receiving one dose in the 9 µg CpG/Alum group and was withdrawn from the study (figure 1). The participant was subsequently found to have a pituitary adenoma, which is a known to potentially cause hyponatraemia.[12–14] Neither event was deemed to be associated with vaccination. One adult decided to withdraw from the 30 µg SCB-2019 (no adjuvant) group for personal reasons before receiving the second vaccination. All other participants completed at least 21 days of follow-up after dose two and up to day 50.

Non-adjuvanted SCB-2019 (S-Trimer protein alone), was generally well tolerated in terms of solicited local adverse events, with only one report of mild pain after the first dose (3 µg) and none after the second dose (figure 2). Formulations with AS03 adjuvant resulted in 54% (26 of 48) of participants having local adverse events after the first vaccination, consisting almost exclusively of transient grade 1 or 2 injection site pain (n=24); cases of redness (n=2) and swelling (n=2) were relatively infrequent (appendix p 6). The frequency and severity of local adverse events increased after the second dose, including one case of grade 3 pain after a 9 µg dose of AS03 adjuvanted SCB-2019. When formulated with CpG/Alum, dose-dependent induction of grade 1 local adverse events was noted, reported by five (31%) of 16 adults after the first dose of 30 µg and by seven (44%) of 16 participants after the second dose of 30 µg. One grade 3 case of pain was reported after the second dose of 9 µg SCB-2019 with CpG/Alum adjuvant. All

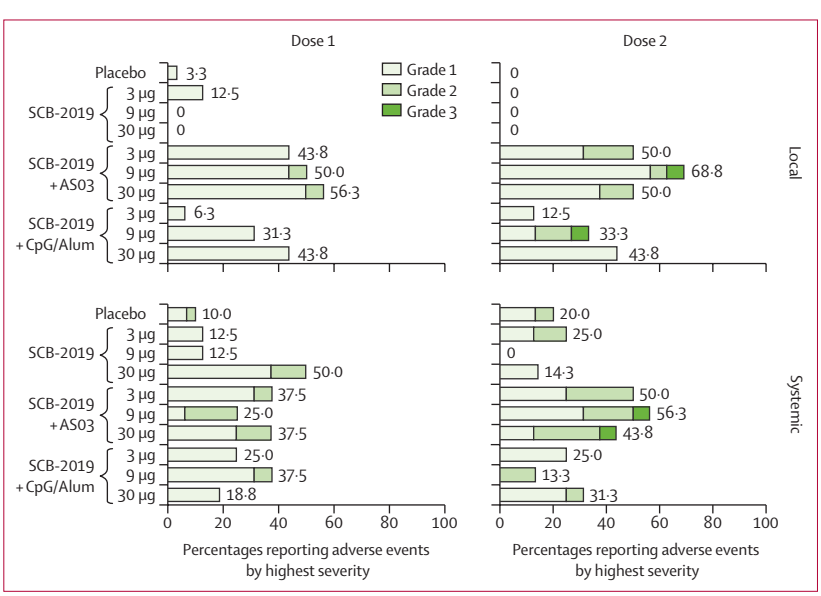


***Figure 2:*** **Incidence and severity of solicited local and systemic adverse events (all ages combined)**
Upper panel shows local events and lower panel shows systemic events. No grade 4 adverse events were reported.

local adverse events were transient and resolved within the reporting period. Younger adults reported local adverse events more frequently (39% of all dose levels combined; 28 of 72) than did older adults (21%; ten of 48) after the first dose, but incidence was similar in the two age groups, 35% (25 of 71) in younger adults and 34% (16 of 47) in older adults after the second dose.

After the first dose of non-adjuvanted SCB-2019, solicited systemic adverse events were infrequent with 3 µg and 9 µg doses (13% in each group; one of eight), but 50% of the 30 µg group (four of eight) reported grade 1 or 2 adverse events (figure 2). Fewer adverse events were recorded after the second dose (appendix p 7). By contrast, when formulated with AS03, the frequency of systemic adverse events was higher and not dose-dependent, reported by 25–38% per group after the first dose and 44–56% after the second dose with a concomitant increase in the proportion described as grade 2 (figure 2). Two participants, one each in the 9 µg and 30 µg groups, reported grade 3 fatigue and myalgia. The most frequently reported systemic adverse events were headache, fatigue, and myalgia, with six reports of fever (appendix pp 7–8); all events were grade 1 or 2 after the second dose of SCB-2019 with AS03 adjuvant. Frequencies of systemic adverse events in participants who received SCB-2019 with CpG/Alum adjuvant (19–38%) were similar to those with the AS03 adjuvant after the first dose, but unlike the AS03 group there was no consistent trend to increased frequency or severity after the second dose of SCB-2019 with CpG/Alum adjuvant (13–31%). As with local adverse events, the frequency of reported systemic adverse events was lower in older adults after their first dose (17%) than in younger adults (38%), and overall rates were similar after second doses, 30% in older and 34% in younger adults. No participants took prophylactic paracetamol or non-steroidal anti-inflammatory drugs.

