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Safety and immunogenicity report from the Com-COV study – A single-blind randomised non-inferiority trial comparing heterologous and homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine.

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Abstract

Background

Use of heterologous prime-boost COVID-19 vaccine schedules could facilitate mass COVID-19 immunisation, however we have previously reported that heterologous schedules incorporating an adenoviral-vectored vaccine (ChAd, Vaxzevria, Astrazeneca) and an mRNA vaccine (BNT, Comirnaty, Pfizer) at a 4-week interval are more reactogenic than homologous schedules. Here we report the immunogenicity of these schedules.

Methods

Com-COV (ISRCTN: 69254139, EudraCT: 2020-005085-33) is a participant-blind, non-inferiority trial evaluating vaccine reactogenicity and immunogenicity. Adults ≥ 50 years, including those with well-controlled comorbidities, were randomised across eight groups to receive ChAd/ChAd, ChAd/BNT, BNT/BNT or BNT/ChAd, administered at 28- or 84-day intervals.

The primary endpoint is the geometric mean ratio (GMR) of serum SARS-CoV-2 anti-spike IgG levels (ELISA) at one-month post boost, when comparing ChAd/BNT with ChAd/ChAd, and (separately) BNT/ChAd with BNT/BNT. The heterologous schedules were considered non-inferior to the approved homologous schedules if the lower limit of the one-sided 97.5% confidence interval of the GMR of these comparisons was above 0.63. The primary analysis was on a per-protocol population, who were seronegative at baseline. Safety analyses were performed amongst participants receiving at least one dose of study vaccines.

Findings

In February 2021, 830 participants were enrolled and randomised, including 463 with a 28-day prime-boost interval whose results are reported in this paper. Participant mean age was 57.8 years, 45.8% were female, and 25.3% from ethnic minorities.

The geometric mean concentration (GMC) of day 28 post-boost SARS-CoV-2 anti-spike IgG in ChAd/BNT recipients (12,906 ELU/ml) was non-inferior to that in ChAd/ChAd recipients (1,392 ELU/ml) with a geometric mean ratio (GMR) of 9.2 (one-sided 97.5% CI: 7.5, ∞). In participants primed with BNT, we failed to show non-inferiority of the heterologous schedule (BNT/ChAd, GMC 7,133 ELU/ml) against the homologous schedule (BNT/BNT, GMC 14,080 ELU/ml) with a GMR of 0.51 (one-sided 97.5% CI: 0.43, ∞). Geometric mean of T cell response at 28 days post boost in the ChAd/BNT group was 184 SFC/10^6 PBMCs (spot forming cells/10^6 peripheral blood mononuclear cells) compared to 48,

80 and 97 SFC/10^6 PBMCs for ChAd/ChAd, BNT/BNT, and BNT/ChAd, respectively. There were four serious adverse events across all groups, none of which were considered related to immunisation.

Interpretation

Despite the BNT/ChAd regimen not meeting non-inferiority criteria, the GMCs of both heterologous schedules were higher than that of a licensed vaccine schedule (ChAd/ChAd) with proven efficacy against COVID-19 disease and hospitalisation. Along with the higher immunogenicity of ChAd/BNT compared with ChAD/ChAd, these data support flexibility in the use of heterologous prime-boost vaccination using ChAd and BNT COVID-19 vaccines.

Funding

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Introduction

COVID-19 has severely impacted the world in terms of health, society and economy.[1] Immunity through vaccination is fundamental to reducing the burden of disease, the emergence from current public health measures and the subsequent economic recovery. Multiple vaccines with proven effectiveness are being deployed globally, including the mRNA vaccine Comirnaty (BNT, Pfizer) and the adenoviral vectored vaccine Vaxzevria (ChAd, AstraZeneca), both of which are approved as two-dose homologous schedules in the UK and elsewhere.[2]

As of June 2021, around 2 billion COVID-19 vaccines were administered worldwide,[3] but many more people remain unimmunised. Heterologous vaccine schedules may ease logistical problems inherent in some national and international vaccine programmes. This could prove of particular importance in low- and middle-income countries[4] as well as in countries which have adopted age-specific restrictions for the use of ChAd.[5–7]

While the Sputnik V vaccine programme, which deploys a heterologous prime-boost schedule using Ad26 and Ad5 vectored COVID-19 vaccines, induces a robust humoral and cellular response and has shown 91.6% efficacy against symptomatic disease,[8,9] there are currently no efficacy data using heterologous schedules incorporating COVID-19 vaccines across different platforms. Nevertheless, pre-clinical studies support evaluation of this approach,[10,11] and a randomised study in Spain suggested that there is an increase in binding and neutralising antibody after boosting ChAd primed participants with BNT, compared with not having a boost dose.[12] Additionally, early results from an observational study in Germany show that humoral responses are similar in the cohort receiving BNT/BNT at a 3-week interval to those receiving ChAd/BNT at 10-week interval, with cellular responses appearing to be higher in the ChAd/BNT cohort.[13]

Robust data on the safety and immunogenicity of heterologous vaccine schedules will help inform the use of these schedules in individuals who develop a contraindication to a specific vaccine after their first dose, and for vaccine programmes looking to mitigate vaccine supply chain disruption or changes in guidance for vaccine usage. In addition, there remains the possibility that mixed schedules may induce an enhanced or more durable humoral and/or cellular immune response compared to licensed schedules, and may do so against a greater range of SARS-CoV-2 variants.

Accordingly, we have undertaken a randomised controlled trial to determine whether the immune responses to heterologous schedules deploying ChAd and BNT are non-inferior to their equivalent homologous schedules.

Methods

Trial Design

Com-COV is a participant-blinded, randomised, phase II, UK multi-centre, non-inferiority study investigating the safety, reactogenicity and immunogenicity of heterologous prime-boost COVID-19 vaccine schedules (See supplementary or https://comcovstudy.org.uk/ for full protocol). Four permutations of prime-boost schedules using the ChAd and BNT vaccines are compared, at two different prime-boost intervals (28 and 84 days) to reflect both 'short' and 'long' interval approaches to immunisation. The majority of participants were enrolled into the 'General cohort' in which participants could be randomised to receive the four vaccine schedules at either a 28 or 84 day interval, while a subset (N=100, selected on the basis of site capacity and participant availability) were enrolled into an immunology cohort that only randomised individuals to vaccine schedules with a 28 day interval and had four additional blood tests to explore the kinetics of the immune responses.

Here we report data from all participants randomised to vaccine schedules with a prime/boost interval of 28 days.

Participants

COVID-19 vaccine-naïve adults aged 50 years and over, with no or well-controlled mild-moderate comorbidities were eligible for recruitment. Key exclusion criteria were previous laboratory confirmed SARS-CoV-2 infection, history of anaphylaxis, history of allergy to a vaccine ingredient, pregnancy, breastfeeding or intent to conceive, and current use of anticoagulants. Full details of the inclusion and exclusion criteria can be found in the protocol (supplementary file).

Interventions and Procedures

Participants who met the inclusion and exclusion criteria via the online screening and/or the telephone screening were invited to the baseline visits (D0), where randomisation occurred for those passing the final eligibility assessment and providing informed consent.

Two COVID-19 vaccines were used in this study. ChAd is a replication-deficient chimpanzee adenovirus vectored vaccine, expressing the SARS-CoV-2 spike surface glycoprotein with a leading tissue plasminogen activator signal sequence. Administration is via 0.5ml intramuscular (IM) injection into the upper arm. BNT is a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine encoding trimerised SARS-CoV-2 spike glycoprotein. Administration is via a 0.3ml IM injection into the upper arm.

Vaccines were administered by appropriately trained trial staff at trial sites. Participants were observed for at least 15 minutes after vaccination. During the D0 visit, participants were given an oral thermometer, tape measure and diary card (electronic or paper) to record solicited, unsolicited, and medically attended adverse events (AEs) with instructions. The study sites' physicians reviewed the diary card regularly to record AEs, adverse events of special interest (AESIs), and serious adverse events (SAEs). The time-points for subsequent visits for immunogenicity blood sampling are shown in the supplementary protocol. During the study visits, AEs, AESIs and SAEs that had not been recorded in the diary card were also collected.

Participants testing positive for SARS-CoV-2 in the community were invited for an additional visit for clinical assessment, collection of blood samples and throat swab, and completion of a COVID-19 symptom diary.

