Safety and immunogenicity of SARS-CoV-2 recombinant protein vaccine formulations in healthy adults: interim results of a randomised, placebo-controlled, phase 1–2, dose-ranging study

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Summary

Background CoV2 preS dTM is a stabilised pre-fusion spike protein vaccine produced in a baculovirus expression system being developed against SARS-CoV-2. We present interim safety and immunogenicity results of the first-in-human study of the CoV2 preS dTM vaccine with two different adjuvant formulations.

Methods This phase 1–2, randomised, double-blind study is being done in healthy, SARS-CoV-2-seronegative adults in ten clinical research centres in the USA. Participants were stratified by age (18–49 years and ≥50 years) and randomly assigned using an interactive response technology system with block randomisation (blocks of varying size) to receive one dose (on day 1) or two doses (on days 1 and 22) of placebo or candidate vaccine, containing low-dose (effective dose 1·3 μg) or high-dose (2·6 μg) antigen with adjuvant AF03 (Sanofi Pasteur) or AS03 (GlaxoSmithKline) or unadjuvanted high-dose antigen (18–49 years only). Primary endpoints were safety, assessed up to day 43, and immunogenicity, measured as SARS-C0V-2 neutralising antibodies (geometric mean titres), assessed on days 1, 22, and 36 serum samples. Safety was assessed according to treatment received in the safety analysis set, which included all randomly assigned participants who received at least one dose. Neutralising antibody titres were assessed in the per-protocol analysis set for immunogenicity, which included participants who received at least one dose, met all inclusion and exclusion criteria, had no protocol deviation, had negative results in the neutralisation test at baseline, and had at least one valid post-dose serology sample. This planned interim analysis reports data up to 43 days after the first vaccination; participants in the trial will be followed up for 12 months after the last study injection. This trial is registered with ClinicalTrials.gov, NCT04537208, and is ongoing.

Findings Between Sept 3 and Sept 29, 2020, 441 individuals (299 aged 18–49 years and 142 aged ≥50 years) were randomly assigned to one of the 11 treatment groups. The interim safety analyses included 439 (>99%) of 441 randomly assigned participants (299 aged 18–49 years and 140 aged ≥50 years). Neutralising antibody titres were analysed in 326 (74%) of 441 participants (235 [79%] of 299 aged 18–49 years and 91 [64%] of 142 aged ≥50 years). There were no vaccine-related unsolicited immediate adverse events, serious adverse events, medically attended adverse events classified as severe, or adverse events of special interest. Among all study participants, solicited local and systemic reactions of any grade after two vaccine doses were reported in 81% (95% CI 61–93; 21 of 26) of participants in the low-dose plus AF03 group, 93% (84–97; 74 of 80) in the low-dose plus AS03 group, 89% (70–98; 23 of 26) in the high-dose plus AF03 group, 95% (88–99; 81 of 85) in the high-dose plus AS03 group, 29% (10–56; five of 17) in the unadjuvanted high-dose group, and 21% (8–40; six of 29) in the placebo group. A single vaccine dose did not generate neutralising antibody titres above placebo levels in any group at days 22 or 36. Among participants aged 18–49 years, neutralising antibody titres after two vaccine doses were 13·1 (95% CI 6·40–26·9) in the low-dose plus AF03 group, 20·5 (13·1–32·1) in the low-dose plus AS03 group, 43·2 (20·6–90·4) in the high-dose plus AF03 group, 75·1 (50·5–112·0) in the high-dose plus AS03 group, 5·00 (not calculated) in the unadjuvanted high-dose group, and 5·00 (not calculated) in the placebo group. Among participants aged 50 years or older, neutralising antibody titres after two vaccine doses were 8·62 (1·90–39·0) in the low-dose plus AF03 group, 12·9 (7·09–23·4) in the low-dose plus AS03 group, 12·3 (4·35–35·0) in the high-dose plus AF03 group, 52·3 (25·3–108·0) in the high-dose plus AS03 group, and 5·00 (not calculated) in the placebo group.

Interpretation The lower than expected immune responses, especially in the older age groups, and the high reactogenicity after dose two were probably due to higher than anticipated host-cell protein content and lower than planned antigen doses in the formulations tested, which was discovered during characterisation studies on the final bulk drug substance. Further development of the AS03-adjuvanted candidate vaccine will focus on identifying the optimal antigen formulation and dose.

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Introduction

COVID-19, caused by SARS-CoV-2, emerged in December, 2019, in Wuhan, China, and a global pandemic was declared in March, 2020. More than 2·8 million deaths and 128·6 million confirmed cases have been reported worldwide (as of March 31, 2021).[1]

Vaccination against SARS-CoV-2 will probably provide the most effective interventional long-term means of preventing and controlling SARS-CoV-2 infection, and it has become an urgent global priority. Of more than 60 vaccines in clinical development, several have reached phase 3 testing,[2] with interim efficacy results already available for some of these through peer-reviewed publications[3–5] or public statements.[6–9] At the time of writing, a number of vaccines had received authorisation for conditional, emergency, or temporary use in countries worldwide, including two mRNA-based vaccines, BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), a chimpanzee adenovirus-vectored vaccine, ChAdOx1 nCoV-19 (Oxford-AstraZeneca), a non-replicating viral vector vaccine, Sputnik V (Gamaleya Research Institute),

and two inactivated vaccines, CoronaVac (Sinovac Biotech) and BBIBP-CorV (Sinopharm).

The SARS-CoV-2 spike (S) glycoprotein on the virion surface mediates host-cell entry, making the S protein a key target in vaccine development. The introduction of double proline substitutions at the beginning of the central helix of the S2 subunit of the closely related MERS-CoV S protein stabilised the protein in a pre-fusion conformation and allowed induction of potent neutralising antibody responses in mice.[10] Wrapp and colleagues[11] successfully applied this stabilising strategy to the SARS-CoV-2 S protein. Sanofi Pasteur has developed a candidate SARS-CoV-2 recombinant protein vaccine containing the stabilised SARS-CoV-2 pre-fusion S protein (CoV2 preS dTM). Another adjuvanted recombinant protein vaccine candidate (Novavax) containing the stabilised SARS-CoV-2 pre-fusion S protein showed robust immunogenicity and efficacy results, against COVID-19 mild, moderate, and severe illness in adults, including older adults (aged 65 years or older), with acceptable safety.[8,12]

Although recombinant protein vaccines offer the advantages of fewer potential safety concerns and lower

Research in context

Evidence before this study

We searched PubMed from database inception to Feb 10, 2021, with no language restrictions, for studies reporting the safety and immunogenicity of adjuvanted recombinant protein vaccine candidates against SARS-CoV-2 using the search terms "vaccine", "clinical trial", "SARS-CoV-2", "recombinant protein", and "adjuvant".

One phase 1–2 human trial was retrieved, reporting the preliminary safety and immunogenicity of a trimeric recombinant full-length spike antigen delivered with Matrix-M adjuvant. The antigen shares design similarities with the antigen evaluated in our study and was manufactured using the same insect-cell expression system. The adjuvant in the phase 1–2 human trial was a saponin-containing adjuvant, distinct from the oil-in-water based adjuvants evaluated in this trial.

Two additional vaccine candidates were known to the authors through joint development activities, both of which have reported preliminary interim results in preprints that were not retrieved through the search. One of the studies tested a virus-like particle vaccine manufactured in plants, which was found to induce higher antibody titres and cellular responses when adjuvanted with AS03 than with a second adjuvant. The second study investigated the safety and immunogenicity of a range of doses of a recombinant trimeric spike-protein antigen vaccine manufactured in a mammalian cell line, administered without adjuvant or adjuvanted with AS03 or a second adjuvant. For both of these AS03-adjuvanted vaccine

candidates, the antibody responses were higher than antibody concentrations in panels of sera from patients recovering from COVID-19.

