Immunogenicity and safety of beta variant COVID-19 vaccine AZD2816 and AZD1222 (ChAdOx1 nCoV-19) as primary-series vaccination for previously unvaccinated adults in Brazil, South Africa, Poland, and the UK: a randomised, partly double-blinded, phase 2/3 non-inferiority immunobridging study

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Summary

Background AZD2816 is a variant-adapted COVID-19 vaccine that expresses the full-length SARS-CoV-2 beta variant spike protein but is otherwise similar to AZD1222 (ChAdOx1 nCoV-19). This study aimed to evaluate the safety and immunogenicity of AZD1222 or AZD2816 (or both) primary-series vaccination in a cohort of adult participants who were previously unvaccinated.

Methods In this phase 2/3, randomised, multinational, active-controlled, non-inferiority, immunobridging study, adult participants previously unvaccinated for COVID-19 were enrolled at 16 study sites in Brazil, South Africa, Poland, and the UK. Participants were stratified by age, sex, and comorbidity and randomly assigned 5:5:5:2 to receive a primary series of AZD1222 (AZD1222 group), AZD2816 (AZD2816 [4-week] group), or AZD1222-AZD2816 (AZD1222-AZD2816 group) at 4-week dosing intervals, or AZD2816 at a 12-week interval (AZD2816 [12-week] group) and evaluated for safety and immunogenicity through 180 days after dose 2. Primary outcomes were safety (rates of solicited adverse events occurring during 7 days and unsolicited adverse events occurring during 28 days after each dose) and immunogenicity (non-inferiority of pseudovirus neutralising antibody geometric mean titre [GMT], GMT ratio margin of 0·67, and seroresponse rate, rate difference margin of –10%, recorded 28 days after dose 2 with AZD2816 [4-week interval] against beta *vs* AZD1222 against ancestral SARS-CoV-2) in participants who were seronegative at baseline. This trial is registered with ClinicalTrials.gov, NCT04973449, and is completed.

Findings Between July 7 and Nov 12, 2021, 1449 participants were assigned to the AZD1222 group (n=413), the AZD2816 (4-week) group (n=415), the AZD1222-AZD2816 group (n=412), and the AZD2816 (12-week) group (n=209). Ten (2·6%) of 378 participants who were seronegative at baseline in the AZD1222 group, nine (2·4%) of 379 in the AZD2816 (4-week) group, eight (2·1%) of 380 in the AZD1222-AZD2816 group, and 11 (5·8%) of 191 in the AZD2816 (12-week) group had vaccine-related unsolicited adverse events. Serious adverse events were recorded in one (0·3%) participant in the AZD1222 group, one (0·3%) in the AZD2816 (4-week) group, two (0·5%) in the AZD1222-AZD2816 group, and none in the AZD2816 (12-week) group. Co-primary immunogenicity endpoints were met: neutralising antibody GMT (ratio 1·19 [95% CI 1·08–1·32]; lower bound greater than 0·67) and seroresponse rate (difference 1·7% [–3·1 to 6·5]; lower bound greater than –10%) at 28 days after dose 2 were non-inferior in the AZD2816 (4-week) group against beta versus in the AZD1222 group against ancestral SARS-CoV-2. Seroresponse rates were highest with AZD2816 against beta (12-week interval 94·3% [95% CI 89·4–97·3]; 4-week interval 85·7% [81·5–89·2]) and with AZD1222 (84·6% [80·3–88·2]) against ancestral SARS-CoV-2.

Interpretation Primary series of AZD1222 and AZD2816 were well tolerated, with no emergent safety concerns. Both vaccines elicited robust immunogenicity against beta and ancestral SARS-CoV-2 with greater responses demonstrated when testing against SARS-CoV-2 strains that matched those targeted by the respective vaccine. These findings demonstrate the continued importance of ancestral COVID-19 vaccines in protecting against severe COVID-19 and highlight the feasibility of using the ChAdOx1 platform to develop COVID-19 vaccines against future SARS-CoV-2 variants.

Funding AstraZeneca.

Lancet Microbe 2024

Published Online https://doi.org/10.1016/S2666-5247(24)00078-8

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Introduction

The COVID-19 pandemic comprised the initial spread of ancestral SARS-CoV-2 followed by successive waves of variant-driven infection, notably omicron and its subvariants. Vaccination and previous infection have been found to provide variable levels of protection against variants with enhanced transmissibility and immune-evasiveness. The resultant continued circulation of SARS-CoV-2 has led to the virus becoming endemic in many regions.[1]

COVID-19 vaccination strategies have evolved from initial two-dose ancestral SARS-CoV-2-based primary-series vaccinations, which were instrumental to early pandemic mitigation[2,3] and in preventing severe disease,[2] to subsequent repeated dosing strategies, which have been widely adopted to maintain protection.[3,4] Such repeated dosing strategies were limited to doses of ancestral-based vaccines, which demonstrated effectiveness against severe disease caused by newer variants, including omicron,[5–7] until the development and authorisation of variant-updated vaccines, including bivalent (against ancestral SARS-CoV-2 and omicron subvariants)[8] and, most recently, monovalent omicron XBB.1.5-adapted mRNA vaccines.[9] As of November 2023, based on evidence of widespread protection conferred by hybrid immunity from previous SARS-CoV-2 infection plus vaccination,[10,11] WHO recommends a single dose of any authorised ancestral-based or, where available, variant-updated monovalent vaccine in individuals who are previously unvaccinated, followed by additional doses in vaccinated individuals deemed at high risk of severe outcomes.[4]

The additional protection against severe COVID-19 afforded by variant-updated versus ancestral-based vaccines was limited for bivalent mRNA vaccines.[12–15] This limited additional protection might be due to immune imprinting following ancestral-based vaccines or high underlying immunity from a combination of natural infection and vaccination.[16] The effectiveness of omicron XBB.1.5-adapted vaccines have not yet been comprehensively evaluated.[4] Therefore, a greater understanding of immune dynamics of variant-updated versus ancestral-based vaccination, particularly in the context of hybrid immunity, is needed to inform future vaccination strategies.

The adenovirus-vectored, ancestral-based vaccine, AZD1222 (ChAdOx1 nCoV-19), has demonstrated high efficacy[17–20] and effectiveness[5,6] against severe disease caused by ancestral SARS-CoV-2 and subsequent variants, including omicron.[5–7] The safety and immunogenicity of AZD2816, a variant-adapted ChAdOx1-vectored vaccine that uses the same platform as AZD1222 but expresses the full-length beta spike protein, as a third-dose booster in a

Research in context

Evidence before this study

Before this study, no clinical data were available on primary-series vaccination with AZD2816, a beta variant-adapted COVID-19 vaccine, but AZD1222 (ChAdOx1 nCoV-19) had been investigated in multiple studies, both as a primary series and a booster. To provide context to the findings in this report, we searched PubMed for research articles published from July 1, 2021, to April 30, 2023, using the search terms "COVID-19" OR "primary series", OR "hybrid immunity" OR "variant vaccine". The available published literature demonstrated that AZD1222 and other COVID-19 vaccines developed against ancestral SARS-CoV-2 have been instrumental in mitigating the health burden of the COVID-19 pandemic by providing continued protection against severe outcomes from COVID-19. Hybrid immunity, resulting from the combination of previous SARS-CoV-2 infection plus vaccination, has been demonstrated to further enhance protection against COVID-19. Variant-updated vaccines, targeted against specific SARS-CoV-2 variants, offer the potential for additional enhancement of the magnitude, duration, and breadth of immunity conferred by vaccination, although the degree of added benefit is unknown in populations with high rates of underlying hybrid immunity.

Added value of this study

We report results from the first clinical trial of primary-series vaccination with AZD2816, a vaccine that was developed with the ChAdOx1 adenovirus-vector platform used for AZD1222. This study, evaluating the safety and immunogenicity of a two-dose primary series of AZD2816 or AZD1222 (or both), showed that both homologous and heterologous primary series were well tolerated, and, although both vaccines were highly immunogenic against beta and ancestral SARS-CoV-2, variant-matched vaccines evoked greater neutralising antibody responses. Additionally, our findings of increased immunogenicity in a cohort of participants who were seropositive for SARS-CoV-2 and of high neutralising antibody titres that exhibited differences over time according to country of enrolment (Brazil and South Africa) support the existing evidence for enhanced immunogenicity due to hybrid immunity.