Unsolicited adverse events reported over the 50-day study period mainly consisted of cases of grade 1 or 2 headache or gastrointestinal disorders (nausea or abdominal pain) that were recorded after the 7-day period for solicited adverse events. None of the two grade 3 unsolicited adverse events (dizziness with 3 µg SCB-2019 and AS03, and presyncope with 9 µg SCB-2019 and CpG/Alum) or two grade 4 unsolicited adverse events (hot flushes with 30 µg SCB-2019 and AS03, and hyponatraemia associated with a pituitary adenoma with 9 µg SCB-2019 and CpG/Alum) were causally related to vaccination. No consistent trends or clinically significant laboratory safety abnormalities were noted in any group at any timepoint. No cases of SARS-CoV-2 infection were reported during the study. No adverse events of special interest, including potential immune-mediated diseases, were seen.

Anti-SCB-2019 IgG antibodies did not increase after the first dose of non-adjuvanted SCB-2019 by day 22, irrespective of dose level (figure 3). By day 50, three SCB-2019 recipients seroconverted, one (13%) of eight in the 3 µg group and two (29%) of seven in the 30 µg group (table 2), although GMTs were low. In both adjuvanted cohorts, SCB-2019 dose-dependent IgG

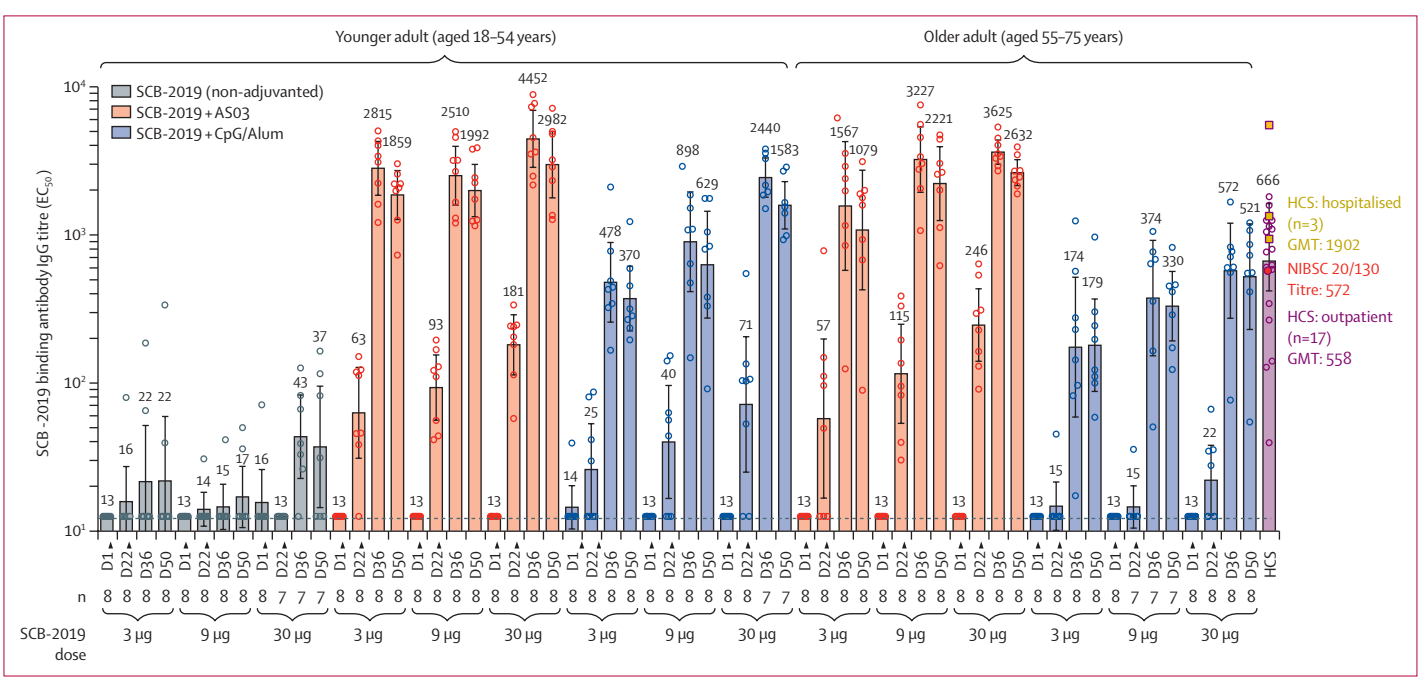


*Figure 3:* **SCB-2019 binding antibody IgG titres**

Titres are shown in the different study groups and human convalescent serum samples from patients with COVID-19 measured by ELISA ($EC_{50}$). Bars show GMTs per group with 95% CIs at days 1, 22, 36, and 50. Circles represent values for individual participants. Small arrows indicate study vaccinations at day 1 (dose 1) and day 22 (dose 2). GMT=geometric mean titre. D=day. HCS=human convalescent serum samples. NIBSC=National Institute for Biological Standards and Control.

| | Placebo | 3 μg SCB-2019 | | | 9 μg SCB-2019 | | | 30 μg SCB-2019 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant |
| **Anti-SCB-2019 IgG antibodies: younger adults (aged 18–54 years)** | | | | | | | | | | |
| Day 22 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 |
| Seroconversion rate | 0 | 0 | 4 (50%; 15·7–84·3) | 0 | 0 | 5 (63%; 24·5–91·5) | 2 (25%; 3·0–65·1) | 0 | 7 (88%; 47·3–99·7) | 5 (63%; 24·5–91·5) |
| Day 36 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Seroconversion rate | 0 | 1 (13%; 0·3–52·7) | 8 (100%; 63·1–100) | 8 (100%; 63·0–100) | 0 | 8 (100%; 63·1–100) | 8 (100%; 63·1–100) | 1 (14%; 0·4–57·9) | 8 (100%; 63·1–100) | 7 (100%; 59·0–100) |
| Day 50 | | | | | | | | | | |
| Number of participants | 17 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Seroconversion rate | 0 | 1 (13%; 0·3–52·7) | 8 (100%; 63·1–100) | 8 (100%; 63·1–100) | 0 | 8 (100%; 63·1–100) | 7 (88%; 7·3–99·7) | 2 (29%; 7–71·0) | 8 (100%; 3·1–100) | 7 (100%; 9·0–100) |