Added value of this study

This interim analysis represents the first-in-human evaluation of our adjuvanted recombinant protein vaccine against SARS-CoV-2, CoV2 preS dTM. In this study, although the vaccine raised antibodies capable of neutralising wild-type SARS-CoV-2 strains in vitro, it had suboptimal immunogenicity, probably due to administration of lower antigen doses than planned due to use of an externally procured reagent in testing. Cytokine profiling using ex-vivo whole blood stimulation showed a more robust induction of T-helper-1 cell cytokines than T-helper-2 cell cytokines. Reactogenicity after the second dose was greater than expected, especially among participants receiving the adjuvanted formulations. This greater than expected reactogenicity was probably due to higher than expected levels of host cell proteins in the vaccine due to use of an externally procured reagent in testing. No other specific safety concerns were noted.

Implications of all the available evidence

Protein vaccines are widely used for vaccine-preventable diseases and represent a reliable and highly scalable technology, with products that can be distributed at 2–8°C, offering clear advantages for global distribution. The present vaccine candidate will require optimisation of the vaccine antigen formulation and dose before testing in phase 3 trials.

production costs than other traditional (eg, attenuated or inactivated) vaccines, they often require the use of an adjuvant to enhance the magnitude, quality, and persistence of the immune response.[13] The antigen dose-sparing qualities of adjuvanted formulations, allowing a reduced quantity of vaccine antigen to achieve a robust immune response compared with antigen alone, are particularly pertinent in a pandemic situation, in which there might be potential constraints on antigen supply. We used two different oil-in-water emulsions as vaccine adjuvant components for the candidate SARS-CoV-2 recombinant protein vaccine, CoV2 preS dTM: the AF03 adjuvant (Sanofi Pasteur, Marcy l'Etoile, France)[14] and the AS03 Adjuvant System (GlaxoSmithKline, Wavre, Belgium).[15]

This interim analysis evaluated the safety and immunogenicity, including binding and neutralising antibody responses and cell-mediated immunity, of CoV2 preS dTM, with the goal of informing selection of an adjuvant formulation, antigen dose, and immunisation schedule to proceed to further clinical development.

Methods
Study design and participants
This is an ongoing phase 1–2, randomised, modified double-blind (unmasked vaccine preparer or adminis-trator, thus person has no role in evaluating the participant for safety outcomes; masked participant; and masked outcome assesor), first-in-human, parallel group, placebo-controlled, dose-ranging study, with a sentinel safety cohort and early safety data review. The study is ongoing across ten clinical research centres in the USA, with a planned duration of approximately 12 months after the last study injection. Here, we present interim safety and immunogenicity data up to 43 days after first vaccination with the stabilised pre-fusion S protein vaccine, CoV2 preS dTM.

Healthy adults aged 18 years and older were eligible for inclusion. A lateral flow immunochromatographic assay (COVID-19 IgG/IgM Rapid Test Cassette; Healgen Scientific, Houston, TX, USA) was used to identify those with recent or previous SARS-CoV-2 infection; the test was done at each clinical site by trained personnel, according to the manufacturer's instructions. Individuals testing negative for SARS-CoV-2 antibodies were included in the study. Exclusion criteria included chronic illness or medical conditions considered to potentially increase the risk for severe COVID-19 illness; women who were pregnant or lactating; women of childbearing potential who were not using an effective method of contraception or abstinence from at least 4 weeks before the first vaccination until at least 12 weeks after the last vaccination; participation, or planned participation, in another clinical trial during the study period; receipt or planned receipt of any vaccine in the 30 days before the first, or up to 30 days after the last, study vaccination (except for influenza vaccination, which could be received at least 2 weeks

before or after study vaccines); receipt of immunoglobulins, blood, or blood-derived products in the past 3 months; and active or previously documented autoimmune disease (appendix 1). Participants who received another authorised COVID-19 vaccine were not withdrawn from the study. No participant received another COVID-19 vaccine during the interim analysis period.

The study was done in compliance with the International Conference on Harmonisation guidelines for Good Clinical Practice and the principles of the Declaration of Helsinki. The protocol and amendments were approved by applicable Independent Ethics Committees and Institutional Review Boards and the regulatory agency as per local regulations. Written informed consent was obtained from the participants before any study procedures were done.

Randomisation and masking
Participants were stratified by age (18–49 years and ≥50 years) and randomly assigned to one of 11 different treatment groups to receive one of five candidate vaccine formulations or placebo, as a single-dose or two-dose schedule. The candidate vaccine formulations were low-dose antigen with AF03 or AS03 adjuvant, high-dose antigen with AF03 or AS03 adjuvant, or unadjuvanted high-dose antigen (appendix 2 p 3). No participant aged 50 years or older was allocated to the unadjuvanted high-dose antigen group as older adults are less likely than younger adults to respond without the presence of an adjuvant and to minimise the theoretical risk of vaccine-enhanced disease. More participants in the two-dose cohort were allocated to receive AS03-adjuvanted formulations than AF03-adjuvanted formulations due to the substantial clinical and post-marketing experience, readiness for large-scale supply, and the extensive and multinational regulatory experience with AS03. Groups were randomly assigned using an interactive response technology system by a contract research organisation, (Calyx, Nottingham, UK). Block randomisation was used, with blocks of varying sizes.

Initially, 30 participants aged 18–49 years were enrolled into a safety sentinel cohort and received a single dose of the intervention to which they were randomly assigned. A review of the safety data up to 9 days after the first dose, unmasked to treatment group, was done by the Sanofi Pasteur internal safety committee. Only upon demonstration of acceptable safety were the remaining participants enrolled.

Only the study site staff who prepared and administered the vaccine knew which vaccine was administered, and they were not involved in assessment of study data.

Procedures
The preS dTM was produced from a Sanofi Pasteur proprietary cell-culture technology based on the insect-cell baculovirus expression vector system (appendix 2 p 5). Purity was determined by sodium dodecyl sulphate

polyAcrylamide gel electrophoresis and scanning densitometry, as described in the appendix 2 (p 5). The target quantities of the SARS-CoV-2 preS-antigen per vaccine dose were 5 µg for the low-dose formulation and 15 µg for the high-dose formulation. However, during characterisation studies (appendix 2 p 5) on the final bulk drug substance, a key polyclonal antibody reagent used to detect the SARS-CoV-2 preS protein was found to also recognise glycosylated host-cell proteins. As a result, the purity and host-cell protein levels reported for the phase 1–2 clinical trial materials were inaccurate, and the concentration of SARS-CoV-2 preS protein in the formulated vaccine product was substantially lower (approximately four to six times) than planned. Upon recalculation, the effective dose levels administered in a 0·5 mL vaccine dose in this study were 1·3 µg (low dose) and 2·6 µg (high dose) of functional SARS-CoV-2 preS protein. The underdosing of the vaccine formulation was discovered after the study was fully enrolled and all participants had received at least one dose of their assigned product. The differences between the targeted and the effective dose levels correspond to an excess in host-cell protein content in the clinical materials (recalculated host-cell protein content was 3·7 µg in the low dose formulation and 12·4 µg high dose formulation). The AF03 (Sanofi Pasteur)[14] and AS03 (GlaxoSmithKline)[15] adjuvants are oil-in-water emulsions, described in detail in the appendix 2 (p 5).

Vaccine formulations were supplied in two separate vials, one vial containing antigen suspension and another containing the adjuvant emulsion or phosphate-buffered saline (PBS) diluent. These were mixed before injection to give a final dose volume for injection of 0·5 mL, containing 0·25 mL antigen and 0·25 mL adjuvant emulsion or PBS diluent. Placebo recipients received 0·5 mL 150 mM NaCl. Vaccine formulations and placebo were prepared by qualified and trained study personnel and administered into the deltoid region of the upper arm by intramuscular injection on day 1 and day 22 (for the two-dose groups only; appendix 2 p 2).

Laboratory assessments included serum biochemistry tests, haematology (complete blood count with differential and platelets), and urine analyses. Adverse events were assessed for intensity (grade 1 to grade 3) and their relationship to the study intervention by the investigator at each site.