Implications of all the available evidence

Our findings demonstrate that AZD2816 is well tolerated and highlight the feasibility of using the ChAdOx1 platform to develop COVID-19 vaccines capable of provoking enhanced targeted immune responses against specific SARS-CoV-2 variants. These findings are relevant given the recent WHO recommendations for the use of monovalent variant-adapted vaccines. Additionally, the persistent, high levels of immunogenicity observed after AZD1222 vaccination, likely enhanced by omicron infections, support the continued importance of ancestral COVID-19 vaccines in protecting against severe COVID-19 outcomes.

cohort of previously vaccinated participants has been demonstrated in this multinational phase 2/3 study.[21] In this Article, we report final analysis results from a separate cohort of adults who were previously unvaccinated and enrolled specifically to evaluate the safety and immunogenicity of homologous or heterologous two-dose primary series of AZD1222 and AZD2816. We also explore the effect of timing of vaccinations relative to COVID-19 waves in the main countries of enrolment (Brazil and South Africa) and the effect of potential hybrid immunity.

Methods

Study design and participants

This primary-series component of a phase 2/3, randomised, multinational, active-controlled, non-inferiority immunobridging study evaluated the safety and immunogenicity of AZD1222 and AZD2816 primary-series vaccination in a cohort of adult (aged ≥18 years) participants who were previously unvaccinated enrolled at 16 study sites in Brazil, Poland, South Africa, and the UK between July 7 and Nov 12, 2021. The last study visit was on Aug 2, 2022. The clinical database lock was Sept 29, 2022.

Participants aged 18 years or older (UK protocol amendment ≥30 years) who had no history of COVID-19 vaccination and were healthy or had medically stable conditions (no significant change in therapy or hospitalisation for worsening disease within 90 days of enrolment) were eligible. Individuals with childbearing potential were required to test negative for pregnancy at screening and dosing visits and use a qualifying birth control method from at least 28 days before day 1 (first vaccine dose) through 30 days after dose 2. Participants with any confirmed or suspected immunosuppressive or immunodeficient state, or history of thrombocytopenia or thrombosis were excluded. Participants could be SARS-CoV-2 seronegative or seropositive at baseline. For inclusion in the seronegative population for evaluation of the primary and secondary objectives, participants were required to have no pre-study medical history of laboratory-confirmed SARS-CoV-2 infection and to be seronegative for SARS-CoV-2 at screening based on a lateral flow test detecting spike protein and nucleocapsid antibodies. Participants who were seropositive were eligible for the seropositive population (enrolment capped at 10% of the seronegative population) for evaluation of exploratory objectives. All eligibility criteria are detailed within the protocol.

Baseline data collected from participants included both self-reported (age, sex [male, female], race [American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, or White], ethnicity [Hispanic or Latino, Not Hispanic or Latino], and physician reviewed comorbidity categories [significant cardiovascular disease, chronic lung disease, or diabetes]) and data that was not self-reported (country was based on enrolment site location, SARS-CoV-2 status was assessed via lateral flow test, and BMI categories were assigned based on vital signs at screening).

This study was done in accordance with ethical principles originating in the Declaration of Helsinki and consistent with International Council for Harmonisation Good Clinical Practice guideline, applicable regulatory requirements, and AstraZeneca's policy on Bioethics. The protocol and amendments were approved by the ethics committee or institutional review board at each centre. The full protocol and statistical analysis plan (SAP) have been published online. All participants provided written informed consent. This trial is registered with ClinicalTrials.gov, NCT04973449, and is completed.

For the full protocol and statistical analysis plan see https://www.astrazenecaclinicaltrials.com/study/D7220C00001/

Randomisation and masking

Eligible participants were randomly assigned in a 5:5:5:2 ratio to receive two intramuscular doses (5×10^{10} viral particles) of (1) AZD1222 or (2) AZD2816 on days 1 and 29 (4-week interval) referred to as the AZD1222 and AZD2816 (4-week) groups, (3) AZD1222 on day 1 followed by AZD2816 on day 29 (the AZD1222-AZD2816 group), or (4) AZD2816 on days 1 and 85 (12-week interval; AZD2816 [12-week] group). Participants were centrally assigned using an Interactive Response Technology or Randomisation and Trial Supply Management system. Randomisation was stratified by three factors: age (<65 years and ≥65 years), sex, and presence or absence of at least one predefined comorbidity (appendix p 4). Participants who were seronegative and seropositive were randomly assigned separately. For groups with a 4-week dosing interval, participants, investigators, and sponsor staff members involved in study conduct were masked. AZD1222 and AZD2816 dose preparation was done by an unmasked pharmacist, or designee, at each site due to visually distinct packaging. Due to the different dosing intervals, vaccine administration in the AZD2816 (12-week) group was open-label.

See Online for appendix

Procedures

Participants assigned to groups with a 4-week interval attended study visits on days 1 (first vaccine dose), 8, 15, 29 (second vaccine dose), 36, 43, 57, and 209. Participants assigned to the 12-week interval group attended study visits on days 1 (first vaccine dose), 8, 15, 29, 85 (second vaccine dose), 92, 99, 113, and 265. Participants reported predefined local and systemic solicited adverse events for 7 days after each dose via electronic diaries (e-diaries, appendix p 4). Clinical laboratory assessments for safety were undertaken at multiple study visits (appendix p 4). Unsolicited adverse events were recorded at study visits through 28 days after each dose. Serious adverse events, medically attended adverse events, and adverse events of special interest (appendix p 4) were recorded throughout the study. Participants were assessed at all study visits for COVID-19 symptoms or post-vaccination diagnosis. Participants presenting with symptoms were tested via RT-PCR. The

relatedness of adverse events to study intervention was assessed by the site investigator.

Serum samples for immunogenicity analyses and SARS-CoV-2 serology assessments were collected just before dosing, at 14 and 28 days after each dose, and at 180 days after dose 2. SARS-CoV-2 neutralising antibody levels were measured using validated pseudovirus neutralisation assays (appendix p 4). SARS-CoV-2 nucleocapsid antibody levels were measured via multiplexed electrochemiluminescence-based assay (appendix p 5). For a listing of reagents and resources used see the appendix (p 7).

Outcomes

The primary safety objective was to characterise the safety and tolerability of two-dose primary-series vaccination with AZD2816 (4-week) in participants who were seronegative at enrolment. Endpoints included the incidence of local and systemic solicited adverse events for 7 days after each dose and incidence of unsolicited adverse events, including serious adverse events, medically attended adverse events, and adverse events of special interest, for 28 days after each dose as well as the incidence of changes in clinical safety laboratory values. Secondary safety objectives are summarised in the appendix (p 5). The co-primary immunogenicity objectives (appendix p 8) were to evaluate whether model-adjusted neutralising antibody geometric mean titre (GMT) responses and seroresponse (defined as a ≥4-fold increase from baseline) rate with AZD2816 (4-week) against beta were non-inferior to those with AZD1222 against ancestral SARS-CoV-2, as assessed by GMT ratio and difference in seroresponse rate, respectively, in the seronegative immunogenicity population at 28 days after dose 2. Key secondary objectives and other secondary immunogenicity objectives, including those that were either not performed or not reported in this Article due to lack of clinical relevance, are outlined in the appendix (appendix p 8). Exploratory objectives in the seronegative immunogenicity population included evaluation of responses against beta with AZD2816 (12-week) compared with AZD2816 (4-week), the incidence of SARS-CoV-2 infections (nucleocapsid serostatus) after dose 2, and the incidence of COVID-19 (SARS-CoV-2 RT-PCR-positive symptomatic illness). An additional exploratory objective was to evaluate immunogenicity in the seropositive immunogenicity population.

The primary countries of enrolment (Brazil and South Africa) had asynchronous COVID-19 waves throughout the study. To evaluate the impact of potential hybrid immunity, exploratory and post-hoc immunogenicity analyses by country over time and by nucleocapsid serostatus were done as described in the appendix (p 5).

Statistical analysis

The safety analysis population included all participants who received at least one dose of AZD2816 or AZD1222, analysed according to treatment received. Primary and secondary safety objectives were evaluated in the seronegative safety analysis population (participants seronegative at screening). Rates of solicited adverse events were analysed using the numbers of participants for whom e-diary data were available as the denominators.

The immunogenicity analysis population included all participants who had received at least one vaccination, had baseline and post-dose antibody measurements, had at least one post-dose quantifiable serum titre, and had no protocol deviations that potentially could have interfered with generating or interpreting an antibody response. Primary and secondary immunogenicity objectives were evaluated in the seronegative immunogenicity analysis population. Immunogenicity was also explored in the seropositive immunogenicity analysis population. Participant immunogenicity data were censored at the time of a missed second dose, non-study COVID-19 vaccination, or confirmed post-vaccination SARS-CoV-2 infection.

