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Heterologous COVID-19 vaccine schedule with protein-based prime (NVX-CoV2373) and mRNA boost (BNT162b2) induces strong humoral responses: Results from COV-BOOST trial

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SUMMARY

Background: Heterologous schedules of booster vaccines for COVID-19 following initial doses of mRNA or adenoviral vector vaccines have been shown to be safe and immunogenic. There are few data on booster doses following initial doses of protein nanoparticle vaccines.
Methods: Participants of the phase 3 clinical trial of the COVID-19 vaccine NVX-CoV2373 (EudraCT 2020–004123-16) enroled between September 28 and November 28, 2020, who received 2 doses of NVX-CoV2373 administered 21 days apart were invited to receive a third dose booster vaccine of BNT162b2 (wild type mRNA vaccine) as a sub-study of the COV-BOOST clinical trial, and were followed up for assessment of safety, reactogenicity and immunogenicity to day 242 post-booster.
Results: The BNT162b2 booster following two doses of NVX-COV2373 was well-tolerated. Most adverse events were mild to moderate, with no serious vaccine-related adverse events reported. Immunogenicity analysis showed a significant increase in spike IgG titres and T-cell responses post-third dose booster. Specifically, IgG levels peaked at day 14 with a geometric mean concentration (GMC) of 216,255 ELISA laboratory units (ELU)/mL (95% CI 191,083–244,743). The geometric mean fold increase from baseline to day 28 post-boost was 168.6 (95% CI 117.5–241.8). Spike IgG titres were sustained above baseline levels at day 242 with a GMC of 58,686 ELU/mL (95% CI 48,954–74,652), with significant decay between days 28 and 84 (geometric mean ratio 0.58, 95% CI 0.53–0.63). T-cell responses also demonstrated enhancement post-booster, with a geometric mean fold increase of 5.1 (95% CI 2.9–9.0) at day 14 in fresh samples and 3.0 (95% CI 1.8–4.9) in frozen samples as measured by ELISpot. In an exploratory analysis, participants who received BNT162b2 after two doses of NVX-COV2373 exhibited higher anti-spike IgG at Day 28 than those who received homologous three doses of BNT162b2, with a GMR of 5.02 (95% CI: 3.17–7.94). This trend remained consistent across all time points, indicating a similar decay rate between the two schedules.
Conclusions: A BNT162b2 third dose booster dose in individuals primed with two doses of NVX-COV2373 is safe and induces strong and durable immunogenic responses, higher than seen in other comparable studies. These findings support the use and investigation of heterologous booster strategies and early investigation of heterologous vaccine technology schedules should be a priority in the development of vaccines against new pathogens.

Research in context

We searched PubMed for clinical trials in non-immunocompromised adults published between database inception and January 23rd 2025, using the search terms "(COVID) AND (vaccin*) AND (booster) AND (NVX OR protein)", with no language restrictions. We found no clinical trials of heterologous COVID-19 booster vaccines following a primary vaccination course with purified protein vaccines. One clinical trial assessed the immunity and reactogenicity of a third dose, homologous booster vaccine with a purified protein vaccine (NVX-CoV2373), and one clinical trial assessed a fourth dose, homologous booster vaccine (NVX-CoV2373). The third dose demonstrated incremental reactogenicity compared to the primary series with boost to humoral immunity. The fourth dose did not appear to increase reactogenicity and provided a further boost to humoral immunity.

Added value of this study

To our knowledge, this study is the only clinical trial to report the outcomes of heterologous third-dose booster vaccines for COVID-19 with mRNA vaccines following a primary vaccination course with a purified protein vaccine (NVX-CoV2373). This study demonstrates a robust immune response to a heterologous third dose booster, which significantly exceeds those found in the original COV-BOOST clinical trial of third dose boosters following a primary vaccination course with mRNA (mRNA1273 or BNT162b2) or adenovirus vector vaccines (ChAdOx-nCoV19).

Implications of all the available evidence

The most effective three dose vaccine combination at inducing humoral immunity against COVID-19 may be a two dose, primary vaccination course with purified protein vaccine followed by an mRNA booster vaccine. This study provides important data to guide future pandemic vaccine policy and research into heterologous vaccine schedules.

Introduction

Following the development of several highly effective vaccines for COVID-19, additional booster doses to the initial primary series have been administered in many countries to counteract the effect of waning immunity. Recently, new variant vaccine boosters have been used due to the emergence of new variants which exhibit immune escape. Evidence from randomised controlled trials has shown boosters to be highly safe and immunogenic both as third[1] and fourth doses,[2] and although antibodies wane rapidly, T cell responses do not wane as significantly.[3] These findings have been supported by observational studies of the "real world" effectiveness of booster vaccines.[4,5]

Most evidence comes from boosters administered to people who received mRNA vaccines (such as BNT162b2 [Pfizer] and mRNA-1273 [Moderna]), adenovirus vector vaccines (such as Ad.26. CoV2. S [J&J], and ChAdOx1-nCoV19 [AstraZeneca]), or inactivated vaccines as these vaccines were deployed as the prime vaccination for the majority of the worldwide population. Protein nanoparticle vaccines (such as NVX-CoV2373 [Novavax]) have been shown to be highly effective in phase 3 clinical trials[6] and received WHO Emergency Use Listing in December 2021. The vaccine was subsequently authorised for use as a primary vaccine series and as a booster dose in the UK, European Union and globally.[7,8]

Following the deployment of protein nanoparticle vaccines as a primary vaccine series for COVID-19, there remains an evidence gap regarding the safety and immunogenicity of third-dose booster vaccines in people primed with protein nanoparticle vaccines. Previous evidence from heterologous prime[9] and heterologous boost vaccine schedule studies[1] have demonstrated that heterologous schedules can be more immunogenic, but also more reactogenic. In addition, some heterologous schedules may provide more durable immune responses.[10] A secondary analysis of a randomised phase 2 trial of NVX-CoV2373 including third homologous dose boosters found immune responses to be similar to or in excess of those associated with high efficacy in the phase 3 trial. However, incremental reactogenicity compared to the primary series was observed.[11] A further, fourth dose of NVX-CoV2373 appeared to further boost humoral immunity with no further increase to reactogenicity.[12]

To evaluate the safety and immunogenicity of heterologous schedules for COVID-19 where protein nanoparticle vaccines are given as the prime dose, we invited participants who had taken part in the phase 3 randomised trial of NVX-COV2373 to receive a booster (third) dose of BNT161b2, an mRNA vaccine, and followed them up to 8 months after vaccination.

Methods

Trial design & oversight

The Novavax substudy of the COV-BOOST trial was a single-arm trial conducted to generate additional data on the safety and immunogenicity of a single full dose of BNT162b2 vaccine following two previous doses of NVX-CoV2373. Participants were recruited from the pivotal NVX-CoV2373 vaccine clinical trial, external to the COV-BOOST trial.[6] This substudy was part of the COV-BOOST trial, a multicentre, randomised, phase 2 trial of third-dose booster vaccination against COVID-19. The substudy was conducted at 6 UK sites, in a mixture of community and secondary care settings.

