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The Platform Trial In COVID-19 Priming and BOOsting (PICOBOO): The immunogenicity, reactogenicity, and safety of different COVID-19 vaccinations administered as a second booster (fourth dose) in AZD1222 primed individuals aged 50-<70 years old

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SUMMARY

Objectives: PICOBOO is a randomised, adaptive trial evaluating the immunogenicity, reactogenicity, and safety of COVID-19 booster strategies. We report data for second boosters among individuals 50- < 70 years old primed with AZD1222 (50- < 70y-AZD1222) until Day 84.

Methods: Immunocompetent adults who received any first booster ≥ three months prior were eligible. Participants were randomly allocated to BNT162b2, mRNA-1273 or NVX-CoV2373 1:1:1. The concentrations of ancestral anti-spike immunoglobulin were summarised as the geometric mean concentrations (GMC). Reactogenicity and safety outcomes were captured. Additional analyses including neutralising antibodies were performed on a subset. ACTRN12622000238774.

Results: Between Mar 2022 and Aug 2023, 743 participants were recruited and had D28 samples; 155 belonged to the 50- < 70y-AZD1222 stratum. The mean adjusted GMCs (95% credible intervals) were 20,690 (17 555–23 883), 23,867 (20 144–27 604) and 8654 (7267–9962) U/mL at D28 following boosting with BNT162b2, mRNA-1273 and NVX-CoV2372, respectively, and 10,976 (8826–13 196), 15,779 (12 512–19 070) and 6559 (5220–7937) U/mL by D84. IgG against Omicron BA.5 was 2.7–2.9 times lower than the ancestral

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strain. Limited neutralisation against Omicron subvariants was found following all vaccines. Severe reactogenicity events were < 4%.

Conclusions: All vaccines were immunogenic with more rapid waning after mRNA vaccines. These data support boosting with vaccines with greater specificity for circulating Omicron subvariants.

Research in context

Evidence before this study

We searched PubMed from inception until 24 April 2024, using the search terms "COVID*" AND "VACCIN*" AND "booster OR fourth dose" and restricted this search to randomised controlled trials (RCTs). We did not identify any RCTs evaluating second booster messenger ribonucleic acid (mRNA) and protein subunit vaccines in individuals primed with AZD1222. A phase two RCT (COV-BOOST) evaluated the immune responses to second boosters with mRNA vaccines (BNT162b2 or mRNA-1273) following a first booster (third dose) of BNT162b2, after priming with two doses of AZD1222 or BNT162b2 in individuals aged ≥30 years old. This trial found mRNA vaccines were well tolerated and boosted humoral and cellular responses. These responses were similar or better than the responses observed following first booster doses. An open-label phase two RCT evaluated immune responses to monovalent or bivalent variant mRNA and protein based vaccines targeting Ancestral, Beta (B.1.351), Delta (B.1.617.2), and Omicron BA.1 strains in individuals who had received three doses of an mRNA vaccine (94–100%) or two doses of an mRNA vaccine and one dose of Ad26. COV2.S. This study found that vaccines targeting Beta or Omicron BA.1 elicited broadly cross-protective neutralising antibody responses against SARS-CoV-2 variants (including B.1.351 and Omicron subvariants BA.1 and BA.4–5) as well as the Ancestral strain. An RCT evaluating second booster doses with monovalent or bivalent Omicron BA.1-adapted BNT162b2 vaccines in adults who had previously received three doses of BNT162b2 and who were aged 55 years or older found monovalent or bivalent Omicron BA.1-adapted vaccines induced neutralising responses against both Ancestral and Omicron BA.1 strains. Neutralisation responses for Omicron subvariants BA.4, BA.5 and BA.2.75 were lower. Adverse events were more common in the 30 μg monovalent-BA.1 and 60 μg bivalent-BA.1 recipients than in the other groups.

Added value of this study

These are the first randomised clinical trial data of the immunogenicity, reactogenicity and safety of second booster (fourth doses) of mRNA and protein subunit COVID-19 vaccines in adults previously primed with two doses of AZD1222. BNT162b2, mRNA-1273 and NVX-CoV2372 were well tolerated and boosted humoral immune responses. Higher binding and neutralising antibodies against Ancestral SARS-CoV-2 were observed following boosting with mRNA vaccines (BNT162b2 and mRNA-1273) compared to NVX-CoV2372 at all time points. Lower neutralising antibody responses were observed against Omicron subvariants BA.5 and XBB.1.5 following all vaccines until Day 84 highlighting the need for boosting with vaccines with greater specificity for Omicron subvariants.

Implication of all the available evidence

BNT162b2, mRNA-1273 and NVX-CoV2372 are suitable for boosting humoral immune responses among adults who were primed with AZD1222. The clinical relevance of the higher antibody responses to the mRNA vaccines is uncertain. Limited neutralisation against Omicron subvariants following vaccination with vaccines targeting Ancestral virus support the need for boosting with vaccines with greater specificity for circulating subvariants.

Introduction

The past four years have seen the rapid development and deployment of Coronavirus 2019 (COVID-19) vaccines, changing epidemiology of COVID-19 disease, viral evolution and the widespread development of infection-induced vaccine and hybrid immunity.[1] Most countries, including Australia, have now lifted non-pharmacological prevention measures including restrictions on travel.[2] Robust evidence exists that vaccination provides strong protection against severe disease, hospitalisations, and death.[3–5] However, further evidence of comparative effectiveness and cost-effectiveness are still required to inform whether periodic boosting should be recommended, and if so, in whom and with which vaccines and schedules.

The Platform trial In COVID-19 priming and BOOsting (PICOBOO) was established on 29 Mar 2022 to generate evidence of the immunogenicity, reactogenicity and safety of first and subsequent COVID-19 booster dose strategies against Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) and its variants to inform practice and policy. Recruitment commenced in Australia shortly after the national recommendation for second booster doses in select populations[6] and when Omicron BA.2 was the predominant circulating variant; by late 2022, Omicron subvariants BA.4 and BA.5 were dominant.[2] Here, we report data on second booster (fourth) doses among the pre-defined stratum of individuals aged 50- < 70 years old who had been primed with two doses of AZD1222 vaccine (50- < 70y-AZD1222) up to Day 84 (D84).

