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Title

Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (Alveavax-v1.2): Results of a first-in-human, open-label, active-controlled, randomized dose-finding study of intradermal and subcutaneous application in primary Ad26.COV2.S vaccinated healthy individuals.

Clinical Trial.gov / SA Registry number

South African National Clinical Trials Registry Identifier: DOH-27-062022-5157
ClinicalTrials.gov Identifier: NCT05844202

Cover Letter

Cambridge, MA, October 2023

Dear Editor,

On behalf of the co-authors I am excited to share this manuscript with you.

We present the results of an open-label, active-controlled, randomized phase I trial conducted in 2022 in South Africa. We tested a room-temperature stable SARS-CoV-2 Omicron BA.2 booster DNA vaccine candidate, Alveavax-v1.2, in 130 primary vaccinated participants across five different arms including comparator.

We believe this manuscript is a valuable advance in the understanding of DNA vaccines. DNA vaccine platforms are of particular interest to low- and middle income countries due to their shelf stability and low manufacturing complexity. The safety and immunogenicity of SARS-CoV-2 Omicron DNA boosters are unknown. To our knowledge this study is the first time where

1. A naked DNA based SARS-CoV-2 booster candidate was studied in preimmunized humans (with ~80% having hybrid immunity from previous infections).
2. Unusually high doses of up to 8 mg DNA plasmid were administered intradermally / subcutaneously during a single visit.
3. A SARS-CoV-2 vaccine candidate was compared during its first Phase-1 safety study against a licensed comparator (Janssen's Ad26.COV2.S).

We appreciate your consideration and look forward to your response.

Kind regards,

Tobias Odendahl, MD
Head of Trial, Alvea

Research in context

Evidence before this study

DNA vaccines are being developed and tested for a diverse set of infectious diseases and cancer immunotherapy, with the SARS-CoV-2 vaccine ZyCoV-D being the first ever approved DNA vaccine in humans. WHO recognizes in their guidelines on the quality, safety and efficacy of plasmid DNA vaccines that DNA vaccines have great potential to address priority pathogens during public health emergencies, particularly in resource limited settings due to design and inherent stability characteristics. A recent review of the COVID-19 DNA vaccine literature identified eleven DNA based vaccine candidates for primary vaccination against SARS-CoV-2 tested in clinic and were able to elicit both humoral and cellular immune responses. Searches for “DNA COVID-19 / SARS-CoV-2 vaccine booster/omicron clinical trial” in PubMed, Medline, Google scholar and clinicaltrials.gov did yield three registered DNA based SARS-CoV-2 booster vaccine clinical trials which have not yet reported their results (NCT05182567, NCT05171946, NCT05904054).

Added value of this study

Findings from this phase I trial comparing a naked Omicron BA.2 DNA plasmid vaccine Alveavax-v1.2 to Janssen Ad26.COV2.S in 130 healthy, primary Janssen vaccinated South African participants most of which also had a previous infection, show that dose levels from 0·5 mg to 8 mg administered intradermally or subcutaneously were safe, and well tolerated. Participants experienced less local injection pain with the DNA vaccine candidate. Neither of the tested vaccine candidate dose levels nor the Janssen Ad26.COV2.S elicited a substantial humoral immune response. The geometric mean titer fold change for SARS-CoV-2 BA.2 at day 28 measured by ELISA ranged between 0·94 - 1·15 across the vaccine candidate arms. The Janssen Ad26.COV2.S comparator showed a fold-rise of 1·33.

Implications of all the available evidence

In preimmunized populations with high baseline titers, naked DNA vaccines seem insufficient to further boost humoral immune responses, even when very high doses are administered. Subcutaneous administration of naked DNA did not seem to provide additional benefits. Even in early clinical development of booster candidates, randomization against an approved vaccine can be used for comparison of primary and secondary endpoints.

Abstract

Background

The safety and immunogenicity of SARS-CoV-2 Omicron DNA boosters are unknown.

Methods

A phase I open-label, active-controlled, randomized safety and dose-finding study for the naked DNA Omicron BA.2 booster vaccine Alveavax-v1.2 was conducted. Healthy participants previously immunized with a single Janssen Ad26.COV2.S vaccine were recruited from seven non-hospital study sites in South Africa. Primary outcome was safety and tolerability on Day 28 after vaccination; secondary endpoints were humoral immunogenicity, clinical efficacy and success rate of intradermal (ID) injections. A central randomization system allocated participants into the groups in blocks ranging from 1 to 5: low dose - 0·5 mg ID; standard dose - 2 mg ID; high dose - 8 mg (4 ID of 2 mg each); subcutaneous injection - 8 mg; control - Janssen Ad26.COV2.S booster as a single intramuscular injection. All analyses were based on a modified Intent-To-Treat (mITT) population. ClinicalTrials.gov Identifier: NCT05844202.

Findings

130 participants were enrolled between 30th June and 14th September 2022 and followed up until 28th February 2023. Nine enrolled participants were lost to follow-up (low: 2; standard: 2; high: 3; subcutaneous: 1; control: 1), one withdrew (standard arm). 105/130 (81%) were positive for nucleocapsid antibodies. 8/20 (40%), 21/40 (52·5%) (16/20 (80%), 7/10 (70%) and 29/40 (72·5%) experienced adverse events during the study, for low, standard, high, subcutaneous and control, respectively. Three SAEs reported for a single participant in the high arm were judged unrelated to vaccination. ELISA anti-SARS-CoV-2 Omicron BA.2 geometric mean fold increase at Day 28 for low, standard, high, subcutaneous, and control was, 1·15 (95% CI, 0·72 to 1·83), 0·94 (95% CI, 0·66 to 1·33), 1·01 (95% CI, 0·61 to 1·66), 1·04 (95% CI, 0·41 to 2·62), and 1·33 (95% CI, 0·91 to 1·95), respectively, with geometric mean baseline titers of 576·4 (95% CI, 534·3 to 621·9) across arms.

Interpretation

While safe, well tolerated, and shelf stable for >6 months at room temperature, a naked DNA SARS-CoV-2 booster candidate in doses up to 8 mg administered intradermally or subcutaneously, as well as Janssen Ad26.COV2.S comparator, did not significantly increase BA.2 antibody titers in a preimmunized, largely pre-infected population.

Funding

Alvea Holdings LLC, Cambridge, Massachusetts.