The trial was reviewed and approved by the South-Central Berkshire Research Ethics Committee, University Hospital Southampton, and the Medicines and Healthcare Products Regulatory Agency (EudraCT 2021-002175-19, IRAS 299180, REC reference 21/SC/0171).

Participants

Participants eligible for this sub-study were individuals from the phase 3 trial of the NVX-CoV2373 vaccine who had received two doses of NVX-CoV2373 and had not yet been administered a third COVID-19 vaccine dose. Participants were aged 30 years or older and in generally good physical health (with mild to moderately well-controlled comorbidities permitted), who had received two doses of NVX-CoV2373.

Procedures

Eligible participants were invited to a baseline visit (day 0) following online or telephone screening (or both).

Following informed consent, vaccines were administered by appropriately trained trial staff at trial sites, and participants were observed for at least 15 minutes after vaccination.

During the baseline visit, participants were given an oral thermometer, tape measure, and diary card (electronic or paper) to record solicited adverse events from day 0-7, unsolicited adverse events from day 0–28, and medically attended adverse events up to three months post immunisation. The study sites' physicians reviewed the diary card regularly to record adverse events, adverse events of special interest, and serious adverse events. During the study visits, adverse events, adverse events of special interest, and serious adverse events that had not been recorded in the diary card were also collected. Troponin levels were measured at baseline and day 14 due to the potential for rare COVID-19 vaccine cardiac side effects such as myocarditis.

Blood was taken for immunogenicity analyses at day 0, 14, 28, 84, and 242 post-booster vaccination. A separate immunology subgroup comprised of 25 participants had additional blood taken at day 14 (to detect the T-cell response). Sera were analysed at Nexelis (Laval, QC, Canada) to determine SARS-CoV-2 anti-spike IgG concentrations reported as ELISA laboratory units [ELU]/mL. IFN-γ-secreting T cells specific to whole spike protein epitopes, designed based on the Wuhan-Hu-1 sequence (YP_009724390.1), were detected using a modified T-SPOT-Discovery test done at Oxford Immunotec (Abingdon, UK) within 32 h of venepuncture, using the addition of T-Cell Xtend reagent to extend peripheral blood mononuclear cell (PBMC) survival. T-cell frequencies were reported as spot-forming cells (SFC) per 250,000 PBMCs with a lower limit of detection of one in 250,000 PBMCs. Cellular immune response assessment was only conducted at centres collecting Lithium Heparin Blood samples (approximately 50% of participants). T-cell assays were conducted using fresh peripheral blood mononuclear cells (PBMCs) and frozen PMBCs (for validation). ELISA tests were also conducted using an in-house standardised total IgG ELISA against trimeric SARS-CoV-2 spike protein, previously used to assess immunogenicity in other clinical trials. Both sets of initial and additional assay results for anti-spike IgG and T-cell responses are presented in the paper. Sera from day 0 were analysed at Porton Down, UK Health Security Agency, by ECLIA (Cobas® platform, Roche Diagnostics) to determine anti-SARS-CoV-2 nucleocapsid IgG status (reported as negative if below a cutoff index of 1.0).

Outcomes

The co-primary outcomes encompassed safety, reactogenicity, and immunogenicity. Safety and reactogenicity were evaluated based on the occurrence of solicited and unsolicited adverse events, adverse events of special interest, or serious adverse events following vaccination. These events were documented in participant electronic diaries or identified during follow-up visits. The primary immunogenicity outcome was anti-spike protein IgG at day 28. Secondary immunogenicity outcomes included T-cell responses (measured by ELISpot) against wild-type and SARS-CoV-2 virus variants of concern: Beta (B.1.351) and Delta (B.1.617.2).

Statistical analysis

No formal sample size calculation was conducted, as this was a single arm study designed to rapidly inform policymakers. We planned to recruit 111 participants in order to match the sample size per arm in the main COV-BOOST study.

Baseline characteristics and immunogenicity outcomes are presented for the entire study population and are further stratified by cohorts (general and immunology).

The analysis population for reactogenicity and safety included all participants who received a study vaccine and logged onto the

eDiary record for at least one in 7 days. The primary outcome of reactogenicity examined solicited adverse events (local and systemic) within the first 7 days. The proportion with at least one severe episode (grade 3 and grade 4) is presented using radial graphs. An additional view of reactogenicity outcomes displays the severity of solicited events over 7 days using stacked bar charts. Unsolicited adverse events reported within 28 days post-third dose were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) and tabulated at System Organ Class level. Adverse events of special interest and serious adverse events were reported until the data lock date of 9th October 2023.

The primary and secondary analysis population for immunogenicity outcomes included all participants with available endpoint data. The primary immunogenicity outcome, anti-spike IgG at day 28, along with measurements at day 14, day 84, and day 242, is reported as geometric mean concentration (GMC) and 95% confidence interval (CI). Additionally, the geometric mean fold change between day 28 post-third dose and day 0 pre-third dose, between day 84 post-third dose and day 28 post-third dose, and between day 242 post-third dose and day 84 post-third dose are presented. We also present the fold changes for day 14, 84, and 242 post-third dose compared to day 0.

Pre-specified subgroup analyses of immunogenicity outcomes were performed based on gender and serostatus before receiving the booster vaccine. Subgroup analysis was also planned but not conducted for participants with co-morbidities as there were < 25 participants per group (12 with cardiovascular disease, 16 with respiratory disease, and 5 with diabetes, as indicated in Table 1). Despite the limited sample size, subgroup analysis for serostatus was conducted due to its potential clinical significance. Previous infection at baseline was defined as a cutoff index ≥1.0 for anti-nucleocapsid IgG by the Roche Elecsys anti-SARS-CoV-2 assay prior to the third dose or self-reported SARS-CoV-2 infection prior to the third dose (confirmed by PCR).

Included in the statistical analysis plan were two additional exploratory analyses:

- To investigate the relationship between baseline and day 14/28 antibody GMC with the occurrence of Grade 3 solicited adverse events to explore whether reactogenicity is reflective of higher baseline or higher post-booster antibodies.
- To compare this cohort with the original COV-BOOST participants who had received BNT162b2 following initial 2 doses of BNT162b2 or ChAdOx1-nCoV19.

In conducting the second exploratory analysis, we reported the GMRs along with 99% confidence intervals (CIs). The use of 99% confidence intervals aligns with the main study statistical analysis plan and reflects a more conservative approach for exploratory analysis, which included two comparisons at each of the three time points.

All analyses were performed using R version 4.3.0 (2023–04-21).

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

There were 116 participants who had received two doses of NVX-CoV2373 as their first two doses of COVID-19 vaccine and who were evaluated for eligibility; 115 were then enroled between November 15, 2021, and November 25, 2021 (Fig. 1). The median interval between the first two doses was 21.0 days (IQR 20.0–22.0). The immunology cohort comprised 25 participants, while the remaining 90 participants constituted the general cohort. All 115 participants who received a standard dose of BNT162b2 as the third dose booster vaccination completed at least one day of the 7-day diary entries and were included in the safety and reactogenicity analysis. All participants with antibody data available were included in the primary immunogenicity analysis.