Randomisation and Blinding

Computer-generated randomisation lists were prepared by the study statistician. Participants were block randomised (block size four) 1:1:1:1 within the immunology cohort to ChAd/ChAd, ChAd/BNT, BNT/BNT and BNT/ChAd schedules (boost interval of 28 days). General Cohort participants were block randomised (block size eight) 1:1:1:1:1:1:1:1 to ChAd/ChAd, ChAd/BNT, BNT/BNT and BNT/ChAd schedules at boosting intervals of both 28 and 84 days. Besides the stratification by cohort, randomisation was further stratified by study site. Clinical research nurses who were not involved in safety endpoint evaluation performed the randomisation using REDCap™ (the electronic data capture system) and prepared and administered vaccine.

Participants and laboratory staff processing the immunogenicity endpoints were blinded to vaccines received, but not to prime-boost interval. Participant blinding to vaccines was maintained by concealing randomisation pages, preparing vaccines out of sight and applying masking tape to vaccine syringes to conceal dose volume and appearance. The clinical team assessing the safety endpoints were not blinded.

Outcomes

The primary outcome is serum SARS-CoV-2 anti-spike IgG concentration at 28 days post boost for those with a prime-boost interval of 28 days in participants who were seronegative for COVID infection at baseline.

Secondary outcomes include reactogenicity, as measured by solicited local and systemic events for 7 days after immunisation (reported previously for the 28-day prime-boost interval groups)[14] and safety, as measured by unsolicited AEs for 28 days after immunisation, medically attended AEs for 3

months after immunisation, AESIs and SAEs were collected throughout the study. Blood biochemistry and haematology assessments were measured at baseline (day 0), on day of boost and 28 days post-boost, with an additional day 7 post-boost time-point (D35) for the immunology cohort only. The detailed definition of safety outcomes can be found in the protocol as a supplementary file.

Immunological secondary outcomes include SARS-CoV-2 anti-spike binding IgG concentration, cellular responses (measured by IFN-gamma ELISpot) in peripheral blood, and pseudotype virus neutralisation titres at D0, D28 and D56. The immunology cohort had additional visits at D7, D14, D35 and D42 to explore the kinetics of the immune responses further.

Laboratory methods

Sera were analysed at Nexelis, (Laval, Canada) to determine SARS-CoV-2 anti-spike IgG concentrations by ELISA (reported as ELISA laboratory unit (ELU)/ml) and the 50% neutralising antibody titre (NT_{50}) for SARS-CoV-2 pseudotype virus neutralisation assay (PNA), using a vesicular stomatitis virus backbone adapted to bear the 2019-nCOV SARS-CoV-2 spike protein.[15] The conversation factors to international standard units can be found in the supplementary file. Sera from day 0 were analysed at Porton Down, Public Health England, by ECLIA (Cobas platform, Roche Diagnostics) to determine anti-SARS-CoV-2 nucleocapsid IgG status (reported as negative if below a cut off index of 1.0). Normalised NT_{50} for live SARS-CoV-2 virus (Victoria/01/2020) was determined by micro-neutralisation assay (MNA) also at Porton Down, on day 0 and 56 samples in the ChAd-primed groups only due to the limitation of laboratory capacity.[15] Interferon-gamma 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 performed at Oxford Immunotec (Abingdon, UK) within 32 hours of venepuncture, using the addition of T-Cell Xtend reagent to extend peripheral blood mononuclear cell (PBMC) survival.[16] 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, and these results multiplied by four to express frequencies per 10^6 PBMCs.

Statistical analysis

The primary analysis of SARS-CoV-2 anti-spike IgG was carried out in participants boosted at D28 on a per-protocol basis. The analysis population was participants who were seronegative for COVID at baseline (defined by anti-nucleocapsid IgG negativity at Day 0 and no confirmed SARS-CoV-2 infection within 14 days post prime vaccination), whose primary endpoint data were available and who had no protocol deviations. The geometric mean ratio (GMR) was calculated as the antilogarithm of the difference between the mean of the $\log_{10}$ transformed SARS-CoV-2 anti-spike IgG in the heterologous arm and that in the homologous arm (as the reference), after adjusting for study site and cohort

(immunology/general) as randomisation design variables in the linear regression model. The GMRs were reported separately for participants primed with ChAd and those with BNT with a one-sided 97.5% confidence interval to adjust for multiple testing as two primary comparisons were made. The criteria for non-inferiority of heterologous boost compared to the homologous boost was for the lower limit of the one-sided 97.5% CI of the GMR to lie above 0.63; this was chosen on a pragmatic basis to approach the WHO criterion of 0.67 for licencing new vaccines when using GMR as the primary endpoint, while still allowing rapid study delivery.[17]

According to recommended practice for non-inferiority trials,[18] we also present the two-sided 95% CI of the adjusted GMRs among the modified intent-to-treat (mITT) population, which follows the per-protocol population definition but included participants whose visit timelines fell outside protocol windows to allow a conservative estimation for superiority comparison (Figure 1), as secondary analyses. The heterologous arm was considered superior to the homologous arm if the lower limit of the two-sided 95% CI lies above one, and the homologous boost arm superior to the heterologous boost arm if the upper limit of the two-sided 95% CI lies below one. The geometric means of secondary immunological outcomes were reported in the mITT population. The proportions of participants with responses higher than the lower limit of detection (LLOD) or lower limit of quantification (LLOQ) were calculated by vaccine schedule, with 95% CIs calculated by the binomial exact method for each secondary immunological outcome, and compared between heterologous and homologous arms using Fisher's exact test. Censored data reported as below the LLOD/LLOQ were imputed with a value equal to half of the threshold before transformation. Between-schedule comparisons of immunological outcomes were evaluated by linear regression models adjusting for study site and cohort as secondary analyses. Correlations between different immunological outcomes were evaluated by Pearson correlation coefficients.

As an exploratory analysis, subgroup analyses were conducted for primary and secondary immunogenicity outcomes by age (50-59, and 60+), sex (male and female) and baseline comorbidity (presence/absence of cardiovascular disease, respiratory disease or diabetes). P values for interaction were reported using Wald test, and the significance level for interaction was set to be two-sided 0.0024 using Bonferroni correction.

Participants who received at least one dose of study vaccines were included in the safety analysis. The proportion of participants with at least one safety event was reported by vaccine schedule. Fisher's exact test was used to compare the difference between schedules.

The sample size calculation was done assuming the standard deviation (SD) of the primary endpoint to be 0.4 at $\log_{10}$ scale and the true GMR to be one. The study needed to recruit 115 participants per

arm to achieve 90% power at a one-sided 2.5% significance level, after adjusting for an attrition rate of 25% due to baseline SARS-CoV-2 seropositivity or loss to follow-up.

All the statistical analyses were carried out using R version 3.6.2 (2019-12-12).

Trial oversight and safety monitoring

The trial was reviewed and approved by the South-Central Berkshire Research Ethics Committee (21/SC/0022), the University of Oxford, and the Medicines and Healthcare Products Regulatory Agency (MHRA). An independent data safety monitoring board (DSMB) reviewed safety data, and local trial-site physicians provided oversight of all adverse events in real-time. The trial is registered at www.isrctn.com as ISRCTN: 69254139.

Funder

The study is funded by the UK Government through the National Institute for Health Research (NIHR) and the Vaccine Task Force (VTF). The funder had no role data collection, analysis, interpretation, manuscript writing or decision to submit.

Results

Between 11th February 2021 and 26th February 2021, 978 participants were screened at eight study sites across England, among whom 830 were enrolled and randomised into the study. 463 participants were randomised to the four arms with a 28-day prime-boost interval reported here including 100 participants enrolled into the immunology cohort. The mean age of the participants was 57.8 years (SD 4.7) with 45.8% female participants and 25.3% from ethnic minorities. Baseline characteristics were well balanced across the four arms in both the general and immunology cohorts (Table 1). At baseline, 20 (4.3%) participants were positive for anti-nucleocapsid IgG (cut-off index ≥1.0), evenly distributed across groups. The numbers of participants included in the modified intent-to-treat and per-protocol analyses were 432 and 426, respectively (Figure 1).

Immune responses at 28 days post boost vaccination: Primary outcome and key secondary outcomes.

Among participants primed with ChAd, the GMCs of SARS-CoV-2 anti-spike IgG at 28 days post boost vaccination was 1,392 ELU/ml (95%CI: 1,188-1,630) and 12,906 ELU/ml (95%CI: 11,404-14,604) in the homologous arm (ChAd/ChAd) and heterologous arm (ChAd/BNT), respectively, with a GMR of 9.2 (one-sided 97.5% CI: 7.5, ∞) between heterologous and homologous arms in the per-protocol analysis (Table 2). Similar GMCs were observed in the modified ITT analysis with a GMR of 9.3 (two-sided 95% CI: 7.7-11). The GMRs of MNA NT_{50} and PNA NT_{50} (secondary outcomes) between heterologous and homologous arms were 6.4 (two-sided 95% CI: 5.2, 7.8) and 8.5 (two-sided 95% CI: 6.5, 11) in the modified ITT analysis. The secondary outcome of cellular responses by T-cell ELISpot revealed 48 SFC/10^6 PBMCs (37-61) for ChAd/ChAd and 184 SFC/10^6 PBMCs (152-223) with a GMR of 3.9 (2.9-5.3) (Table 2). These results indicate that the ChAd/BNT schedule was not only non-inferior, but also statistically superior to ChAd/ChAd schedule for the SARS-CoV-2 anti-spike IgG, MNA NT_{50}, PNA NT_{50}, and cellular responses.