Main text

Introduction

Naked DNA vaccine platforms are of particular interest due to their shelf stability and low manufacturing complexity. Like mRNA vaccines, DNA vaccines can be designed quickly and therefore have great potential to address priority pathogens during public health emergencies.[1] One naked DNA SARS-CoV-2 vaccine (ZyCoV-D) has received emergency use authorisation (EUA) in India,[2] and several other DNA vaccine candidates were in development during the COVID-19 pandemic.[3]

The SARS-CoV-2 Omicron variant (B.1.1.529 lineage) was reported by the WHO in November 2021 as a novel variant of concern with a number of immune evasive mutations.[4] Besides high cost and limited dose availability, the worldwide distribution of mRNA vaccine candidates for booster shots to resource constrained settings was hampered by the requirement for cold chain storage and shipment.[5] At the point of design of Alveavax-v1.2, 15 months after the EUA of the first COVID-19 vaccine, more than five billion people world-wide (>60%) had received at least one dose of a COVID-19 vaccine – but only 13·7% of people in low-income countries.[6]

To address vaccine delivery challenges and ensure equitable and rapid access to COVID-19 booster vaccines, Alvea LLC developed a plasmid DNA booster vaccine, Alveavax-v1.2. The vaccine comprises double-stranded plasmid DNA carrying the gene for the SARS-CoV-2 spike protein containing Omicron/BA.2-specific mutations, as well as K986P and V987P ("2P") proline pre-fusion conformation mutations.[7] Alveavax-v1.2 uses the well-known pVAX1 backbone.[8] The plasmid is formulated in preservative-free, sterile phosphate buffer saline (PBS) at a concentration of 5 ± 0·5 mg/mL and administered intradermally (0·1 ml or 0·4 ml). At release >95% of the plasmid were circular, >90% supercoiled, with stability at room temperature for at least six months based on an acceptance criterion of ≥ 80% supercoiled. To simplify manufacturing processes and supply chain bottlenecks in resource constrained settings no additional adjuvants were used, analogous to the ZyCoV-D vaccine. Three unpublished preclinical studies in mice demonstrated neutralizing antibody responses against SARS-CoV-2 BA.2 after intradermal injection of Alveavax-v1.2 (see Supplements for animal study reports). The aim of the clinical phase I study was to evaluate the safety and tolerability of Alveavax-v1.2 in healthy participants, compared with a control booster vaccine (the Janssen Ad26.COV2.S COVID-19 vaccine), as a booster vaccine against SARS-CoV-2.

Methods

Study design

An open-label, active-controlled, randomized safety and dose-finding study was chosen to evaluate Alveavax-v1.2. Participants were recruited from seven non-hospital study sites in South Africa. Approval by the South African Health Products Regulatory Authority and South African Medical Association Research Ethics Committee were obtained (South African National Clinical Trials Registry Identifier: DOH-27-062022-5157. ClinicalTrials.gov Identifier: NCT05844202). The study protocol can be found in the Supplementary Materials.

Participants

The main inclusion criteria were healthy volunteers (sex self-reported with the options “male”, “female”, and “undifferentiated”) between the age of 18 and 65 years (inclusive) having received a single primary Janssen Ad26.COV2.S COVID-19 vaccine ≥ 60 days prior to receiving the study vaccine. Volunteers were excluded when they had received any other form of SARS-CoV-2 vaccination or planned to receive any additional SARS-CoV-2 vaccination within 90 days after the study vaccine administration. The full list of eligibility criteria in the study protocol and the informed consent forms can be found in the supplements. A participant was considered a screen failure if the informed consent form was signed but they were ineligible at the screening visit or withdrew before receiving the study vaccine.

Procedures

The arms of the study were (see Figure 1):

- Low dose - 0·5 mg intradermal (ID), 20 participants;
- Standard dose - 2 mg ID, 40 participants;
- High dose - 8 mg as four ID injections of 2 mg each, 20 participants;
- Subcutaneous (SC) injection - 8 mg as a single SC injection, 10 participants;
- Control - Janssen Ad26.COV2.S booster as a single intramuscular (IM) injection, 40 participants.

The vaccine candidate was stored in temperature controlled refrigerators at 2-8 °C. Each Alveavax-v1.2 vial contained sufficient volume for two standard doses (2 mg, 0·4 mL), with 30% excess fill volume. The Janssen Ad26.COV2.S vaccine was stored and administered according to the package leaflet. After successful screening, participants had one enrollment and vaccine administration visit. Local staff were provided with video training of correct intradermal administration and measurement of intradermal bleb size. No additional training for subcutaneous or intramuscular injections was provided. After administration of the study vaccine on Day 1 participants were monitored on-site for local or systemic reactions to the vaccine either 4 h (early safety cohorts) or 0·5 h (remaining study participants). Subsequent visits included a telephone call on Day 3 and in person follow-up visits on days 7, 14, 28, 84 and 168. Each visit included safety and laboratory assessments, in addition to inquiries regarding pregnancy and COVID-19 infections or vaccinations. The full schedule of events can be found in the study protocol in the supplements.

Randomisation and masking

Initially, participants were randomized in two sequential safety cohorts and the remaining cohorts were opened for randomization thereafter. An illustration of the randomization flow is presented in Figure 2. The randomization lists and specifications are provided in the supplements.

The first 10 participants were randomized into the low dose arm (1.a) and the control arm (1.e). No more than five participants were vaccinated on the first day, and subsequent recruitment of the study groups or escalation between dose levels was allowed only after an independent medical monitor had reviewed at least 24-hour post-dose safety data. Then, the remaining participants assigned to the low and standard dose (1.b) cohorts, in addition to those assigned to the control arm, were recruited. In parallel, the first five participants of the high dose (1.c) arm were enrolled. Further recruitment and the subcutaneous injection arm (1.d) were started after an independent medical monitor reviewed 24-hour safety data of the high dose arm.

A central interactive response technology (IRT) randomization system generated the sequence and allocated participants into the respective arms of the trial. Block sizes ranged between one and five. An open-label design with no blinding for study site staff or participants was selected. Safety and immunogenicity laboratory personnel were blinded.

Outcomes

Primary endpoints for the assessment of safety were solicited local and systemic adverse events (AEs) within seven days of dose administration via patient diary cards, unsolicited AEs within 28 days of vaccination, serious adverse events (SAEs), adverse events of special interest (AESIs), and AEs leading to withdrawal during the 6 month post vaccination follow-up period. Secondary endpoints included humoral immune response on Day 1 and Day 28 measured by geometric mean titer (GMT) and geometric mean fold rise (GMFR) of anti-spike protein (S) immunoglobulin G (IgG) antibody for SARS-CoV-2 BA.2/Omicron, clinical efficacy measured using the WHO clinical progression scale for COVID-19 on Day 7 / 14 / 28 / 84 / 168, and the success rate of ID injections as measured by the absolute number and fraction of ID injections that generated a clearly demarcated bleb, of ≥ 1 mm and ≥ 7 mm in diameter, clearly visible for at least 20 seconds, for 0·5 mg (0·1 mL) and 2 mg (0·4 mL) Alveavax-v1.2, respectively. All humoral assays were performed by the National Institute for Communicable Diseases (NICD) in Johannesburg, South Africa. Details on the assays are described in the supplements. Additional exploratory endpoints listed in the protocol, including cellular immune responses, were not performed.

Statistical analysis

As this was a phase I study, all data were analyzed descriptively without a formal statistical hypothesis and sample sizes were not based on a statistical power calculation. Figure 1 illustrates the participants included in the safety and immunogenicity analyses.

The safety population was the set of all study participants who were administered with a dose of the vaccine candidate. Participants were grouped as treated. All enrolled participants who received a study vaccine and experienced at least one post-baseline immunogenicity readout comprised the modified intent-to-treat (mITT) population. Missing or non- evaluable measurements were not replaced. The mITT and Safety populations were identical. The mITT population was used instead of the per protocol population for the immunogenicity analyses as the differences to the Janssen control were small and the former included more participants.