Methods

Study design

PICOBOO is a parallel group, randomised, Bayesian adaptive platform trial. PICOBOO is currently randomising eligible participants to alternative first or subsequent COVID-19 booster dose strategies; participants are stratified by age group and their primary COVID-19 vaccination schedule. Recruitment is occurring in Perth, Adelaide, and Launceston (Australia).

The PICOBOO protocol is modular and hierarchical, including the Core Protocol,[7] the Booster Vaccination Substudy Protocol (unpub, McLeod), and the Statistical Appendix.[8] The master protocol structure aims to ensure efficiency and standardised study procedures, data collection and endpoints for all substudies nested within the trial platform.

PICOBOO is registered with Australia's Therapeutic Goods Administration (CTN-00603–1) and was approved by the Child and Adolescent Health Service Human Research Ethics Committee (RGS5222). Approval for the use of the vaccines evaluated in this study was provided by the vaccine manufacturers and the Commonwealth Government of Australia.

Participants

Eligibility criteria for the PICOBOO platform are documented elsewhere.[7] The pre-specified 50- < 70y-AZD1222 stratum included individuals aged 50- < 70 years old who had received two priming doses of AZD1222 followed by a single dose of any licensed first

booster vaccine dose at least three months prior to randomisation. Immunocompromised individuals and those with an established contraindication to any study vaccine (e.g., history of anaphylaxis or myocarditis attributable to prior receipt of COVID-19 vaccine) were excluded.

Recruitment strategies are detailed in the PICOBOO Core Protocol.[7] Recruitment to PICOBOO commenced on 29 Mar 2022 and is ongoing. 50- < 70y-AZD1222 stratum participants were randomised to second COVID-19 booster vaccine doses between 12 May 2022 and 6 Oct 2022. Screening for eligibility was performed via an online questionnaire or telephone interview. At the baseline visit (D0), after discussing the study and confirming eligibility, online or written informed consent was obtained from all participants. The immunological and safety data cut-off date was 29 Aug 2023.

Randomisation and masking

Allocation to a single COVID-19 booster vaccine dose (study vaccine) was centrally determined, irrespective of study site, using computer-generated random sequences prepared by an unblinded trial statistician. Equal assignment probabilities were used for all study vaccines and randomisation was stratified by stratum and booster dose number. Randomisation was performed by an unblinded research nurse using REDcap version 12.2.4. Further details regarding randomisation, blinding and concealment are supplied in the PICOBOO Core Protocol.[7] Participants were blinded to their assignment until at least six weeks after randomisation, at which time the detailed vaccination record was uploaded to the Australian Immunisation Register and was thereby accessible to participants. The unblinded nurse had no role in collecting outcome data after vaccination. Blinding was achieved by drawing up the study vaccine out of sight, covering the vaccine label, and instructing participants to look away at the time of vaccine administration. Research staff involved in collecting or processing specimens and reviewing adverse events were blinded to the study vaccine. The trial statistician preparing interim analyses and members of the Data Safety Monitoring Committee were unblinded. All study staff became unblinded to the results of the first interim analysis following its release on 22 May 2023.

Procedures

PICOBOO is designed to evaluate up to three COVID-19 booster vaccines at any given time, including a maximum of one vaccine subtype from each vaccine manufacturer. During the reported study period, seven vaccines were evaluated in the platform. These included BNT162b2, mRNA-1273 and NVX-CoV2372 initially, and subsequently tozinameran/riltozinameran, elasomeran/imelasomeran, tozinameran/famtozinameran and elasomeran/davesomeran. Three vaccines were evaluated as second boosters in the 50- < 70y-AZD1222 stratum: (i) BNT162b2, a messenger ribonucleic acid (mRNA) vaccine, encoding the full-length SARS-CoV-2 spike protein administered as 30 μg (0·3 mL) intramuscularly; (ii) mRNA-1273, another mRNA vaccine, also encoding the full-length SARS-CoV-2 spike protein, administered as 50 μg (0·25 mL) intramuscularly and (iii) NVX-CoV2372, a nano-particle vaccine constructed from the full-length Ancestral strain pre-fusion trimers of the SARS-CoV2 spike glycoprotein, administered as 5 μg (0·5 mL) intramuscularly.

On the day of enrolment, participants were randomised to receive a single dose of one of the three study vaccines administered by a trained, unblinded study nurse per Australian guidelines; participants were observed for at least 15 min afterwards. Participants were also given an oral thermometer, tape measure, and a diary card to be used as a memory aid to record solicited and unsolicited adverse events (AEs). Participants were encouraged to present for polymerase chain reaction (PCR) symptoms concerning for active SARS-CoV-2 infection during follow-up. Two rapid antigen test (RAT) kits were supplied to participants to expedite self-testing; those with a positive RAT were requested to confirm with a PCR test.

Study visits were scheduled on day 0 (D0), day 6–8 (D7), day 21–31 (D28) and day 70–98 (D84) post-randomisation. Bloods were collected prior to randomisation and at all subsequent study visits. Saliva samples were collected at all study visits except D7.

Electronic surveys were sent to participants on days 1 to 7 following randomisation, on D28 and three-monthly throughout study follow-up to capture patient-reported outcome (PRO) data.