| **Anti-SCB-2019 IgG antibodies: older adults (aged 55–75 years)** | | | | | | | | | | |
| Day 22 | | | | | | | | | | |
| Number of participants | 12 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 16 |
| Seroconversion rate | 0 | .. | 3 (38%; 8·5–75·5) | 0 | .. | 4 (50%; 15·7–84·3) | 0 | .. | 7 (88%; 47·3–99·7) | 0 |
| Day 36 | | | | | | | | | | |
| Number of participants | 12 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 8 (100%; 63·1, 100) | 5 (63%; 24·5–91·5) | .. | 8 (100%; 63·1–100) | 6 (86%; 42·1–99·6) | .. | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) |
| Day 50 | | | | | | | | | | |
| Number of participants | 29 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 7 (88%; 47·3–99·7) | 6 (75%; 34·9–96·8) | .. | 8 (100%; 63·1–100) | 7 (100%; 59·0–100) | .. | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) |
| **ACE2-receptor competitive-blocking IgG antibodies: younger adults (aged 18–54 years)** | | | | | | | | | | |
| Day 22 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 |
| Seroconversion rate | 0 | 0 | 1 (13%; 3·0–52·7) | 0 | 0 | 0 | 2 (25%; 3·3–65·1) | 0 | 3 (38%; 8·5–75·5) | 1 (13%; 0·3–52·7) |
| Day 36 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Seroconversion rate | 0 | 0 | 8 (100%; 63·1–100) | 6 (75%; 34·9–96·8) | 0 | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) | 1 (14%; 0·4–57·9) | 8 (100%; 63·1–100) | 7 (100%; 59·0–100) |
| Day 50 | | | | | | | | | | |
| Number of participants | 17 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Seroconversion rate | 0 | 0 | 8 (100%; 63·1–100) | 5 (63%; 24·5–91·5) | 0 | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) | 0 | 8 (100%; 63·1–100) | 7 (100%; 59·0–100) |
| **ACE2-receptor competitive-blocking IgG antibodies: older adults (aged 55–75 years)** | | | | | | | | | | |
| Day 22 | | | | | | | | | | |
| Number of participants | 12 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 3 (38%; 8·5–75·5) | 0 | .. | 2 (25%; 3·3–65·1) | 0 | .. | 3 (38%; 8·5–75·5) | 0 |
| Day 36 | | | | | | | | | | |
| Number of participants | 12 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 7 (88%; 47·3–99·7) | 2 (25%; 3·3–65·1) | .. | 8 (100%; 63·1–100) | 5 (71%; 29·0–96·3) | .. | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) |
| Day 50 | | | | | | | | | | |
| Number of participants | 12 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 7 (88%; 47·3–99·7) | 3 (38%; 8·5–75·5) | .. | 8 (100%; 63·1–100) | 5 (71%; 29·0–96·3) | .. | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) |