Categorical variables were summarized as frequencies and percentages. Continuous variables were summarized using descriptive statistics (number of participants with an observation [n], mean, standard deviation [SD], median, and range). Where partial dates (missing day or missing day and month) were recorded on the electronic case report form (CRF) and where these could not be resolved by queries, dates were estimated for the purpose of calculating durations. Statistical analysis was performed using SAS® software (version 9.4 or higher; SAS Institute Inc., USA). AEs were coded using MedDRA version

25.1. Coding included the system organ class and preferred term. The statistical analysis plan as well as the code conducted to run the analysis are included in the supplements.

Role of the funding source

There was no funding source for this study other than the sponsor, Alvea Holdings, LLC. The funding source was involved in the study design, collection of data, analysis, interpretation of data, writing of the study report, writing of this paper and the decision to submit the paper for publication.

Results

Between 30th June 2022 and 14th September 2022, 238 patients were screened, 91 did not meet eligibility criteria during screening, 17 met the criteria but were not enrolled in the study. A total of 130 participants were enrolled and randomized and followed up until 28th February 2023 (see Figure 1). Nine enrolled participants were lost to follow-up, one withdrew from participation, and three participants did not have a Day 28 immunogenicity result. The mean age (SD) was 32·9 (11·21) years for the Alveavax-v1.2 groups combined, 33·3 (11·82) for the Janssen control arm, mean BMI (SD) was 23·9 (4·23) and 23·6 (3·97), respectively. 39/90 female participants (43·3%) were enrolled across the Alveavax-v1.2 arms and 15/40 (37·5%) were enrolled in the control arm. Approximately 90% of all participants were from black African descent, the remainder from southern African and mixed race descent. Table 1 details the baseline demographics of the participants across arms.

The medical exams and history confirmed a healthy participant population without significant prior or current illness. None of the laboratory and vital sign values post vaccination were deemed clinically significant. Solicited AEs reported within the week after vaccination included mostly mild to moderate signs (see Figure 3). Mild to moderate injection pain on the day of injection was present in 12/80 (15%) for ID, 1/10 (10%) for SC and 17/40 (42·5%) for IM injections, and lasted up to Day 6 in three Alveavax-v1.2 and one control participant. Individual participants experienced severe arthralgia, fatigue, nausea or local tenderness in the Alveavax-v1.2 groups and severe pain in the Janssen control arm. Symptoms resolved quickly for most participants. Moderate to severe fatigue was experienced by a subset of participants in the Alveavax-v1.2 high dose group at Day 5 and Day 6 (moderate N=2; severe, N=1). Two participants in the high dose arm and two in the subcutaneous arm showed clinically significant abnormal findings at the physical examination at Day 7 (Skin and head, eyes, ears, nose, and throat [HEENT] and HEENT and lymphatic, respectively) which in the further course returned to normal.

Unsolicited AEs up until Day 28 were experienced by 8/20 (40%), 21/40 (52·5%) (16/20 (80%), 7/10 (70%) and 29/40 (72·5%), for low, standard, high, subcutaneous and control, respectively. Common events, both across Alveavax-v1.2 and control, included aforementioned injection site reactions, fatigue (14/90 [15·6%], 1/40 [2·5%]), and headache (24/90 [26·7%], 7/40 [17·5%]). Three SAEs judged unrelated to vaccination were recorded for a single Alveavax-v1.2 participant in the high dose group (a severe lower respiratory tract infection requiring hospitalization, a fecaloma which resolved, and a pregnancy that resulted in a complication-free vaginal birth with a healthy newborn). There were no AESIs or deaths during the study. A table summarizing all adverse events classified by organ system can be found in the supplementary material (Table S1 in the Supplements).

The administration of the full dose of the vaccine candidate was successful in 136/140 (97·1%) intradermal injections, 9/10 (90%) subcutaneous injections and 40/40 (100%) intramuscular injections. The median bleb size for intradermal injections was 6·9 mm (SD 2·44) and 10·7 mm in diameter (SD 2·66, see Table 2) for 0·1 mL and 0·4 mL, respectively.

GMT for BA.2 spike at baseline assessed by ELISA for low, standard, high, subcutaneous and control was 556 (95% CI, 441 to 701), 562 (95% CI, 467 to 676), 600 (95% CI, 451 to 798), 722 (95% CI, 472 to 1107), 518 (95% CI, 425 to 632) (see Figure 4A). ELISA BA.2 GMT fold increase in the mITT population based on Day 28 ELISA data for low, standard, high, subcutaneous, and control was, 1·15 (95% CI, 0·72 to 1·83), 0·94 (95% CI, 0·66 to 1·33), 1·01 (95% CI, 0·61 to 1·66), 1·04 (95% CI, 0·41 to 2·62), and 1·33 (95% CI, 0·91 to 1·95), respectively. A subset of Day 84 participant samples were tested and confirmed the magnitude of geometric mean fold increase (GMFI). ELISA fold rises were validated via neutralizing antibodies for Omicron BA.2 in a subset of 27/130 (20·8%) participants of the low dose, standard dose and control arm. Figure 4B plots the BA.2 antibodies of the

subgroups of participants 25/130 (19%) who were not positive for SARS-CoV-2 nucleocapsid antibodies at baseline, Figure 4C those who had a previous SARS-CoV-2 infection prior to the administration of the vaccine. Baseline GMTs for the group of uninfected participants were lower when compared to the infected population and GMFI tended to be higher for the low dose and the Janssen control arm. Only one participant in the standard group experienced a COVID-19 infection during the study, thus no statistical analysis using the WHO clinical progression scale was performed.

Discussion

The review of Shafaati et al identified eleven DNA based vaccine candidates for primary vaccination in clinical testing.[3] To our knowledge this phase I safety, tolerability and immunogenicity study represents the first report of a DNA COVID-19 booster vaccine candidate evaluated in humans. Searches for “DNA COVID-19 / SARS-CoV-2 vaccine booster/omicron clinical trial” in PubMed, Medline, Google scholar and clinicaltrials.gov yielded three registered DNA based SARS-CoV-2 booster vaccine clinical trials which have not yet reported their results (NCT05182567, NCT05171946, NCT05904054). DNA vaccines for primary SARS-CoV-2 vaccination were able to elicit both humoral and cellular immune responses in SARS-CoV-2 naive populations when administered IM or ID.[3] Typical dose levels per vaccination are in the 1-3 mg range and included up to three administrations for primary vaccination and GMTs of 952·7 based on ELISA at Day 84.[8]

This study provided a single boost with a BA.2 optimized naked DNA vaccine to healthy adults with a single primary Janssen Ad26.COV2.S vaccination. Approximately 80% of those randomized had also previously experienced an infection, which is in accordance with South African seroprevalence studies conducted end of 2021.[9] It was hypothesized that the potency of a single DNA shot could be sufficient in context of preimmunized participants most of which have hybrid immunity from past infections. Preclinical experiments before and after the clinical trial with single- and escalating dose regimens similar to HIV vaccines[10,11] showed potent development of neutralizing antibodies against various SARS-CoV-2 strains (see Supplements). The candidate vaccine was randomized against Janssen’s Ad26.COV2.S to provide comparative data about the expected effectiveness at a very early stage of clinical development and to inform future sample size calculations.[12] Despite active comparisons with approved vaccines in early clinical trials being common for other conditions,[13,14] randomization against approved SARS-CoV-2 vaccines have been astonishingly rare for COVID-19 vaccine candidates.[15]