Outcomes

All immunological analyses were performed on participants without evidence of intercurrent SARS-CoV-2 infection between D0 and D28 (or between D0 and D7 for D7 outcomes). The $\log_{10}$ ancestral SARS-CoV-2 anti-spike receptor binding domain (RBD) total immunoglobulin (Ig) was captured using a quantitative total Ig assay ((Elecsys anti-SARS-CoV-2 S (RBD) Roche, Basel, Switzerland). SARS-CoV-2 anti-spike Ig was summarised for each study arm at each time point as a geometric mean concentration (GMC; U/mL) with 95% credible intervals (95% CrI) provided for the purposes of publication to facilitate comparison to data published previously.[9]

The concentration of anti-spike IgG (captured by Meso Scale Discovery [MSD] ECL assay in AU/mL) against Ancestral SARS-CoV-2 and Omicron BA.5 was tested on all participant samples at D0, D7, D28 and D84. Additional assays were performed on a dedicated immunological subset comprising the first 20 participants per vaccine intervention, for each booster dose number and stratum with D28 samples. The additional tests included the dilution of serum that neutralises 50% (normal neutralisation or NF_{50}) of Ancestral SARS-CoV-2 virus, Omicron subvariants BA.5 (D28) and XBB.1.5 at D28 and D84 (measured by microneutralisation assay measured in IU/mL) and the percentage inhibition of virus measured by GenScript Surrogate Virus Neutralisation test (Ancestral SARS-CoV-2 virus and Omicron BA.5).

Prior COVID-19 infections captured at baseline were based on self-reported infections and/or positive anti-nucleocapsid protein antibody status (anti-NCP). PROs comprised reactogenicity and safety endpoints including solicited local and systemic reactions on D1–7, solicited and unsolicited adverse events (AEs) up to D28, intercurrent SARS-CoV-2 infections and associated time off school, work or usual activities and unplanned hospitalisations. Serious AEs (SAEs), AEs of special interest (AESIs) and serious adverse reactions (SARs) are defined in the core protocol.[7]

Statistical analysis

Statistical analyses were pre-specified using the estimands framework[10] and are detailed in the Statistical Appendix[8] and supplementary materials. Unadjusted summary statistics are presented as median (interquartile range [IQR]) for continuous variables and frequency (percentage) for categorical variables.

A Bayesian three-level hierarchical linear model was used as it was anticipated that immune responses to each study vaccine were likely to be, in part, mutually informative across COVID-19 booster dose number, age groups, and across the mRNA vaccines.[10] The model estimated the posterior distribution of the mean outcome for each study vaccine in each stratum and for each booster dose number, conditional on a set of predefined covariates including sex, outcome at baseline, previous COVID-19 infection, and time epoch. All posterior distributions presented have been marginalised (averaged) over time epoch and therefore can be interpreted as representing the mean during the respective period of recruitment.

A maximum sample size of 50 participants per study vaccine per stratum and booster dose number was targeted, allowing for up to 5% attrition from loss to follow-up or intercurrent COVID-19 infection before D28. This was based on simulations used to estimate the probability of achieving desirable precision on the D28 anti-spike Ig GMC estimates as described in the Statistical Appendix.[8]

Unsolicited AEs were coded and reported according to the Medical Dictionary for Regulatory Activities (MEDRA). For further detail regarding the statistical methods, including the pre-trial simulations, please refer to the Statistical Appendix.[8] All statistical analyses were performed using STAN,[11] via the R package cmdstanr[12] in R,[13] version 4.2.2.

Role of the funding source

Funders who supported this trial had no role in the study design, data collection, analysis, interpretation or writing of this manuscript.

Results

There were 743 participants recruited to the PICOBOO platform during the reported study period (S1). Of these, 155 participants belonging to the 50- < 70y-AZD1222 primed stratum were randomised to receive a second booster vaccine dose (Fig. 1). These participants were recruited in Perth (n = 137, 88%) and Adelaide (n = 18, 12%). The median (IQR) age of participants was 60·4 (56·8, 63·3) years. There were 117 females (75%). Baseline characteristics for participants were balanced between study arms (Table 1). Overall, 23 of the 155 participants had a history of prior COVID-19 infection at baseline (15%); these data are broken down by site in S2. The immunological subset comprised 60 participants; the baseline characteristics for these participants are presented in S3.

Raw anti-spike Ig concentrations against Ancestral SARS-CoV-2 at D0, D7, D28 and D84 are presented in S4. The posterior distributions of the GMCs of Ancestral anti-spike IgG at D7, D28 and D84 are presented in Fig. 2 and additional modelled data are provided in S5. At D7, the mean adjusted Ig GMCs (95% CrI) were 21 293 (17 762, 24 663), 21 170 (18 016, 24 169) and 6 674 (5 714, 7 751) U/mL for those who received BNT162b2, mRNA-1273 and NVX-CoV2372, respectively. At D28, these values were 20 690 (17 555, 23 883), 23 867 (20 144, 27 604) and 8654 (7267, 9962) U/mL, respectively. By D84, the adjusted GMCs had fallen to 10 976 (8826, 13 196), 15 779 (12 512, 19 070) and 6559 (5220, 7937) U/mL, respectively. At the time of the first scheduled analysis, the D28 estimates each met the pre-specified precision criteria for the stopping criterion (0·13 for BNT162b2 and NVX-CoV2372 and 0·14 for mRNA-1273).

Raw data regarding neutralisation is presented in S6 and posterior distributions for neutralisation against Ancestral SARS-CoV-2 are presented in Table 2 and S7. The posterior mean of the adjusted geometric mean NF_{50} for Ancestral SARS-CoV-2 at D28 was 159, 213 and 75 IU/mL following receipt of BNT162b2, mRNA-1273 and NVX-CoV2372, respectively. At D84, these values were 100, 156 and 72 IU/mL. The neutralisation activity detected against Omicron BA.5 at D28 and Omicron XBB.1.5 at D28 or D84 following any study vaccine dose was minimal. The proportion of participant samples with neutralisation activity against Omicron BA.5 below the lower limit of detection (10 IU/mL) at D28 was 32% (6/19), 35% (6/17) and 85% (17/20) following receipt of BNT162b2, mRNA-1273 and NVX-CoV2372, respectively. The proportion of participants with neutralisation activity below the lower limit of detection for Omicron XBB.1.5 was 90% (18/20), 71% (12/17) and 95% (19/20) at D28 and 95% (18/19), 79% (15/19) and 89% (16/18) at D84, respectively.