SARS-CoV-2 neutralising antibodies were measured in serum samples with a microneutralisation assay at Sanofi Pasteur Global Clinical Immunology Swiftwater, PA, USA, using the SARS-CoV-2 USA-WA1/2020 strain (BEI Resources; catalog number NR-52281). The reduction in SARS-CoV-2 infectivity, compared with that in the control wells, indicated the presence of neutralising antibodies in the serum sample. The 50% neutralisation titre was recorded[16] (appendix 2 p 6). Binding antibody concentrations were measured using indirect ELISA done at Nexelis, Laval, Quebec, Canada (appendix 2 p 6),

in which the reference standard was human serum with known concentration of anti-S protein IgG antibodies; quantitative results were reported in EU/mL.

A participant subset was randomly selected from the two-dose cohort for evaluation of cell-mediated immunity using an interactive response technology system. Immune responses mediated by T-helper-1 (Th1) and T-helper-2 (Th2) cells were measured from blood samples obtained on days 1, 22, and 36, after ex-vivo stimulation, using the TruCulture system (Myriad Biosciences, Austin, TX, USA). Blood samples (1 mL) were drawn directly into the TruCulture tubes, containing 2 mL of buffered media without stimulation (negative control), SARS-CoV-2 S protein for specific stimulation (S 2P-GCN4, GeneArt), or staphylococcal enterotoxin B plus anti-cluster of differentiation 28 for unspecific stimulation (positive control; appendix 2 p 6). Validated cytokine profiling panels were used to evaluate concentrations of interferon-γ (IFNγ), tumour necrosis factor-α (TNF-α), interleukin (IL)-2, IL-4, IL-5, and IL-13 (appendix 2 pp 6–7). When the initial regulatory guidances were made public, an emphasis was placed on the need to characterize polarization of T-helper responses as Th1 or Th2 predominant. This was viewed as a safety concern. In this spirit, we have considered the ratio of cytokines as the most efficient way to summarise these data and address this concern.

Outcomes

The primary objective was to describe the safety profile of the candidate vaccine formulations up to 12 months after the last dose in all participants. Safety endpoints included immediate unsolicited systemic adverse events (occurring within 30 min after each dose); solicited injection-site reactions (pain, erythema, and swelling) and solicited systemic reactions (fever, headache, malaise, and myalgia) up to 7 days after each dose; clinical safety laboratory measures 8 days after the last dose (on day 9 for the single-dose cohort and day 30 for the two-dose cohort); unsolicited adverse events up to 21 days after each dose; and medically attended adverse events, serious adverse events, and adverse events of special interest, documented throughout the study. Adverse events of special interest included anaphylactic reactions and potential immune-mediated diseases. Potential immune-mediated diseases are a subset of adverse events that include autoimmune diseases and other inflammatory or neurological disorders of interest that might have an autoimmune aetiology.[17] In this interim analysis, we describe primary safety endpoints up to day 43.

The primary immunogenicity objective was to describe the neutralising capacity of vaccine-induced antibodies at days 2, 22, and 36 for each study group. Secondary objectives for immunogenicity included descriptions of the neutralising antibody profile at days 181 and 366 (single-dose cohort) or days 202 and 387 (two-dose cohort) and the binding antibody profile on days 1, 22,

	Low-dose plus AF03 group (n=34)	Low-dose plus AS03 group (n=34)	High-dose plus AF03 group (n=34)	High-dose plus AS03 group (n=34)	Placebo group (n=34)
Aged 18–49 years					
Sex					
Female	14/24 (58%)	9/24 (38%)	13/24 (54%)	14/24 (58%)	12/24 (50%)
Male	10/24 (42%)	15/24 (62%)	11/24 (46%)	10/24 (42%)	12/24 (50%)
Age, years	36·0 (10·0)	35·4 (8·66)	32·0 (9·69)	29·7 (8·88)	31·1 (8·45)
Race or ethnicity					
White	20/24 (83%)	21/24 (88%)	22/24 (92%)	16/24 (67%)	20/24 (83%)
Asian	1/24 (4%)	2/24 (8%)	1/24 (4%)	4/24 (17%)	1/24 (4%)
American Indian or Alaska Native	0	0	0	1/24 (4%)	1/24 (4%)
Black or African American	1/24 (4%)	0	0	1/24 (4%)	0
Multiple	0/24	1/24 (4%)	1/24 (4%)	0	1/24 (4%)
Not reported or unknown	2/24 (8%)	0/24	0/24	2/24 (8%)	1/24 (4%)
Hispanic or Latino	6/24 (25%)	2/24 (8%)*	3/24 (13%)	3/24 (13%)	7/24 (29%)
Aged 50 years or older					
Sex					
Female	8/10 (80%)	6/10 (60%)	4/10 (40%)	7/10 (70%)	6/10 (60%)
Male	2/10 (20%)	4/10 (40%)	6/10 (60%)	3/10 (30%)	4/10 (40%)
Age, years	59·3 (7·07)	57·3 (5·70)	58·5 (9·29)	61·3 (10·1)	60·1 (7·74)
Race or ethnicity					
White	9/10 (90%)	7/10 (70%)	8/10 (80%)	9/10 (90%)	10/10 (100%)
Asian	1/10 (10%)	1/10 (10%)	0	1/10 (10%)	0
Black or African American	0	1/10 (10%)	0	0	0
American Indian or Alaska Native	0	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	1/10 (10%)	0	0	0
Multiple	0	0	1/10 (10%)	0	0
Not reported or unknown	0	0	1/10 (10%)	0	0

Data are n/N (%) or mean (SD). *Hispanic or Latino ethnicity was not reported for one participant in the high-dose plus AF03 group, in the 18–49 years stratum.

Table 1: Participant demographic characteristics (full analysis set) per treatment group for participants in the one-dose cohort

36, 181, and 366 (single-dose cohort) or days 1, 22, 36, 202, and 387 (two-dose cohort) for each study group. In this interim analysis, we describe cell-mediated immunogenicity endpoints up to day 36 (exploratory endpoint).

In an exploratory analysis, neutralising antibody titres were measured in a panel of human convalescent serum samples (Sanguine Biobank, iSpecimen, and PPD). Convalescent samples were obtained from 93 donors between days 17 and 47 after PCR-positive diagnosis of COVID-19. Donors had recovered (with clinical severity ranging from mild to severe) and were asymptomatic at the time of sample collection.

Other study objectives include efficacy endpoints, specifically the occurrence of virologically confirmed COVID-19-like illness and serologically confirmed SARS-CoV-2 infection, and the association between antibody responses to SARS-CoV-2 recombinant protein and the risk of virologically confirmed COVID-19-like illness or serologically confirmed SARS-CoV-2 infection (full list of study objective in appendix 1). These prespecified efficacy endpoints will be assessed as part of the ongoing study and reported separately.

Statistical analysis
All analyses were descriptive; there was no hypothesis testing. No sample size calculations were done. Approximately 440 participants were planned to be enrolled into this study (appendix 2 p 3), with 300 participants aged 18–49 years (20 participants in each group, except AS03-adjuvanted groups in the two-dose cohort, with 60 participants in each group) and 140 participants aged 50 years or older (ten participants in each group, except in AS03-adjuvanted groups in the two-dose cohort, with 30 participants in each group).