(Table 2 continues on next page)

| | Placebo | 3 μg SCB-2019 | | | 9 μg SCB-2019 | | | 30 μg SCB-2019 | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant | No adjuvant | AS03 adjuvant | CpG/Alum adjuvant |
| (Continued from previous page) | | | | | | | | | | |
| **Wild-type SARS-CoV-2 neutralising antibodies: younger adults (aged 18–54 years)** | | | | | | | | | | |
| Day 22 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 8 |
| Seroconversion rate | 0 | 0 | 0 | 0 | 0 | 3 (38%; 8·5–75·5) | 2 (25%; 3·3–65·1) | 0 | 6 (75%; 34·9–96·8) | 4 (50%; 15·7–84·3) |
| Day 36 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Seroconversion rate | 0 | 0 | 8 (100%; 63·1–100) | 8 (100%; 63·1–100) | 0 | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) | 1 (14%; 0·4–57·9) | 8 (100%; 63·1–100) | 7 (100%; 59·0–100) |
| Day 50 | | | | | | | | | | |
| Number of participants | 18 | 8 | 8 | 8 | 8 | 8 | 8 | 7 | 8 | 7 |
| Seroconversion rate | 0 | 0 | 8 (100%; 63·1–100) | 8 (100%; 63·1–100) | 0 | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) | 0 | 8 (100%; 63·1–100) | 7 (100%; 59·0–100) |
| **Wild-type SARS-CoV-2 neutralising antibodies: older adults (aged 55–75 years)** | | | | | | | | | | |
| Day 22 | | | | | | | | | | |
| Number of participants | 30 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 2 (25%; 3·3–65·1) | 0 | .. | 4 (50%; 15·7–84·3) | 0 | .. | 1 (13%; 0·3–52·7) | 0 |
| Day 36 | | | | | | | | | | |
| Number of participants | 30 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 7 (88%; 47·3–99·7) | 6 (75%; 34·9–96·8) | .. | 8 (100%; 63·1–100) | 6 (86%; 42·1–99·6) | .. | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) |
| Day 50 | | | | | | | | | | |
| Number of participants | 28 | 0 | 8 | 8 | 0 | 8 | 7 | 0 | 8 | 8 |
| Seroconversion rate | 0 | .. | 7 (88%; 47·3–99·7) | 4 (50%; 15·7–84·3) | .. | 8 (100%; 63·1–100) | 6 (86%; 42·1–99·6) | .. | 8 (100%; 63·1–100) | 7 (88%; 47·3–99·7) |

Data are number or number (%; 95% CI). SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

*Table 2:* **Antibody seroconversion rates, by age and vaccine group**

responses were evident after a single dose in both age groups (figure 3). All participants at each dose level of SCB-2019 with AS03 adjuvant seroconverted by day 36 (table 2). After a second dose of SCB-2019 with AS03 adjuvant, striking increases in GMTs were seen, to higher levels than those observed in the convalescent serum samples and NIBSC reference serum 20/130 (GMT 666 $EC_{50}$ [95% CI 272–1628]; n=21) with a range of GMTs from 2510 to 4452 across dose levels in young adults and 1567 to 3625 in older adults (figure 3). Antibody titres persisted at high levels at day 50. Small dose-dependent IgG responses against SCB-2019 with CpG/Alum adjuvant were seen at all dose levels at day 22 after one dose in young adults (GMTs 25–71), which greatly increased after the second dose, with GMTs of 478–2440 on day 36. GMTs were lower in the equivalent older adult groups (15–22 at day 22), with GMTs of 174–572 at day 36 (figure 3). High GMTs were maintained to day 50, when seroconversion rates were 87·5–93·8% in both age groups combined across doses (table 2).

When ACE2 receptor-competitive blocking antibodies were assessed (figure 4), little or no response to non-adjuvanted SCB-2019 was seen, but robust responses were noted to the first and second doses of SCB-2019 with AS03 adjuvant, for all dose levels. Similar $EC_{50}$ GMTs were achieved in both age groups at day 36, ranging from 435 to 754 in younger adults and from 288 to 688 in older adults across all dose levels. Seroconversion rates for both age groups combined were 94% for 3 μg and 100% for 9 μg and 30 μg. All groups had GMTs that were higher than those seen with convalescent serum samples, including the NIBSC reference serum 20/130 (GMT 144 [95% CI 54–386]; n=21), and titres remained higher than convalescent serum samples at day 50 (figure 4). After vaccination with SCB-2019 plus the CpG/Alum adjuvant, dose-dependent responses were noted in younger adults that were higher than in older adults and that only matched the levels seen in convalescent serum samples with 9 μg and 30 μg doses of SCB-2019 with CpG/Alum adjuvant in younger adults, who had seroconversion rates of 50–93% after two doses (table 2).