Surrogate neutralisation (percentage inhibition) testing against Ancestral SARS-CoV-2 was similar across all study arms at D28, with sustained responses observed until D84. At all timepoints, surrogate neutralisation against Omicron BA.5 was lower for all study vaccines compared to Ancestral SARS-CoV-2 (S8–9).

The posterior distributions of the adjusted GMC of anti-spike IgG (by MSD assay) against Ancestral SARS-CoV-2 and Omicron BA.5 at D7, D28 and D84 are presented in Fig. 3 and were lower at all timepoints for the BA.5 spike protein after all vaccines. The raw MSD data is presented in S10, and unadjusted fold-changes are presented in S11–12.

Reactogenicity and safety data are detailed in S13. Severe reactions were uncommon across all study vaccines (< 4%). No local severe reactions were elicited. Fever was reported in < 6% of

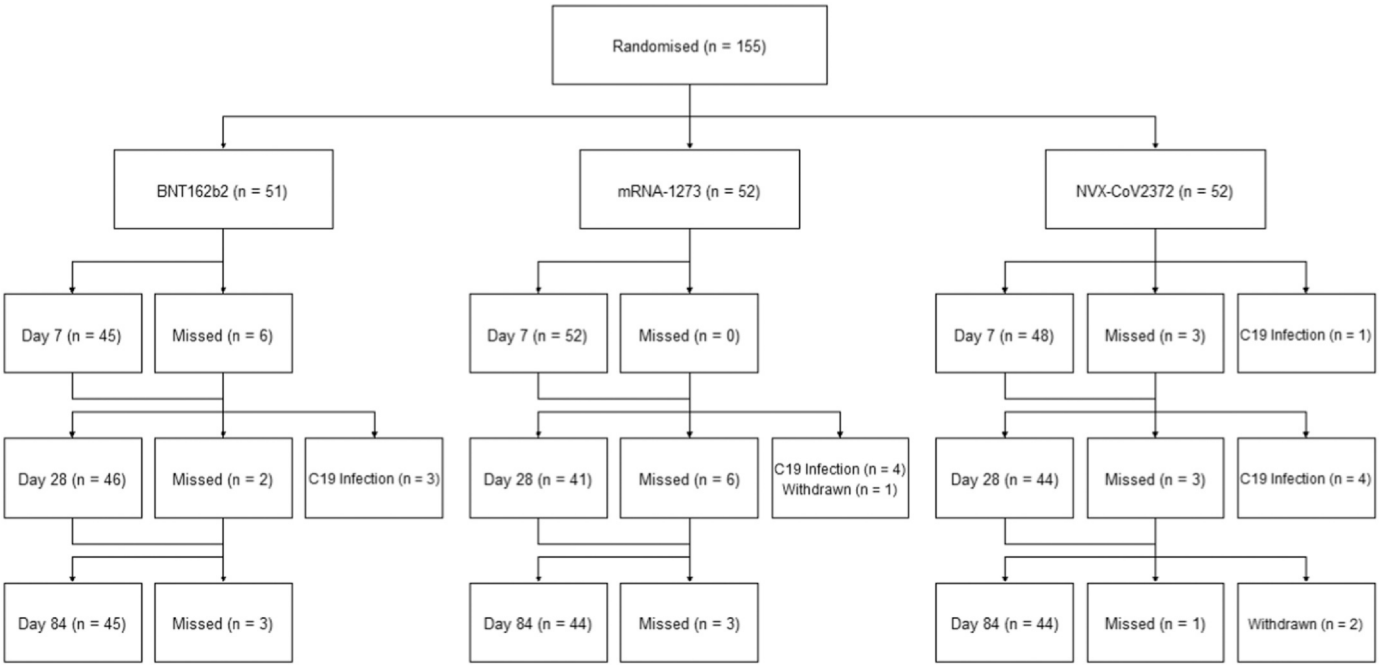

Fig. 1. CONSORT diagram for participants recruited to the 50- < 70y-AZD1222 stratum for second booster vaccines. Participants were excluded from subsequent analyses if they were infected with COVID-19 (C19 Infection) or had withdrawn. Participants who missed visits (Missed) were eligible to be included in subsequent analyses.

Table 1
Baseline characteristics for study participants recruited to the 50- < 70y-AZD1222 stratum for second booster vaccines summarised according to study arm.

	BNT162b2 (N = 51)	mRNA-1273 (N = 52)	NVX-CoV2372 (N = 52)
Age (years)[a]	60·1 (56·3, 62·4)	61.3 (57·0, 63·3)	61.9 (57·4, 63·6)
Sex[b]			
Male	14 (27%)	9 (17%)	15 (29%)
Female	37 (73%)	43 (83%)	37 (71%)
Ethnicity[b]			
European Caucasian	51 (100%)	51 (98%)	50 (96%)
Asian	0 (0%)	1 (2%)	2 (4%)
Comorbidity[b]			
Any	30 (59%)	28 (54%)	21 (40%)
Allergy	6 (12%)	6 (12%)	1 (2%)
Diabetes	2 (4%)	1 (2%)	2 (4%)
Hypertension	11 (22%)	4 (8%)	9 (17%)
Cardiovascular disorder	1 (2%)	2 (4%)	5 (10%)
Respiratory disease	7 (14%)	9 (17%)	4 (8%)
Blood disorder	2 (4%)	1 (2%)	2 (4%)
Neurological condition	2 (4%)	4 (8%)	0 (0%)
Immunocompromising disorder	1 (2%)	3 (6%)	0 (0%)
Liver disease	1 (2%)	2 (4%)	1 (2%)
Previous COVID-19 vaccine received[b]			
AZD1222	1 (2%)	0 (0%)	2 (4%)
BNT162b2	31 (61%)	35 (67%)	30 (58%)
mRNA-1273	19 (37%)	17 (33%)	20 (38%)
Days since previous COVID-19 vaccine[a]	167 (154, 189)	173 (160, 188)	169 (150, 190)
Previous COVID-19 infection[b]			
Any	5 (10%)	11 (21%)	7 (13%)
Anti-NCP	5 (10%)	10 (19%)	6 (12%)
Self-reported	5 (10%)	8 (15%)	7 (13%)
Days since previous COVID-19 infection[a,c]	57 (57, 67)	94 (55, 112)	85 (68, 101)
Ancestral anti-spike Ig (Roche assay, U/mL)[a]	3460 (2374, 5647)	4203 (2377, 7366)	3620 (1753, 5562)
Ancestral anti-spike IgG MSD (AU/mL)[a]	59 956 (41 802, 95 339)	63 780 (44 030, 113 013)	66 836 (45 517, 114 255)
Omicron BA.5 anti-spike IgG MSD (AU/mL)[a]	16 561 (11 325, 27 417)	19 863 (13 421, 30 098)	20 872 (11 841, 29 425)