Participant demographic characteristics were described table 1 and table 2 for the full analysis set, which include all randomly assigned participants who received at least one dose; participants were analysed according to the treatment group to which they were randomly assigned. Safety objectives were assessed in the safety analysis set, which included all participants who received at least one dose; participants were analysed according to the study treatment received. The per-protocol analysis set was defined as the subset of participants who received at least one dose, met all inclusion and exclusion criteria, had no protocol deviation, and had negative results in the

	Low-dose plus AF03 group (n=28)	Low-dose plus AS03 group (n=82)	High-dose plus AF03 group (n=27)	High-dose plus AS03 group (n=85)	High-dose plus unadjuvanted group (n=18)	Placebo group (n=29)
Aged 18–49 years						
Sex	..	..	..	..	..	..
Female	9/18 (50%)	25/54 (46%)	11/17 (65%)	23/54 (43%)	10/18 (56%)	8/18 (44%)
Male	9/18 (50%)	29/54 (54%)	6/17 (35%)	31/54 (57%)	8/18 (44%)	10/18 (56%)
Age, years	35·3 (8·67)	33·7 (8·99)	32·5 (9·12)	34·9 (8·77)	34·8 (10·2)	32·0 (8·41)
Race or ethnicity	..	..	..	..	..	..
White	15/18 (83%)	46/54 (85%)	16/17 (94%)	48/54 (89%)	16/18 (89%)	16/18 (89%)
Asian	2/18 (11%)	4/54 (7%)	0/17	4/54 (7%)	1/18 (6%)	1/18 (6%)
Black or African American	1/18 (6%)	4/54 (7%)	1/17 (6%)	2/54 (4%)	1/18 (6%)	1/18 (6%)
American Indian or Alaska Native	0	0	0	0	0	0
Hispanic or Latino	4/18 (22%)	9/54 (17%)	2/17 (12%)	6/54 (11%)	3/18 (17%)	3/18 (17%)
Aged 50 years or older						
Sex	..	..	..	..	..	..
Female	5/10 (50%)	11/28 (39%)	7/10 (70%)	21/31 (68%)	NA	6/11 (55%)
Male	5/10 (50%)	17/28 (61%)	3/10 (30%)	10/31 (32%)	NA	5/11 (45%)
Age, years	58·8 (7·69)	59·8 (6·53)	58·7 (7·92)	61·7 (7·86)	NA	61·5 (4·41)
Race or ethnicity	..	..	..	..	..	..
White	9/10 (90%)	24/28 (86%)	9/10 (90%)	30/31 (97%)	NA	10/11 (91%)
Asian	1/10 (10%)	3/28 (11%)	0	0	NA	0
Black or African American	0	0	0	1/31 (3%)	NA	1/11 (9%)
American Indian or Alaska Native	0	1/28 (4%)	0	0	NA	0
Multiple	0	0	1/10 (10%)	0	NA	0
Hispanic or Latino	0	2/28 (7%)	1/10 (10%)	3/31 (10%)	NA	0

Data are n/N (%) or mean (SD). NA=not applicable.

Table 2: Participant demographic characteristics (full analysis set) per treatment group for participants in the two-dose cohort

ELISA or neutralisation test at baseline. Neutralising and binding antibody profiles were assessed in the per-protocol analysis set for immunogenicity, which included participants who had at least one valid post-dose serology sample within the predefined time window (appendix 1). Cell-mediated immunity analyses were done in a subset of participants in the per-protocol analysis set who provided at least one cell-mediated immunity sample within the predefined time window as per previous response. Immunogenicity analyses were done according to the treatment group to which a participant was randomly assigned.

Neutralising antibody profiles were described based on geometric mean titres (GMTs) and 95% CIs. Fold rises in serum antibody neutralisation titres after vaccination relative to day 1 were calculated, whereby pre-vaccination titres below the lower limit of quantification (LLOQ) were converted to half the LLOQ. We calculated the percentage of participants with a four-fold rise in serum neutralisation titres relative to day 1 at days 22 and 36 (before and after dose). We also calculated the proportion of participants with seroconversion, which was defined as detectable neutralisation titres at days 22 and 36 in participants with baseline values below the LLOQ.
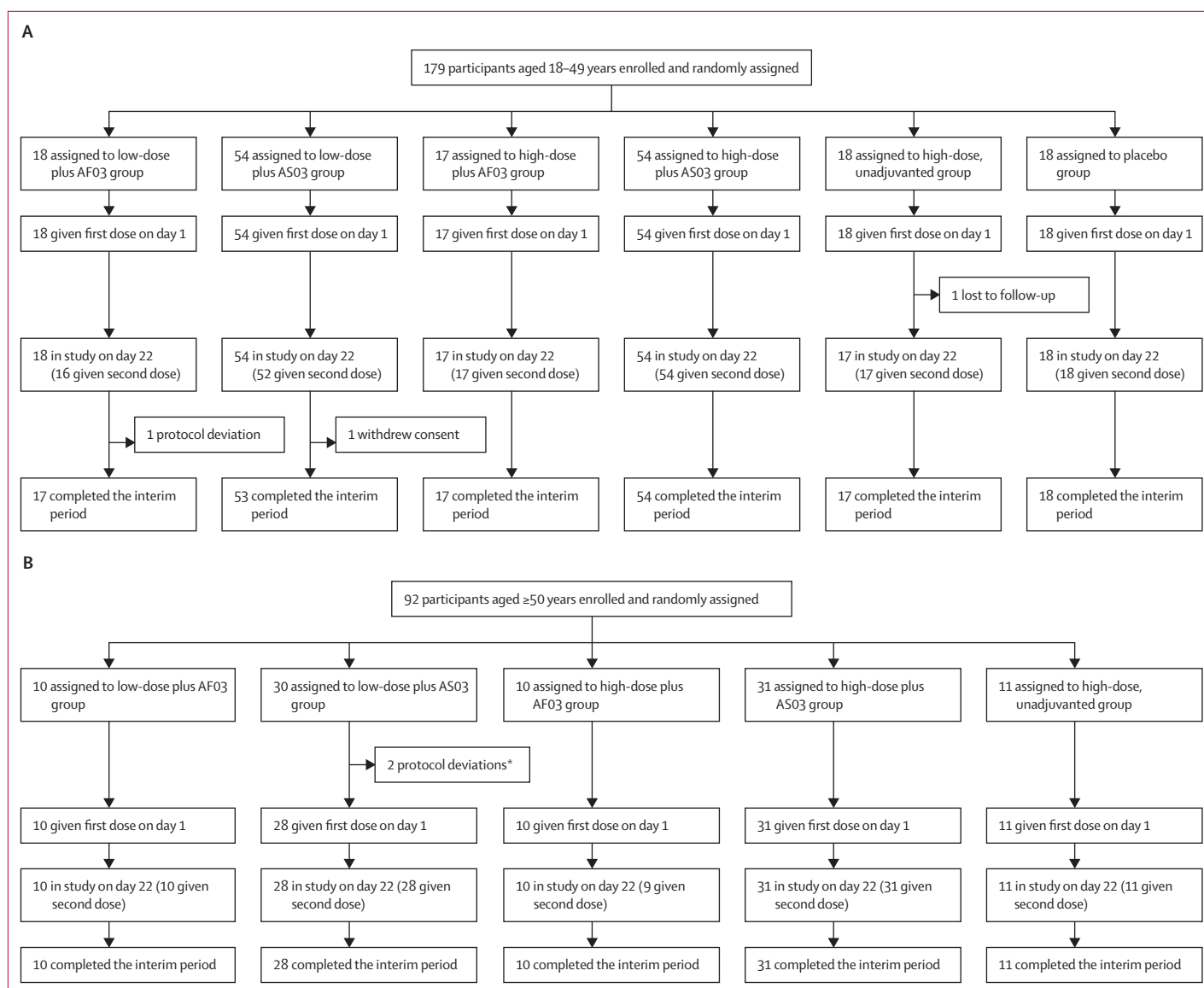
Binding antibody profiles were described based on S-specific antibody geometric mean concentrations measured at days 22 and 36. Antibody concentrations less than the LLOQ were converted to half the LLOQ (appendix pp 6–7).

95% CIs for GMTs, geometric mean concentrations, and GMT ratios were calculated using normal approximation of $\log_{10}$-transformed titres. 95% CIs for proportions were calculated using the Clopper-Pearson method.[18] The differences in seroconversion rates between groups were computed along with the two-sided 95% CIs using the Wilson-Score method without continuity correction.[18]

To characterise Th cell polarisation, the pre-vaccination (day 1) to post-vaccination (days 22 or 36) fold cytokine rises were computed by treatment group, and ratios of fold rises for cytokine pairs (IFNγ and IL-4, IL-5, or IL-13; IL-2 and IL-4, IL-5, or IL-13; and TNFα and IL-4, IL-5, or IL-13) were computed (appendix pp 6–7). Statistical analyses were done using SAS (version 9.4). This study is ongoing and is registered with ClinicalTrials.gov, NCT04537208.