Serum neutralising activity of wild-type SARS-CoV-2 showed a similar pattern of response to SCB-2019-binding IgG antibodies (figure 5). No increase in neutralising activity was seen with non-adjuvanted SCB-2019, with

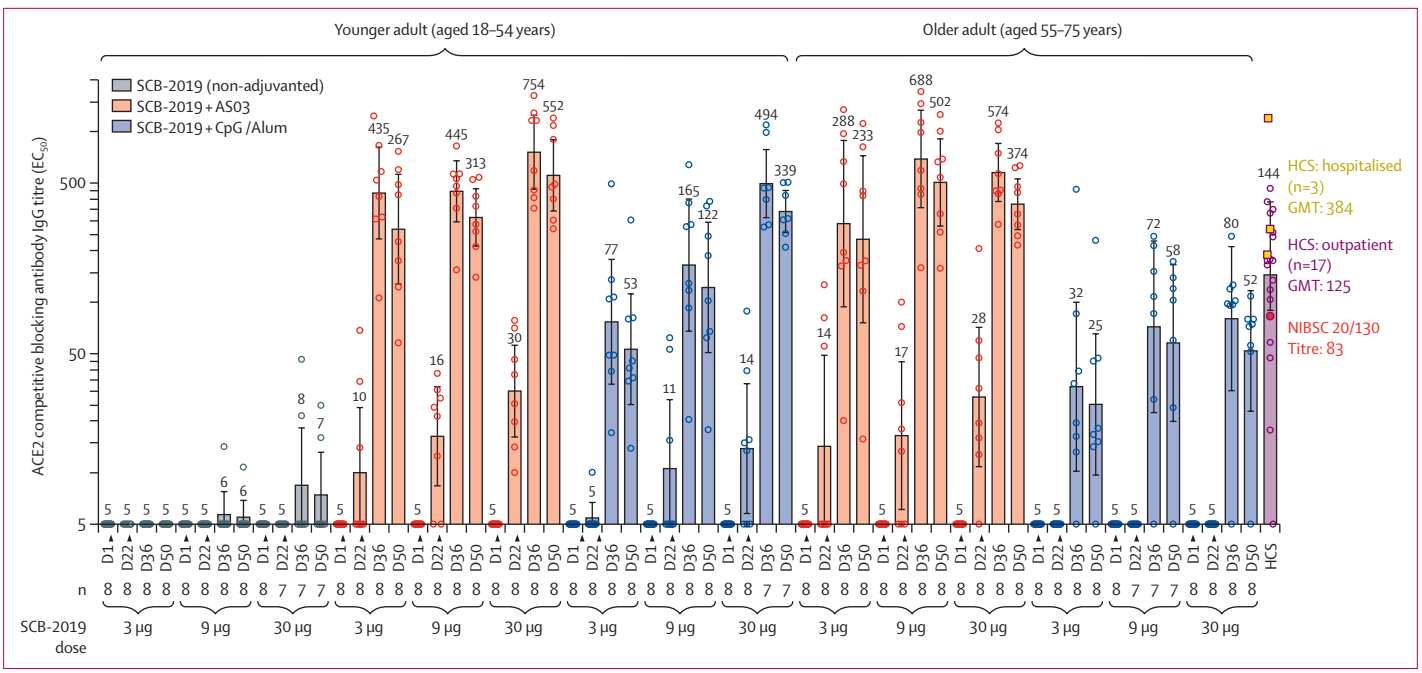


***Figure 4:*** **ACE2-competitive blocking antibody IgG titres**

Titres are shown in the different study groups and human convalescent serum samples from patients with COVID-19 measured by ELISA ($EC_{50}$). Bars show GMTs per group with 95% CIs at days 1, 22, 36, and 50. Circles represent values for individual participants. Small arrows indicate study vaccinations at day 1 (dose 1) and day 22 (dose 2). GMT=geometric mean titre. D=day. HCS=human convalescent serum samples. NIBSC=National Institute for Biological Standards and Control.