[a] Median (interquartile range).
[b] Frequency (percentage).
[c] Days since previous COVID-19 infection is summarised only for participants with a self-reported previous COVID-19 infection.

participants across all study vaccines. Reported systemic severe reactions included headache (BNT162b2 n = 1, mRNA-1273 n = 3), fatigue (BNT162b2 n = 2, MRNA-1273 n = 2), chills (BNT162b2 n = 1, mRNA-1273 n = 1), myalgia (BNT162b2 n = 1, mRNA-1273 n = 1) and joint pain (mRNA-1273 n = 1). There were seven solicited AEs; none were attributed to a study vaccine. There were three unsolicited AEs thought to be possibly (n = 2) or probably (n = 1) related to a study vaccine. The latter event was heart palpitations reported by a 60-year-old female participant occurring on day 1 to day 4 after mRNA-1273.

PRO data including reports of intercurrent COVID-19 infection after randomisation are presented in Table 3. There were no hospitalisations due to COVID-19. There were two SAEs, and both were deemed unrelated to study vaccine: an admission for lower respiratory tract infection and an admission for surgical management of breast cancer.

Discussion

Here we present the first randomised controlled trial data globally of the immunogenicity, reactogenicity, and safety of mRNA and protein subunit COVID-19 vaccines delivered as second booster (fourth dose) in immunocompetent adults aged 50- < 70 years old who had received two priming doses of with AZD1222. We also present immunogenicity data for this population against Omicron subvariants BA.5 and XBB.1.5. This builds on the data reported from the COV-BOOST trial which generated evidence of the immunological responses to second booster doses of mRNA vaccines in individuals aged ≥30 years primed with AZD1222.[9]

There are several important findings from this study. First, higher binding and neutralising antibodies against Ancestral SARS-CoV-2 were observed following boosting with mRNA vaccines (BNT162b2 and mRNA-1273) compared to NVX-CoV2372 at all time points. However, the difference in anti-spike Ig GMCs between mRNA vaccines and NVX-CoV2372 were less marked at D84 compared to D7 and D28. The kinetics of the increase in anti-spike Ig appeared slower in the NVX-CoV2372 recipients when comparing D7 and D28 levels in our study, whereas Ig levels were similar at D7 and D28 in participants who received mRNA vaccines. The antibody responses observed in our study population were lower than the responses observed in the COV-BOOST cohort after second booster vaccine doses with the same vaccines. However, these results are not directly comparable. The primary endpoint in COV-BOOST was measured at day 14 rather than D28 in PICOBOO. This earlier time point was selected to expedite the generation of data to inform policy, and because maximum anti-spike Ig responses had been observed before D28 following first booster vaccine doses following third doses of mRNA COVID-19 vaccines.[14] The primary analysis for COV-BOOST was also restricted to SARS-CoV-2 seronegative participants (modified intention to treat population). Our study population included individuals with a history of SARS-CoV-2 infection prior to trial enrolment (10%, 21% and 13% for BNT162b2, mRNA-1273 and NVX-CoV2372 study arms, respectively). National seroprevalence of SARS-CoV-2 anti-spike Ig increased from 17% in March 2022[14] to 65% in August-September 2022,[15] corresponding to the window period for recruitment; in Western Australia, where 88% of participants were enrolled, seroprevalence increased from 0.5%[14] to 61.7% (58.6–64.6%)[15] over this period. There was a median of 7 months between first and second booster doses in COV-BOOST participants compared to ~5.5 months in our study population. The second booster dose vaccine responses in our study population at D28 were similar to the responses observed in the COV-BOOST population at D28 following first booster dose vaccination.[9] The PICOBOO data must also be considered in the context of vaccine effectiveness data. One large observational study from Korea found there was no difference in the risk of clinical COVID-19 infection after receipt of