Role of the funding source

Sanofi Pasteur was involved in the study design, data collection, data analysis, data interpretation, writing of

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179 participants aged 18–49 years enrolled and randomly assigned

| 18 assigned to low-dose plus AF03 group | 54 assigned to low-dose plus AS03 group | 17 assigned to high-dose plus AF03 group | 54 assigned to high-dose plus AS03 group | 18 assigned to high-dose, unadjuvanted group | 18 assigned to placebo group |

| 18 given first dose on day 1 | 54 given first dose on day 1 | 17 given first dose on day 1 | 54 given first dose on day 1 | 18 given first dose on day 1 | 18 given first dose on day 1 |

1 lost to follow-up

| 18 in study on day 22 (16 given second dose) | 54 in study on day 22 (52 given second dose) | 17 in study on day 22 (17 given second dose) | 54 in study on day 22 (54 given second dose) | 17 in study on day 22 (17 given second dose) | 18 in study on day 22 (18 given second dose) |

1 protocol deviation   1 withdrew consent

| 17 completed the interim period | 53 completed the interim period | 17 completed the interim period | 54 completed the interim period | 17 completed the interim period | 18 completed the interim period |

B

92 participants aged ≥50 years enrolled and randomly assigned

| 10 assigned to low-dose plus AF03 group | 30 assigned to low-dose plus AS03 group | 10 assigned to high-dose plus AF03 group | 31 assigned to high-dose plus AS03 group | 11 assigned to high-dose, unadjuvanted group |

2 protocol deviations*

| 10 given first dose on day 1 | 28 given first dose on day 1 | 10 given first dose on day 1 | 31 given first dose on day 1 | 11 given first dose on day 1 |

| 10 in study on day 22 (10 given second dose) | 28 in study on day 22 (28 given second dose) | 10 in study on day 22 (9 given second dose) | 31 in study on day 22 (31 given second dose) | 11 in study on day 22 (11 given second dose) |

| 10 completed the interim period | 28 completed the interim period | 10 completed the interim period | 31 completed the interim period | 11 completed the interim period |

Figure 1: Participant flow through the study for those randomised to receive two vaccine doses in the 18–49 years (A) and ≥50 years (B) age strata
*Two participants who were found not to meet all inclusion criteria after randomisation were withdrawn from the study before receiving the first dose.

the report, and the decision to submit the paper for publication.

Results

Between Sept 3 and Sept 29, 2020, 441 participants were randomly assigned (299 aged 18–49 years and 142 aged ≥50 years). Two participants aged 50 years or older from the low-dose plus AS03 group (two-dose cohort) did not meet eligibility criteria. Therefore, 439 participants received at least one dose and were included in the full analysis set: 269 in the two-dose cohort (179 participants aged 18–49 years and 90 participants aged ≥50 years; figure 1) and 170 in the single-dose cohort (120 participants aged 18–49 years and 50 participants

aged ≥50 years; appendix pp 8–9). During randomisation, the specification of the maximum number of participants allocated to the single-dose cohort in the younger age stratum did not account for the allocation to the sentinel safety cohort. Therefore, among participants in the younger age stratum, more were allocated to the single-dose cohort and fewer to the two-dose cohort than planned. The male to female participant ratio was balanced overall, and across most treatment groups. Most enrolled participants were White (table 1, table 2).

The reactogenicity profiles (solicited injection-site and systemic events) were similar across the adjuvanted groups in the single-dose cohort (appendix pp 12–13, 16–17). Among the adjuvanted vaccine groups in the
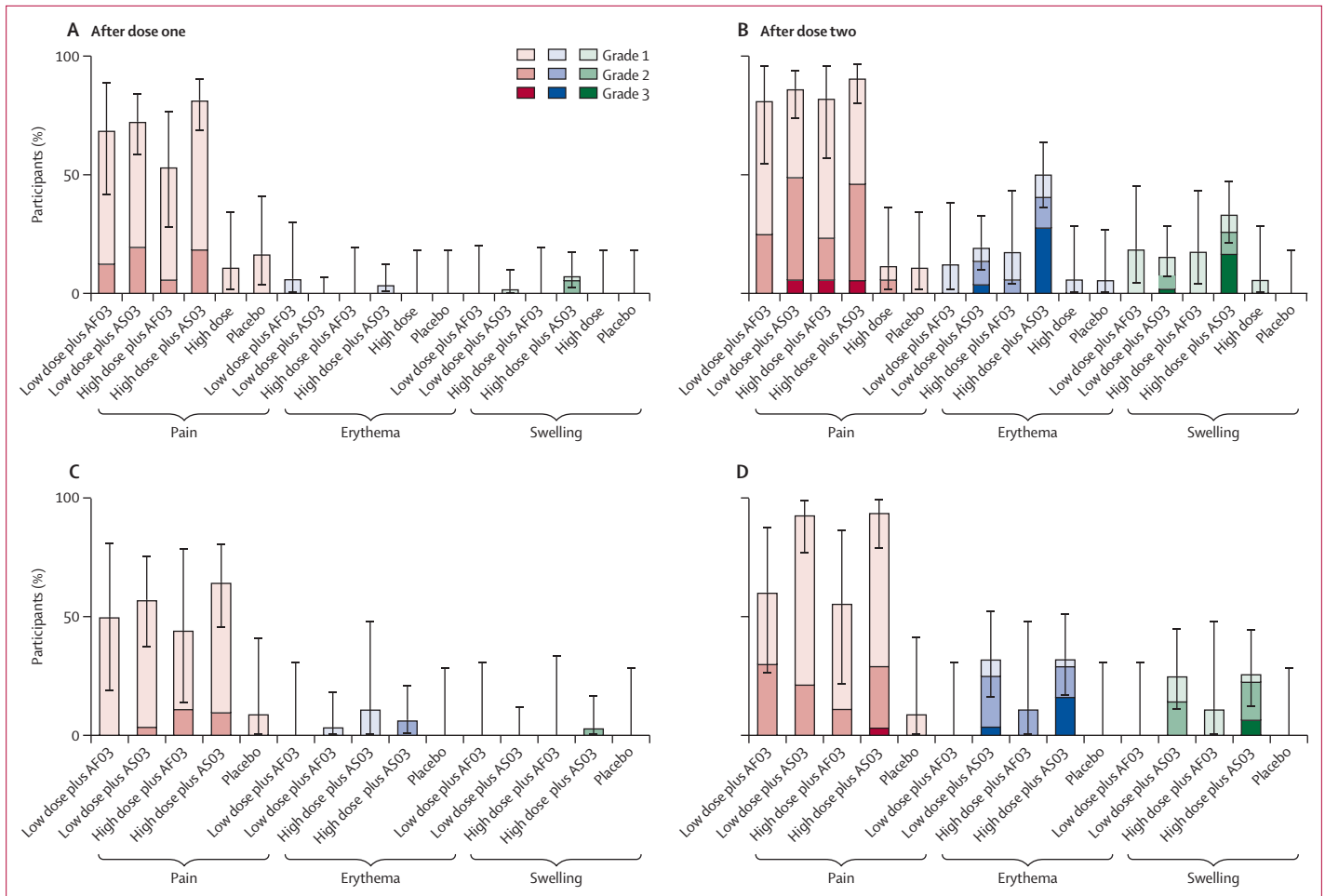
Figure 2: Frequency of solicited injection-site reactions after the first or second dose in participants from the two-dose cohort