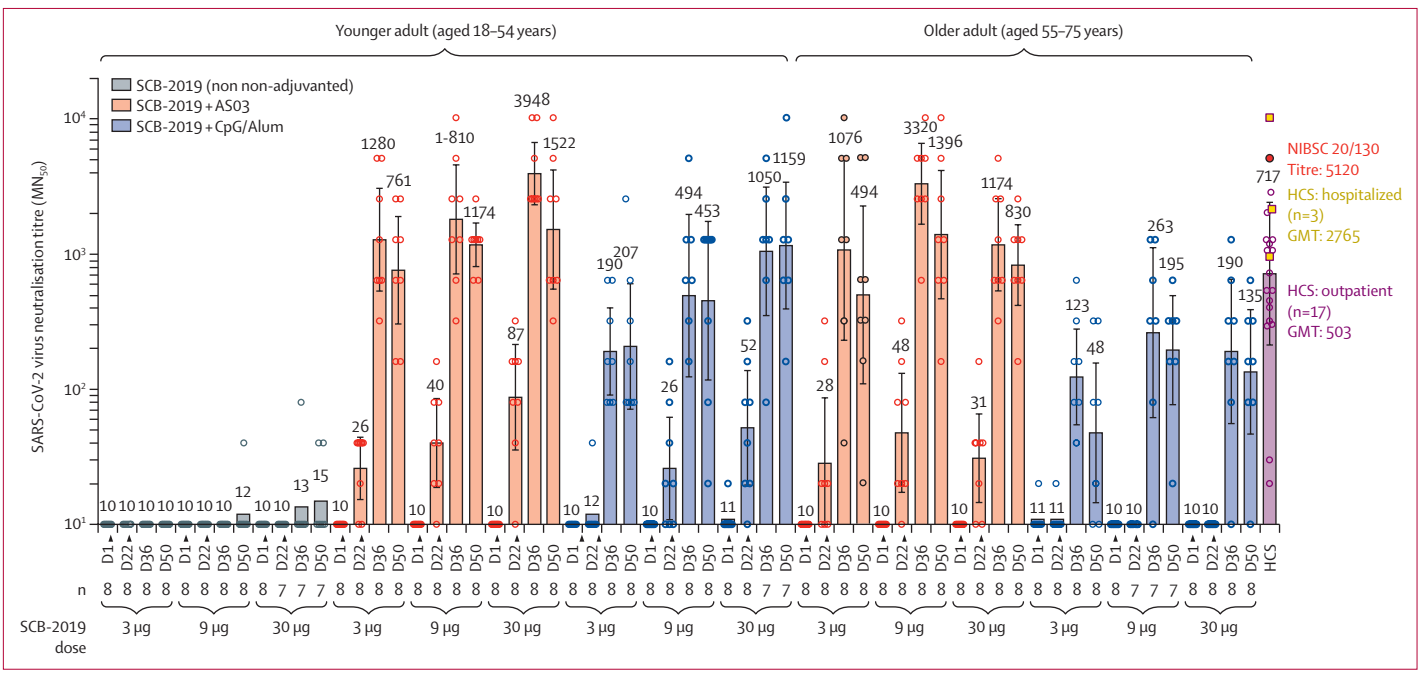


***Figure 5:*** **Wild-type SARS-CoV-2 neutralisation titres**

Titres are shown in the different study groups and human convalescent serum samples from patients with COVID-19 measured by microneutralisation based on cytopathic effect ($MN_{50}$). Bars show GMTs per group with 95% CIs at days 1, 22, 36, and 50. Circles represent values for individual participants. Small arrows indicate study vaccinations at day 1 (dose 1) and day 22 (dose 2). GMT=geometric mean titre. D=day. HCS=human convalescent serum samples. NIBSC=National Institute for Biological Standards and Control. SARS-CoV-2=severe acute respiratory syndrome coronavirus 2.

only one (14%) of seven participants responding by day 36 in the 30 µg group (table 2). After the first dose of SCB-2019 with AS03 adjuvant, 16 (33%) of 48 recipients across doses had seroconverted, increasing to 47 (98%) of 48 by day 36, after the second dose. This increase in neutralising antibodies was SCB-2019 dose-dependent,

shown by the geometric means of 1280–3948 $MN_{50}$ across dose levels. Importantly the range of $MN_{50}$ GMTs seen in older adult groups (1076–3320) were similar to those in the younger adult groups for all dose levels and were higher than convalescent serum samples ($MN_{50}$ GMT 717 [95% CI 213–2417]; n=21). Some decline in GMTs was seen, but high levels of neutralising antibodies persisted to day 50 in both age groups (figure 5), with a range of titres that overlapped those observed in convalescent serum samples from hospitalised COVID-19 patients and the NIBSC reference serum 20/130.