In alignment with the broad safety literature already available for DNA vaccines[1] Alveavax-v1.2 was safe and well tolerated among all treatment groups, with frequency and intensity of solicited and unsolicited AEs matching those of the comparator, DNA primary series[8] and mRNA booster vaccine programs.[16] Both the Janssen comparator as well as Alveavax-v1.2 were not able to elicit meaningful GMT fold rises against Omicron/BA.2 on top of the already high antibody titers. This is noticeable, as there was a 16x dose difference between the low and high dose Alveavax-v1.2 arms and the control, the Janssen Ad26.COV2 vaccine, was a known effective vaccine. It is possible that there were limitations in the design and conduct of the study, whereby the baseline antibody titers may have been too high to show a titer increase after treatment. The analysis of the small subgroup of uninfected participants with lower baseline titers and slightly higher GMFI’s is in agreement with this hypothesis. The overall baseline titers of the study’s South African population were comparable to post-boost titers (606 and 896 for age groups 18-55 and >55, respectively) of Pfizer's BA4/5 trial.[17] Zhou et al collected neutralization data across multiple SARS-CoV-2 variants showing that geometric mean factor increase is generally substantially greater in individuals without previous infection compared to individuals with previous infection, even at the fourth booster dose.[18] DNA vaccine neutralizing humoral immune responses have been small even in primary vaccination.[8] While a lack of humoral response does not imply absence of additional protection - for instance via cellular response mechanisms[19] - it still led to a hold on further exploratory testing and the clinical program for this drug candidate. Participants with low titers were informed and additional vaccination with a licensed vaccine was recommended.

Further study limitations were that the study population was a potential lack of heterogeneity in the study population recruited only from South Africa, and that they were a healthy population that excluded HIV patients. The results may not be transferable to other

vaccination regimens, as only people who had received the Janssen vaccine as a first vaccination and had not received a mRNA-based vaccine as primary vaccination, were examined. Alternative ways of administration than ID with needles were not studied, due to the widespread availability of needles even in resource limited contexts. Reliance on ID injections with needles makes direct immunogenicity comparison to other DNA vaccine trials difficult, where proprietary needle-free injection systems or electroporation were studied to improve the immune response.[20]

The strengths of this study included the multicenter and the active controlled study design. The total and per arm population was relatively large for a phase I study and a representative region for low- and middle income countries was selected. Alveavax-v1.2 was targeting the dominant Omicron strain at the time.

To our knowledge for the first time an ID dosing up to 8 mg and SC administration routes in DNA vaccines were evaluated in humans.

In spite of not attaining the desired immunological endpoints, it is a noteworthy accomplishment that a drug development initiative guided by principles of vaccine equity has showcased arguably the most expeditious transition of a pharmaceutical entity to the clinical phase in comparison to analogous trials. This contrasts with the prevailing public perception that the drug development process is marked by languor, bureaucratic impediments, and inaccessibility for nascent enterprises.[21] A mere 174 days subsequent to the establishment of Alvea at the advent of the Omicron wave, the human trial of Alveavax-v1.2 started, without compromising on best practices. This reinforces the imperativeness of persistent investments in broadly protective and equitable medical countermeasure platforms. Furthermore, it confirms how operational excellence and financial risk-taking can enable clinical trials to be realized in the shortest possible time.

Figures and Tables

Figure 1: Trial flow diagram

Flow diagram for participants in the trial showing screening, group allocation, follow-up and analysis groups.

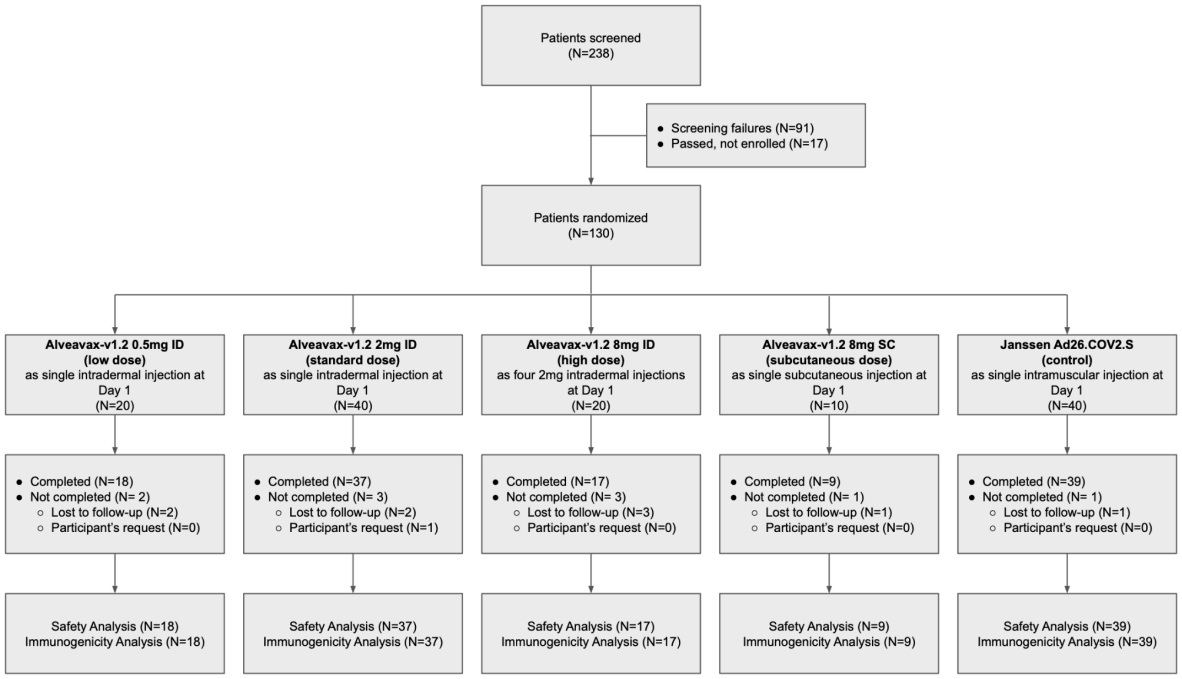


Figure 2: Study Design and Randomization Schema

Randomization flow of the study. Red text represents safety events before opening up additional arms. Study groups were as follows. low dose: 0·5 mg intradermal (ID); standard dose: 2 mg ID; high dose: 8 mg as four ID injections of 2 mg each; subcutaneous (SC) injection: 8 mg as a single SC injection; control arm - Janssen Ad26.COV2.S booster as a single intramuscular (IM) injection.