We planned to enrol 1290 participants who were seronegative and 129 participants who were seropositive to result in 380 participants who were seronegative in groups using 4-week intervals (appendix p 4). Under the assumption of no difference between cohorts in neutralising antibody GMTs (ie, a GMT ratio of 1), this sample size provided >90% power to demonstrate non-inferiority in terms of GMT ratio, with a non-inferiority margin of 0·67 (log-scale difference of 0·405). Similarly, under the assumption of no difference between cohorts in neutralising antibody seroresponse rate and an observed seroresponse rate of 59·7%, a sample size of 380 participants per cohort provided 80·5% power to demonstrate non-inferiority in terms of seroresponse difference, with a non-inferiority margin of –10%.

Based on a request from the European Medicines Agency's Committee for Medicinal Products for Human Use, before database lock and analysis, the immunogenicity analyses testing hierarchy that was specified in the protocol was amended to promote a previous key secondary endpoint to a co-primary endpoint (comparison of seroresponse rates). All analyses and statistical comparisons were reported per the revised SAP (appendix p 8). Per the revisions, non-inferiority of AZD2816 (4-week) against beta versus AZD1222 against ancestral SARS-CoV-2 would only be concluded if both co-primary endpoints were met.

For immunogenicity assessment, raw and model-adjusted GMTs and geometric mean fold-rise (GMFR) values were calculated as described in the appendix (p 5). Model-adjusted values were derived via analysis of covariance models to control for potential between-group confounders. All analyses were done using SAS (9.4 or higher). For all primary and key secondary immunogenicity non-inferiority comparisons, we used model-adjusted GMT values and compared GMT ratios using the lower bound of two-sided score-based CIs (α=0·05) with a non-inferiority margin of 0·67, per standard guidelines.[22] All non-inferiority comparisons of seroresponse rates were made using the Newcombe score method without continuity correction to compute CIs (α=0·05), with a non-inferiority margin of –10%. We used a hierarchical approach to

control for multiplicity of testing of the co-primary and key secondary immunogenicity endpoints, with a type I error rate of 5%. Testing of each subsequent endpoint only occurred on rejection of the null hypothesis of each previous endpoint (appendix p 8). All analyses of other secondary endpoints and exploratory endpoints, as well as post-hoc analyses, were tested for nominal significance only at the 5% level. Study data was monitored throughout the duration of the study by an independent Data and Safety Monitoring Board (DSMB).

Role of the funding source

AstraZeneca authors and the International Coordinating Investigator (AJP) designed the trial. The investigators collected the data in collaboration with AstraZeneca and IQVIA (contract research organisation). Authors from AstraZeneca and ClinChoice (contract research organisation) analysed the data. All authors had access to the full dataset and interpreted the data alongside authors from AstraZeneca. AstraZeneca funded medical writing assistance for the development of the manuscript under the direction of the authors.

Results

Between July 7 and Nov 12, 2021, 1449 participants who were previously unvaccinated were enrolled, all but 11 (0·8%) participants were from Brazil (966 [67·0%]) or South Africa (464 [32·2%]); 413 participants were randomly assigned to the AZD1222 group, 415 to the AZD2816 (4-week) group, 412 to the AZD1222-AZD2816 group, and 209 to the AZD2816 group (12-week; figure 1). Of these participants, eight did not receive any intervention, leaving 1441 participants in the overall safety population (409 in AZD1222, 413 in AZD2816 [4-week], 411 in AZD1222-AZD2816, and 208 in AZD2816 [12-week]). The seronegative immunogenicity analysis population included 1318 participants (376 in AZD1222, 375 in AZD2816 [4-week], 378 in AZD1222-AZD2816, and 189 in AZD2816 [12-week]) who were seronegative for SARS-CoV-2 on lateral flow test at baseline and had post-vaccination antibody measurements. The seropositive immunogenicity analysis population comprised 113 participants (31 in AZD1222, 34 in AZD2816 [4-week], 31 in AZD1222-AZD2816, and 17 in AZD2816 [12-week]; figure 1).

Baseline characteristics of the overall safety population were similar between groups (table 1); the median age was 24·0 years (IQR 20–33), 850 (59·0%) were male and 591 (41·0%) were female, 706 (49·0%) were Black, and 320 (22·2%) had a comorbidity of interest, most commonly obesity (BMI ≥30 kg/m^2; 283 [19·6%]). Compared with participants who were seronegative, participants who were seropositive were slightly older (median age 26·0 [IQR 20–40] for seropositive participants *vs* 24·0 [20–32] years for seronegative participants), included fewer men than women (58/113 [51·3%] *vs* 784/1318 [59·5%]) and were recruited more frequently in Brazil than in other countries (103/113 [91·2%] *vs* 857/1318 [65·0%]; appendix pp 9–10).

The primary safety objective was to characterise the safety and tolerability of AZD2816 (4-week) in the seronegative safety population. Local and systemic solicited adverse events through 7 days after each dose were less frequent following dose 2 than following dose 1 in each of the groups using a 4-week dosing interval (table 2). In the AZD2816 (12-week) group, rates of local solicited adverse events were similar after each dose; systemic solicited adverse events were numerically lower after dose 2. Solicited adverse events were mostly reported within 3 days after dosing. Numerically higher rates of solicited adverse events were observed in the AZD2816 (12-week) group than in the 4-week interval groups (appendix p 24). In most participants in each group, solicited adverse events were mild or moderate. More participants reported grade 3 or greater adverse events after dose 1 than they did after dose 2 (table 2). Across all groups, the most common local solicited adverse events were pain and tenderness and the most common systemic solicited adverse events were headache and muscle pain (table 2). Findings were similar in the overall safety population, which included participants who were seropositive (appendix p 11).

In the seronegative safety population, unsolicited adverse events through 28 days after each dose were reported by 93 (24·6%) of 378 participants in the AZD1222 group, 96 (25·3%) of 379 in the AZD2816 (4-week) group, 93 (24·5%) of 380 in the AZD1222-AZD2816 group, and 67 (35·1%) of 191 in the AZD2816 (12-week) group (table 3). Grade 3 or higher adverse events were reported by one (0·30%) of 378 participants in the AZD1222 group, eight (2·1%) of 379 in the AZD2816 (4-week) group, eight (2·1%) of 380 in the AZD1222-AZD2816 group, and four (2·1%) of 191 in the AZD2816 (12-week) group. Serious adverse events were reported by one (0·3%) of 378 in the AZD1222 group, one (0·3%) of 379 in the AZD2816 (4-week) group, two (0·5%) of 380 in the AZD1222-AZD2816 group, and no participants in the AZD2816 (12-week) group. The most common unrelated unsolicited adverse event was headache (35 [2·6%] of 1328). The rate of unsolicited adverse events considered related to vaccination was ten (2·6%) of 378 in the AZD1222 group, nine (2·4%) of 379 in the AZD2816 (4-week) group, eight (2·1%) of 380 in the AZD1222-AZD2816 group, and 11 (5·8%) of 191 in the AZD2816 (12 week) group (table 3). Data for the overall safety population are summarised in the appendix (p 12) and related unsolicited adverse events are listed in appendix (p 13). Rates of serious adverse events through 180 days after dose 2 were low and similar across groups (table 3); individual serious adverse events were each reported in only one participant (appendix p 14). Medically attended adverse events were reported in 68 (18·0%) participants in the AZD1222 group, 69 (18·2%) in the AZD2816 (4-week) group, 82 (21·6%) in the AZD1222-AZD2816 group, and 50 (26·2%) in the AZD2816 (12-week) group (appendix p 15). Adverse events of special interest were reported in