Dose-dependent increases in neutralising activity were also observed in the SCB-2019 with CpG/Alum adjuvant groups, but these responses were lower in magnitude than in the AS03 adjuvant groups (figure 5). In the older adult groups, the range of $MN_{50}$ GMTs (123–263) was lower than in convalescent serum samples. High titres were maintained up to day 50, the last timepoint tested in this interim analysis.

When correlations between immune responses assessed by the three different immunological assays were investigated in convalescent serum samples and samples from vaccinated participants, significant linear relationships were seen between each of the three assays (appendix pp 9–10). The calculated Pearson correlation coefficients ($R$) were 0·88 ($p<0·0001$) for ACE2-competitive blocking antibodies versus SCB-2019 binding IgG antibodies, 0·70 ($p<0·0001$) for SCB-2019 binding IgG antibodies versus WT-$MN_{50}$, and 0·67 ($p<0·0001$) for WT-$MN_{50}$ versus ACE2-competitive blocking antibodies (appendix p 10). Further, the calculated ratios of neutralising antibodies to SCB-2019 binding IgG antibodies in serum samples from vaccinated participants obtained at day 36 and day 50 after two doses of vaccine fell in the same range for SCB-2019 with AS03 adjuvant and SCB-2019 with CpG/Alum adjuvant and convalescent serum samples (appendix p 11).

Assessment of T-helper (Th)-1-biased cell-mediated immune responses specific to the SARS-CoV-2 S-protein were recorded in both adjuvanted vaccine groups with increases in IFN-γ-positive $CD4^+$ T-cells, IL-2-positive $CD4^+$ T-cells, or both, after the first dose, which further increased after the second dose (appendix pp 12–13). There were no cell-mediated immune responses with non-adjuvanted SCB-2019 and no observable increases in Th-2 (IL-4-positive or IL-5-positive $CD4^+$ cells) or Th-17 (IL-17-positive $CD4^+$ cells) cellular immune responses in any group.

## Discussion

The primary objective of this study was to assess the safety and reactogenicity of SCB-2019 when administered as the S-Trimer protein alone (non-adjuvanted) or as one of two adjuvanted formulations with either AS03 or CpG/Alum. For this phase 1 study, antigen and adjuvant were mixed before administration. Although a ready-mixed single vial formulation would be preferable, this practice was successfully used for administration of approximately 90 million doses of the influenza vaccine Pandemrix (GSK, Wavre, Belgium) during the H1N1 influenza pandemic in 2008.[15] All formulations seemed to have an acceptable safety profile, with no vaccine-related serious adverse events or study withdrawals. Non-adjuvanted SCB-2019 was well tolerated and, when administered in combination with either adjuvant, showed acceptable reactogenicity with few grade 3 solicited adverse events. Higher reactogenicity was noted with AS03, unaffected by the dose level of SCB-2019, which consisted of mainly transient grade 1 or 2 adverse events, which all resolved spontaneously without intervention. No prophylactic paracetamol was used in this study and would not seem necessary for general use. All local adverse events resolved within the reporting period of 7 days post-vaccination. When the age of participants was considered, no overall effect on safety or reactogenicity was seen. Although older adults (aged 55–75 years) had fewer local and systemic adverse events than did younger adults (aged 18–54 years) after the first dose, the incidence of solicited adverse events was similar in both age groups after the second dose. Use of AS03 in pandemic H5N1 influenza vaccines showed its general safety,[15] whereas large trials of the same vaccine revealed a higher local reactogenicity than we noted in our trial, with injection pain in 89% of adults aged 18–64 years.[16] Overall, this reactogenicity profile compares favourably with those of the mRNA SARS-CoV-2 vaccines, which had incidences of local pain approaching or reaching 100% in adults, and similar to those of a chimpanzee adenovirus-vectored vaccine.[17–19] The rates of solicited adverse events in the CpG/Alum-adjuvanted vaccine groups were lower and consistent with licensed CpG-adjuvanted vaccines.[20,21]

Non-adjuvanted SCB-2019 (S-Trimer protein alone) at the tested dose levels was poorly immunogenic, but when combined with ether adjuvant system (AS03 or CpG/Alum) there were robust increases in functional immune responses detected as SARS-CoV-2 neutralising activity that correlated well with IgG antibodies against SCB-2019 or ACE2-competitive blocking antibodies. Neutralising responses were already seen after the first dose in the higher dose AS03 groups. Highest responses were seen with AS03 and, after completion of the two-dose series, GMTs peaked at day 36 at levels that were higher than those recorded in convalescent serum samples from patients hospitalised with COVID-19, and the NIBSC reference serum sample. These high levels persisted until the end of this interim analysis at day 50. Little meaningful difference was seen between the immune responses to SCB-2019 with AS03 between younger and older adults. When adjuvanted with CpG/Alum, immune responses were lower than with SCB-2019 with AS03 adjuvant, and were dose-dependent. Further, the response to SCB-2019 with CpG/Alum were lower in the older age group. Further investigation of the cellular immune responses showed increases in Th1-polarised responses after both first and second doses

for both AS03-adjuvanted and CpG/Alum-adjuvanted SCB-2019. $CD4^+$ T-cell responses have been suggested to complement humoral antibody responses in overcoming SARS-CoV-2 infection.[20]