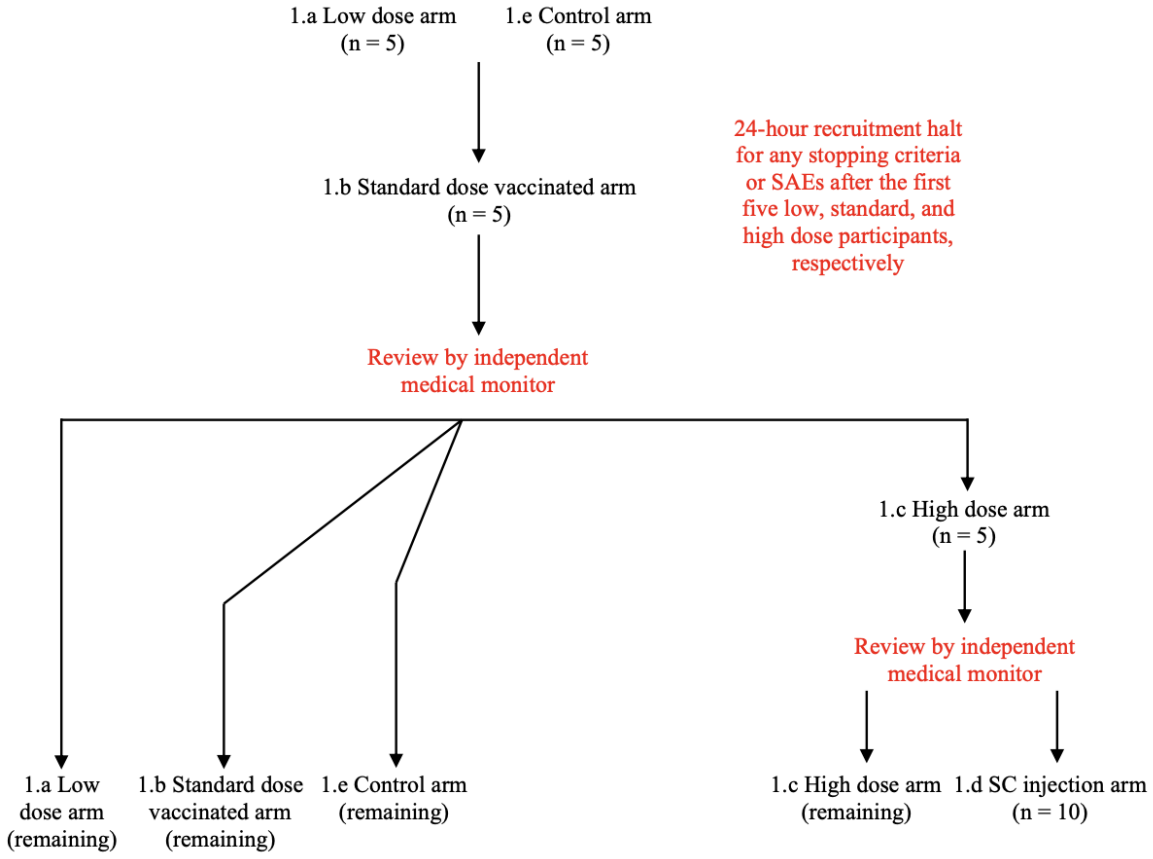


Table 1: Baseline and Demographic Characteristics

ID = intradermal, SC = subcutaneous

	Alveavax -v1.2 0·5 mg ID (low dose) N = 20	Alveavax-v1.2 2 mg ID (standard dose) N = 40	Alveavax -v1.2 8 mg ID (high dose) N = 20	Alveavax-v1.2 8 mg SC (subcutaneous dose) N = 10	Alveavax-v1.2 all participants (combined) N = 90	Janssen Ad26.COV2.S (Control) N = 40
Gender n (%)						
Male	11 (55·0%)	26 (65·0%)	10 (50·0%)	4 (40·0%)	51 (56·7%)	25 (62·5%)
Female	9 (45·0%)	14 (35·0%)	10 (50·0%)	6 (60·0%)	39 (43·3%)	15 (37·5%)
Age (y)						
Mean (SD)	35·5 (13·30)	32·2 (11·00)	31·0 (9·19)	34·2 (11·83)	32·9 (11·21)	33·3 (11·82)
Median	34·5	29·5	31·0	36·5	31·0	29·5
Range	19·0 – 61·0	18·0 – 58·0	19·0 – 57·0	21·0 – 57·0	18·0 – 61·0	19·0 – 60·0
Race n (%)						
Black African	16 (80·0%)	39 (97·5%)	18 (90·0%)	8 (80·0%)	81 (90·0%)	37 (92·5%)
Mixed race- Colored	1 (5·0%)	0 (0·0)	0 (0·0)	0 (0·0)	1 (1·1%)	0 (0·0)
Southern African- Colored	3 (15·0%)	1 (2·5%)	2 (10·0%)	2 (20·0%)	8 (8·9%)	3 (7·5%)
Height (cm)						
Mean (SD)	165·3 (9·36)	166·3 (7·84)	165·1 (8·21)	165·4 (8·65)	165·7 (8·24)	165·7 (9·31)
Median	167·5	166·5	161·9	164·2	165·9	167·5
Range	145·5 – 176·2	147·0 – 182·4	146·4 – 182·0	155·0 – 178·4	145·5 – 182·4	145·0 – 181·0
Weight (kg)						
Mean (SD)	68·2 (11·67)	65·1 (10·69)	65·6 (11·13)	60·8 (11·14)	65·4 (11·06)	64·7 (12·14)
Median	68·1	67·3	62·4	57·2	65·9	61·6
Range	48·9 – 86·0	43·1 – 87·0	46·3 – 85·8	45·3 – 79·9	43·1 – 87·0	42·4 – 90·0
BMI (kg/m^2)						
Mean (SD)	25·0 (4·19)	23·6 (4·07)	24·1 (4·03)	22·5 (5·30)	23·9 (4·23)	23·6 (3·97)
Median	24·9	23·0	22·6	20·9	23·2	22·6
Range	19·1 – 32·0	17·7 – 31·6	19·8 – 32·9	17·5 – 31·6	17·5 – 32·9	18·1 – 31·5

Figure 3. Solicited Local and Systemic Adverse Reactions

Shown are the percentages of participants in whom solicited local or systemic adverse reactions occurred within seven days of the vaccine dose administered in the trial. (20, 40, 20, and 10 participants in the Alveavax-v1.2 0·5 mg [low], 2mg [standard], 8 mg [high] and 8 mg subcutaneous [subcutaneous] group; 40 participants in the Janssen Ad26.COV2.S [control] group)

Grade 1 (Mild): Transient or mild discomfort (< 48 hours); no medical intervention/therapy required. Grade 2 (Moderate): Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required Grade 3 (Severe): Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.