In the two schedules with BNT as the prime vaccine, the GMCs of SARS-CoV-2 anti-spike IgG at 28 days post boost vaccination were 14,080 ELU/ml (95%CI: 12,491-15,871) and 7,133 ELU/ml (95%CI: 6,415-7,932) for the homologous and heterologous arms in the per-protocol analysis. The GMR in the per-protocol analysis was 0.51 (one-sided 97.5% CI: 0.43, ∞). The study therefore failed to show non-inferiority of the heterologous arm (BNT/ChAd) to its corresponding homologous arm (BNT/BNT). In addition, BNT/ChAd was statistically inferior for both SARS-CoV-2 anti-spike IgG ($p<0.0001$) and PNA NT_{50} ($p=0.0041$), compared with BNT/BNT. The geometric mean SFC frequency (T-cell ELISpot) was higher in the heterologous arm compared with the homologous arm (97 vs 80 SFC/10^6 PBMCs), though did not reach a level of statistical significance (GMR: 1.2, two-sided 95% CI: 0.87-1.7).

Similar patterns of GMRs were seen in all subgroup analyses with SARS-CoV-2 anti-spike IgG and PNA NT_{50} consistently higher in the ChAd/BNT compared with ChAd/ChAd and BNT/BNT higher than BNT/ChAd (Figure 2). Strong correlations were seen between SARS-CoV-2 anti-spike IgG and PNA NT_{50}, and between SARS-CoV-2 anti-spike IgG and MNA NT50 at 28 days post boost (Pearson correlation coefficients of 0.6-0.7), while the correlations between humoral responses and cellular response were weak (Pearson correlation coefficients <0.4) (Figure 3).

Additional secondary outcomes

Immunology cohort: Humoral & cellular immune responses at 7 and 14 days post boost vaccination

Across all four schedules an increase in SARS-CoV-2 anti-spike IgG was seen from day 28 to day 35 (day 7 post boost), contrasting with a lack of response at day 7 post prime, suggesting that both vaccines induced immunological priming that was augmented by either homologous or heterologous boost (Figure 4 and Appendix Figure 1). No further increase in SARS-CoV-2 anti-spike IgG was seen at day 28 post boost, suggesting the peak response post-boost is likely to be earlier than 28 days. For all schedules except ChAd/ChAd, peak T cell response was observed at 14 days post boost; no further increase was seen in ChAd/ChAd post boost. (Appendix Figure 1).

Humoral & cellular immune responses: Post-prime vaccination

In participants primed with ChAd and BNT, the SARS-CoV-2 anti-spike IgG GMCs were 129 (95% CI: 83-200) and 843 (95% CI: 658-1,081) ELU/ml at 14 days post prime ($p<0.0001$), and 555 (95% CI: 469-657) and 1,597 (1,407-1,812) ELU/ml at 28 days post prime ($p<0.0001$), respectively .

In contrast, ChAd induced significantly higher cellular responses at 14 days ($p<0.0001$) and 28 days ($p<0.0001$) post prime vaccination compared with BNT: Geometric mean at 14 days was 159 (95% CI: 119-211) vs 32 SFC/10^6 PBMCs (95%CI: 22-47), and at 28 days was 53 (95% CI: 44-63) vs 15 SFC/10^6 PBMCs (95%CI: 13-18), respectively.

Humoral & cellular immune responses: Cross-schedule comparisons

When BNT was given as the boost vaccine, similar levels of SARS-CoV-2 anti-spike IgG ($p=0.44$) and PNA NT_{50} ($p=0.40$) at 28 days post-boost were observed among participants primed with ChAd (ChAd/BNT) and BNT (BNT/BNT). Participants boosted with ChAd following BNT prime (BNT/ChAd) had significantly higher SARS-CoV-2 anti-spike IgG ($p<0.0001$) and PNA NT_{50} ($p<0.0001$) than those primed with ChAd (ChAd/ChAd). Homologous BNT/BNT immunisation generated higher binding antibodies at day 7 ($p<0.0001$) and day 28 ($p<0.0001$) post boost compared with ChAd/ChAd, with a difference also observed in PNA at day 28 post boost ($p<0.0001$).

In contrast to the lack of further response following a homologous second dose of ChAd (Figure 4, Appendix Figure 1), a significant increase in cellular response was seen after a homologous boost with BNT, such that those receiving BNT/BNT had significantly higher number of SARS-CoV-2 specific T cells per 10^6 PBMCs than ChAd/ChAd (p=0.0028) at 28 days post boost with a four week interval (Figure 4).

Safety

The results of the solicited adverse events in the week following immunisation have been reported previously.[14] In summary, we observed an increase in systemic reactogenicity after boost in participants receiving heterologous schedules in comparison to homologous schedules with the same prime vaccine. In participants randomised to 28-day interval groups there were 316 adverse events from 178 participants up to 28 days following boost immunisation (Supplementary Table 1). No significant difference was observed between the vaccine schedules in the proportion of participants with at least one AE (p=0.89). Adverse events of Grade ≥3 are described in Supplementary Table 2.

Amongst all participants up to 6th Jun 2021 (date of data-lock) there were seven AESIs, of which four were COVID-19 diagnoses (Supplementary Tables 3 & 4). The non-COVID-19 AESIs were not considered related to immunisation. Four participants across all groups developed COVID-19. Three were within 7 days of prime immunisation, one was 54 days later, and had not received their planned 28 day boost due to travel. (Supplementary Table 4)

There were four SAEs across all groups in the study up to the data lock, and none was considered related to immunisation (Supplementary table 5).

Discussion

We present here, for the first time in a randomised controlled clinical trial, the immunogenicity of heterologous and homologous ChAd and BNT vaccine schedules with a 28-day prime-boost interval. The findings demonstrate that all the schedules studied induced concentrations of SARS-CoV-2 anti-spike IgG concentrations at least as high as those induced after a licensed ChAd/ChAd schedule, which is effective in preventing symptomatic COVID-19 when administered at a 4-12 week prime-boost interval.[19] Nevertheless, it is notable that the BNT containing schedules were more immunogenic than the homologous ChAd/ChAd schedule, and none of the heterologous schedules generated binding or pseudotype virus neutralising antibodies above those induced by BNT/BNT immunisation. Cellular immune responses in the BNT vaccine containing schedules were likewise all at least as high as ChAd/ChAd group with BNT/ChAd showing the greatest expansion of vaccine-antigen responsive T-cells in the peripheral circulation at 28 days post boost.

Although the 28-day homologous ChAd/ChAd was the least immunogenic of the four schedules in our trial, data from a phase 3 randomised clinical trial showed this 4-week interval regimen to be 76% (95%CI: 68%-82%) efficacious against symptomatic disease, and 100% against severe disease.[20] This schedule is known to be more immunogenic when administered at an 8 to 12 week schedule,[19] and when deployed in this manner, it has been shown to be 86% (95% CI 53%-96%) and 92% (95% CI 75%-97%) effective against hospitalisation,[21] and 66% (95%CI: 54%-75%) and 60% (95%CI: 29%-77%) against symptomatic infection,[22] due to the Alpha (B.1.1.7) and Delta (B.1.617.2) variants, respectively. Given the established associations between humoral responses and vaccine efficacy,[19] our findings indicate the two heterologous schedules in this trial are also likely to be highly effective, and could be considered, in some circumstances, for national vaccine programmes.