The median age of the cohort was 46.0 years (IQR 39.0–56.0), 93.9% of the cohort was aged under 70 years (Table 1). The median interval between the second dose and third dose booster was 349.0 days (IQR 200.5–365.5) (Table 1). 50.4% of the study population were male and 90.4% identified as white (Table 1).

Pain was the most commonly reported solicited local adverse event (AE) by participants, and 85.2 were reported as grade 1 or 2 severity (Supplementary Table 1, Supplementary Figure 1). Two grade 4 local solicited AEs were reported, including one episode of pain and one episode of warmth from the same participant (Supplementary Table 1, Supplementary Figure 1). Redness was the most frequently reported local solicited AE graded as 3 or above (Supplementary Table 1, Fig. 2). Fatigue, feverish, and malaise were the most reported systemic solicited AEs at grade 3 or above with no grade 4 systemic solicited AEs reported (Supplementary Table 2, Fig. 2).

Table 1
Baseline Characteristics by cohort.

		General Cohort N=90	Immunology Cohort N=25	Total N=115
Age (years)	Mean (SD)	47.9 (11.7)	48.4 (13.6)	48.0 (12.1)
	Median (Q1-Q3)	46.0 (39.0–55.0)	47.0 (37.0–63.0)	46.0 (39.0–56.0)
Intervals between 1st and 2nd doses (days)	Median (Q1-Q3)	21.0 (21.0–21.0)	23.0 (22.0–25.0)	21.0 (20.0–22.0)
Intervals between 2nd and 3rd doses (days)	Median (Q1-Q3)	342.5 (200.2–361.0)	359.0 (201.0–366.0)	349 (200.5–365.5)
Age groups (years)	< 70	84 (93.3%)	24 (96.0%)	108 (93.9%)
	≥70	6 (6.7%)	1 (4.0%)	7(6.1%)
Gender	Male	45 (50.0%)	13 (52.0%)	58 (50.4%)
	Female	45 (50.0%)	12 (48.0%)	57 (49.6%)
Occupation	Health worker	16 (17.8%)	1 (4.0%)	17 (14.8%)
	Other	74 (82.2%)	24 (96.0%)	98 (85.2%)
Ethnicity	White	83 (92.2%)	21 (84.0%)	104 (90.4%)
	Black	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Asian	5 (5.6%)	2 (8.0%)	7 (6.1%)
	Mixed	2 (2.2%)	1 (4.0%)	3 (2.6%)
	Other	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Not given	0 (0.0%)	1 (4.0%)	1(0.9%)
Comorbidities	Cardiovascular	6 (6.7%)	6 (24.0%)	12 (10.4%)
	Respiratory	13 (14.4%)	3 (12.0%)	16 (13.9%)
	Diabetes	4 (4.4%)	1 (4.0%)	5 (4.3%)

Data are median (IQR) or N (%) unless otherwise stated.

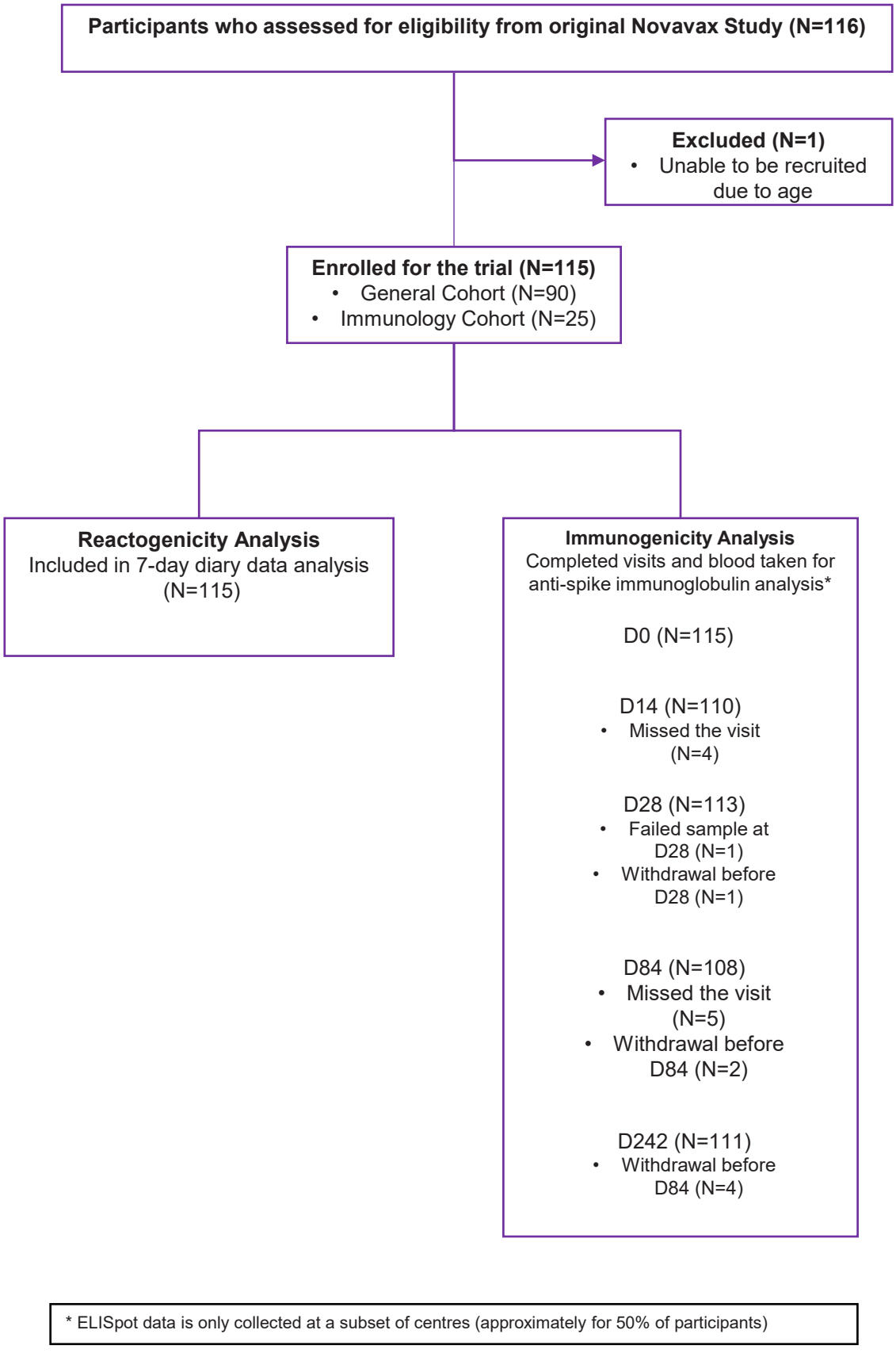

Fig. 1. Study profile for participants who received BNT162b2 booster following two doses of NVX-COV2373.