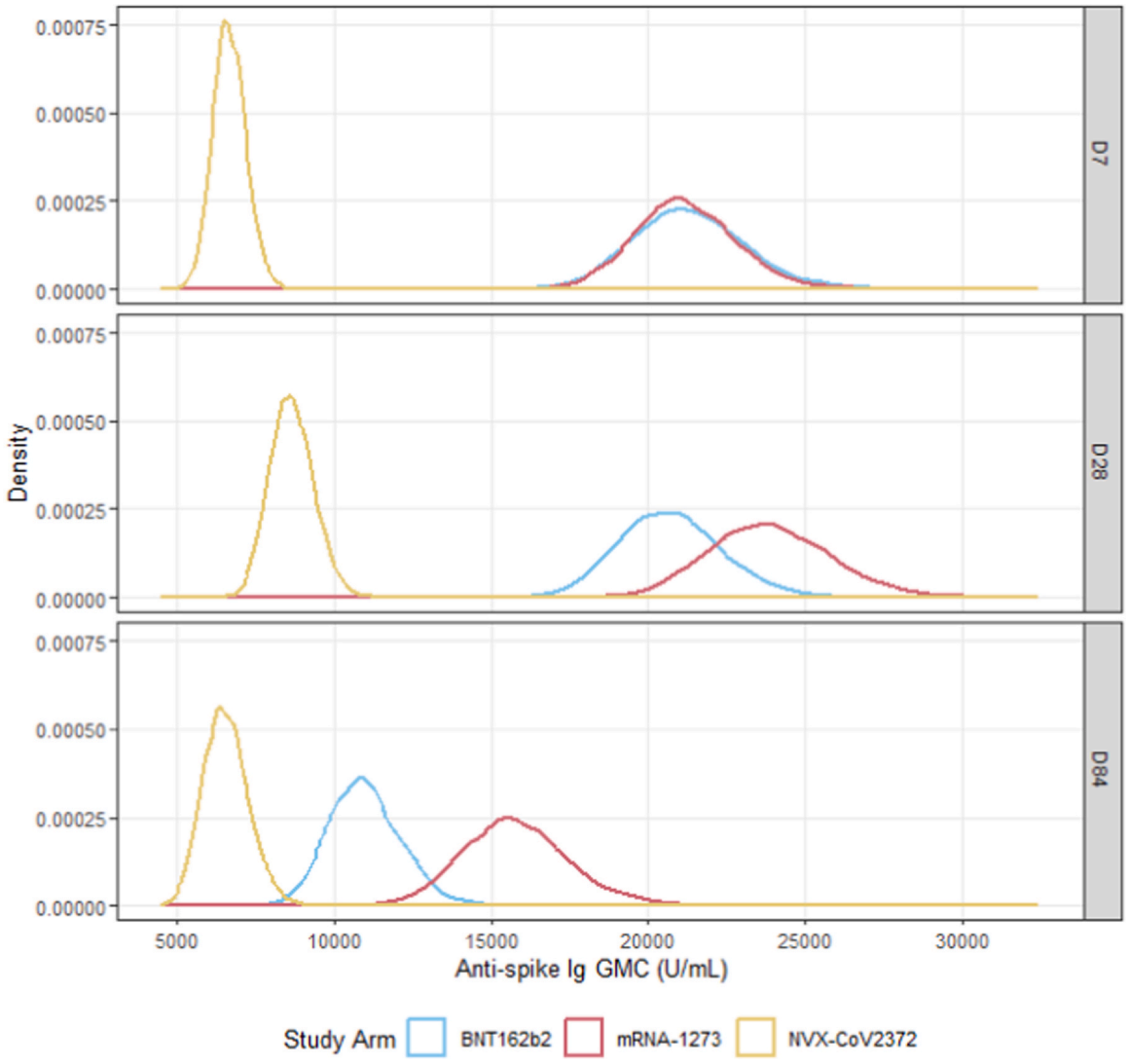

Fig. 2. Posterior distributions of the anti-spike Ig adjusted GMC against Ancestral SARS-CoV-2 at D7, D28 and D84 for each study arm in participants recruited to the 50- < 70y-AZD1222 stratum for second booster vaccines without COVID-19 infection after randomisation and before D28 (D7 for D7 distributions).

Table 2
Posterior distributions of the adjusted geometric mean NF_{50} of Ancestral SARS-CoV-2 at D28 and D84 for each study arm in participants recruited to the 50- < 70y-AZD1222 stratum for second booster vaccines without COVID-19 infection after randomisation and before D28 in the immunological subset.

	Study Arm	N	Mean (SD)	95% HDI	Mean GMC	GMC 95% HDI
D28	BNT162b2	20	2·20 (0·07)	(2·07, 2·33)	159	(112, 208)
	mRNA-1273	17	2·32 (0·07)	(2·18, 2·45)	213	(149, 281)
	NVX-CoV2372	20	1·87 (0·07)	(1·73, 2·01)	75	(53, 102)
D84	BNT162b2	19	1·99 (0·08)	(1·83, 2·15)	100	(65, 139)
	mRNA-1273	19	2·19 (0·08)	(2·02, 2·34)	156	(100, 210)
	NVX-CoV2372	17	1·85 (0·09)	(1·67, 2·03)	72	(45, 102)

SD: standard deviation
HDI: highest density interval
GMC: geometric mean concentration

NVX-XoV2372 compared to BNT162b2 in a cohort of more than 47 000 people over a 22 week period which may relate to the differential waning of antibodies between vaccines.[16]

Second, all vaccines were well-tolerated and had acceptable reactogenicity profiles, including the full dose mRNA-1273 booster vaccine. We note that the mRNA vaccines have been uncommonly associated with myocarditis and pericarditis and this trial was too small to assess this association; the reported association is greatest in young males and following second dose priming vaccination, with fewer events noted after a first priming dose or following third or subsequent booster vaccine doses.[17]

Further data are required to advance our understanding of the relationship between the immune responses after vaccination and protection against SARS-CoV-2 infection and COVID-19 disease. Data suggest SARS-CoV-2 binding and neutralising antibody concentrations against the circulating variant correlate with protection against symptomatic infection.[5] A GMC of anti-spike IgG (95% confidence interval) by the MSD assay of 17 538 (no value provided, 37 929) and 40 923 (16 748, 125 017) AU/mL have been correlated with 70% and