Our results for the ChAd/BNT schedule build on preliminary data from a Spanish randomised trial in which 18-60 year olds received a dose of BNT two to three months after priming with ChAd and demonstrated a 37-fold increase in SARS-CoV-2 anti-spike IgG at 14 days post-boost, higher than the 22-fold and 19-fold rises at 7 days and 28 days post boost in this study.[12] Potential explanations for these differences include the longer prime-boost interval, the different sampling time-points and a younger population in the Spanish study.[12] Fold rises in the cellular response were, however, similar (4-fold vs. 3.7-fold). Early results from a prospective cohort study in Germany, which compared healthcare workers immunised with BNT/BNT at a 3-week interval or ChAd/BNT at an 8-12 week interval, showed similar concentrations of binding antibody at 3 weeks post-boost and higher cellular responses in the ChAd/BNT recipients.[13] Another German cohort study of 26 participants aged 25-46 years receiving a ChAd/BNT schedule with an 8-week prime-boost interval also reported a robust

humoral immune response, with a suggestion of better retention of neutralising activity against Beta and Delta variants than that observed in a non-randomised cohort receiving BNT/BNT.[23]

Together with the evidence that the T cell ELISpot readouts are similar between schedules, the immunological data presented here provide reassurance that ChAd/BNT and BNT/ChAd are acceptable options. However, in contrast with recent non-randomised and non-blinded studies, we did observe increased reactogenicity in the 28-day ChAd/BNT schedule,[14] compared with ChAd/ChAd. This discrepancy may be due to the variation in the prime-boost interval, and the forthcoming data from the 84-day prime-boost interval participants in this trial will help to delineate this difference. Although these mild-moderate symptoms were transient, this does need to be taken into consideration when deploying this schedule, especially in those younger than the participants enrolled in this study, given the reported trend towards increased reactogenicity with decreasing age.[24,25] Additional considerations for deployment of mixed schedules include potential logistical challenges within the healthcare infrastructure as well as the complex public communications surrounding this.

Numerous other randomised heterologous prime/boost COVID-19 vaccine studies are now underway or planned,[26] including Com-COV2, which incorporates vaccines manufactured by Moderna and Novavax.[27] Crucially, several of these studies include vaccines manufactured by CanSinoBIO, Gamaleya Research Institute and Sinovac that are extensively used in low- and middle-income countries, which are potentially more likely to rely on mixed schedules. These data on heterologous vaccination will also inform '3rd dose' booster immunisation programmes, currently being considered in preparation for the Northern Hemisphere 2021/2022 winter[28] and being studied in the ongoing 'Cov-Boost' study.[29]

There are a number of limitations of this study. Firstly, as an immunogenicity and reactogenicity study the sample size is not adequate to assess vaccine schedule efficacy. Although there is evidence that both binding and neutralising antibodies correlate well with protection against symptomatic disease,[19,30,31] it is less clear to what extent variations in these measures above a certain, unknown, threshold impact on protection against severe disease. Similarly, we are unable, at this point, to determine whether higher antibody concentrations measured at 28 days post boost immunisation will result in a more sustained elevation of vaccine-induced antibodies (as may be expected), and this will be evaluated at ongoing study visits up to one-year post enrolment. An additional limitation is the generalisability of these results to a younger population given the age (50 – 70 years old) of participants in this trial. Previous RCTs on homologous schedules of viral vector and mRNA vaccines reported similar post boost immunogenicity between younger (18-55 years) and older (>55 years) adults, and higher reactogenicity in younger cohorts,[24,32,33] and there is no reason to expect this would

be different for the heterologous schedules but this has not been extensively demonstrated. Lastly, the data presented here were from schedules with a 28-day prime-boost interval, whereas the WHO recommended interval for ChAd/ChAd is 8-12 weeks.[34] There is evidence that a longer prime-boost interval results in a higher post-boost SARS-CoV-2 anti-spike IgG response for ChAd/ChAd,[19] and for BNT/BNT[35] but it is unknown how lengthening the prime-boost interval will affect the heterologous schedules in this study. This question will be addressed when the immunogenicity data for the schedules including boosting at 84 days become available.

In conclusion, our study confirms the heterologous and homologous schedules of ChAd and BNT can induce robust immune responses with a 4-week prime boost interval. These results argue for allowing for flexibility in deploying mRNA and viral vectored vaccines, subject to supply and logistical considerations, and emphasise the importance of obtaining information on other mixed schedules with different prime boost intervals, especially using vaccines being deployed in low- and middle-income countries.

Research in context

Evidence before this study

National regulatory authorities have granted emergency use authorizations for more than 15 vaccines, among which six vaccines have been approved for emergency use by the World Health Organisation.[2] Although >2 billion COVID-19 vaccines have been administered as of June 2021,[3] only approximately 20% of the global population has received at least one dose of COVID-19 vaccine, with less than 1% of the population in low-income countries having received a vaccine dose.[36] Heterologous COVID-19 vaccine schedules have the potential to accelerate vaccine roll-out worldwide, especially in low and middle income countries. We searched PubMed for research articles published between database inception and 22nd June 2021 using the search terms (COVID) AND (Heterologous) AND (Vaccin*) NOT (BCG) with no language restrictions. Beside our previously published reactogenicity results,[14] we identified two animal studies using combinations of messenger RNA, adenoviral vectored, inactivated and recombinant protein vaccines as prime boost schedules. Both studies showed robust humoral and cellular responses induced by heterologous schedules in mice.[10,11] In addition, there were two clinical trials on the rAd26 and rAd5 vector-based heterologous prime-boost schedule (Sputnik V, Gamaleya Research Institute of Epidemiology and Microbiology), showing good safety profiles, strong humoral/cellular responses and a 91.6% vaccine efficacy.[8,9] A further clinical trial, which randomised participants primed with ChAd to received BNT as the boost vaccine or no boost vaccination, reported robust immune response and acceptable reactogenicity profile, but with no comparison to a homologous vaccine schedule.[12] There were another two cohort studies evaluating ChAd prime and BNT boost schedules on medRxiv, showing similar results.[13,23]

Added Value of this study

We report the results on the safety and immunogenicity of the first participant-blinded randomised clinical trial using two vaccines approved by WHO for emergency use, ChAd and BNT, when administered at a 28-day interval in heterologous and homologous vaccine schedules (ChAd/ChAd, ChAd/BNT, BNT/BNT, BNT/ChAd). The cellular and humoral responses at 28 days post-boost of the two heterologous vaccines schedules are no lower than the ChAd/ChAd schedule, which has shown to be highly effective in preventing severe COVID-19 disease, and no safety concerns were raised.

Implications of all the available evidence

In the era of multiple COVID-19 vaccines having approval for emergency use, the paramount issue in solving the COVID-19 pandemic is now to optimise global vaccine coverage rate using the currently available vaccines. The positive results from our study support flexibility in use of heterologous prime-

458 boost schedules using ChAd and BNT, which can contribute to the acceleration of vaccine roll-out.
459 Further studies are needed examining more heterologous schedules, especially those vaccines being
460 deployed in low and middle-income countries.

461

Author Contributions

MDS and JSN-V-T conceived the trial and MDS is the chief investigator. MDS, AS, RHS, and XL contributed to the protocol and design of the study. AS, EP and RHS led the implementation of the study. XL and MG conducted the statistical analysis and have verified the underlying data. AS, RHS, MG, XL and MDS drafted the report. All other authors contributed to the implementation and data collection. All authors reviewed and approved the final report.

Declaration of interests

MDS acts on behalf of the University of Oxford as an Investigator on studies funded or sponsored by vaccine manufacturers including AstraZeneca, GlaxoSmithKline, Pfizer, Novavax, Janssen, Medimmune, and MCM vaccines. He receives no personal financial payment for this work. JSN-V-T is seconded to the Department of Health and Social Care, England. AMC and DMF are investigators on studies funded by Pfizer and Unilever. They receive no personal financial payment for this work. AF is a member of the Joint Committee on Vaccination and Immunisation and Chair of the WHO European Technical Advisory Group of Experts (ETAGE) on Immunisation. He is an investigator and/or provides consultative advice on clinical trials and studies of COVID-19 vaccines produced by AstraZeneca, Janssen, Valneva, Pfizer and Sanofi and of other vaccines from these and other manufacturers including GSK, VPI, Takeda and Bionet Asia. He receives no personal remuneration or benefits for any of 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. PTH acts on behalf of St. George's University of London as an Investigator on clinical trials of COVID-19 vaccines funded or sponsored by vaccine manufacturers including Janssen, Pfizer, AstraZeneca, Novavax and Valneva. He receives no personal financial payment for this work. CAG acts on behalf of University Hospitals Birmingham NHS Foundation Trust as an Investigator on clinical trials and studies of COVID-19 and other vaccines funded or sponsored by vaccine manufacturers including Janssen, Pfizer, AstraZeneca, Novavax, CureVac, Moderna, and Valneva vaccines, and receives no personal financial payment for this work. 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. TL is named as an inventor on a patent application covering this SARS-CoV-2 vaccine and is an occasional

consultant to Vaccitech unrelated to this work. Oxford University has entered into a partnership with AstraZeneca for further development of ChAdOx1 nCoV-19

Data sharing

The study protocol is provided in the appendix. Individual participant data will be made available when the trial is complete, upon requests directed to the corresponding author; after approval of a proposal, data can be shared through a secure online platform.