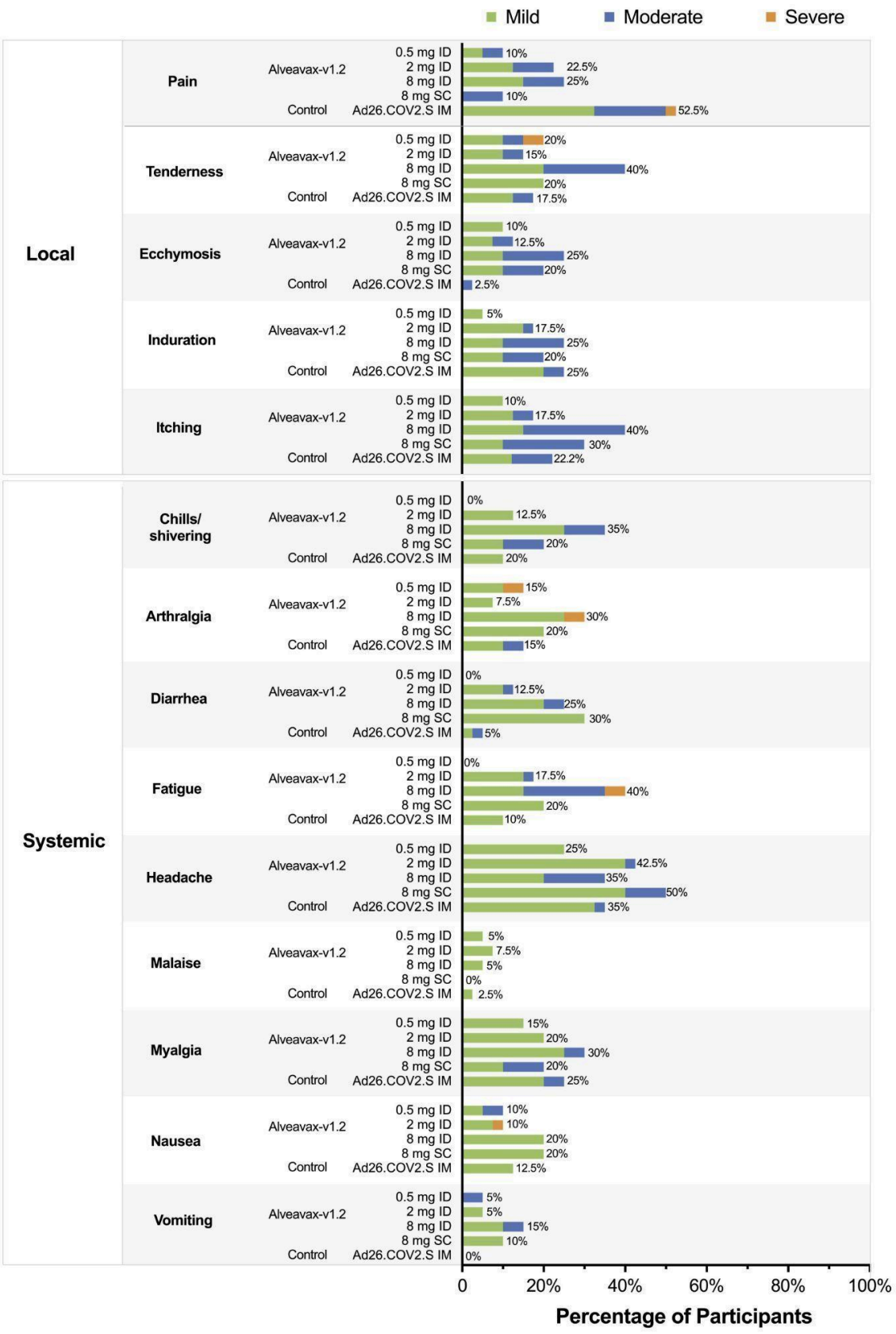
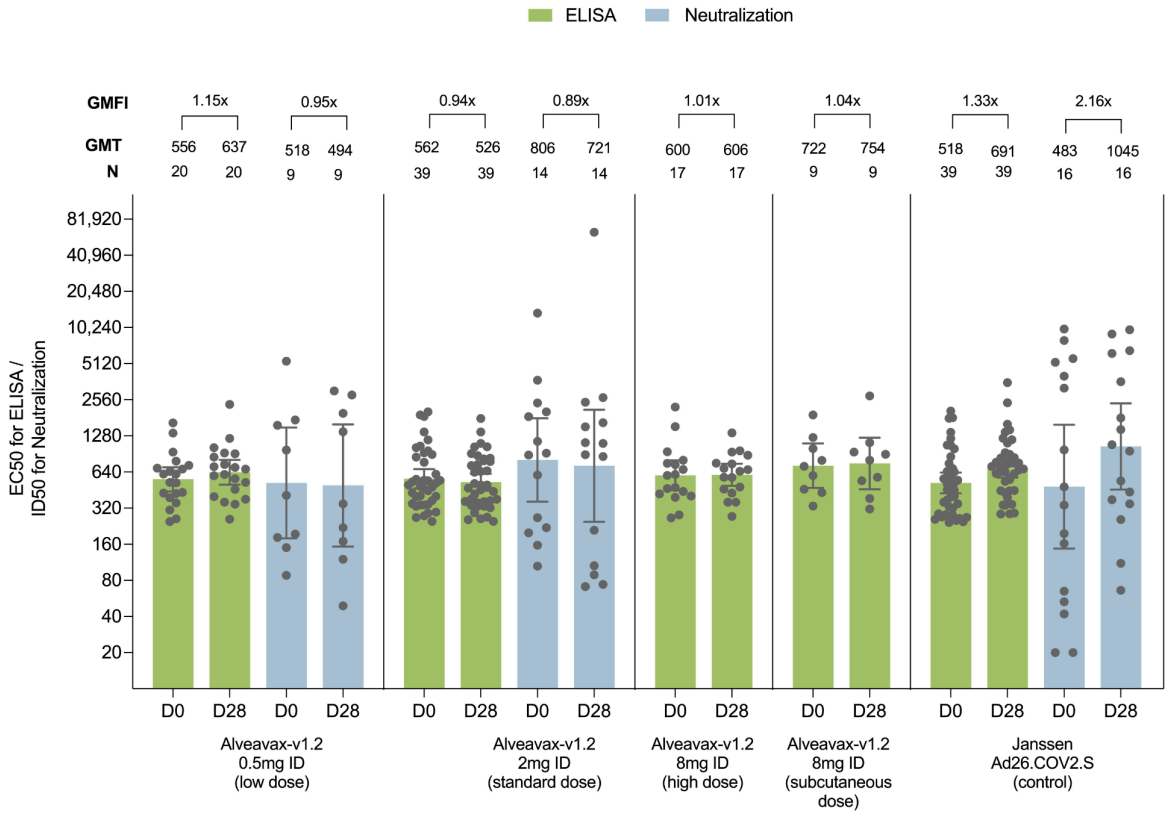

Mild
Moderate
Severe
Local
Pain
Tenderness
Ecchymosis
Induration
Itching
Systemic
Chills/ shivering
Arthralgia
Diarrhea
Fatigue
Headache
Malaise
Myalgia
Nausea
Vomiting
Alveavax-v1.2
Control
0.5 mg ID
2 mg ID
8 mg ID
8 mg SC
Ad26.COV2.S IM
0
20%
40%
60%
80%
100%
Percentage of Participants