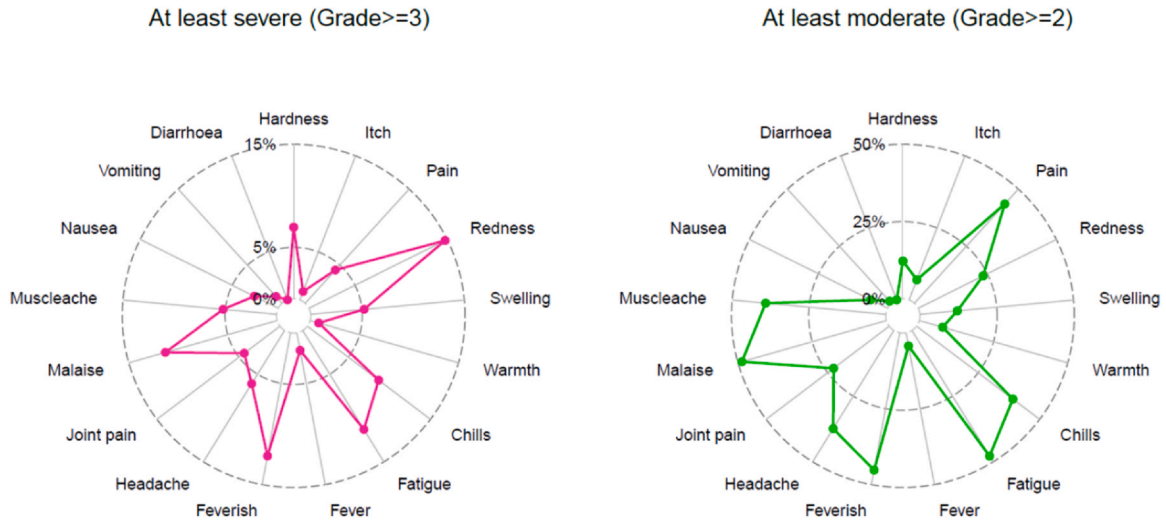

Fig. 2. Radial graph for the occurrence of 'at least severe' and 'at least moderate' solicited adverse events in the first 7 days post vaccination.

Males had slightly higher serum troponin levels than females at baseline and day 14. There was no increase detected between baseline and day 14 (110 of 115 participants had serum levels at baseline and at day 14; baseline troponin 2.5 ng/mL [IQR 2.1–3.6] in males, 2.1 ng/mL [IQR 1.6–2.7] in females; day 14 troponin 2.4 ng/mL [IQR 1.9–3.5] in males, 2.3 ng/mL [IQR 1.4–2.9] in females) (Supplementary Table 4, Supplementary Figure 3). One male participant had troponin greater than the upper limit of normal (> 34 ng/mL) at baseline, which was reduced to normal by day 14 (Supplementary Figure 3).

There were 120 unsolicited adverse events reported for 68 participants (Supplementary Table 3). Six were reported by the investigators as 'definitely' related to the vaccine. Two serious adverse events were reported: one hospitalisation due to cellulitis at the injection site treated with oral antibiotics, which resolved without complications, and a diagnosis of abdominal aortic aneurysm without sequelae during the trial (Supplementary Table 3).

Anti-spike protein IgG was measured at day 0, 14, 28, 84, and 242 with a trend to peaking at day 14 (GMC 216,255 ELISA laboratory units (ELU)/mL (95% CI 191,083–244,743)) before reducing by day 242 (58,686 ELU/mL, 95% CI 48,954–74,652) (Table 2, Fig. 3). There was a 168.6 geometric mean fold increase (95% CI 117.5–241.8) between baseline and day 28 (Table 2) and a geometric mean fold change of 0.58 (95% CI 0.53–0.63) from day 28 to day 84 post vaccination (Table 2).

To explore whether reactogenicity is correlated with antibody levels post-booster, an exploratory analysis was conducted to compare the day 0/day 14/day 28 anti-spike IgG levels among participants with or without at least one grade 3 solicited adverse events following booster dose. Participants who reported grade 3 or above solicited adverse events within 7 days following booster were found to have higher levels of anti-spike protein IgG post-booster (Supplementary Table 6).

The BNT162b2 booster vaccine induced cellular responses against wild-type SARS-CoV-2. A geometric mean fold increase of 5.1 (95% CI 2.9–9.0) was observed on day 14 in the fresh samples and 3.0 (95% CI 1.8–4.90) in the frozen samples (Table 3, Fig. 4). Following a decay between day 14 and day 28, the cellular responses plateaued (Table 3 and Fig. 4).

Pre-planned subgroup analysis of immune response (SARS-CoV-2 anti-spike IgG, ELU/mL) was carried out by gender and serostatus before receiving the booster vaccine (Supplementary Table 5). Females had higher anti-spike protein IgG levels than males at all time points. However, the geometric mean fold change between day 0 and day 28 was 186.2 (95%CI 120.3–288.1) in males and 152.9 (95%CI 86.0–271.8) in females. The decay between day 84 and 28 was similar in males and females (fold change 0.59 (95%CI 0.52–0.67) and 0.57 (95%CI 0.51–0.64) respectively).

Of 115 participants, 16 showed evidence of previous infection based on anti-nucleocapsid IgG before receiving the booster vaccine. Higher anti-spike protein IgG levels were observed in the seropositive population at baseline: 28,694 (95% CI 9305–88,488) (n=16) compared with 662 (95% CI 526–834) (n=99) in seronegative individuals (Supplementary Table 5). At 14 and 28 days following booster vaccination, the anti-spike protein IgG titres were similar between the two groups. The rate of decay was faster in seropositive population than seronegative populations between day 28 and day

Table 2
Immune responses (SARS-CoV-2 anti-spike IgG, ELU/mL) by cohort at Day 0 and Days 14, 28, 84 and 242 after booster vaccine.

	Full Cohort	General	Immunology
SARS-CoV-2 anti-spike IgG, ELU/mL (95%CI)			
Day 0	1119(791–1582) (n=115)	1201(799–1805) (n=90)	867(464–1619) (n=25)
Day 14	216,255(191,083–244,743) (n=110)	226,839(199,994–257,288) (n=88)	178,632(125,222–254,822) (n=22)
Day 28	193,351(169,649–220,365) (n=113)	195,200(170,795–223,093) (n=90)	186,281 (127,280–272,634) (n=23)
Day 84	112,135(95,087–132,239) (n=108)	120,093(101,424–142,197) (n=86)	85,772 (54,116–135,944) (n=22)
Day 242	58,686(48,954–74,652) (n=111)	66,416(52,306–77,615) (n=89)	42,077 (27,412–64,590) (n=22)
Fold change ay14/Day0	189.2 (132.6–270.1) (n=110)	191.3 (126.9–288.3) (n=88)	181.3 (89.7–366.5) (n=22)
Fold change Day28/Day0	168.6(117.5–241.8) (n=113)	162.5 (107.2–246.3) (n=90)	194.6(95.2–398) (n=23)
Fold change Day84/Day0	114.8 (79.3–166.3) (n=108)	114.7 (74.8–175.8) (n=86)	115.4 (55.0–241.8) (n=22)
Fold change Day242/Day0	54.1 (36.5–80.3) (n=111)	54.4 (34.7–85.1) (n=89)	53.3 (23.0–123.3) (n=22)
Fold change Day84/Day28	0.58 (0.53–0.63) (n=107)	0.60 (0.55–0.67) (n=86)	0.49 (0.42–0.58) (n=21)
Fold change Day242/Day84	0.52 (0.49–0.57) (n=107)	0.53 (0.49–0.58) (n=86)	0.49 (0.42–0.57) (n=21)