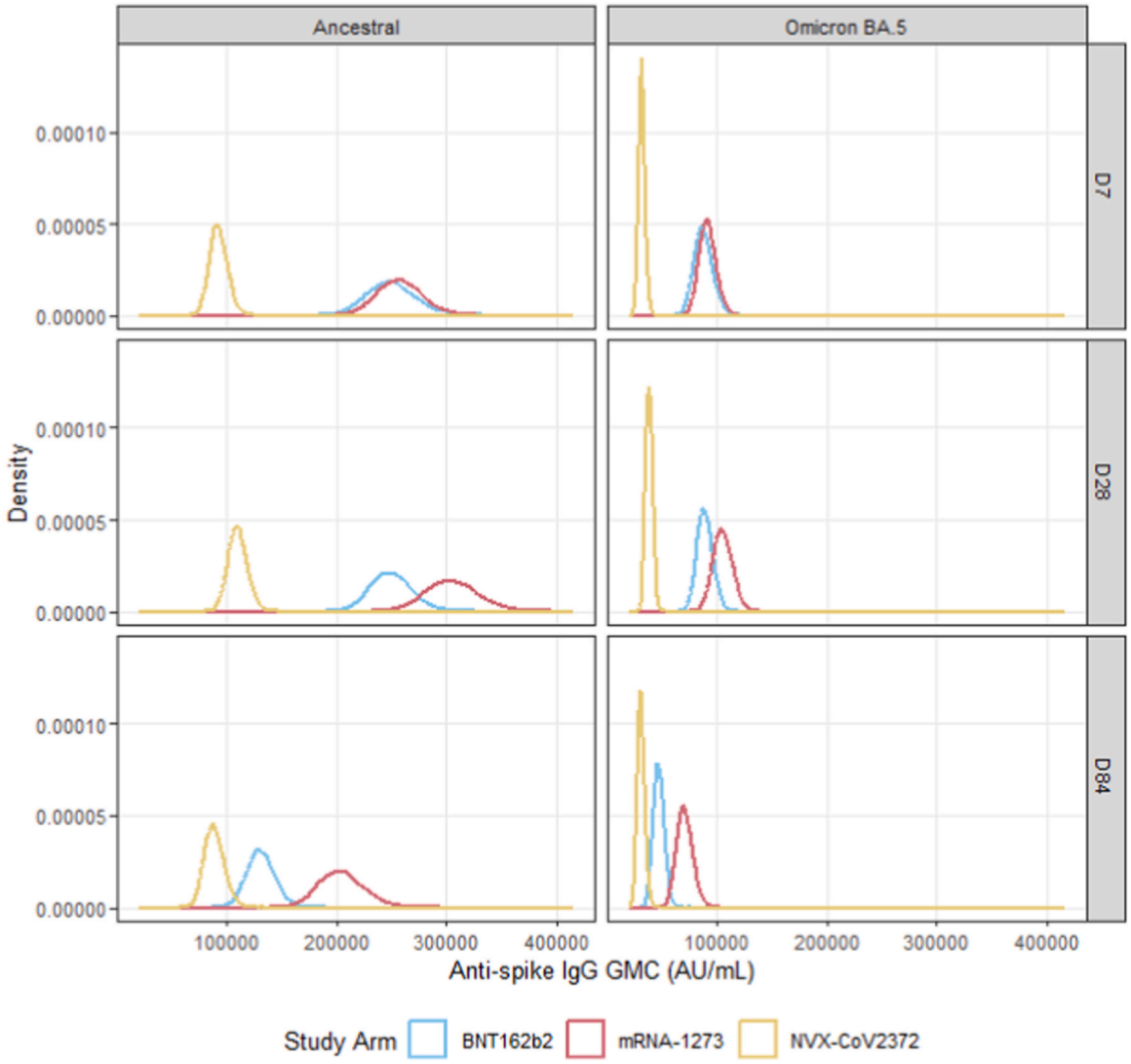

Fig. 3. Posterior distributions of the anti-spike IgG adjusted GMC (by MSD assay) against Ancestral SARS-CoV-2 and Omicron BA.5 at D7, D28 and D84 for each study arm in participants recruited to the 50- < 70y-AZD1222 stratum for second booster vaccines without COVID-19 infection after randomisation until D28 (D7 for D7 distributions).

80% vaccine efficacy, respectively.[5] NF_{50} titres against the B.1.1.7 variant corresponding to 70% and 80% efficacy were 135 (48, 267) and 247 (101, no value provided) IU/mL, respectively.[5] Khoury et al. reported an NF_{50} titre against Ancestral virus to be 54 IU/mL (95% confidence interval 30–96 IU/mL). The estimated neutralisation response required for protection against symptomatic infection was found to be sixfold higher than for protection against severe infection.[18] It is unclear however, how these identified correlates apply for newer, antigenically distinct variants such as Omicron XBB.1.5. Notably in this study, the level of neutralising antibodies against the Omicron BA.5 and XBB.1.5 variants were not boosted by a fourth dose of the ancestral derived monovalent vaccines. There was a 2–3-fold reduction in binding IgG titres against BA.5 spike protein at D28 compared to the ancestral spike in the MSD assay. The finding that binding antibody levels vary with different variants and correlate less well with neutralising antibody levels might be explained by the presence of multiple mutations in the binding domains. These data suggest that in Australia, older adults who may have already received a fourth dose of COVID-19 vaccine, would still benefit from receiving the current XBB.1.5 booster vaccines which have been shown to elicit better neutralising antibody against circulating variants.[19]

This study has two main limitations. First, PICOBOO was not designed to evaluate vaccine effectiveness against clinical disease. Differences in post-randomisation SARS-COV-2 infection between sites should be interpreted in the context of differences in local transmission, non-pharmacological prevention measures (such as mask wearing and border closures) and testing approaches in different jurisdictions at different stages of the pandemic.[20] SARS-CoV-2 infections ascertained after randomisation are likely to represent an underestimate of the true number of infections, with a declining propensity for people with symptoms to test for COVID-19 as the pandemic evolved.[21] Second, we only report on short-term humoral responses for immunocompetent older Australian adults who had received priming doses of AZD1222; we will report on cellular and longer term humoral immune responses to these vaccines in this and other age and priming subgroups as these data become available.