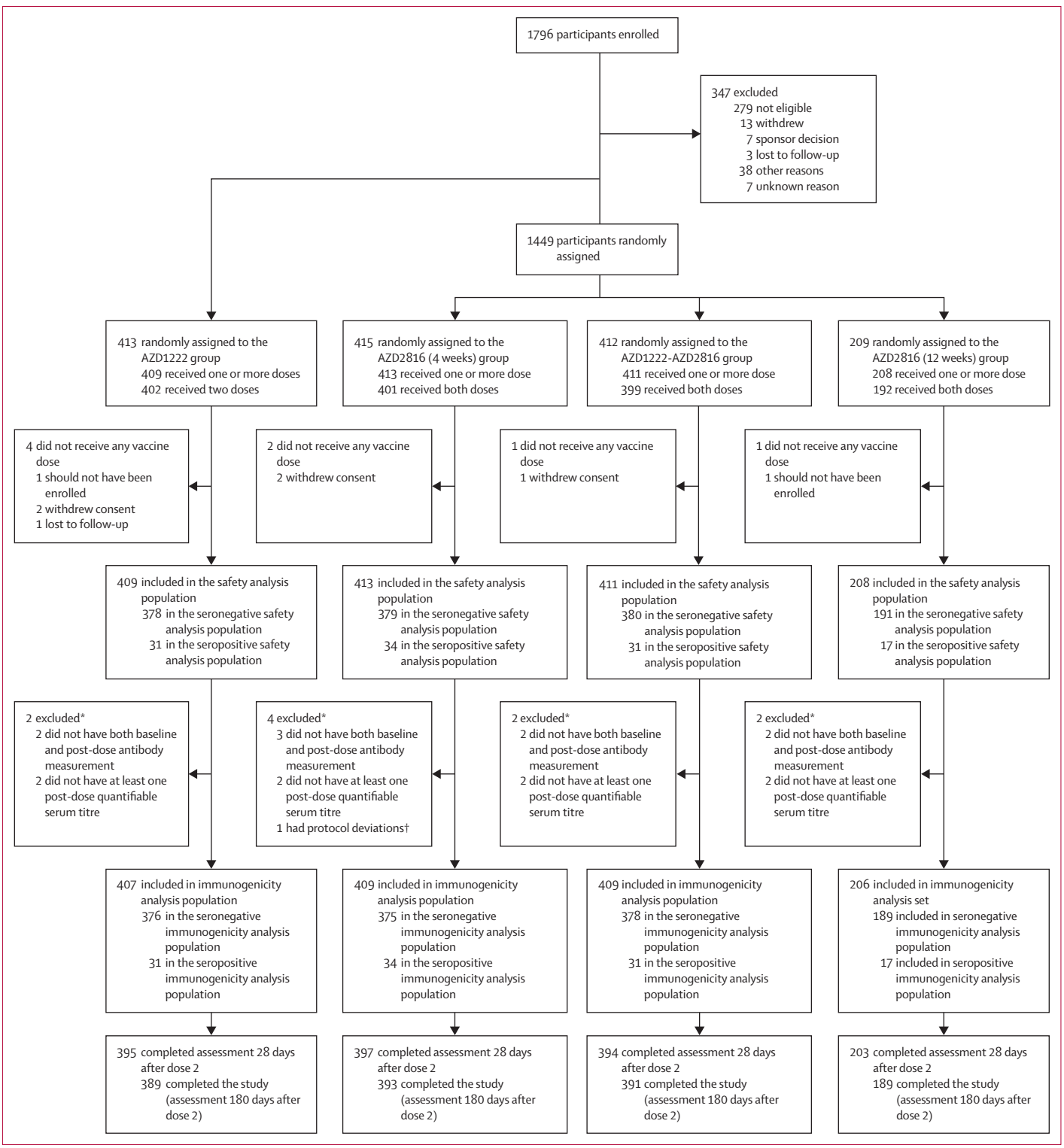


Figure 1: **Trial profile**

The number of participants who were screened and who were screen failures includes some participants who were previously vaccinated. These participants may have been eligible to participate in the booster cohort of the study, but they were considered screen failures for the purposes of this previously unvaccinated cohort. *Participants could be excluded for more than one reason. †Protocol deviations judged to have the potential to interfere with antibody response evaluation.

	AZD1222 (n=409)	AZD2816 (4-week interval; n=413)	AZD1222-AZD2816 (n=411)	AZD2816 (12-week interval; n=208)	All (n=1441)
Age, years	25·0 (20–33)	25·0 (21–34)	23·0 (20–31)	24·5 (20–33)	24·0 (20–33)
18–64 years	392 (95·8%)	391 (94·7%)	394 (95·9%)	198 (95·2%)	1375 (95·4%)
≥65 years	17 (4·2%)	22 (5·3%)	17 (4·1%)	10 (4·8%)	66 (4·6%)
Sex					
Male	238 (58·2%)	244 (59·1%)	245 (59·6%)	123 (59·1%)	850 (59·0%)
Female	171 (41·8%)	169 (40·9%)	166 (40·4%)	85 (40·9%)	591 (41·0%)
Race					
Black	202 (49·4%)	192 (46·5%)	214 (52·1%)	98 (47·1%)	706 (49·0%)
White	161 (39·4%)	166 (40·2%)	151 (36·7%)	86 (41·3%)	564 (39·1%)
Mixed	11 (2·7%)	23 (5·6%)	10 (2·4%)	6 (2·9%)	50 (3·5%)
Asian	3 (0·7%)	5 (1·2%)	4 (1·0%)	0	12 (0·8%)
American Indian or Alaska Native	4 (1·0%)	0	2 (0·5%)	2 (1·0%)	8 (0·6%)
Not reported	23 (5·6%)	20 (4·8%)	18 (4·4%)	13 (6·3%)	74 (5·1%)
Unknown	5 (1·2%)	7 (1·7%)	12 (2·9%)	3 (1·4%)	27 (1·9%)
Ethnicity					
Hispanic or Latino	239 (58·4%)	246 (59·6%)	257 (62·5%)	131 (63·0%)	873 (60·6%)
Not Hispanic or Latino	143 (35·0%)	148 (35·8%)	137 (33·3%)	70 (33·7)	498 (34·6)
Not reported	19 (4·6)	15 (3·6)	12 (2·9)	7 (3·4)	53 (3·7)
Unknown	8 (2·0)	4 (1·0)	5 (1·2)	0	17 (1·2)
Country					
Brazil	275 (67·2%)	274 (66·3%)	279 (67·9%)	138 (66·3%)	966 (67·0%)
South Africa	131 (32·0%)	135 (32·7%)	128 (31·1%)	70 (33·7%)	464 (32·2%)
Poland	3 (0·7%)	4 (1·0%)	3 (0·7%)	0	10 (0·7%)
UK	0	0	1 (0·2%)	0	1 (0·1%)
SARS-CoV-2 serostatus at baseline					
Negative	378 (92·4%)	379 (91·8%)	380 (92·5%)	191 (91·8%)	1328 (92·2%)
Positive	31 (7·6%)	34 (8·2%)	31 (7·5%)	17 (8·2%)	113 (7·8%)
Comorbidities					
Any	95 (23·2%)	91 (22·0%)	89 (21·7%)	45 (21·6%)	320 (22·2%)
BMI ≥30 kg/m^2	86 (21·0%)	79 (19·1%)	77 (18·7%)	41 (19·7%)	283 (19·6%)
Significant cardiovascular disease	19 (4·6%)	18 (4·4%)	21 (5·1%)	10 (4·8%)	68 (4·7%)
Chronic lung disease	3 (0·7%)	5 (1·2%)	3 (0·7%)	2 (1·0%)	13 (0·9%)
Diabetes	8 (2·0%)	4 (1·0%)	7 (1·7%)	1 (0·5%)	20 (1·4%)
None	314 (76·8%)	322 (78·0%)	322 (78·3%)	163 (78·4%)	1121 (77·8%)

Data are n (%) or median (IQR). *The safety analysis population included all participants who received at least one dose of AZD2816 or AZD1222, analysed according to treatment received.

Table 1: **Baseline characteristics of the overall safety population***

37 (9·8%) participants in the AZD1222 group, 48 (12·7%) in the AZD2816 (4-week) group, 51 (13·4%) in the AZD1222-AZD2816 group, and 32 (16·8%) in the AZD2816 (12-week) group (appendix p 18), respectively. One death was reported in the AZD1222 group following an adverse event of acute myocardial infarction on study day 16 (assessed as unrelated to vaccination).

Events of COVID-19 through 180 days after dose 2 were recorded in the seronegative safety population (table 3). COVID-19 cases were recorded in 32 (8·5%) of 378 participants in the AZD1222 group, 42 (11·1%) of 379 in the AZD2816 (4-week) group, 41 (10·8%) of 379 in the AZD1222-AZD2816 group, and 29 (15·2%) of 191 in the AZD2816 (12-week) group.