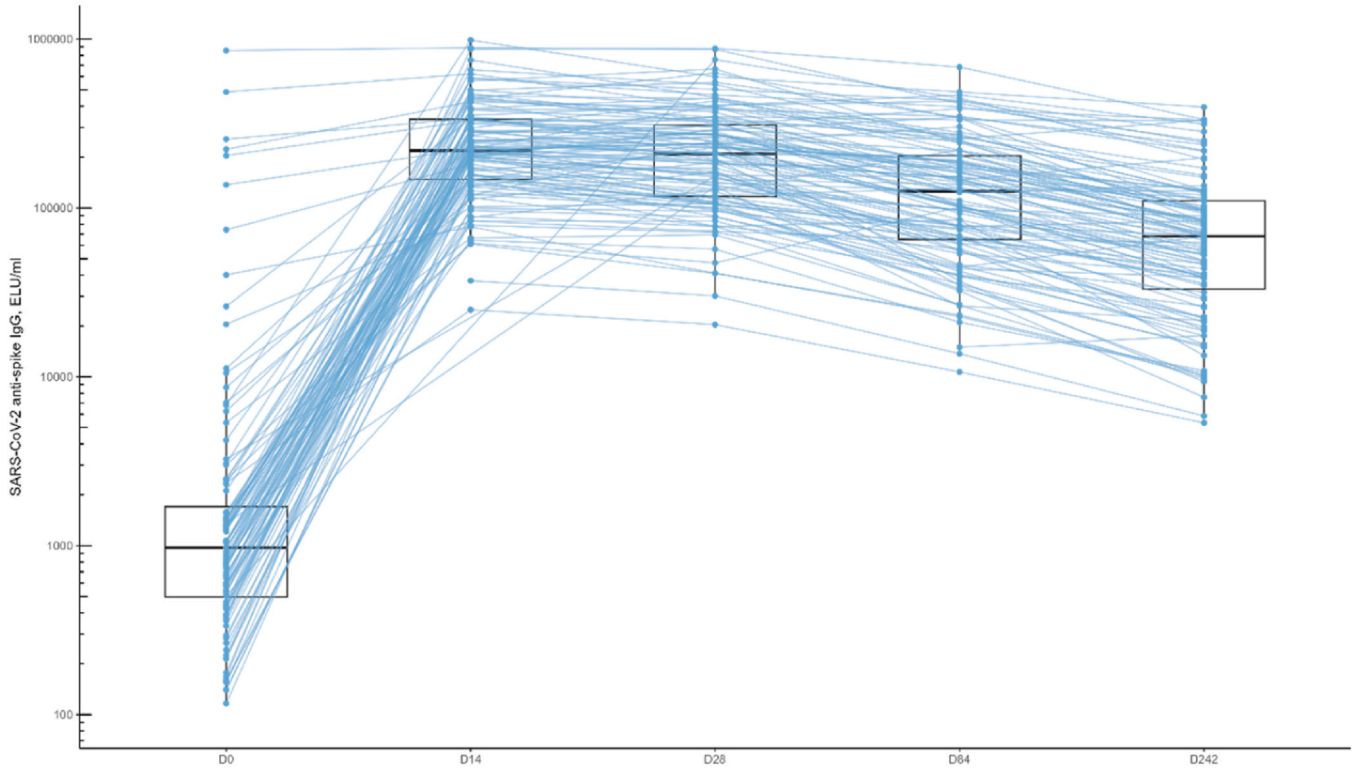

Fig. 3. Kinetic of IgG immunogenicity in patients receiving the booster vaccination. Boxplots represent the median and 25th and 75th percentiles. Each data point is one participant. Solid lines connect samples from the same participant at multiple timepoints. D0=pre-booster. D14=14 days after booster. D28=28 days after booster. D84=84 days after booster. D242=242 days after booster. ELU=ELISA laboratory units.

84: fold change of 0.42 (95% CI 0.34–0.52) in seropositive vs. 0.61 (95% CI 0.55–0.66) in seronegative participants but became similar between day 84 and day 242 in the two groups (seropositive fold change 0.45 (95% CI 0.39–0.52) compared to 0.53 (95% CI 0.49–0.58) seronegative).

Supplementary Figure 4 compares SARS-CoV-2 anti-spike IgG levels for participants from the current study (NVX-CoV2373/NVX-CoV2373/BNT162b2) with those who received BNT162b2 following two initial doses of either BNT162b2 or ChAdOx1-nCoV19 and a third dose of BNT162b2 in the original COV-BOOST study. The graph includes comparisons for NVX-CoV2373/NVX-CoV2373/BNT162b2 and ChAdOx1-nCoV19/ChAdOx1-nCoV19/BNT162b2, using BNT162b2/BNT162b2/BNT162b2 as the reference group.

Participants who received BNT162b2 after two doses of NVX-CoV2373 exhibited significantly higher anti-spike IgG levels at day 28 compared to those who received two doses of BNT162b2: 193,350.8 (95% CI 169,648.7–220,364.5) vs. 28,205.0 (95% CI 25,123.3–31,664.4), with a GMR of 5.02 (99% CI 3.17–7.94). This trend remained consistent across all time points, indicating a similar decay rate between the two schedules.

A similar trend was observed for participants who received BNT162b2 after two doses of ChAdOx1-nCoV19. Participants who received BNT162b2 after two doses of NVX-CoV2373 showed higher anti-spike IgG levels at day 28 compared to those who received two doses of ChAdOx1-nCoV19, with a GMR of 4.52 (99% CI 2.62–7.79). This trend also remained consistent across all time points.

The results from the in-house ELISA assay revealed a similar pattern between the NVX-CoV2373/NVX-CoV2373/BNT162b2 and BNT162b2/BNT162b2/BNT162b2 groups (Supplementary Table 7).

Discussion

To our knowledge, this is the only published study of participants who have received a primary series of the NVX-CoV2373 protein-based vaccine followed by a heterologous third dose booster with the BNT162b2 mRNA vaccine. We showed that boosting with BNT162b2 following NVX-CoV2373 was associated with high rates of reactogenicity. It also produced a remarkably strong humoral immunity response measured by anti-spike IgG, higher than that of any other reported third-dose trial. Whilst not directly comparable to the main COV-BOOST study, peak GMC IgG levels in this cohort of 216,255 (95% CI 191,083–244,743) at 14 days were nearly five-fold higher than those measured in recipients of three doses of BNT162b2 (27,242 (95% CI 24,148–30,731).[1]

Because of the significant differences in levels compared to other third-dose trials, we investigated carefully with the reporting laboratory and subsequently repeated anti-spike IgG ELISA at a different laboratory to validate the results. Results from the different ELISA analyses were consistent with the original data (Nexelis, Canada) reported here.