two-dose cohort, solicited injection-site reactions (pain, erythema, and swelling) during the first 7 days after vaccination, including grade 3 reactions, occurred more frequently after the second dose than after the first dose (figure 2; appendix pp 10–12). Most injection-site reactions occurred on day 0 (pain) or days 1 and 2 (erythema and swelling; appendix pp 14–15). The unadjuvanted high-dose formulation had a low frequency of injection-site reactions, similar to the placebo group (appendix pp 10–12). Overall, pain was the most frequently reported injection-site reaction: after dose two, 19 (73%) of 26 participants in the low-dose plus AF03 group, 70 (89%) of 79 in the low-dose plus AS03 group, 19 (73%) of 26 in the high-dose plus AF03 group, and 78 (92%) of 85 in the high-dose plus AS03 group reported injection-site pain. Solicited injection-site reactions were generally less frequent and less severe in participants aged 50 years or older than in participants aged 18–49 years across adjuvanted groups, in participants who received the AF03-adjuvanted formulations than in participants who received the AS03-adjuvanted formulations, and in

participants who received the low-dose formulation than in participants who received the high-dose formulation (two-dose cohort; figure 2; appendix pp 10–12). No grade 3 solicited injection-site reactions were observed after the first dose in any treatment group, and none were observed in the unadjuvanted high-dose or placebo groups after one or two doses. After the second dose, the highest frequency of grade 3 injection-site reactions was seen in the high-dose plus AS03 group; the most frequent grade 3 injection-site reaction was erythema (observed in 20 [24%] of 85 participants in the high-dose plus AS03 group) and then swelling (11 [13%] of 85 participants in the high-dose plus AS03 group). No grade 3 injection-site reactions were considered serious, and all resolved within a median duration of 2 days.

Among the adjuvanted vaccine groups in the two-dose cohort, solicited systemic reactions (fever, headache, malaise, and myalgia) during the first 7 days after vaccination, including grade 3 reactions, were reported more frequently after the second dose than after the first dose in both age strata (figure 3; appendix pp 10–12).
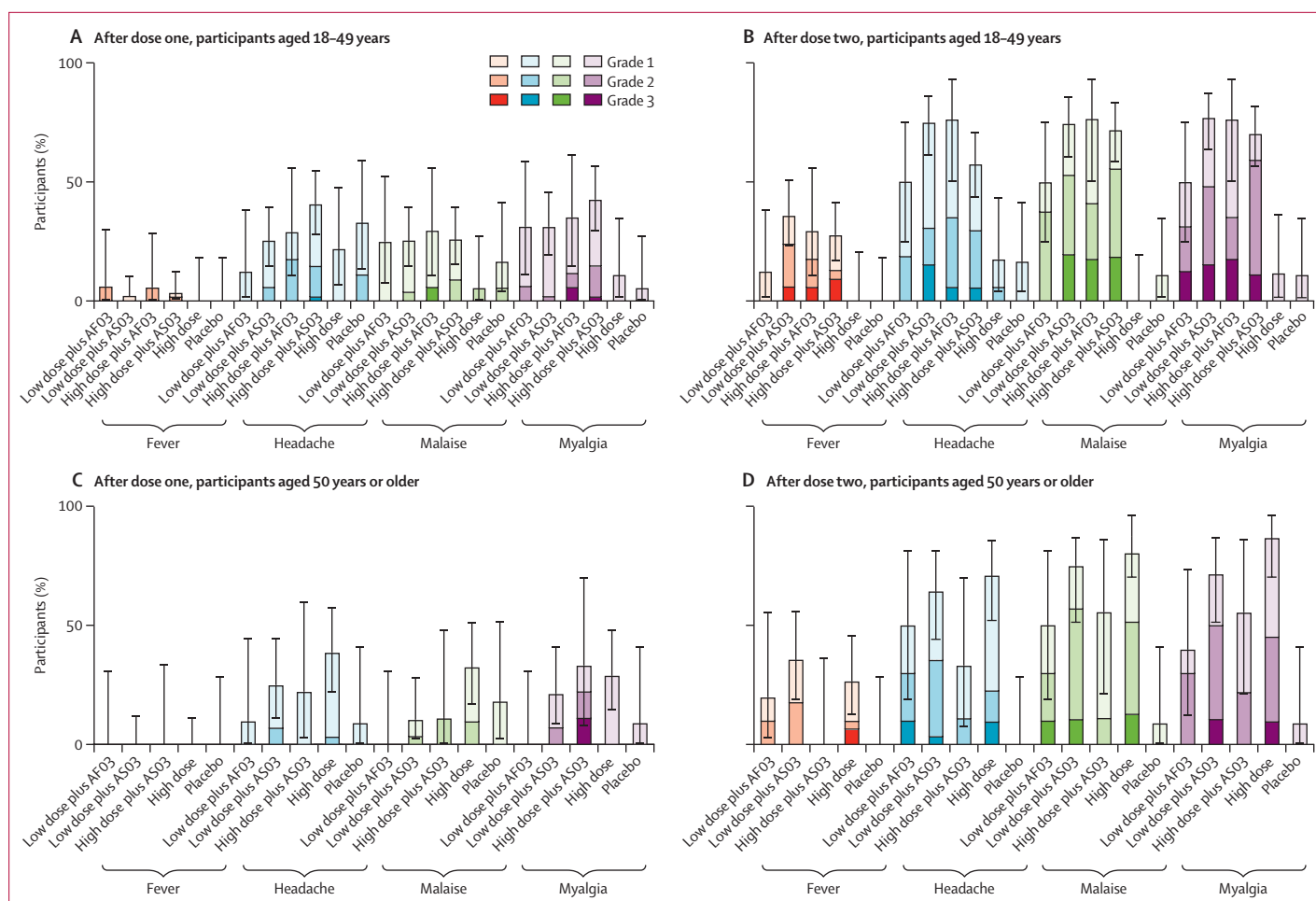
Figure 3: Frequency of solicited systemic reactions after the first or second dose in participants from the two-dose cohort

Systemic reactions occurred most frequently on day 2 after the second dose (appendix pp 14–15). Systemic reactions were generally less frequent and less severe in participants aged 50 years or older than in those in the younger age group, and in participants who received AF03-adjuvanted formulations than in those who received the AS03-adjuvanted formulations. The frequency of solicited systemic reactions among the unadjuvanted high-dose vaccine and placebo groups in the two-dose cohort was low and similar in both groups (figure 3; appendix pp 10–12). Myalgia, malaise, and headache were the most commonly reported solicited systemic reactions after the second dose of adjuvanted vaccine, with frequencies ranging from 46% (12 of 26 participants; for myalgia in the low-dose plus AF03 group) to 76% (65 of 85 participants; for myalgia in the high-dose plus AS03 group; appendix pp 11–12). Fever was reported after the second dose in four (15%) of 26 participants in the low-dose plus AF03 group, 28 (36%) of 78 in the low-dose plus AS03 group, five (19%) of 26 in the high-dose plus AF03 group, and 23 (27%) of 84 in the

high-dose plus AS03 group. Grade 3 systemic reactions also occurred more frequently after the second dose than after the first dose among the adjuvanted formulations, with the highest frequency in the low-dose plus AS03 group (grade 3 headache, malaise, and myalgia occurred in nine [11%] of 80 participants, 13 [17%] of 79 participants, and 11 [14%] of 80 participants, respectively), high-dose plus AS03 group (grade 3 headache, malaise, and myalgia occurred in six [7%], 14 [17%], and nine [11%] of 85 participants, respectively), and high-dose plus AF03 group (grade 3 headache, malaise, and myalgia occurred in one [3%], three [12%], and three [12%] of 26 participants, respectively; figure 3; appendix pp 11–12). No grade 3 solicited systemic reactions were considered serious, and all resolved with a median duration of 2 days.