Because a strong correlation between neutralising activity and ELISA IgG antibody responses to S-protein and RBD has been seen in convalescent serum samples from patients with PCR-confirmed SARS-CoV-2 infection,[22] we investigated these ratios in our assays. We confirmed strong correlations between neutralising activity and IgG measured in either the SCB-2019 or ACE2-receptor assays. This observation is important because it has been suggested that low neutralising to binding antibody ratios could contribute to increased risk of antibody-enhanced disease.[23] The magnitude of neutralising antibody titres and ratio to binding antibodies after two doses are similar to those seen in adults after adjuvanted recombinant protein nanoparticle vaccine[24] and compare favourably with those to mRNA-based vaccines with reported efficacy[17–19,25–27] when accounting for different assay methodologies by comparing GMTs in human convalescent serum samples. This direct comparison between different vaccine candidates in different studies must be interpreted with caution. Immune responses against different vaccines were done in different populations using various assay formats and, different panels of convalescent serum samples. To mitigate bias, we compared ratios of neutralising antibodies to binding antibodies generated by different vaccine candidates from the reported studies as the ratio is mostly independent of the above factors. Moreover, we included the NIBSC reference serum 20/130 in the validated assays, and titres obtained for this reference serum are presented. Both adjuvanted formulations were protective in preclinical non-human primate and rodent animal models,[11] but AS03 formulations seemed to induce superior humoral immunogenicity with an apparent lack of age effect on the response. Although the AS03 formulation had higher reactogenicity than did CpG/Alum, severity seemed to be lower than with the mRNA and some vector-derived SARS-CoV-2 vaccines[17,18,25,26] and mainly consisted of transient mild-to-moderate local and systemic adverse events. Cases of narcolepsy were reported after the vaccination campaign with AS03-adjuvanted H1N1 vaccine during the 2009–10 H1N1 influenza pandemic, but evidence suggests these cases were attributable to the H1N1 viral antigen, and AS03 adjuvant does not have a role in this observed increased risk of narcolepsy.[28,29]

Our study has several limitations, including the enrolment of seronegative participants only and its relative small numbers of participants per vaccine formulation per age group. These limitations hamper observation of any true effect of age on tolerability or immunogenicity but allows observation of apparent safety and general tolerability of the different formulations. Immune responses have only been assessed up to day 50, approximately 4 weeks after the second vaccination. Antibody persistence and the ability to boost, as well as safety, are being followed up in a long-term study. Because study recruitment was done in Australia, ethnic diversity was limited, these factors will be addressed in the phase 2/3 studies, and the extension of this study in Panama will provide an additional information in a Hispanic population. Although we have compared immune responses elicited by vaccination with those measured in convalescent serum samples of patients with PCR-confirmed SARS-CoV-2 infection, in the absence of an established serological correlate of protection, we cannot extrapolate our data to infer protection.

In conclusion, we have shown high neutralising antibody responses, with a Th1-biased cellular immune response, and an acceptable safety profile. Based on these results, 9 µg SCB-2019 adjuvanted with AS03 and 30 µg SCB-2019 adjuvanted with CpG/Alum are the preferred candidates to be taken into the phase 2/3 trial; the final selection will be determined by manufacturing considerations.

#### Contributors

MD, JL, RC, PR, BM, BH, PeL, IS, and HHH designed the study. Data collection was by LH. Data analysis support was by PiL. Interpretation and writing of the manuscript was done by all authors, led by RC and JL. All authors approved the final version and made the decision to submit for publication. All authors had access to the statistically analysed data. PR, PiL, JL, and RC had access to and verified the raw data.

#### Declaration of interests

MD, BM, BH, IS, PeL, PiL, HHH and JL are full-time employees of Clover Biopharmaceuticals. RC and PR are scientific advisers for Clover Biopharmaceuticals. LH declares no competing interests.

#### Data sharing

The datasets, including the redacted study protocol, redacted statistical analysis plan, and individual participants data supporting the results reported in this article, will be available three months from initial request, to researchers who provide a methodologically sound proposal, at the discretion of the company governing body. The data will be provided after its de-identification, in compliance with applicable privacy laws, data protection and requirements for consent and anonymisation.

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