Our results suggest that an mRNA vaccine following a protein nanoparticle vaccine primary series is a highly immunogenic combination. Even by the end of follow-up at day 242, the absolute GMC (58,686, 95% CI 48,954–74,652) was almost double the maximum levels observed at day 28 for three doses of BNT162b2 in the main COV-BOOST study (27,242, 95% CI 24,148–30,731).[1] Antibody titres decay relatively quickly over the study period, roughly halving from day 28 to day 84, then again from day 84 to day 242, mirroring other studies on the durability of humoral immunity following other COVID-19 vaccines.[10] A longer interval between primary COVID-19 immunisation doses has previously demonstrated an augmented humoral response.[13] Whilst the longer duration between the primary series and third dose booster is likely to result in a higher fold change post-booster and higher peak antibody response, this is unlikely to fully explain the magnitude of the difference observed. The cellular response following BNT162b2 booster among people primed with protein nanoparticle vaccine is similar to that of those primed with two doses of BNT162b2 at 28 days post-booster.[10] We

Table 3
Immune responses (Cellular) response at Day 0 and Days 14, 28, 84 and 242 after booster vaccine in patients primed with NVX.

	Fresh Samples			Frozen Samples		
	Full Cohort	General	Immunology	Full Cohort	General	Immunology
Cellular response (WT), SFC/106 PBMCs (95% CI)						
Day 0	36.0 (23.4–55.4) (n=54)	33.3 (18.2–61.0) (n=30)	39.5 (21.3–73.6) (n=24)	51.8 (32.2–83.3) (n=38)	66.4 (36.1–122.1) (n=16)	43.2 (21.6–86.4) (n=22)
Day 14	207.4 (120.5–356.9) (n=21)	NA	207.4 (120.5–356.9) (n=21)	127.6 (67.3–241.9) (n=19)	NA	127.6 (67.3–241.9) (n=19)
Day 28	96.2 (65.5–141.1) (n=54)	91.4 (54.8–152.3) (n=32)	103.6 (57.3–187.1) (n=22)	54.5 (32.1–92.7) (n=51)	129.2 (81.9–203.9) (n=29)	17.5 (7.3–42.1) (n=22)
Day 84	146.1 (95.8–222.9) (n=51)	175.6 (109.9–280.7) (n=29)	114.7 (53.6–245.3) (n=22)	64.2 (40.3–102.4) (n=46)	92.2 (51.3–165.6) (n=24)	43.3 (21.2–88.6) (n=22)
Day 242	96.4 (54.1–172.0) (n=33)	168.9 (71.3–400.0) (n=11)	72.9 (34.8–152.4) (n=22)	141.6 (87.2–230.0) (n=32)	281.5 (152.1–520.9) (n=12)	93.8 (50.2–175.2) (n=20)
Fold change Day14/Day0	5.1 (2.9–9.0) (n=20)	NA	5.1 (2.9–9.0) (n=20)	3.0 (1.8–4.9) [n=17]	NA	3.0 (1.8–4.9) (n=17)
Fold change Day28/Day0	2.6 (1.9–3.5) (n=51)	2.9 (1.9–4.3) (n=30)	2.2 (1.4–3.5) (n=21)	0.9 (0.5–1.8) (n=36)	2.6 (1.8–3.8) (n=16)	0.4 (0.1–1.1) (n=20)
Fold change Day84/Day0	3.4 (2.5–4.6) (n=48)	4.1 (2.7–6.2) (n=27)	2.7 (1.7–4.2) (n=21)	1.4 (0.9–2.0) (n=33)	2.0 (1.3–2.9) (n=13)	1.1 (0.6–1.9) (n=20)
Fold change Day242/Day0	2.5 (1.6–4.0) (n=32)	3.3 (1.7–6.6) (n=11)	2.2 (1.2–4.1) (n=21)	2.6 (1.9–3.6) (n=28)	2.5 (1.8–3.6) (n=10)	2.7 (1.7–4.3) (n=18)
Fold change Day84/Day28	1.4 (1.1–1.7) (n=49)	1.5 (1.1–2.0) (n=29)	1.2 (0.9–1.7) (n=20)	1.4 (0.8–2.3) (n=42)	0.8 (0.5–1.2) (n=22)	2.5 (0.9–6.6) (n=20)
Fold change Day242/Day84	0.7 (0.5–1.2) (n=32)	0.8 (0.5–1.2) (n=11)	0.7 (0.4–1.4) (n=21)	2.2 (1.6–3.0) (n=30)	1.6 (1.2–2.2) (n=11)	2.6 (1.6–4.2) (n=19)
Cellular response (Delta), SFC/106 PBMCs (95% CI)						
Day 0	38.7 (25.5–58.8) (n=54)	35.0 (19.4–63.2) (n=30)	44.0 (24.3–79.6) (n=24)	39.6 (23.7–66.1) (n=38)	47.0 (23.0–96.1) (n=16)	34.9 (16.9–72.2) (n=22)