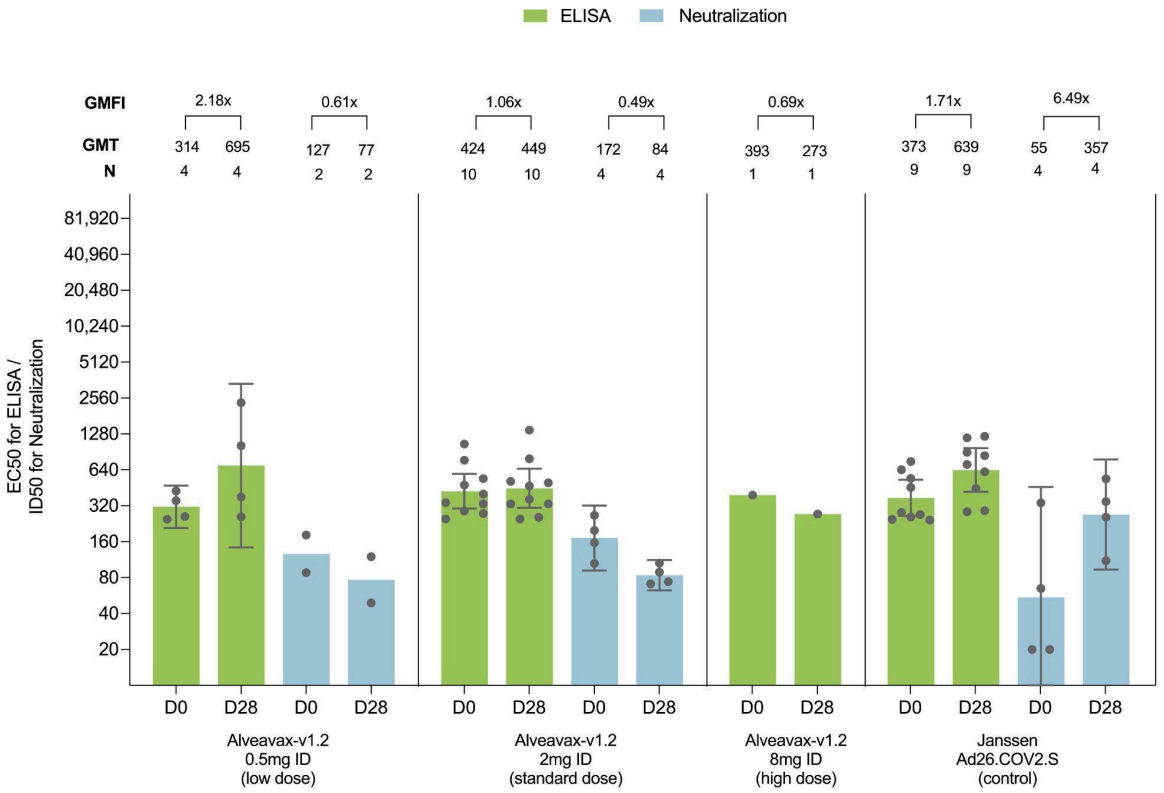
Figure 4: Humoral immune response

ELISA and neutralization data on Day 0, Day 28, and Day 84 measured by geometric mean titer (GMT) of anti-spike protein (S) immunoglobulin G antibody for SARS-CoV-2 BA.2/Omicron are shown

A) All participants pooled



B) Subset of participants without prior infection based on nucleocapsid antibody titers.



C) Subset of participants with prior infection based on nucleocapsid antibody titers.

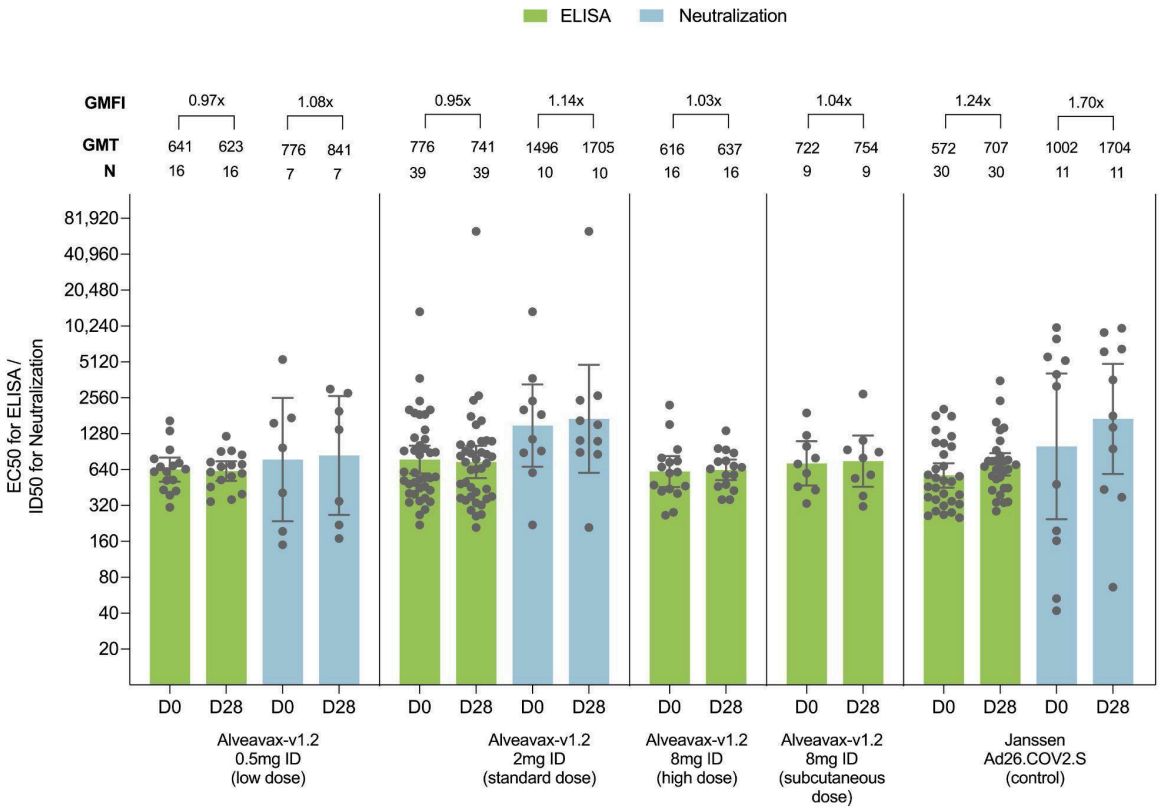


Table 2: A) Administration success of vaccine and B) bleb size following intradermal vaccination

A)

	Alveavax-v1.2 0·5 mg ID (low dose) N = 20	Alveavax-v1.2 2 mg ID (standard dose) N = 40	Alveavax-v1.2 8 mg ID (high dose) N = 20	Alveavax-v1.2 8 mg SC (subcutaneous dose) N = 10	Alveavax-v1.2 all participants (combined) N = 90	Janssen Ad26.COV2.S (Control) N = 40
Was the Full Dose Administered						
n	20	40	80	10	150	40
Yes	20 (100%)	39 (97·5%)	77 (96·3%)	9 (90·0%)	145 (96·7%)	40 (100%)
No	0	1 (2·5%)	3 (3·8%)	1 (10·0%)	5 (3·3%)	0

The denominator for the number of vaccinations and the number administered a full dose is the total number of vaccinations not the number of participants

B)

	Alveavax-v1.2 0·5 mg ID (low	Alveavax-v1.2 2 mg ID (standar	Alveavax-v1.2 8 mg ID (high dose)	Alveavax-v1.2 8 mg ID (high dose)	Alveavax-v1.2 8 mg ID (high dose)	Alveavax-v1.2 8 mg ID (high dose)

	dose) N= 20	d dose) N= 40	Injection 1 N= 20	Injection 2 N= 20	Injection 3 N= 20	Injection 4 N= 20
Size of Bleb (mm) when it Lasted 20 seconds or more						
n	19	39	20	20	20	20
Mean (SD)	6·9 (2·44)	10·7 (2·66)	10·7 (2·30)	11·1 (2·10)	10·7 (2·25)	10·6 (1·70)
Median	8·0	10·0	12·0	12·0	11·0	10·0
Min-Max	3·0 to 10·0	7·0 to 16·0	6·0 to 14·0	7·0 to 14·0	7·0 to 14·0	7·0 to 14·0

Note: only two participants had blebs lasting less than 20 seconds. They are not included in the table.