The co-primary immunogenicity endpoints were met. Model-adjusted neutralising antibody GMT (ratio 1·19; 95% CI 1·08 to 1·32) and seroresponse rate (difference 1·7%; 95% CI −3·1 to 6·5) at 28 days after dose 2 were non-inferior for AZD2816 (4-week) against beta versus AZD1222 against ancestral SARS-CoV-2 in the seronegative immunogenicity analysis population (figure 2). Of the key secondary immunogenicity endpoints, neutralising antibody GMT was superior with AZD2816 (4-week) versus AZD1222 against beta (ratio 3·21; 95% CI 3·06 to 3·36), but non-inferiority was not demonstrated for comparisons of AZD1222-AZD2816 against beta versus AZD1222 against ancestral SARS-CoV-2 (0·50; 0·45 to 0·56) and AZD2816 (4-week) versus AZD1222 against ancestral SARS-CoV-2 (0·31; 0·27 to 0·35; descriptive statistic only; figure 2A). For other secondary immunogenicity endpoints (descriptive statistics only), the neutralising antibody seroresponse rate was non-inferior for AZD2816 (4-week) versus

	After first dose				After second dose			
	AZD1222 (n=358)	AZD2816 (4-week; n=365)	AZD1222-AZD2816 (n=366)	AZD2816 (12-week; n=184)	AZD1222 (n=353)	AZD2816 (4-week; n=356)	AZD1222-AZD2816 (n=351)	AZD2816 (12-week; n=165)
Any solicited adverse events	278 (77·7%)	299 (81·9%)	303 (82·8%)	153 (83·2%)	217 (61·5%)	222 (62·4%)	215 (61·3%)	122 (73·9%)
Any grade ≥3	58 (16·2%)	81 (22·2%)	76 (20·8%)	40 (21·7%)	11 (3·1%)	11 (3·1%)	17 (4·8%)	12 (7·3%)
Any local solicited adverse events	235 (65·6%)	256 (70·1%)	269 (73·5%)	126 (68·5%)	183 (51·8%)	181 (50·8%)	177 (50·4%)	114 (69·1%)
Any grade ≥3	22 (6·1%)	32 (8·8%)	22 (6·0%)	14 (7·6%)	5 (1·4%)	4 (1·1%)	4 (1·1%)	4 (2·4%)
Local solicited adverse events								
Pain	210 (58·7%)	234 (64·1%)	242 (66·1%)	121 (65·8%)	183 (51·8%)	181 (50·8%)	177 (50·4%)	114 (69·1%)
Redness	7 (2·0%)	3 (0·8%)	6 (1·6%)	5 (2·7%)	2 (0·6%)	2 (0·6%)	1 (0·3%)	2 (1·2%)
Tenderness	170 (47·5%)	209 (57·3%)	210 (57·4%)	95 (51·6%)	141 (39·9%)	130 (36·5%)	136 (39·0%)	88 (53·3%)
Swelling	7 (2·0%)	5 (1·4%)	7 (1·9%)	5 (2·7%)	2 (0·6%)	2 (0·6%)	2 (0·6%)	2 (1·2%)
Any systemic solicited adverse events	244 (68·2%)	269 (73·7%)	272 (74·3%)	138 (75·0%)	146 (41·4%)	150 (42·1%)	156 (44·4%)	92 (55·8%)
Any grade ≥3	51 (14·2%)	72 (19·7%)	70 (19·1%)	36 (19·6%)	10 (2·8%)	10 (2·8%)	14 (4·0%)	9 (5·5%)
Systemic solicited adverse events								
Fever	59 (16·5%)	72 (19·7%)	59 (16·1%)	36 (19·6%)	16 (4·5%)	11 (3·1%)	13 (3·7%)	10 (6·1%)
Chills	131 (36·6%)	154 (42·2%)	155 (42·3%)	66 (35·9%)	27 (7·6%)	35 (9·8%)	33 (9·4%)	20 (12·1%)
Muscle pain	167 (46·6%)	192 (52·6%)	204 (55·7%)	109 (59·2%)	79 (22·4%)	74 (20·8%)	85 (24·2%)	60 (36·4%)
Fatigue	123 (34·4%)	145 (39·7%)	148 (40·4%)	77 (41·8%)	54 (15·3%)	65 (18·3%)	50 (14·2%)	43 (26·1%)
Headache	182 (50·8%)	207 (56·7%)	194 (53·0%)	108 (58·7%)	90 (25·5%)	99 (27·8%)	92 (26·2%)	56 (33·9%)
Malaise	113 (31·6%)	126 (34·5%)	138 (37·7%)	70 (38·0%)	33 (9·3%)	46 (12·9%)	48 (13·7%)	26 (15·8%)
Nausea	52 (19·5%)	73 (20·0%)	81 (22·1%)	39 (21·2%)	28 (7·9%)	27 (7·6%)	33 (9·4%)	14 (8·5%)
Vomiting	11 (3·1%)	6 (1·6%)	21 (5·7%)	11 (6·0%)	5 (1·4%)	7 (2·0%)	7 (2·0%)	5 (3·0%)

Data are n (%). Table includes solicited adverse events with an onset date on or after the date of either vaccine dose through 7 days after each dose. Electronic diary data was not available for five participants in the AZD1222 group, nine in the AZD2816 (4-week) group, three in the AZD1222-AZD2816 group, and one in the AZD2816 (12-week) group.

Table 2: **Summary of solicited adverse events reported through 7 days after each dose of vaccine in the seronegative safety population with available electronic diary data**

AZD1222 against beta, but non-inferiority was not demonstrated for seroresponse rates with AZD1222-AZD2816 against beta versus AZD1222 against ancestral SARS-CoV-2 and AZD2816 (4-week) versus AZD1222 against ancestral SARS-CoV-2 (figure 2B).

In the seronegative immunogenicity analysis populations, of the groups using 4-week intervals, neutralising antibody GMTs against beta increased at 14 days and decreased slightly at 28 days after each dose, and then increased from 28 days to 180 days after dose 2 (figure 3A; appendix p 19). In the groups using a 4-week interval, GMFRs were highest after AZD2816 (4-week) for all timepoints (figure 3A). In the AZD2816 (12-week) group, GMT was highest at 14 days (1980·6) and 28 days (1744·0) after dose 2, with some waning observed at 180 days (figure 3A). For the exploratory immunogenicity endpoint comparing neutralising antibody responses against beta in participants who were seronegative in the AZD2816 (4-week) and AZD2816 (12-week) groups, GMTs were 666·1 and 1744·0 at 28 days and 1531·5 and 1368·5 at 180 days after dose 2, respectively (figure 3; appendix p 18).

Neutralising antibody GMTs against ancestral SARS-CoV-2 were numerically highest at 14 days after dose 2 in the AZD1222 (GMT 1049·9), AZD1222-AZD2816 (663·7), and AZD2816 (12-week; 409·9) groups, with some waning at 180 days after dose 2 (figure 3B; appendix p 19). In the AZD2816 (4-week) group, GMTs increased at 14 days and decreased slightly at 28 days after each dose, and then increased from 28 days to 180 days after dose 2; GMFRs were highest in the AZD1222 group for all timepoints (figure 3B; appendix p 19).

Neutralising antibody seroresponse rates at 28 days after dose 2 and were 51·5% (95% CI 46·0–56·9) in the AZD1222, 85·7% (81·5–89·2) in the AZD2816 (4-week) group, 58·1% (52·6–63·4) in the AZD1222-AZD2816 group, and 94·3% (89·4–97·3) in the AZD2816 (12-week) group against beta and 84·6% (80·3–88·2), 49·4% (44·0–54·8), 75·8% (70·8–80·2), and 67·5% (59·6–74·8), respectively, against ancestral SARS-CoV-2.

In the seropositive immunogenicity analysis population, neutralising antibody GMTs against both beta and ancestral SARS-CoV-2 were numerically higher at all timepoints than in the seronegative immunogenicity analysis population across all groups (appendix pp 21, 25). In the groups using 4-week intervals, GMTs were increased at 14 days after dose 1 and were consistent through 28 days after dose 2; waning was observed at 180 days after dose 2 except against beta in the AZD1222 group. In the AZD2816 (12-week) group, GMTs were generally highest at 14 days after dose 1 and decreased thereafter, except for a slight increase against beta following dose 2 (appendix pp 21, 25).