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619 **Table 1. Baseline demographics and characteristics by cohort and study arm in the 28-day boost study arms**

Cohort/ Characteristic	Prime with ChAd		Prime with BNT	
	ChAd/ChAd-28	ChAd/BNT-28	BNT/BNT-28	BNT/ChAd-28
General	**(N=90)**	**(N=90)**	**(N=93)**	**(N=90)**
Age (years)				
Mean (SD)	58.2 (4.81)	58.0 (4.76)	58.2 (4.85)	57.3 (4.56)
Median (range)	57.6 (50.1, 69.1)	57.6 (50.3, 68.1)	57.7 (50.2, 69.3)	56.1 (50.5, 68.9)
Gender				
Female	38 (42.2%)	40 (44.4%)	49 (52.7%)	41 (45.6%)
Male	52 (57.8%)	50 (55.6%)	44 (47.3%)	49 (54.4%)
Ethnicity				
White	70 (77.8%)	65 (72.2%)	76 (81.7%)	66 (73.3%)
Black	1 (1.1%)	1 (1.1%)	-	2 (2.2%)
Asian	13 (14.4%)	15 (16.7%)	7 (7.5%)	9 (10.0%)
Mixed	6 (6.7%)	6 (6.7%)	8 (8.6%)	10 (11.1%)
Other	-	3 (3.3%)	2 (2.2%)	3 (3.3%)
Comorbidities				
Cardiovascular	19 (21.1%)	16 (17.8%)	18 (19.4%)	21 (23.3%)
Respiratory	16 (17.8%)	11 (12.2%)	11 (11.8%)	11 (12.2%)
Diabetes	7 (7.8%)	8 (8.9%)	-	2 (2.2%)
Immunology	**(N=25)**	**(N=24)**	**(N=26)**	**(N=25)**
Age (years)				
Mean (SD)	55.7 (4.26)	58.4 (4.60)	56.7 (5.04)	57.6 (4.65)
Median (range)	55.3 (50.7, 64.1)	58.9 (51.8, 68.3)	54.7 (50.1, 67.2)	55.8 (51.4, 67.0)

Cohort/ Characteristic	Prime with ChAd		Prime with BNT	
	ChAd/ChAd-28	ChAd/BNT-28	BNT/BNT-28	BNT/ChAd-28
Gender				
Female	13 (52.0%)	9 (37.5%)	12 (46.2%)	10 (40.0%)
Male	12 (48.0%)	15 (62.5%)	14 (53.8%)	15 (60.0%)
Ethnicity				
White	17 (68.0%)	17 (70.8%)	17 (65.4%)	18 (72.0%)
Black	-	-	2 (7.7%)	-
Asian	6 (24.0%)	4 (16.7%)	4 (15.4%)	4 (16.0%)
Mixed	2 (8.0%)	3 (12.5%)	2 (7.7%)	3 (12.0%)
Other	-	-	1 (3.8%)	-
Comorbidities				
Cardiovascular	7 (28.0%)	6 (25.0%)	10 (38.5%)	7 (28.0%)
Respiratory	5 (20.0%)	6 (25.0%)	6 (23.1%)	5 (20.0%)
Diabetes	6 (24.0%)	1 (4.2%)	2 (7.7%)	1 (4.0%)

620 SD: standard deviation

621

Table 2. Immune responses between heterologous and homologous prime/boost schedules at 28 days post boost dose in the 28-day boost study arms

	Prime with ChAd			Prime with BNT		
	ChAd/ChAd-28	ChAd/BNT-28	GMR[§]	BNT/BNT-28	BNT/ChAd-28	GMR[§]
Per-protocol analysis	**N=104**	**N=104**		**N=109**	**N=109**	
SARS-CoV-2 anti-spike IgG, ELU/ml	1392 (1188-1630) [n=104]	12906 (11404-14604) [n=104]	9.2 (97.5% CI:7.5, ∞)	14080 (12491-15871) [n=109]	7133 (6415-7932) [n=109]	0.51 (97.5% CI:0.43, ∞)
Modified ITT	**N=105**	**N=108**		**N=110**	**N=109**	
SARS-CoV-2 anti-spike IgG, ELU/ml	1387 (1186-1623) [n=105]	12995 (11520-14660) [n=108]	9.3 (95% CI:7.7,11)	13938 (12358-15719) [n=110]	7133 (6415-7932) [n=109]	0.51 (95% CI:0.44,0.6)
Live virus neutralising antibody, normalised NT_{50}	201(171-235) [n=98]	1269(1107-1454) [n=104]	6.4 (95%CI:5.2,7.8)			
Pseudotype virus neutralising antibody, NT_{50}	61 (50-73) [n=101]	515 (430-617) [n=101]	8.5 (95% CI:6.5,11)	574 (475-694) [n=102]	383 (317-463) [n=104]	0.67 (95% CI:0.51,0.88)
Cellular response, SFC/10^6 PBMCs	48 (37-61) [n=104]	184 (152-223) [n=108]	3.9 (95% CI:2.9,5.3)	80 (63-101) [n=110]	97 (76-125) [n=109]	1.2 (95% CI:0.87,1.7)

* Data shown are geometric mean (95% CI) for continuous variables;

[§] GMRs were adjusted for randomisation stratification variables, including study site and cohort, with one-sided 97.5% CIs in per-protocol analyses and two-sided 95% CIs in the modified ITT analyses; non-inferiority margin is 0.63.

627 **Table 3 Immune responses between heterologous and homologous prime/boost schedules in the 28-day boost study arms***

	Prime with ChAd			Prime with BNT		
	ChAd/ChAd-28 N=105	ChAd/BNT-28 N=108	p value¶	BNT/BNT-28 N=110	BNT/ChAd-28 N=109	p value¶
SARS-CoV-2 anti-spike IgG, ELU/ml						
D7§¥	25 (25-25) [n=21]	25 (25-25) [n=19]	NA	25 (25-25) [n=23]	25 (25-25) [n=23]	0.95
≥50.3 ELU/ml	0/21, 0% (0%, 16%)	0/19, 0% (0%, 18%)	>0.99	2/23, 9% (1%, 28%)	2/23, 9% (1%, 28%)	>0.99
D14§	87 (54-141) [n=21]	198 (96-408) [n=19]	0.041	967 (718-1304) [n=23]	735 (495-1092) [n=23]	0.39
≥50.3 ELU/ml	14/21, 67% (43%, 85%)	16/19, 84% (60%, 97%)	0.28	23/23, 100% (85%, 100%)	23/23, 100% (85%, 100%)	>0.99
D28	501 (394-638) [n=105]	613 (485-776) [n=108]	0.22	1487 (1233-1795) [n=110]	1715 (1447-2033) [n=109]	0.28
≥50.3 ELU/ml	100/105, 95% (89%, 98%)	104/108, 96% (91%, 99%)	0.75	110/110, 100% (97%, 100%)	109/109, 100% (97%, 100%)	>0.99
D35§	1151 (825-1605) [n=22]	15365 (11764-20068) [n=20]	<0.0001	17011 (12446-23248) [n=22]	6798 (5060-9133) [n=24]	<0.0001
≥50.3 ELU/ml	22/22, 100% (85%, 100%)	20/20, 100% (83%, 100%)	>0.99	22/22, 100% (85%, 100%)	24/24, 100% (86%, 100%)	>0.99
Cellular response, SFC/10^6 PBMCs						
D14§	182 (133-251) [n=21]	136 (83-223) [n=19]	0.21	37 (17-64) [n=23]	32 (20-51) [n=23]	0.92
≥4 SFC/10^6 PBMCs	21/21, 100% (84%, 100%)	19/19, 100% (82%, 100%)	>0.99	22/23, 96% (78%, 100%)	23/23, 100% (85%, 100%)	>0.99
≥24 SFC/10^6 PBMCs	21/21, 100% (84%, 100%)	17/19, 89% (67%, 99%)	0.22	12/23, 52% (31%, 73%)	12/23, 52% (31%, 73%)	>0.99
D28	53(41-69) [n=103]	52(41-66) [n=107]	0.98	15(12-18) [n=109]	16(13-20) [n=108]	0.81