Day 14	183.3 (99.8–336.4) (n=21)	NA	183.3 (99.8–336.4) (n=21)	118.3 (64.1–218.3) (n=19)	NA	118.3 (64.1–218.3) (n=19)
Day 28	103.2 (69.7–152.7) (n=54)	97.4 (57.2–165.7) (n=32)	112.2 (62.5–201.6) (n=22)	91.7 (61.1–137.4) (n=51)	91.6 (52.7–159.1) (n=29)	91.7 (49.9–168.6) (n=22)
Day 84	140.2 (92.9–211.5) (n=51)	175.4 (110.8–277.7) (n=29)	104.3 (50.2–216.9) (n=22)	57.3 (35.2–93.4) (n=46)	69.1 (35.4–135.1) (n=24)	46.7 (22.7–96.0) (n=22)
Day 242	113.8 (70.7–183.3) (n=33)	167.2 (68.9–405.3) (n=11)	94.0 (53.8–164.1) (n=22)	128.3 (78.1–210.7) (n=32)	243.7 (136.1–436.7) (n=12)	87.2 (44.8–170.0) (n=20)
Fold change Day14/Day0	3.9 (2.2–7.1) (n=20)	NA	3.9 (2.2–7.1) (n=20)	2.9 (1.7–4.9) (n=17)	NA	2.9 (1.7–4.9) (n=17)
Fold change Day28/Day0	2.7 (2.1–3.6) (n=51)	2.8 (2.0–4.1) (n=30)	2.6 (1.7–3.8) (n=21)	3.2 (2.3–4.6) (n=36)	2.8 (1.7–4.4) (n=16)	3.7 (2.2–6.1) (n=200
Fold change Day84/Day0	3.1 (2.3–4.1) (n=48)	3.7 (2.6–5.3) (n=27)	2.4 (1.5–3.9) (n=21)	1.6 (1.1–2.4) (n=33)	1.9 (1.2–3.1) (n=13)	1.5 (0.8–2.6) (n=20)
Fold change Day242/Day0	2.6 (1.6–4.1) (n=32)	3.0 (1.7–5.4) (n=11)	2.3 (1.2–4.5) (n=21)	3.6 (2.5–5.1) (n=28)	3.4 (2.1–5.5) (n=10)	3.7 (2.3–5.9) (n=18)
Fold change Day84/Day28	1.3 (1.0–1.6) (n=49)	1.4 (1.0–2.0) (n=29)	1.0 (0.8–1.3) (n=20)	0.7 (0.5–0.9) (n=42)	0.9 (0.6–1.3) (n=22)	0.5 (0.3–0.7) (n=20)
Fold change Day242/Day84	1.0 (0.6–1.5) (n=32)	0.9 (0.6–1.4) (n=11)	1.0 (0.5–1.9) [n=21]	2.2 (1.6–3.1) (n=30)	2.0 (1.2–3.2) (n=11)	2.4 (1.6–3.7) (n=19)
Cellular response (Beta), SFC/106 PBMCs (95% CI)						
Day 0	36.8 (23.9–56.5) (n=54)	36.3 (20.0–65.9) (n=30)	37.4 (19.9–70.1) (n=24)	35.6 (21.1–59.9) (n=38)	37.1 (17.4–79.1) (n=16)	34.5 (16.7–71.5) (n=22)
Day 14	195.1 (105.4–361.3) (n=21)	NA	195.1 (105.4–361.3) (n=21)	101.7 (50.2–206.2) (n=19)	NA	101.7 (50.2–206.2) (n=19)
Day 28	106.6 (72.8–156.1) (n=54)	102.6 (61.1–172.3) (n=32)	112.7 (63.7–199.2) (n=22)	89.7 (61.3–131.2) (n=51)	86.4 (49.5–151.0) (n=29)	94.3 (57.2–155.3) (n=22)
Day 84	143.6 (98.5–209.5) (n=51)	163.5 (103.9–257.4) (n=29)	121.1 (63.6–230.7) (n=22)	59.7 (38.3–93.0) (n=46)	79.0 (45.9–135.9) (n=24)	44.0 (21.7–88.9) (n=22)
Day 242	107.0 (64.2–178.1) (n=33)	186.7 (84.2–414.1) (n=11)	81.0 (43.0–152.5) (n=22)	124.4 (74.7–207.0) (n=32)	220.9 (109.7–444.9) (n=12)	88.1 (45.3–171.5) (n=20)
Fold change Day14/Day0	5.7 (3.2–10.1) (n=20)	NA	5.7 (3.2–10.1) (n=20)	3.3 (2.0–5.5) (n=17)	NA	3.3 (2.0–5.5) (n=17)
Fold change Day28/Day0	3.0 (2.2–4.0) (n=51)	3.0 (2.0–4.6) (n=30)	3.0 (2.1–4.4) (n=21)	3.1 (2.4–4.1) (n=36)	3.3 (2.3–4.6) (n=16)	3.0 (2.0–4.6) (n=20)
Fold change Day84/Day0	3.3 (2.5–4.4) (n=48)	3.5 (2.3–5.3) (n=27)	3.2 (2.3–4.4) (n=21)	1.7 (1.2–2.5) (n=33)	2.7 (1.6–4.6) (n=13)	1.3 (0.8–2.2) (n=20)
Fold change Day242/Day0	2.6 (1.6–4.4) (n=32)	3.1 (1.6–6.1) (n=11)	2.4 (1.2–4.8) (n=21)	3.3 (2.3–4.7) (n=28)	3.3 (1.8–6.2) (n=10)	3.3 (2.2–5.1) (n=18)
Fold change Day84/Day28	1.3 (1.0–1.6) (n=49)	1.3 (0.9–1.9) (n=29)	1.2 (1.0–1.5) (n=20)	0.7 (0.6–1.0) (n=42)	1.2 (0.9–1.7) (n=22)	0.4 (0.3–0.6) (n=20)
Fold change Day242/Day84	0.8 (0.5–1.2) (n=32)	1.0 (0.6–1.7) (n=11)	0.7 (0.4–1.3) (n=21)	1.9 (1.4–2.5) (n=30)	1.5 (1.1–2.0) (n=11)	2.2 (1.5–3.2) (n=19)