The strengths of PICOBOO include its adaptive design, which has provided agility to evaluate newly approved COVID-19 vaccines in the platform as soon as they have become available for use. These data are expected to assist policymakers charged with deciding whether further COVID-19 boosters will be required in Australia and globally, and if so, in whom, and what vaccines and schedules should be recommended. Additional immunological analyses including T-cell responses and additional analyses until D365 will be reported once available and are also likely to be informative. However, it has only been possible to evaluate vaccines already approved for use by

Table 3
PRO data including cumulative intercurrent COVID-19 infections and time off usual activities owing to infection by study arm for participants recruited to the 50- < 70y-AZD1222 stratum for second booster vaccines.

	BNT162b2 (N = 51)	mRNA-1273 (N = 52)	NVX-CoV2372 (N = 52)
Intercurrent COVID-19 infection within 7 days			
Any[a]	1 (2%)	0 (0%)	1 (2%)
PCR[a]	0 (0%)	..	0 (0%)
RAT[a]	1 (2%)	..	1 (2%)
Anti-NCP[a]	1 (2%)	..	0 (0%)
Days off work[b,c]	14 (14, 14)	..	0 (0, 0)
Intercurrent COVID-19 infection within 28 days			
Any[a]	3 (6%)	4 (8%)	5 (10%)
PCR[a]	0 (0%)	2 (4%)	2 (4%)
RAT[a]	3 (6%)	4 (8%)	5 (10%)
Anti-NCP[a]	3 (6%)	3 (6%)	4 (8%)
Days off work[b,c]	7 (4, 10·5)	7 (5, 7)	2 (0, 3)
Intercurrent COVID-19 infection within 84 days			
Any	5 (10%)	12 (23%)	11 (21%)
PCR[a]	0 (0%)	7 (13%)	3 (6%)
RAT[a]	5 (10%)	9 (17%)	9 (17%)
Anti-NCP[a]	5 (10%)	7 (13%)	8 (15%)
Days off work[b,c]	7 (7, 10)	7 (0, 7)	2 (0, 3)

[3]Days off work is summarised only for participants with a self-reported COVID-19 infection.
[a] Frequency (percentage).
[b] Median (interquartile range).
[c] Days off work is summarised only for participants with a self-reported COVID-19 infection.

the Australian regulator (TGA) owing to contractual commitments between the Commonwealth Government and vaccine manufacturers. The use of a Bayesian hierarchical model allowed sharing of information across different levels, enhancing the efficiency of antibody GMC estimates, enabling precise and robust inferences to be made.[22] This approach allowed all data to contribute meaningfully to the analyses, including data from a small number of participants who received first booster doses. Less variability was observed in the D28 anti-spike Ig concentrations than anticipated. Our assumptions about the variance in antibody responses which informed the Bayesian model were derived from COV-BOOST following first (rather than second) booster doses. The hierarchical model structure and the similarity in antibody responses across the mRNA vaccines may also have improved our precision more than expected. As a result, the desired precision for the D28 anti-spike Ig GMC estimates may have been reliably exceeded with fewer participants. Future substudies nested within PICOBOO and other trials may require fewer participants.

It is likely that the persistence of humoral antibody response is more important than the early peak humoral antibody response in conferring lasting protection. Policymakers will need to consider the durability of humoral antibody responses as well as cellular immune responses when making immunisation policy decisions. These data will be forthcoming from the PICOBOO trial and are likely to be informative.

Author contributions

TS and PR conceived the trial. The primary design was elaborated by CM, ME, JR, JM, and MD with subsequent input from all PICOBOO investigators. JM, MD, TS conceived the statistical methods. MD was the unblinded statistician, while JM was blinded until the first interim analysis. All authors contributed to protocol development. CM and George Salama coordinated the implementation of this study together with site primary investigators UW, HM and KF. Laboratory assays were conducted by MCT, RT, SN and KS. All authors had access to the data from this study and were responsible for the decision to publish. CM and MD drafted the primary version of this manuscript. All authors reviewed and approved the final version for publication and met the criteria for authorship as per the ICMJE recommendations.

Declaration of Competing Interest

KF and TS are members of the Australian Technical Advisory Group on Immunisation (ATAGI), which advises the government on vaccine policy; their involvement as investigators on this trial has been declared to ATAGI. MP is involved in an ovarian cancer clinical trial that received funding from AstraZeneca. MP was involved in performing immunological assays on biological specimens obtained from participants in this trial, but was not involved in participant recruitment, data collection or the analysis of results. SNF leads the UK National Institute for Health and Care Research funded trial of third and fourth-dose COVID-19 boosters. SNF acts on behalf of University Hospital Southampton NHS Foundation Trust, UK, as an Investigator and/or providing consultative advice on clinical trials and studies of vaccines funded or sponsored by vaccine manufacturers including Moderna, Sanofi, Janssen, Pfizer, AstraZeneca, GlaxoSmithKline, Novavax, Seqirus, Medimmune, Merck and Valneva vaccines and antimicrobials. UW is an investigator on clinical trials of vaccines funded or sponsored by vaccine manufactures including Moderna, Sanofi, Pfizer, Merck and GlaxoSmithKline. PR also reports acting on behalf of University of Western Australia, as an Investigator and/or providing consultative advice on clinical trials and studies of vaccines funded or sponsored by vaccine manufacturers including Moderna, Sanofi, Janssen, Pfizer, AstraZeneca, GlaxoSmithKline, Novavax, Seqirus, Merck and Clover Biopharmaceutical vaccines. They receive no personal financial payment for this work. The other authors declare that they have no competing interests.

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Data availability

The PICOBOO Core Protocol and the Booster Vaccination Substudy protocol, associated Laboratory and Statistical Appendices and interim statistical reports are available on the trial website (picoboo.com.au). All trial protocols and interim statistical reports will be available on the trial website. De-identified participant data that underlie the results reported in this article will be shared with investigators whose proposed use of the data has been approved by the Child and Adolescent Service Human Research Ethics Committee.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2024.106286.

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