≥4 SFC/10^6 PBMCs	101/103, 98% (93%, 100%)	107/107, 100% (97%, 100%)	0.24	101/109, 93% (86%, 97%)	97/108, 90% (83%, 95%)	0.48
≥24 SFC/10^6 PBMCs	74/103, 72% (62%, 80%)	75/107, 70% (60%, 79%)	0.88	34/109, 31% (23%, 41%)	35/108, 32% (24%, 42%)	0.88
D42[§]	97(60-157) [n=22]	375(266-528) [n=18]	0.0022	135(83-219) [n=22]	130 (69-243) [n=23]	0.87
≥4 SFC/10^6 PBMCs	22/22, 100% (85%, 100%)	18/18, 100% (81%, 100%)	>0.99	22/22, 100% (85%, 100%)	22/23, 96% (78%, 100%)	>0.99
≥24 SFC/10^6 PBMCs	19/22, 86% (65%, 97%)	18/18, 100% (81%, 100%)	0.24	19/22, 86% (65%, 97%)	20/23, 87% (66%, 97%)	>0.99

628 * Data shown are geometric mean (95% CIs) for continuous variables, and frequency, percentage (95%CIs) for binary variables; 50.3 ELU/ml is the LLOQ for

629 SARS-CoV-2 anti-spike IgG; 4 SFC/10^6 PBMCs is the LLOD and 24 SFC/10^6 PBMCs is the LLOQ for cellular response;

630 [§] Immunology cohort only;

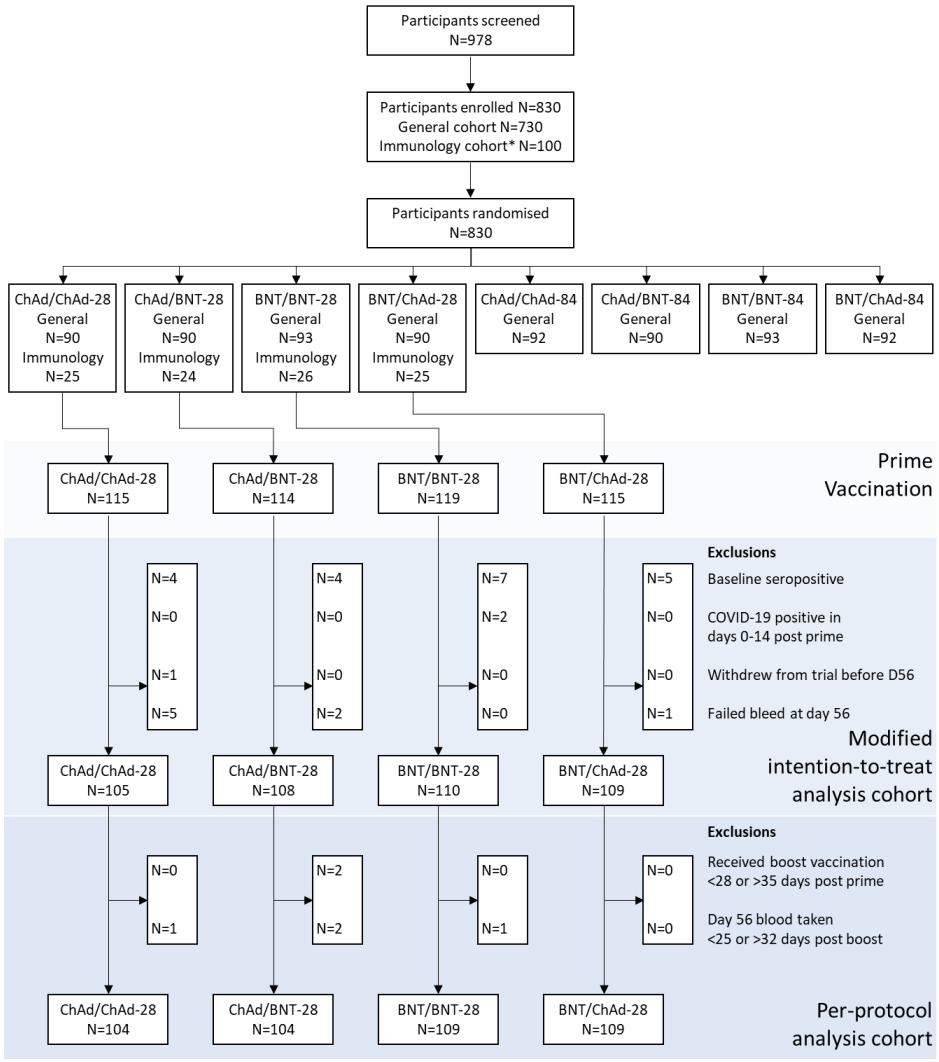
631 [¶] For continuous variables, p values were reported using linear regression model adjusting for age, sex, study site and cohort (where applicable); Fisher's

632 exact test was used to report p values for binary variables;

633 [¥] Data shown are median (IQR) due to high proportion of censored data; p values were reported using Mann-Whitney U test.

634

Figure 1. Consort Diagram



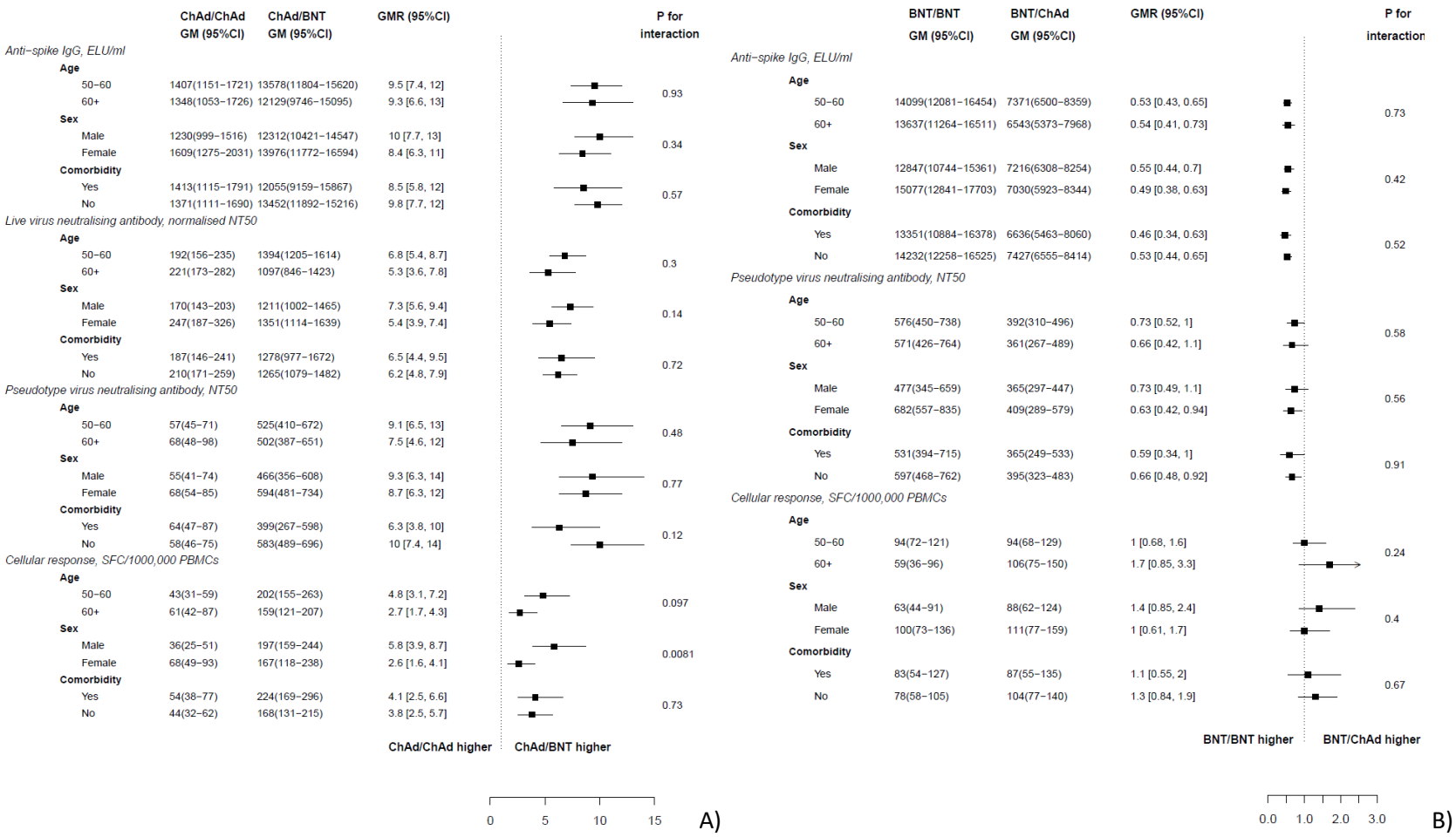
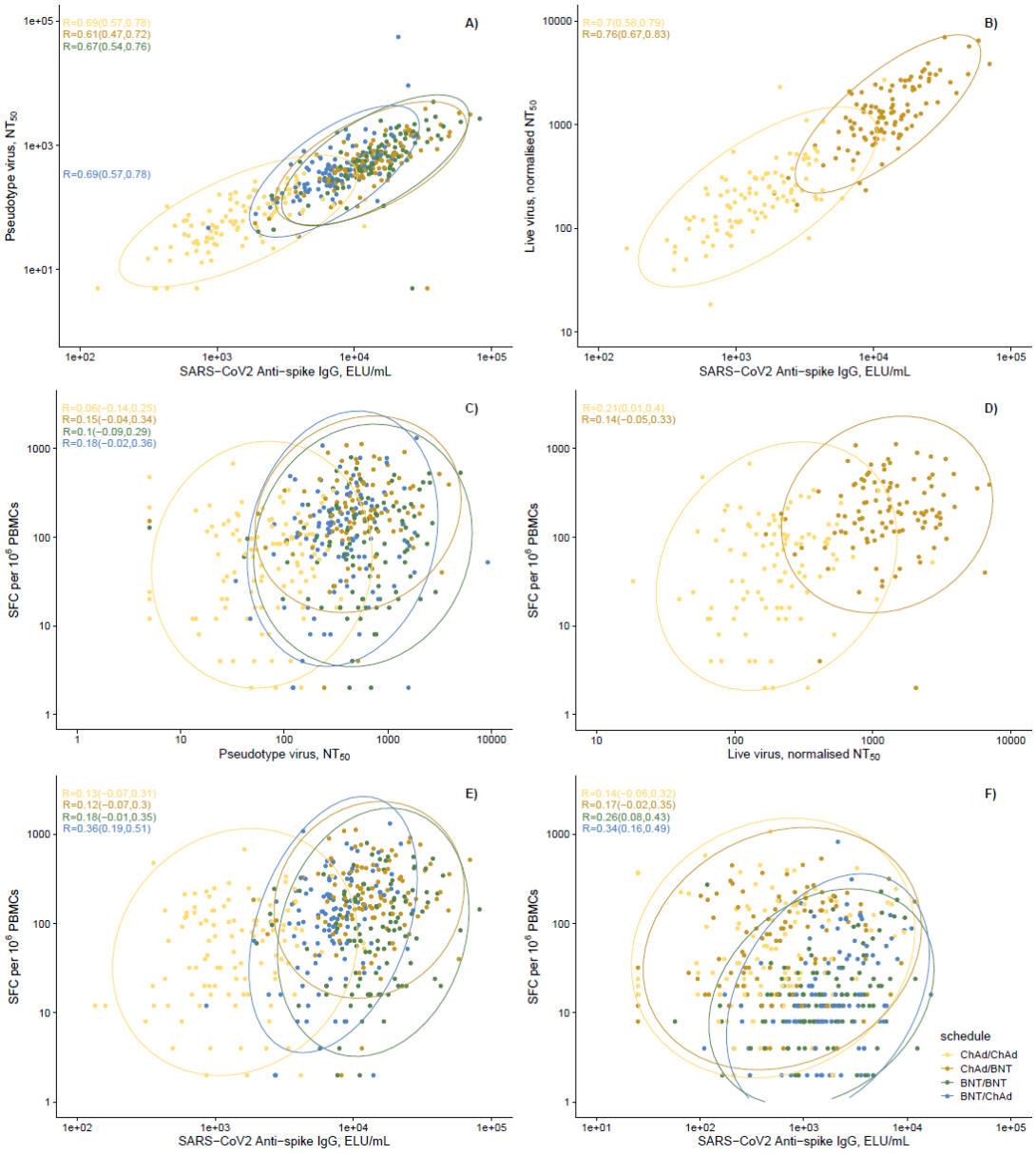