Brazil and South Africa experienced substantial omicron-dominant COVID-19 waves during the study period, the timing of which differed between countries and relative to the vaccination and follow-up periods in each country (appendix p 26). Post-hoc analysis of neutralising antibody

	AZD1222 (n=378)	AZD2816 (4-week; n=379)	AZD1222-AZD2816 (n=380)	AZD2816 (12-week; n=191)
Any unsolicited adverse events through 28 days post dose 1 or 2	93 (24·6%)	96 (25·3%)	93 (24·5%)	67 (35·1%)
Any grade ≥3	1 (0·3%)	8 (2·1%)	8 (2·1%)	4 (2·1%)
Any related unsolicited adverse events through 28 days post dose 1 or 2	10 (2·6%)	9 (2·4%)	8 (2·1%)	11 (5·8%)
Unsolicited adverse events in ten participants or more through 28 days post dose 1 or 2				
Headache	8 (2·1%)	9 (2·4%)	12 (3·2%)	6 (3·1%)
COVID-19	4 (1·1%)	9 (2·4%)	7 (1·8%)	6 (3·1%)
Upper respiratory tract infection	6 (1·6%)	3 (0·8%)	6 (1·6%)	4 (2·1%)
Allergic rhinitis	5 (1·3%)	6 (1·6%)	5 (1·3%)	2 (1·0%)
Influenza	1 (0·3%)	5 (1·3%)	9 (2·4%)	1 (0·5%)
Hypertension	5 (1·3%)	4 (1·1%)	3 (0·8%)	3 (1·6%)
Tension headache	4 (1·1%)	3 (0·8%)	4 (1·1%)	2 (1·0%)
Nasopharyngitis	4 (1·1%)	3 (0·8%)	3 (0·8%)	2 (1·0%)
Events through 28 days after dose 1 or 2				
Any serious adverse events	1 (0·3%)	1 (0·3%)	2 (0·5%)	0
Any medically attended adverse events	34 (9·0%)	31 (8·2%)	37 (9·7%)	25 (13·1%)
Any adverse events of special interest	7 (1·9%)	10 (2·6%)	9 (2·4%)	7 (3·7%)
Any adverse events with outcome of death	1 (0·3%)	0	0	0
Confirmed COVID-19 adverse events	4 (1·1%)	9 (2·4%)	7 (1·8%)	6 (3·1%)
Events through 180 days after dose 2				
Any serious adverse events	3 (0·8%)	2 (0·5%)	5 (1·3%)	2 (1·0%)
Any medically attended adverse events	68 (18·0%)	69 (18·2%)	82 (21·6%)	50 (26·2%)
Any adverse events of special interest	37 (9·8%)	48 (12·7%)	51 (13·4%)	32 (16·8%)
Confirmed COVID-19 adverse events	32 (8·5%)	42 (11·1%)	41 (10·8%)	29 (15·2%)

Data are n (%). Table includes all unsolicited adverse events with an onset date on or after the date of either vaccine dose through 28 days after each dose and through 180 days after dose 2. No adverse events led to study discontinuation in any group. No additional adverse events with outcome of death were reported beyond 28 days after dose 2.

Table 3: **Summary of all unsolicited adverse events reported through 28 days after either dose of vaccine, and serious adverse events, medically attended adverse events, and adverse events of special interest reported through 180 days after dose 2, in the seronegative safety population**

GMTs with AZD2816 (4-week) against beta and AZD1222 against ancestral SARS-CoV-2 showed differing patterns by country. Participants from Brazil had numerically lower GMTs than participants from South Africa at each timepoint through 28 days after dose 2. At 180 days after dose 2, GMTs were further increased in participants from Brazil but showed some waning in participants from South Africa (appendix pp 22, 27).

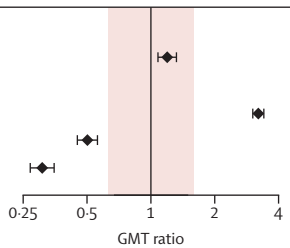
Exploratory and post-hoc analyses of SARS-CoV-2 nucleocapsid antibody titres in the seronegative immunogenicity analysis population showed that the median baseline titre was 390·5 arbitrary units (AU) per mL overall but was 147·5 AU/mL in participants from Brazil and 2353·0 AU/mL in participants from South Africa (appendix p 28). Based on nucleocapsid antibody assay titre threshold (>19 904 AU/mL) at baseline (instead of lateral flow test), 75 (5·7%) participants were classified as seropositive, including 18 (2·1%) from Brazil and 57 (12·8%) from South Africa (appendix p 28). In all groups, nucleocapsid seroresponse rate increased over time from 28 days to 180 days after dose 2 (appendix p 28). In the AZD1222 (appendix p 28) and AZD2816 (4-week; appendix p 28) groups, seroresponse rates were numerically lower in Brazil than those in South Africa at 28 days after dose 2; rates then increased more substantially in Brazil and were higher than those in South Africa at 180 days after dose 2. An analysis that censored participants in the seronegative immunogenicity population at the time of first recorded nucleocapsid antibody titre above the assay seropositivity threshold (>19 904 AU/mL, including those who were assay-seropositive at baseline), resulted in numerically lower neutralising antibody responses against ancestral SARS-CoV-2 at some timepoints compared with the overall seronegative immunogenicity population; however, patterns of responses were similar to those recorded in the overall population (appendix pp 23, 29).

Discussion

In this generally young population who were enrolled primarily in Brazil and South Africa and were unvaccinated as of July 7, 2021, we demonstrated that primary-series vaccination with AZD2816 (4-week) was as immunogenic against the beta variant as was AZD1222 against ancestral SARS-CoV-2. The study's co-primary immunogenicity endpoints were met, with non-inferiority demonstrated for neutralising antibody GMT and seroresponse rate at 28 days after dose 2. We also showed that homologous and heterologous primary series were safe and well tolerated, with no emergent safety concerns. Adverse events were generally consistent with the established safety profile of AZD1222

A

Comparator regimen	SARS-CoV-2 strain	GMT (95% CI)	Reference	SARS-CoV-2 strain	GMT (95% CI)	GMT ratio (95% CI)
Co-primary endpoint						
AZD2816 (4-week)	Beta	790·96 (735·29 to 850·85)	AZD1222	Ancestral	661·29 (617·16 to 708·57)	1·19 (1·08 to 1·32)
Key secondary endpoints						
AZD2816 (4-week)	Beta	865·48 (837·85 to 894·02)	AZD1222	Beta	269·89 (260·72 to 279·37)	3·21 (3·06 to 3·36)
AZD1222-AZD2816	Beta	405·20 (373·64 to 439·43)	AZD1222	Ancestral	800·92 (745·59 to 860·36)	0·50 (0·45 to 0·56)
AZD2816 (4-week)	Ancestral	222·75 (201·60 to 246·12)	AZD1222	Ancestral	719·37 (654·68 to 790·46)	0·31 (0·27 to 0·35)



B

Comparator regimen	SARS-CoV-2 strain	Seroresponse rate (95% CI)	Reference	SARS-CoV-2 strain	Seroresponse rate (95% CI)	Seroresponse rate difference (95% CI)
Co-primary endpoint						
AZD2816 (4-week)	Beta	89·47% (85·73 to 92·52)	AZD1222	Ancestral	87·79% (83·86 to 91·06)	1·7% (–3·1 to 6·5)
Other secondary endpoints						
AZD2816 (4-week)	Beta	100% (98·73 to NE)	AZD1222	Beta	99·42% (97·92 to 99·93)	0·6% (–0·6 to 2·1)
AZD1222-AZD2816	Beta	62·57% (57·14 to 67·78)	AZD1222	Ancestral	87·50% (83·53 to 90·80)	–24·9% (–31·1 to –18·6)
AZD2816 (4-week)	Ancestral	49·42% (44·00 to 54·85)	AZD1222	Ancestral	84·59% (80·34 to 88·24)	–35·2% (–41·5 to –28·4)

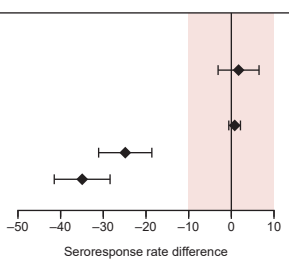


Figure 2: **Co-primary, key secondary, and other secondary immunogenicity endpoint comparisons of pseudovirus-neutralising antibody responses in participants receiving an AZD1222, AZD2816, or AZD1222-AZD2816 primary vaccination series in the seronegative immunogenicity analysis population**

(A) Co-primary and key secondary comparisons of GMT. (B) Co-primary and other secondary comparisons of seroresponse rate. Shaded areas denote non-inferiority margins. GMT units are 1/dilution. Seroresponse is defined as a four-fold or more rise from baseline. Titre values measured as below the lower limit of quantitation (40) are imputed to a value that is half of the lower limit of quantitation. Titre values measured as above upper limit of quantitation (787 339) are imputed at the upper limit of quantitation value (before model adjustment for model-adjusted estimates). GMT ratio was calculated as the ratio of the titre levels in the comparator group to the reference group. The difference in seroresponse was calculated as seroresponse rate of comparator group minus seroresponse rate of reference group. Model-adjusted difference is based on proportions calculated using model-adjusted titre results. Model-adjusted estimates for GMT were derived using ANCOVA models that included the log-transformed value of the titre as the dependent variable and independent variables for vaccine group, visit, baseline comorbidities, sex, and age group as fixed effects as well as participant intercept and slope as random effects that were fitted to the comparator and reference arms group. *The immunogenicity analysis population included all randomly assigned participants who had received at least one vaccination, had baseline and post-dose antibody measurements, had at least one post-dose quantifiable serum titre, and had no protocol deviations that potentially could have interfered with generating or interpreting an antibody response. GMT=geometric mean titre.

through a 6-month follow-up period.[17,18] No clinically meaningful between-group differences in safety were observed, although both reactogenicity following dose 2 and overall rates of unsolicited adverse events were numerically higher in the AZD2816 (12-week) group.