No vaccine-related immediate unsolicited reactions were observed in any group (appendix pp 18–20). We observed an increase in the number of unsolicited adverse events after the second dose compared with placebo, mainly in the AS03 groups, with a small number of grade 3 reactions, primarily due to reporting of
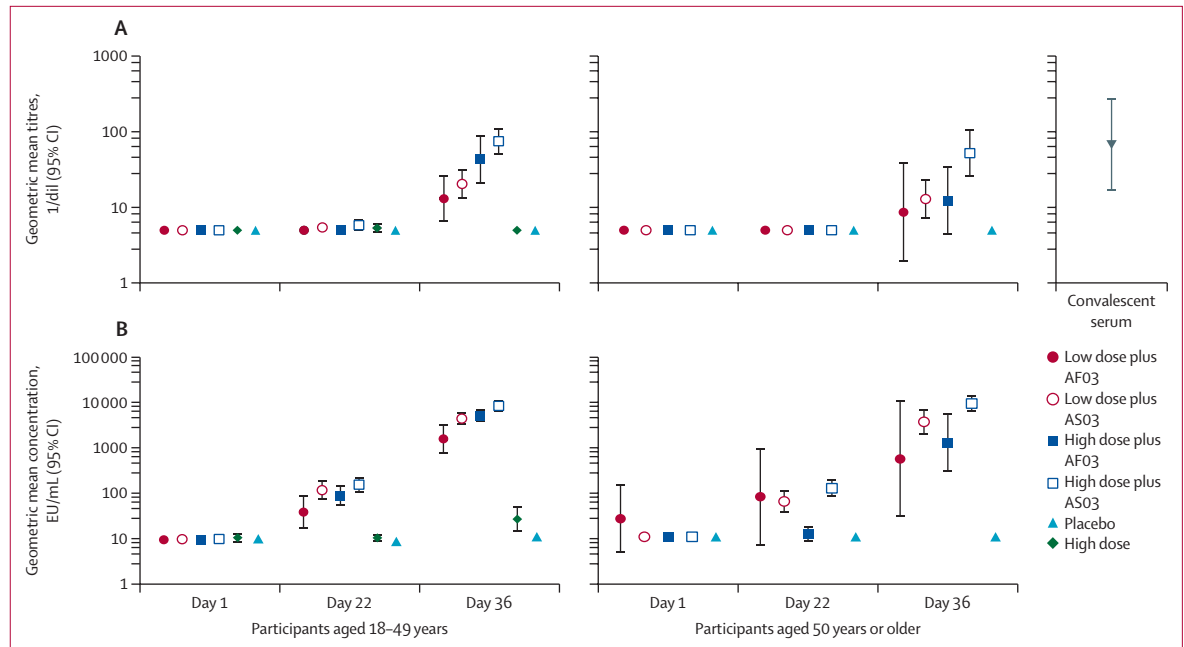
Figure 4: Neutralising (A) and binding (B) antibody responses after the first and second doses by age group
Data for the two-dose cohort; per-protocol analysis set for immunogenicity. Neutralising antibody titres are additionally shown for a panel of 93 convalescent serum samples as indicated in (A).

reactogenicity-type (local and systemic) events (appendix p 18). Three serious adverse events were reported (two in the single-dose cohort, one each in the low-dose plus AF03 and high-dose plus AF03 groups; and one in the two-dose cohort in the high-dose plus AS03 group). All three were deemed not related to the vaccine by the investigator and the sponsor, and none led to discontinuation from the study. These serious adverse events included one transient ischaemic attack in a participant with a history of ocular occlusion and treatment with antiplatelet drugs, who did not receive a second dose; one hip fracture after the first dose in a participant who remained in the study; and one participant who developed breast cancer, who had symptoms before study vaccination, and did not receive a second dose. There were no potential immune-mediated diseases reported and there were no adverse events of special interest, serious adverse events, or severe medically attended adverse events considered to be related to study vaccine by the investigator in any group (up to day 43 of the study).

A single vaccine dose did not generate neutralising antibody titres above placebo levels in any group at days 22 or 36 (appendix p 22). Among participants aged 18–49 years, neutralising antibody GMTs after two doses were 13·1 (95% CI 6·40–26·9) in the low-dose plus AF03 group, 20·5 (13·1–32·1) in the low-dose plus AS03 group, 43·2 (20·6–90·4) in the high-dose plus AF03 group, 75·1 (50·5–112·0) in the high-dose plus AS03 group, 5·00 (not calculated) in the unadjuvanted high-dose group, and 5·00 (not calculated) in the placebo

group (figure 4A). Among participants aged 50 years or older, neutralising antibody titres after two vaccine doses were 8·62 (1·90–39·0) in the low-dose plus AF03 group, 12·9 (7·09–23·4) in the low-dose plus AS03 group, 12·3 (4·35–35·0) in the high-dose plus AF03 group, 52·3 (25·3–108·0) in the high-dose plus AS03 group, and 5·00 (not calculated) in the placebo group. The GMT for the pooled convalescent serum panel was 72·4 (95% CI 17·6–297·5; figure 4A). After dose two, GMTs observed with the unadjuvanted high-dose formulation did not differ substantially from those in the placebo group. AF03-adjuvanted and AS03-adjuvanted high-dose formulations yielded approximately three-fold and four-fold higher GMTs, respectively, than did their low-dose formulation counterparts: GMTs on day 36 were 30·2 (95% CI 16·3–55·9) in the high-dose plus AF03 group and 67·6 (47·9–95·4) in the high-dose plus AS03 group versus 11·7 (6·50–20·9) in the low-dose plus AF03 group and 17·2 (12·1–24·5) in the low-dose plus AS03 group (both age strata combined).

At day 36, the proportion of all participants independently from the age group with a four-fold rise in neutralising antibody titres was 29·4% (95% CI 10·3–56·0; 5 of 17) in the low-dose plus AF03 group, 37% (25–50; 23 of 63) in the low-dose plus AS03 group, 75% (63·0–84·7; 51 of 68) in the high-dose plus AF03 group, and 67% (43·0–85·0; 14 of 21) in the high-dose plus AS03 group (appendix p 23). In the post-hoc analysis, the proportion of participants with a four-fold rise in neutralising antibody titres was lower in the 50 years or older than in the 18–49 years age groups, particularly

for participants aged 60 years or older (range across vaccine groups for ≥60 years was from 0% [0–98; 0 of one] in the low-dose plus AF03 group to 50% [16–84; four of eight] in the high-dose plus AS03 group; appendix p 23). Seroconversion for neutralising antibody titres at day 36 was observed in 47% (95% CI 23–72; eight of 17) of participants in the low-dose plus AF03 group, 52% (39–65; 33 of 63) in the low-dose plus AS03 group, 71% (48–89; 15 of 21) in the high-dose plus AF03 group, and 88% (78–95; 60 of 68) in the high-dose plus AS03 group; appendix p 23). The proportion with seroconversion was lower in participants aged 50 years or older, particularly those aged 60 years or older, than in participants aged 18–49 years (range across vaccine groups for ≥60 years was from 0% [0–98; 0 of 1] in the low-dose plus AF03 group to 63% [25–92; five of eight] in the high-dose plus AS03 group; appendix p 23).

Binding antibody responses were noted in the single dose cohort, although geometric mean concentrations were ten-times lower than the corresponding two-dose group and none of the groups achieved higher than 90% binding antibody seroconversion at D36 whereas 100% binding antibody seroconversion was achieved in all of the two-dose adjuvanted vaccine groups (two dose groups Figure 4B; single dose groups appendix p 22). Some increases in binding antibody geometric mean concentrations were measured after the first dose, but greater increases were evident after the second dose in both age groups (figure 4B). Responses were higher in the AS03-adjuvanted groups than in the AF03-adjuvanted groups, and higher in the high-dose groups than the low-dose groups; a small increase compared with placebo at day 36 was observed in the unadjuvanted high-dose group.

Cell-mediated immunity was assessed in a subset of 87 participants from the two-dose cohort: 60 participants aged 18–49 years (18 per group for the AS03-adjuvanted vaccine groups; six per group for all other study groups) and 27 participants aged 50 years and older (nine per group in AS03-adjuvanted groups; three per group in all other study groups). Increases of IFN-γ, IL-2, and TNFα cytokines from pre-vaccination to days 22 and 36 tended to be more robust than were the increases for IL-4, IL-5, and IL-13, especially in the AS03-adjuvanted groups, suggesting no Th2 cell bias in the cell-mediated responses (appendix p 24).