Figure 2. Subgroup analyses for immune responses between heterologous and homologous prime/boost schedules at 28 days post boost dose in the 28-day boost study arms

GMRs were adjusted for randomisation stratification variables, including study site and cohort; two-sided 95% CI are presented; the vertical dotted line represents a GMR of one.


A)
R=0.69(0.57,0.78)
R=0.61(0.47,0.72)
R=0.67(0.54,0.76)
R=0.69(0.57,0.78)
Pseudotype virus, NT50
SARS−CoV2 Anti−spike IgG, ELU/mL
B)
R=0.7(0.58,0.79)
R=0.76(0.67,0.83)
Live virus, normalised NT50
SARS−CoV2 Anti−spike IgG, ELU/mL
C)
R=0.06(−0.14,0.25)
R=0.15(−0.04,0.34)
R=0.1(−0.09,0.29)
R=0.18(−0.02,0.36)
SFC per 10^6 PBMCs
Pseudotype virus, NT50
D)
R=0.21(0.01,0.4)
R=0.14(−0.05,0.33)
SFC per 10^6 PBMCs
Live virus, normalised NT50
E)
R=0.13(−0.07,0.31)
R=0.12(−0.07,0.3)
R=0.18(−0.01,0.35)
R=0.36(0.19,0.51)
SFC per 10^6 PBMCs
SARS−CoV2 Anti−spike IgG, ELU/mL
F)
R=0.14(−0.06,0.32)
R=0.17(−0.02,0.35)
R=0.26(0.08,0.43)
R=0.34(0.16,0.49)
SFC per 10^6 PBMCs
SARS−CoV2 Anti−spike IgG, ELU/mL
schedule
ChAd/ChAd
ChAd/BNT
BNT/BNT
BNT/ChAd

642

643 **Figure 3. Correlation between A) SARS-CoV-2 anti-spike IgG and Pseudotype virus neutralising antibodies, B) SARS-CoV-2 anti-spike IgG and Live virus**
644 **neutralising antibodies, C) Pseudotype virus neutralising antibodies and Cellular response by IFN-γ ELISpot, D) Live virus neutralising antibodies and**
645 **Cellular response by IFN-γ ELISpot, and E) SARS-CoV-2 anti-spike IgG and Cellular response by IFN-γ ELISpot at 28 days post boost, and F) SARS-CoV-2**
646 **anti-spike IgG and Cellular response by IFN-γ ELISpot at 28 days post prime.**

647 Ellipses show the 95% confidence intervals for different vaccine schedules assuming multivariate normal distributions. Pearson correlation coefficients (95%
648 CI) are presented for each vaccine schedule.

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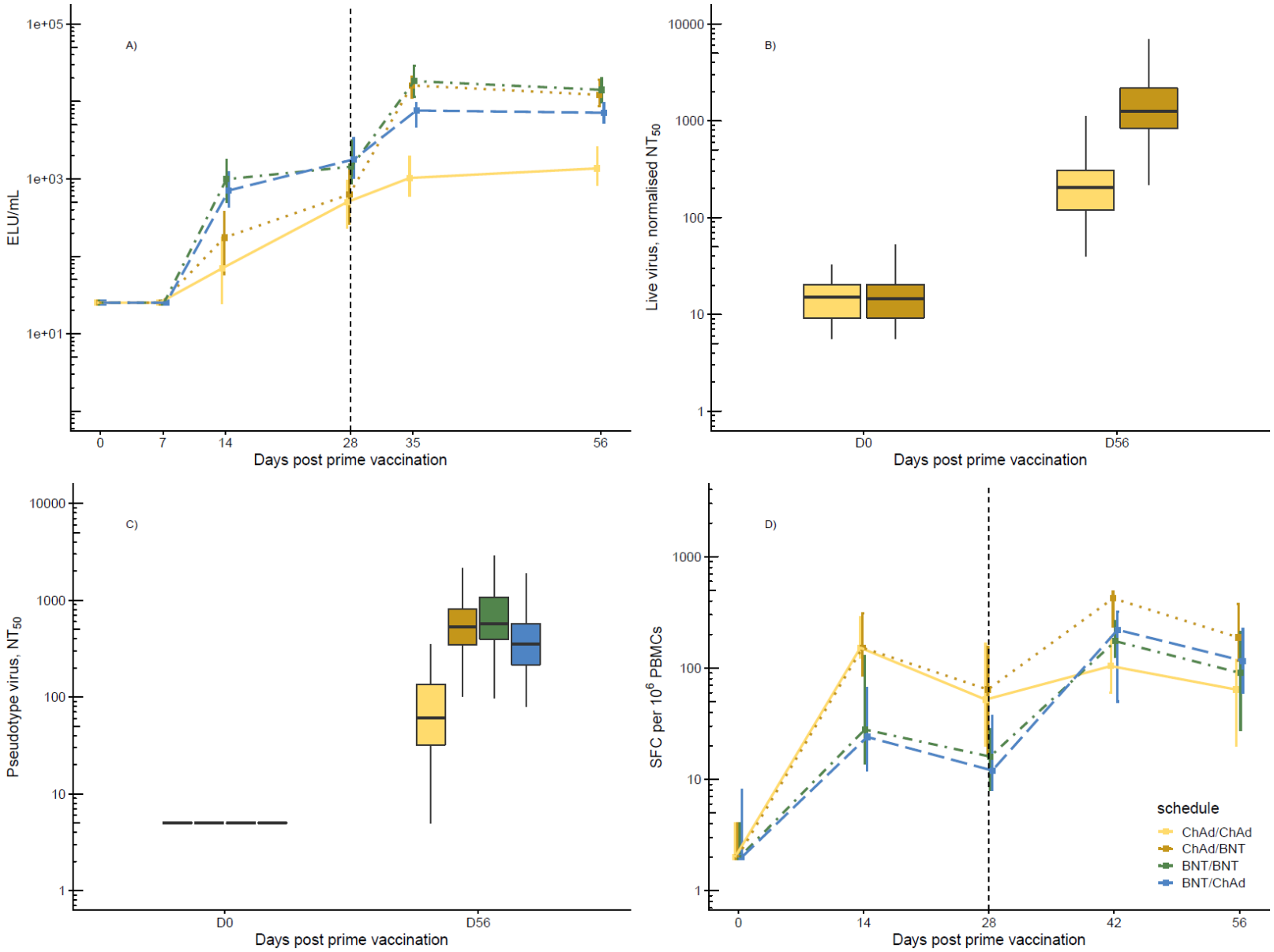