Our results not only demonstrated that both vaccines were highly immunogenic but also showed that neutralising antibody responses were greater for matched vaccine strains, as expected. AZD2816 (4-week) was superior to AZD1222 in terms of neutralising antibody GMT and seroresponse rate against beta at 28 days after dose 2. Conversely, neutralising antibody GMT and seroresponse rate were lower with AZD2816 (4-week) versus AZD1222 against ancestral SARS-CoV-2. Across all groups, seroresponse rates against beta were highest with AZD2816 (12-week and 4-week), whereas seroresponse rates against ancestral SARS-CoV-2 were greatest in the AZD1222 and AZD1222-AZD2816 groups. Similarly, we noted the greatest GMTs against beta in the AZD2816 groups and against ancestral SARS-CoV-2 in the AZD1222 and AZD1222-AZD2816 groups. However, non-inferiority was not demonstrated for the key secondary endpoint of neutralising antibody GMT with AZD1222-AZD2816 against beta versus AZD1222 against ancestral SARS-CoV-2. GMT ratio was inferior and seroresponse rate was lower for AZD1222-AZD2816 against beta than for AZD1222 against ancestral SARS-CoV-2, suggesting that variant-specific immunogenicity was optimised using two doses of the variant-adapted vaccine rather than a single dose following AZD1222, potentially due to immune imprinting. However, similar results were not observed when AZD2816 was evaluated as a third-dose booster as AZD2816 was highly immunogenic against beta following ancestral-based primary vaccination.[21]

Neutralising antibody levels have been shown to correlate with protection against SARS-CoV-2,[23,24] although this was established based on data primarily against ancestral SARS-CoV-2,[23,24] whereas subsequent variants have been more infectious and immune-evasive.[1] Nevertheless, the high neutralising antibody titres seen in the present study, which were greater and more persistent than in previous studies of AZD1222,[17–19] provide immunogenicity findings supportive of real-world data demonstrating that ancestral-based vaccines, such as AZD1222, offer ongoing protection against severe outcomes due to several omicron subvariants.[5,7,20] Such protection is probably also mediated by durable cellular immune protection,[25] although analysis of this was beyond the scope of this study.

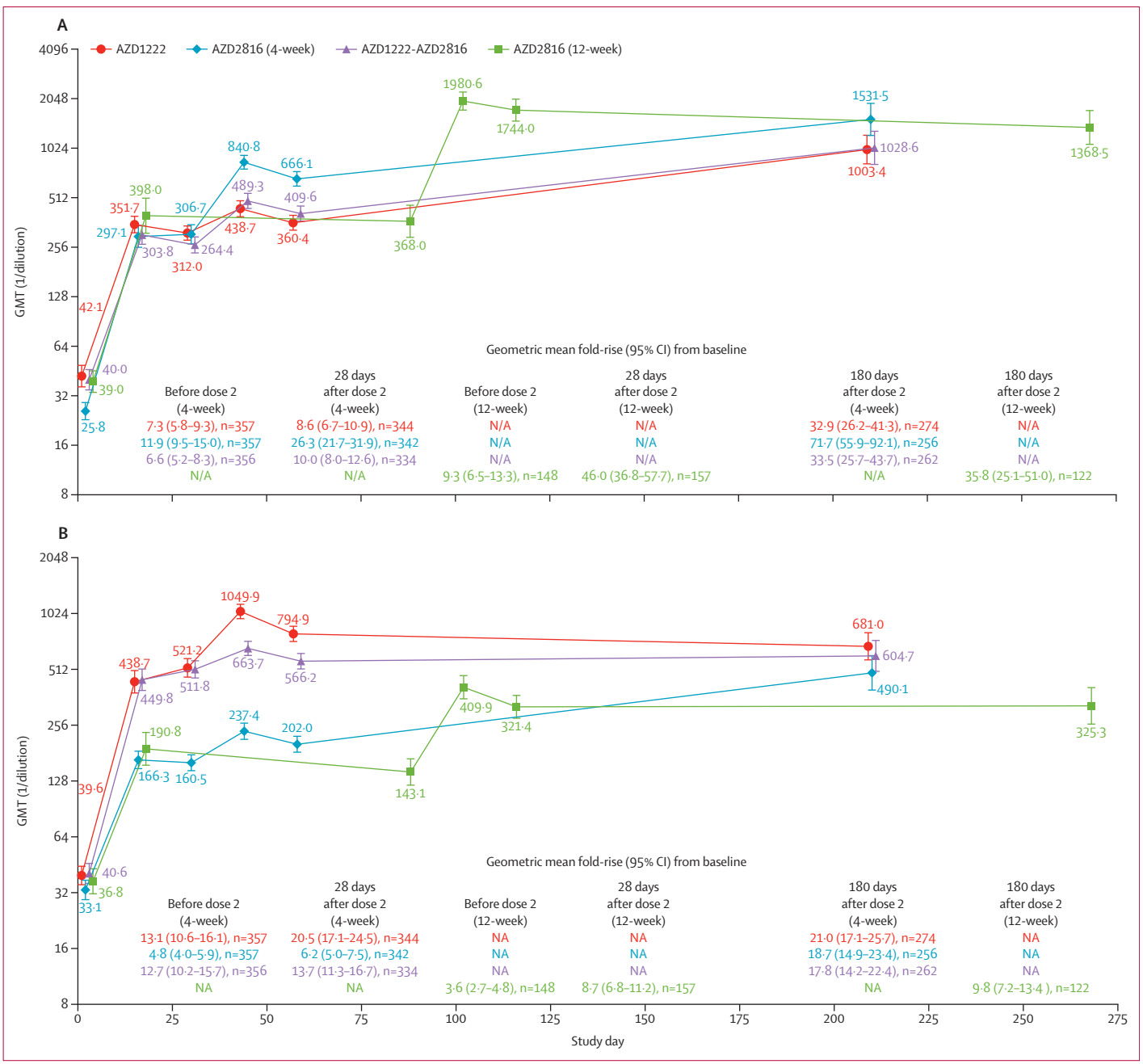


Figure 3: **Pseudovirus neutralising antibody responses over time in participants receiving an AZD2816, AZD1222, or AZD1222-AZD2816 primary vaccination series in the seronegative immunogenicity analysis population**

Note that scales on y axes differ between plots. GMTs (95% CI) and geometric mean fold-rise from baseline by study group against (A) the SARS-CoV-2 beta variant and (B) ancestral SARS-CoV-2. Titre values measured as below the lower limit of quantitation (40) were imputed to a value that is half of the lower limit of quantitation. Titre values measured as above the upper limit of quantitation (787 339) were imputed at the upper limit of quantitation value (before model adjustment). Model-adjusted analyses were derived using ANCOVA models that included the log-transformed titre value as the dependent variable, and independent variables for vaccine group, visit window, baseline co-morbidities, sex, and age group (18–64 years, ≥65 years) as fixed effects as well as participant intercept as a random effect. Plotting of data by study day was offset by adding 1 for AZD2816 (4-week interval), 2 for AZD1222-AZD2816, and 3 for AZD2816 (12-week interval) for clarity at coincident timepoints. Collected data are summarised in the appendix (p 19). GMT=geometric mean titre. NA=not applicable.