References

1. Guidelines on the quality, safety and efficacy of plasmid DNA vaccines, Annex 2, TRS No 1028. Accessed September 11, 2023. https://www.who.int/publications/m/item/plasmid-dna-vaccines-annex-2-trs-no-1028
2. Abbasi J. India's New COVID-19 DNA Vaccine for Adolescents and Adults Is a First. *JAMA*. 2021;326(14):1365. doi:10.1001/jama.2021.16625
3. Shafaati M, Saidijam M, Soleimani M, et al. A brief review on DNA vaccines in the era of COVID-19. *Future Virol*. 2022;17(1):49-66. doi:10.2217/fvl-2021-0170
4. Chen J, Wei GW. Omicron BA.2 (B.1.1.529.2): high potential to becoming the next dominating variant. *ArXiv*. Published online February 10, 2022:arXiv:2202.05031v1.
5. Capua I, Giaquinto C. The unsung virtue of thermostability. *Lancet Lond Engl*. 2021;397(10282):1346. doi:10.1016/S0140-6736(21)00526-2
6. Mathieu E, Ritchie H, Rodés-Guirao L, et al. Coronavirus Pandemic (COVID-19). *Our World Data*. Published online March 5, 2020. Accessed September 11, 2023. https://ourworldindata.org/covid-vaccinations
7. Bos R, Rutten L, van der Lubbe JEM, et al. Ad26 vector-based COVID-19 vaccine encoding a prefusion-stabilized SARS-CoV-2 Spike immunogen induces potent humoral and cellular immune responses. *Npj Vaccines*. 2020;5(1):1-11. doi:10.1038/s41541-020-00243-x
8. Khobragade A, Bhate S, Ramaiah V, et al. Efficacy, safety, and immunogenicity of the DNA SARS-CoV-2 vaccine (ZyCoV-D): the interim efficacy results of a phase 3, randomised, double-blind, placebo-controlled study in India. *The Lancet*. 2022;399(10332):1313-1321. doi:10.1016/S0140-6736(22)00151-9
9. Madhi SA, Kwatra G, Myers JE, et al. Population Immunity and Covid-19 Severity with Omicron Variant in South Africa. *N Engl J Med*. 2022;386(14):1314-1326. doi:10.1056/NEJMoa2119658
10. Cirelli KM, Carnathan DG, Nogal B, et al. Slow Delivery Immunization Enhances HIV Neutralizing Antibody and Germinal Center Responses via Modulation of Immunodominance. *Cell*. 2019;177(5):1153-1171.e28. doi:10.1016/j.cell.2019.04.012
11. Tam HH, Melo MB, Kang M, et al. Sustained antigen availability during germinal center initiation enhances antibody responses to vaccination. *Proc Natl Acad Sci U S A*. 2016;113(43):E6639-E6648. doi:10.1073/pnas.1606050113
12. Schumi J, Wittes JT. Through the looking glass: understanding non-inferiority. *Trials*. 2011;12(1):106. doi:10.1186/1745-6215-12-106
13. Platt H, Omole T, Cardona J, et al. Safety, tolerability, and immunogenicity of a 21-valent pneumococcal conjugate vaccine, V116, in healthy adults: phase 1/2, randomised, double-blind, active comparator-controlled, multicentre, US-based trial. *Lancet Infect Dis*. 2023;23(2):233-246. doi:10.1016/S1473-3099(22)00526-6
14. Ramsauer K, Schwameis M, Firbas C, et al. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. *Lancet Infect Dis*. 2015;15(5):519-527. doi:10.1016/S1473-3099(15)70043-5
15. Corominas J, Garriga C, Prenafeta A, et al. Safety and immunogenicity of the protein-based PHH-1V compared to BNT162b2 as a heterologous SARS-CoV-2 booster vaccine in adults vaccinated against COVID-19: a multicentre, randomised, double-blind, non-inferiority phase IIb trial. *Lancet Reg Health – Eur*. 2023;28. doi:10.1016/j.lanepe.2023.100613
16. Chalkias S, Harper C, Vrbicky K, et al. A Bivalent Omicron-Containing Booster Vaccine against Covid-19. *N Engl J Med*. 2022;387(14):1279-1291. doi:10.1056/NEJMoa2208343
17. Pfizer and BioNTech Announce Updated Clinical Data for Omicron BA.4/BA.5-Adapted Bivalent Booster Demonstrating Substantially Higher Immune Response in Adults Compared to the Original COVID-19 Vaccine | Pfizer. Accessed September 11, 2023. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-and-biontech-ann

ounce-updated-clinical-data-omicron
18. Zou J, Kurhade C, Patel S, et al. Neutralization of BA.4–BA.5, BA.4.6, BA.2.75.2, BQ.1.1, and XBB.1 with Bivalent Vaccine. *N Engl J Med*. 2023;388(9):854-857. doi:10.1056/NEJMc2214916
19. Moss P. The T cell immune response against SARS-CoV-2. *Nat Immunol*. 2022;23(2):186-193. doi:10.1038/s41590-021-01122-w
20. Momin T, Kansagra K, Patel H, et al. Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): Results of an open-label, non-randomized phase I part of phase I/II clinical study by intradermal route in healthy subjects in India. *eClinicalMedicine*. 2021;38. doi:10.1016/j.eclinm.2021.101020
21. Why Are Clinical Trials So Complicated? Accessed September 25, 2023. https://www.science.org/content/blog-post/why-clinical-trials-so-complicated

Statements

Contributors

We want to acknowledge the following individuals who have made substantial contributions to the execution of this clinical trial:

Sashkia Balla, Angela Cai, Christopher Da Costa, Ryan Duncombe, Kevin Esvelt, Connor Flexman, Sumen Govender, Cate Hall, Dang Khanh Ngan Ho, Alwyna Holtzhausen, Stephanie Koehl, Madeleine Lourens, Phumzile Promise Mhlongo, Zanele Makhado, Thandeka Moyo-Gwete, Portia Mutevedzi, Brent Packer, Georg Petrick, Sarah Robinson, Patricia Rutherfoord, Kenza Samlali, Pascal Scheven, Jannik Stemler, Subrahmanian Tarakkad Krishnaji, Adam Trotman, Roland van Rensburg, Brian Wang, Kyle Webster.

Declaration of interests

Tobias Odendahl, James A. Smith, Kyle Fish, Anemone Franz, Miti Saskena, Ethan Alley, Grigory Khimulya have been employed and were shareholders of the sponsor of the study. Sonia Sutherland, Vaughan Reed, Zinhle Zwane, Kgoete Dimakatso, Veronique De Jager, Phumzile Mhlongo, Madeli Kruger, Penny Moore do not report any conflicts of interest.

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Data sharing statement

All of the individual participant data collected during the trial, after de-identification, Study protocol, statistical analysis plan, informed consent form, clinical study report will be made available immediately following publication with no end date to anyone who wishes to access the data for any purpose. Data are available indefinitely at Schons, Maximilian (2023), “Phase I clinical trial dataset: naked DNA SARS-CoV-2 Omicron BA.2 booster vaccine Alveavax-v1.2 against Janssen Ad26.COV2.S comparator, NCT05844202”, Mendeley Data, V1, doi: 10.17632/pjrs3rrnfc.1

Supplementary Materials

- Supplementary Information
- Protocol
- IB
- Informed Consent Forms

- Study Report
- Data and Analysis code
- Randomization lists and specifications