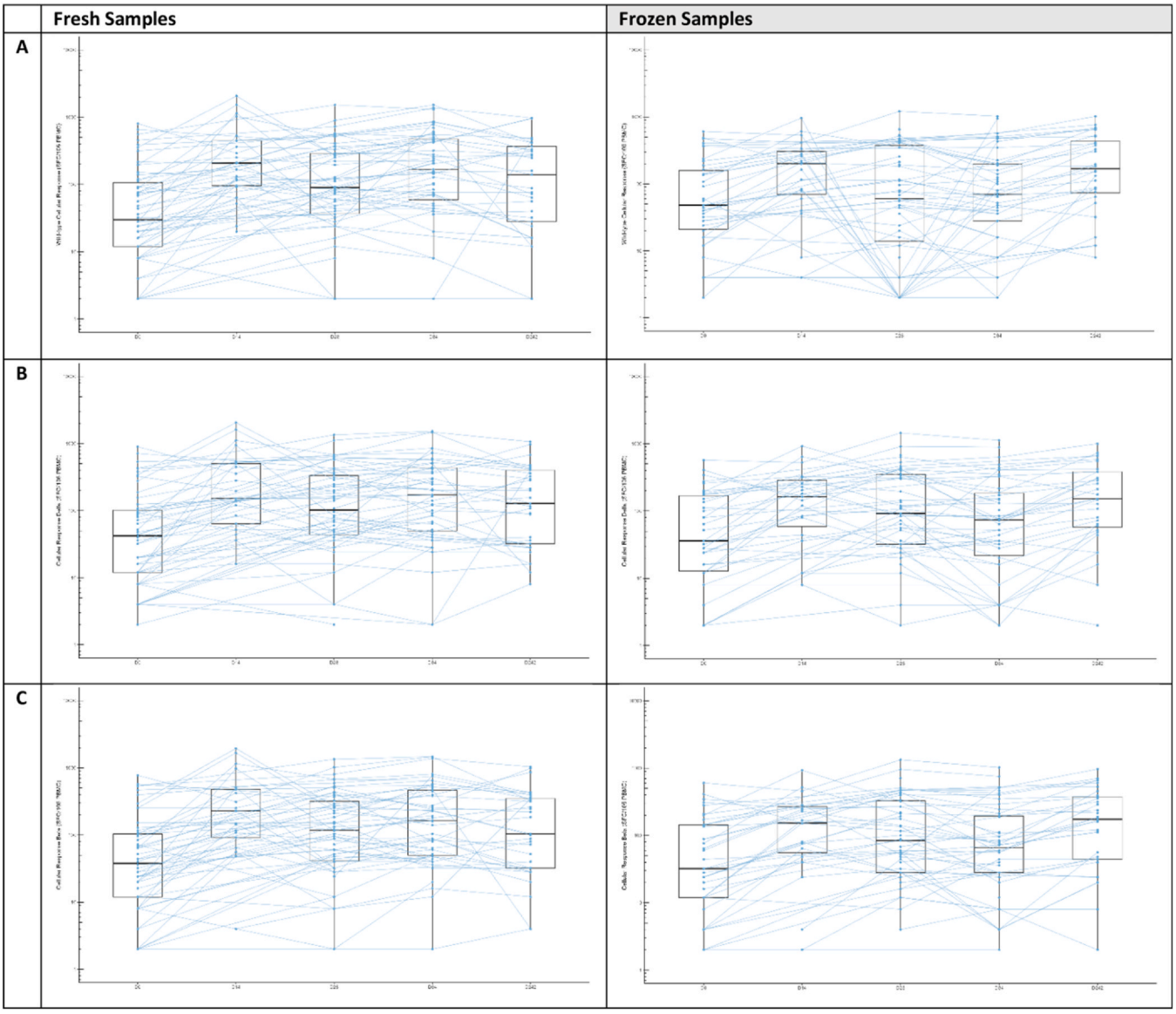

Fig. 4. Kinetics of cellular immune responses in NVX-primed participants receiving the BNT booster vaccination. A) Wild-type, B) Delta C) Beta Cellular responses. Boxplots represent the median and 25th and 75th percentiles. Each data point is one participant. Solid lines connect samples from the same participant at multiple timepoints. D0=pre-booster. D14=14 days after booster. D28=28 days after booster. D84=84 days after booster. D242=242 days after booster. ELU=ELISA laboratory units. SFCs=spot forming cells. PBMCs=peripheral blood mononuclear cells.

did not observe an obvious decay in cellular response. To validate the kinetics, we re-ran the ELISpot assay on frozen PBMCs, which showed a similar result. This observation was different to previous schedules, and further studies would be needed to confirm and understand the biological mechanism for the NVX-CoV2373 / NVX-CoV2373 / BNT162b2 schedule.

A third dose of BNT162b2 following a two-dose NVX-CoV2373 primary series showed greater reactogenicity than was observed for vaccine combinations used during the COV-BOOST main study. In particular, local reactions were noted to be more prominent. Redness at the site of vaccination was the most frequently reported grade 3 or above reaction. In previous trials, heterologous regimens of COVID-19 vaccines have been noted to be more reactogenic overall than homologous regimens.[1,14] Some combinations are also reported to be more reactogenic than others, and the order in which different vaccines are received may also change reactogenicity. For example, a booster dose of NVX-CoV2373 following a primary series with BNT162b2 had a relatively mild reactogenicity profile[1] compared with the high reactogenicity seen in the present study. Of note, a third dose of NVX-CoV2373 as a booster given approximately 6 months after the primary series with NVX-CoV2373 also resulted in incremental reactogenicity,[11] although responses were less than observed in our study (for example, < 60% experiencing ≥grade 1 pain for a third homologous dose of NVX-CoV2373 compared to > 75% for a third dose of BNT162b2 following two doses of NVX-CoV2373).

Our study has some limitations, in particular, that we did not enrol a contemporaneous control group, limiting the conclusions that may be drawn on reactogenicity. Additionally, whilst comparisons can be made to the main COV-BOOST study, these must be considered in the context of a longer duration between the primary series and the booster in this sub-study. Comparative data on persistence are not available. The study period post-vaccination also covered the period of the emergence of the Omicron variant, with associated high numbers of community infections. As such, a number of individuals within the study are likely to have had their immunity also boosted by infection with SARS-CoV-2. This is unlikely to have significantly impacted the peak antibody response in

the four-week window before day 28 but may have influenced the kinetics of antibody decay.

Conclusion

A heterologous third dose booster of BNT162b2 for COVID-19 following a primary series of NVX-COV2373 was highly immunogenic and associated with a high but tolerable level of reactogenicity comparable to that of a homologous booster of NVX-CoV2373. Further research is warranted into the potential benefits of heterologous boosting schedules for protein and mRNA vaccine combinations for all new vaccines. As higher protective antibody levels are consistently shown to be a correlate of protection for vaccines against many diseases, heterologous schedule testing should be considered from the start of new clinical vaccine development in future pandemic emergencies.

Author contributions

SNF, XL and JSN-V-T conceived the trial and SNF is the chief investigator. SNF, AM and XL contributed to the protocol and design of the study. AM, GB and SS led the implementation of the study. XL, AW, LJ and VC designed and conducted the statistical analysis and have verified the underlying data. LJ, XL, AM, AW and SNF drafted the report. All other authors contributed to the implementation and data collection. All authors reviewed and approved the final report.

Data availability

The study protocol is provided in the appendix. Individual participant data will be made available when the study is complete, upon reasonable requests made to the corresponding author; data can be shared through secure online platforms after proposals are approved. All the sequence datasets used in the T-cell analysis are available in the public GISAID database (https://www.gisaid.org).

Declaration of Competing Interest

KC acts on behalf of University Hospital Southampton as an investigator on studies funded or sponsored by vaccine manufacturers including AstraZeneca, GlaxoSmithKline, Janssen, Medimmune, Merck, Pfizer, Sanofi and Valneva. She receives no personal financial payment for this work. SNF acts on behalf of University Hospital Southampton NHS Foundation Trust as an Investigator and/or providing consultative advice on clinical trials and studies of COVID-19 and other vaccines funded or sponsored by vaccine manufacturers including Janssen, Pfizer, AstraZeneca, GlaxoSmithKline, Novavax, Seqirus, Sanofi, Medimmune, Merck and Valneva vaccines and antimicrobials. He receives no personal financial payment for this work. ALG is named as an inventor on a patent covering use of a particular promoter construct that is often used in ChAdOx1-vectored vaccines and is incorporated in the ChAdOx1 nCoV-19 vaccine. ALG may benefit from royalty income paid to the University of Oxford from sales of this vaccine by AstraZeneca and its sublicensees under the University's revenue sharing policy. JH has received payments for presentations for AstraZeneca, Boehringer Ingelheim, Chiesi, Ciple & Teva. VL acts on behalf of University College London Hospitals NHS Foundation Trust as an Investigator on clinical trials of COVID-19 vaccines funded or sponsored by vaccine manufacturers including Pfizer, AstraZeneca and Valneva. He receives no personal financial payment for this work. PM acts on behalf of University Hospital Southampton NHS Foundation Trust and The Adam Practice as an investigator on studies funded or sponsored by vaccine manufacturers including AstraZeneca, GlaxoSmithKline, Novavax, Medicago and Sanofi. He received no personal financial payment for this work. JSN-V-T was seconded to the Department of Health and Social Care, England until 31st March 2022. He has subsequently received lecture fees from AstraZeneca, Sanofi Pasteur and has performed paid consultancy for Janssen and Seqirus. MR has provided post-marketing surveillance reports on vaccines for Pfizer and GSK for which a cost recover charge is made. PTH acts on behalf of City St Georges, University of London as an Investigator on clinical trials and studies of COVID-19 and other vaccines funded or sponsored by vaccine manufacturers including Pfizer, Moderna, AstraZeneca, GlaxoSmithKline, Novavax, Sanofi, Minervax and Valneva vaccines. He receives no personal financial payment for this work.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2025.106576.

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