Immune protection against subsequent infection and severe COVID-19 is enhanced by previous SARS-CoV-2 infection.[10,11,26,27] Our findings in participants who were seropositive reflect this effect: neutralising antibody titres against both beta and ancestral SARS-CoV-2 appeared substantially higher at baseline and through 180 days after

dose 2 compared with those for participants who were seronegative, acknowledging that the values from the former are raw titres while the latter are model-adjusted. Additionally, although censoring participants in the seronegative immunogenicity population based on nucleocapsid assay seropositivity resulted in a reduction of neutralising antibody titres against ancestral SARS-CoV-2 at some study timepoints, a similar pattern of response was still observed to those in the overall seronegative immunogenicity population. Such increased hybrid immunity from vaccination and previous or subsequent infection was suggested as a reason for the decoupling of infection rate from hospitalisation and death rates during the initial omicron wave.[28]

Our data strongly suggest that extensive development of hybrid immunity in participants over the course of the study, associated with widespread SARS-CoV-2 infection, was a key driver of the differential findings from Brazil and South Africa.[10–12,26] During the study period, omicron waves in Brazil and South Africa peaked at different calendar times (appendix p 26). In Brazil, this peak occurred from Jan 29 to Feb 2, 2022, whereas in South Africa the peak was earlier, around Dec 15–18, 2021. Additionally, participant enrolment occurred at different calendar times (appendix p 26). Consequently, whereas Brazil's omicron wave peaked approximately 5 months after study vaccinations, enrolment and vaccinations in South Africa coincided with the onset of its first omicron wave, which lasted for approximately 3 months.

We therefore believe that, despite the low rate of recorded COVID-19 adverse events during the data collection period for this study, undetected or asymptomatic SARS-CoV-2 infections from omicron substantially affected the responses observed in participants who were baseline seronegative. The very likely presence of undetected or asymptomatic infections is a possible limitation of the study but is also an opportunity to examine the data in the context of these real-world circumstances. For instance, despite being SARS-CoV-2 negative on lateral flow testing at screening, participants in South Africa had higher baseline SARS-CoV-2 nucleocapsid antibody titres than participants in Brazil. Additionally, nucleocapsid seroresponse rate was higher in participants from South Africa versus Brazil at 28 days after dose 2, broadly coinciding with the omicron wave peak in South Africa. However, by 180 days after dose 2, nucleocapsid seroresponse rate had increased greatly in Brazil and was higher than in South Africa, associated with peaking omicron infection in Brazil during the study follow-up period. Patterns of neutralising antibody titre over time in each country reflect these seroresponse findings, with greater initial increases in South Africa compared with Brazil through 28 days after dose 2, followed by further increases in Brazil but evidence of some waning in South Africa through 180 days after dose 2. Extending primary-series dosing intervals has been shown to result in increased immunogenicity;[29] however, undetected or asymptomatic SARS-CoV-2 infections occurring during the longer dosing interval in the AZD2816 (12-week) group might also have contributed to the increased responses recorded at 28 days after dose 2. This supposition is supported by the higher rate of COVID-19 adverse events reported in the AZD2816 (12-week) group, which is suggestive of additional undetected SARS-CoV-2 in those participants as they had a longer interval in which they were only partially vaccinated (the 12 weeks between dose 1 and dose 2); a period which featured robust omicron circulation in South Africa.

These findings are also supported by other studies demonstrating enhanced immunogenicity and protection against severe COVID-19 outcomes associated with hybrid immunity arising from previous SARS-CoV-2 infection plus vaccination against ancestral SARS-CoV-2.[26,27] Such effects might help explain the limited additional benefit conferred by bivalent vaccines in the context of previous infection.[12]

WHO has declared an end to the acute phase of the COVID-19 public health emergency and recommended that primary vaccination consist of one dose of a monovalent COVID-19 vaccination, with repeated dosing in individuals at risk of COVID-19 severe outcomes.[4] Our findings, particularly in participants who were seropositive, are supportive of this recommendation in areas of high SARS-CoV-2 seroprevalence. Our findings also support the rationale for future efforts to develop circulating-variant-adapted vaccines—efforts that will almost certainly evolve along with our capabilities for accurately predicting SARS-CoV-2 circulation. This study clearly shows the potential benefit of a variant-matched vaccine against a circulating strain and supports the feasibility of developing variant-adapted COVID-19 vaccines using the ChAdOx1 platform. Nonetheless, our findings and recent literature also support the ongoing use of ancestral-based vaccines in protecting against severe COVID-19 outcomes, notably with AZD1222 in low-income and middle income countries and regions that have more challenging circumstances for vaccine distribution.

Contributors

This study was designed by SS, EJK, and TV, and the International Coordinating Investigator, AJP, in collaboration with the sponsor and regulatory authorities. All trial site investigators gathered the data in collaboration with AstraZeneca and IQVIA, a contract research organisation. The data were analysed by AstraZeneca and ClinChoice, a contract research organisation. The accuracy of the data was verified by the authors from AstraZeneca and AJP. All authors interpreted the data. The manuscript was written under the direction of all authors by medical writers funded by the study sponsor, and all authors reviewed and provided feedback on manuscript drafts. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

SACC declares participation in the clinical development, with University of Oxford, of the vaccines described in this Article, membership on CureVac and Clover advisory boards, and institutional research grants from AstraZeneca. BJ is an employee of Cytel and is currently on assignment to AstraZeneca. QEB declares institutional funding, the provision of study materials, or both from the Wits Health Consortium, the Bill & Melinda Gates Foundation, the South African Medical Research Council, and AstraZeneca, as well as research grants or contracts from Regeneron, Novo Nordisk, Avillion, GlaxoSmithKline,

Novavax, Sinovac, Johnson & Johnson, and Pfizer pharmaceuticals. PKA declares institutional grants to support the conduct of the study from AstraZeneca and the UK Vaccine Taskforce via the UK National Institute for Health Research (NIHR). HB is an employee of Bogier consulting and is currently on assignment to AstraZeneca. EJK is an employee of Sanofi. SM is an employee of Exploristics and is currently on assignment to AstraZeneca. EPM declares no competing interests. TL declares consulting fees from Vaccitech on an unrelated project, an honorarium from Seqirus on an unrelated project, an institutional grant from the UK Vaccine Taskforce via the NIHR, work-related investments, and is a named inventor on a patent application for a vaccine against SARS-CoV-2. JJL declares no competing interests. MNR declares institutional support for the study from AstraZeneca. AJP is Chair of the UK Department of Health and Social Care's Joint Committee on Vaccination and Immunisation (JCVI) but does not participate in the JCVI COVID-19 committee. AJP also declares membership of the WHO's Strategic Advisory Group of Experts on Immunization until January, 2022; provision of advice to Shionogi on COVID-19; and funding from the NIHR, AstraZeneca, the Bill & Melinda Gates Foundation, Wellcome, the UK Research and Innovation Medical Research Council, the Serum Institute of India, and the Coalition for Epidemic Preparedness Innovations. University of Oxford has entered a partnership with AstraZeneca for the development of COVID-19 vaccines; TL, MNR, SB, PKA, and AJP are contributors to the intellectual property licensed by Oxford University Innovation to AstraZeneca. AA, TA, EJK, MK, UO, SS, KS, AS, TV, and JAG are, or were, employees of and may hold (or have held) stock or stock options in AstraZeneca. MSMdOP declares no competing interests.

Data sharing

Data underlying the findings described in this manuscript can be obtained in accordance with AstraZeneca's data sharing policy described at https://astrazenecagrouptrials.pharmacm.com/st/submission/disclosure. Data for studies directly listed on Vivli can be requested through Vivli at www.vivli.org. Data for studies not listed on Vivli can be requested through Vivli at https://vivli.org/members/enquiries-about-studies-not-listed-on-the-vivli-platform/. AstraZeneca Vivli member page is also available outlining further details https://vivli.org/ourmember/astrazeneca/.

Acknowledgments

We thank the participants and their families, and caregivers, health care providers, all investigators, and research staff who contributed to the study. We also thank Johan Vekemans for his contributions to the study design; the Data Safety Monitoring Board (DSMB) Chair, Prof Robert Heyderman, and the DSMB members (Manish Sadarangani, George Bouliotis, Steven Black, Paul Kaye, Bernhards Ogutu, Walt Orenstein, Sonia Regina TS Ramos, Elizabeth Bukusi, and Cornelia L Dekker) for their contributions to this study; and Labcorp-Monogram Biosciences and PPD Vaccines for immunogenicity analysis. AZD2816 Clinical Study Group members involved in this analysis of the previously unvaccinated cohort are listed in the appendix (p 2), together with additional acknowledgments of individuals at the study sites who the authors wish to thank for their contributions to the successful conduct of this study (appendix p 3). Medical writing support for the development of this manuscript, under the direction of the authors, was provided by Steve Hill and Rose Follis of Ashfield MedComms (Macclesfield, UK), an Inizio company, in accordance with Good Publication Practice 2022 guidelines and funded by AstraZeneca.

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