Discussion

In this descriptive, first-in-human study, a recombinant, pre-fusion stabilised trimeric SARS-CoV-2 S-antigen, formulated with either AS03 or AF03 oil-in-water-based adjuvants, elicited neutralising antibodies with no specific safety concerns that preclude further development. Furthermore, a non-Th2 cell biased cytokine response was generated in AS03-adjuvanted vaccine groups, with IFN-γ production consistent with previous observations using the AS03 adjuvant system in influenza, hepatitis B, and

SARS-CoV-2 candidate vaccines.[19–22] In SARS-CoV-2-seronegative participants aged 18–49 years, two doses of the adjuvanted candidate vaccine formulations were needed to generate neutralising antibody titres of a similar magnitude to those measured in patients recovering from PCR-confirmed infection (convalescent serum titres). The need for an adjuvant was shown as the unadjuvanted high-dose group did not elicit a neutralising antibody response. Neutralising antibody titres among participants aged 50 years or older were lower than those in the younger age group, showing an age effect with the formulations evaluated. This effect was further evidenced by lower proportions of participants aged 50 years or older than aged 18–49 years with a four-fold rise in neutralising antibody titres and who seroconverted, particularly those aged 60 years and older, a key population at risk of severe illness after infection with SARS-CoV-2. The high-dose formulation given with either AS03 or AF03 resulted in higher neutralising responses than the corresponding low-dose formulation. The high-dose plus AS03 formulation consistently showed more robust neutralising and binding antibody responses compared with the other candidate vaccine formulations. However, not all participants in the high-dose plus AS03 group had seroconversion of neutralising antibodies after the second dose, especially among older participants (63% [95% CI 24·5–91·5] of those aged ≥60 years).

Previous human experimental coronavirus infection studies identified that the presence of pre-challenge neutralising antibodies was predictive of protection from infection or symptoms after challenge.[23,24] A growing body of evidence from animal models suggests a key role for humoral responses, and specifically neutralising antibody responses, in protection against SARS-CoV-2. A study showed that adoptive transfer of purified IgG from convalescent macaques protected naive recipient rhesus macaques against SARS-CoV-2 challenge in a dose-dependent manner, with reasonably low neutralising antibody titres sufficient to protect against SARS-CoV-2 in this model.[25] High neutralising antibody titres were able to achieve full protection in macaques, whereas ten-fold lower titres could still attain partial protection. These data suggest that neutralising antibodies might be sufficient for protection, even in the absence of cellular and innate immune responses.[25] Our findings indicate that, although the best performing candidate vaccine formulation among younger adults was similar in terms of neutralising titres to the titres seen in convalescent serum titres, the responses in older participants were notably lower than convalescent serum titres.

The poor antibody responses observed in this study are most likely due to the use of substantially lower antigen doses than planned. Although other factors, for example poor characterisation of the expressed antigen or product matrix, or failure during bedside mixing, could theoretically lead to poor immune responses to the candidate vaccine formulations, these are unlikely given

the extensive characterisation undertaken in our study. Nevertheless, further development of the manufacturing process and assays to support characterisation of antigen content were ongoing in parallel to this first-in-human trial. Polyclonal sera used in antigen characterisation were discovered, after the study had commenced, to also bind to host-cell proteins, resulting in an overestimation of the S-antigen content during manufacture and under-estimation of the host-cell protein content. Therefore, although the observed antibody responses support the selection of the AS03 adjuvant for further development, they also indicate that further optimisation of the antigen formulation or purification process and antigen dosage for the selected AS03-adjuvanted candidate vaccine is required. It should be noted that such evolution of manufacturing and production methods is typical during the development pathway but has been accelerated necessarily for COVID-19 vaccine development.

No vaccine-related serious adverse events, adverse events of special interest, or severe medically attended adverse events were reported after either one or two vaccine doses. There was a higher than anticipated number and severity of local and systemic solicited reactions after the second dose of the adjuvanted vaccine formulations, with the highest frequency in the high-dose plus AS03 groups. These reactions were generally less frequent and milder in the older adults than in younger adults. The unadjuvanted high-dose formulation generated similar reactogenicity profiles to placebo. Overall, these solicited local and systemic reactions were not serious, lasted a median of 2 days, and fully resolved. Although we did not assess a potential association of reactogenicity with the magnitude of the immune response, it is notable that both were greatest in the high-dose plus AS03 groups of the two-dose cohort. Although transient, the reactogenicity profile observed after the second dose in the adjuvanted groups showed more frequent and more severe reactions than those reported in previous studies of influenza vaccine candidates (two-dose schedules) containing the same adjuvants[26–29] and the reactogenicity profiles reported for other SARS-CoV-2 vaccine candidates using AS03-adjuvanted recombinant S proteins (Medicago [NCT04450004][22] and Clover Biopharmaceuticals [NCT04405908]). Notably, high levels of reactogenicity have been described during the clinical development of other SAR-CoV-2 vaccines, including the two recently authorised mRNA vaccines (BioNTech-Pfizer and Moderna),[30,31] as well as for the Janssen adenovirus vector vaccine,[32] the Novavax recombinant CoV2 preS vaccine (containing Matrix-M1 adjuvant),[12] and the candidate mRNA-based vaccine CVnCoV (Curevac).[33]

We hypothesised that the high levels and severity of the reactogenicity observed in our study might be explained by the higher than anticipated content of host-cell protein in the clinical material (estimated at approximately 3·7 µg per vaccine dose in the low-dose groups and 12·4 µg per dose in the high-dose groups) resulting from the erroneous characterisation of S protein and host-cell protein content. Although a high host-cell protein content has been administered historically in the context of clinical development of a recombinant influenza vaccine using the same manufacturing platform, no such levels of host-cell proteins have been previously administered in combination with an adjuvant or in a two-dose injection schedule. In future clinical studies with the CoV2 preS dTM vaccine we therefore plan to use clinical material with host-cell protein content below that of the low-dose group in the present study, with the aim of improving the reactogenicity profile. There was no other medically relevant safety observation during the interim study period (up to day 43). Safety monitoring continues for up to 12 months after administration of the second vaccine dose.

A previously postulated theoretical safety concern with SARS-CoV-2 vaccines is the risk of potentiating immunopathology in vaccine recipients upon exposure to wild-type virus. Various hypothetical risk factors have been proposed, including the magnitude of the immune responses, the balance between binding and functional antibodies, the induction of antibodies with functional characteristics such as binding to particular Fc receptors, and the nature of the Th2 cell response, with Th2-polarised cellular responses being proposed to contribute to immunopathology.[34–37] In this interim analysis, there was no evidence of vaccine-mediated disease enhancement. The results from our cell-mediated immunity analysis do not support a bias towards Th2 polarisation after the first or second dose of the AS03-adjuvanted candidate vaccine formulations; rather, we observed a consistent Th1 response, as measured through IFNγ, combined with low levels of Th2 responses, as measured through IL-4, IL-5, and IL-13. Although there is currently no evidence of any candidate SARS-CoV-2 vaccine giving rise to the phenomenon of vaccine-mediated enhanced disease, the observed cell-mediated immunity profile is reassuring.

The main limitation of this study is the erroneous characterisation of the content of protein and host-cell proteins used in the filled clinical materials administered in the trial, resulting in a substantially lower concentration of SARS-CoV-2 S protein in the formulated vaccine product than expected, and a correspondingly higher host-cell protein content. Other limitations include the necessarily small numbers of participants in this phase 1–2 study, such that rare serious adverse events and adverse events of special interest might not have been captured. Furthermore, an error in the specification of the caps on group sizes during randomisation resulted in more participants in the younger age stratum being allocated to the single-dose cohort and fewer participants in the two-dose cohort than planned. Further analysis of the cell-mediated immune responses is ongoing; in particular, cellular profiling will help determine the source of cytokines detected during ex-vivo stimulation of whole blood as described here.

The results from the candidate vaccine formulations tested here are informative in terms of the neutralising and binding antibody responses generated in healthy adults. Further improvement of the preS vaccine antigen formulation is needed to identify the optimal vaccine dose before evaluation in larger-scale phase 3 trials.

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