A)
ELU/mL
1e+05
1e+03
1e+01
0
7
14
28
35
56
Days post prime vaccination
B)
Live virus, normalised NT50
10000
1000
100
10
1
D0
D56
Days post prime vaccination
C)
Pseudotype virus, NT50
10000
1000
100
10
1
D0
D56
Days post prime vaccination
D)
SFC per 10^6 PBMCs
1000
100
10
1
0
14
28
42
56
Days post prime vaccination
schedule
ChAd/ChAd
ChAd/BNT
BNT/BNT
BNT/ChAd

650

Figure 4. Kinetics of immunogenicity by vaccine schedule: A) SARS-CoV-2 anti-spike IgG; B) Live virus neutralising antibodies; C) Pseudotype virus neutralising antibodies; and D) Cellular response by IFN-γ ELISpot.

For A) and D), data points are medians with IQRs. Data presented at D0, D28 and D56 are based on all participants in the modified ITT population, while data at D7, D14, 35 and D42 are for the modified ITT population in the immunology cohort only. For B) and C) boxplots for different schedules are presented at D0 and D56 in the modified ITT population. The boxplot represents the median, 25^{th} and 75^{th} percentiles, the whiskers extend up to the largest value, not greater than 1.5 times the IQR beyond the box. Values greater than this are not shown

Supplementary Appendix

Supplementary Table 1. Summary of adverse events in the 28-day boost study arms within 28 days post boost

	Prime with ChAd		Primed with BNT	
	ChAd/ChAd-28 (N=115)	**ChAd/BNT-28 (N=114)**	**BNT/BNT-28 (N=119)**	**BNT/ChAd-28 (N=115)**
Number of unique participants with at least one adverse event	44 (38.2%)	45 (39.5%)	41 (34.5%)	48 (41.7%)
Number of adverse events	74	71	81	90
Timing of adverse event				
Between prime and boost	41 (55.4%)	42 (59.2%)	40 (49.4%)	48 (53.3%)
Within 28 days post boost	33 (44.6%)	29 (40.8%)	41 (50.6%)	42 (46.7%)
Severity				
Grade 1	41 (55.4%)	40 (56.3%)	48 (59.3%)	49 (54.4%)
Grade 2	26 (35.1%)	23 (32.4%)	32 (39.5%)	34 (37.8%)
Grade 3	6 (8.1%)	8 (11.3%)	1 (1.2%)	7 (7.8%)
Grade 4	1 (1.4%)			
Causality				
No relationship	29 (39.2%)	25 (35.2%)	20 (24.7%)	25 (27.8%)
Unlikely	30 (40.5%)	27 (38.0%)	26 (32.1%)	36 (40.0%)
Possible	6 (8.1%)	9 (12.7%)	30 (37.0%)	18 (20.0%)
Probable	5 (6.8%)	6 (8.5%)	4 (4.9%)	9 (10.0%)
Definite	4 (5.4%)	4 (5.6%)	1 (1.2%)	2 (2.2%)

Supplementary Table 2. Non-serious adverse events of grade ≥3 in the 28-day boost study arms

Days to onset from prime	Days to onset from boost	Study arm	MedDRA Parent Term	MedDRA System Order Class	Duration (days)	Severity	Causality assessment
0	NA	ChAd/Chad-28	Fatigue	General disorders and administration site conditions	2	Grade 3	Possible
3	NA	ChAd/ChAd-28	Organic dust toxic syndrome~	Respiratory, thoracic and mediastinal disorders	3	Grade 3	No relationship
32	3	ChAd/ChAd-28	Limb injury	Injury, poisoning and procedural complications	1	Grade 3	No relationship
33	5	ChAd/ChAd-28	Migraine	Nervous system disorders	2	Grade 3	Unlikely
48	19	ChAd/ChAd-28	Tension headache	Nervous system disorders	1	Grade 3	Unlikely
55	27	ChAd/ChAd-28	Back pain	Musculoskeletal and connective tissue disorders	8	Grade 3	No relationship
0	NA	ChAd/BNT-28	Chills§	General disorders and administration site conditions	2	Grade 3	Definite
1	NA	ChAd/BNT-28	Meniere's disease	Nervous system disorders	2	Grade 3	Probable
15	NA	ChAd/BNT-28	Fatigue	General disorders and administration site conditions	29	Grade 3	Unlikely
38	10	ChAd/BNT-28	Tension headache	Nervous system disorders	5	Grade 3	No relationship
43	15	ChAd/BNT-28	Back pain	Musculoskeletal and connective tissue disorders	3	Grade 3	No relationship

Days to onset from prime	Days to onset from boost	Study arm	MedDRA Parent Term	MedDRA System Order Class	Duration (days)	Severity	Causality assessment
43	14	ChAd/BNT-28	Foot fracture	Musculoskeletal and connective tissue disorders	38	Grade 3	No relationship
48	20	ChAd/BNT-28	Fatigue	General disorders and administration site conditions	2	Grade 3	Unlikely
56	28	ChAd/BNT-28	Abdominal pain	Gastrointestinal disorders	8	Grade 3	No relationship
26	NA	BNT/BNT-28	Pneumonia	Respiratory, thoracic and mediastinal disorders	9	Grade 3	No relationship
28	0	BNT/ChAd-28	Depressed mood	Psychiatric disorders	2	Grade 3	No relationship
28	0	BNT/ChAd-28	Arthralgia	Musculoskeletal and connective tissue disorders	8	Grade 3	Probable
31	1	BNT/ChAd-28	Migraine	Nervous system disorders	1	Grade 3	Probable
33	5	BNT/ChAd-28	Back pain	Musculoskeletal and connective tissue disorders	3	Grade 3	Possible
44	16	BNT/ChAd-28	Viral upper respiratory tract infection*	Respiratory, thoracic and mediastinal disorders	2	Grade 3	No relationship
45	17	BNT/ChAd-28	Influenza like illness*	General disorders and administration site conditions	4	Grade 3	Unlikely

~ Participant developed respiratory irritation after performing DIY

§ Episode of rigors with fever, entered in unsolicited diary

* Tested for COVID-19 and negative

Supplementary Table 3. Adverse events of special interest* in all study arms until data lock

Days since prime	Days since boost	Study arm	MedDRA Parent Term	MedDRA System Organ Class	Duration (days)	Severity	Causality
14	N/A	BNT/ChAd-84	Anaphylactoid reaction	Immune system disorders	2	Grade 3	Unlikely
81	N/A	BNT/ChAd-84	Trigeminal palsy^	Nervous system disorders	34[#]	Grade 3	No relationship
92	64	ChAd/BNT 28	Deep vein thrombosis	Vascular disorders	19[#]	Grade 3	Unlikely

* Excluding SARS-CoV-2 infection/COVID-19

^ Secondary to trauma from dental procedure

[#]Ongoing at time of data-lock

Supplementary Table 4. Adverse event of special interest - COVID-19 cases after prime vaccination in all study arms

Days post prime	Study arm	Severity*
6	BNT/ChAd-84	Mild
1	BNT/BNT-28	Moderate A
54^	ChAd/BNT-28	Mild
3	BNT/BNT-28	Moderate A

*Severity grading as per protocol.

^ Participant had not received boost prior to infection, dose delayed due to travel

± Defined by first symptom meeting government testing criteria at that time

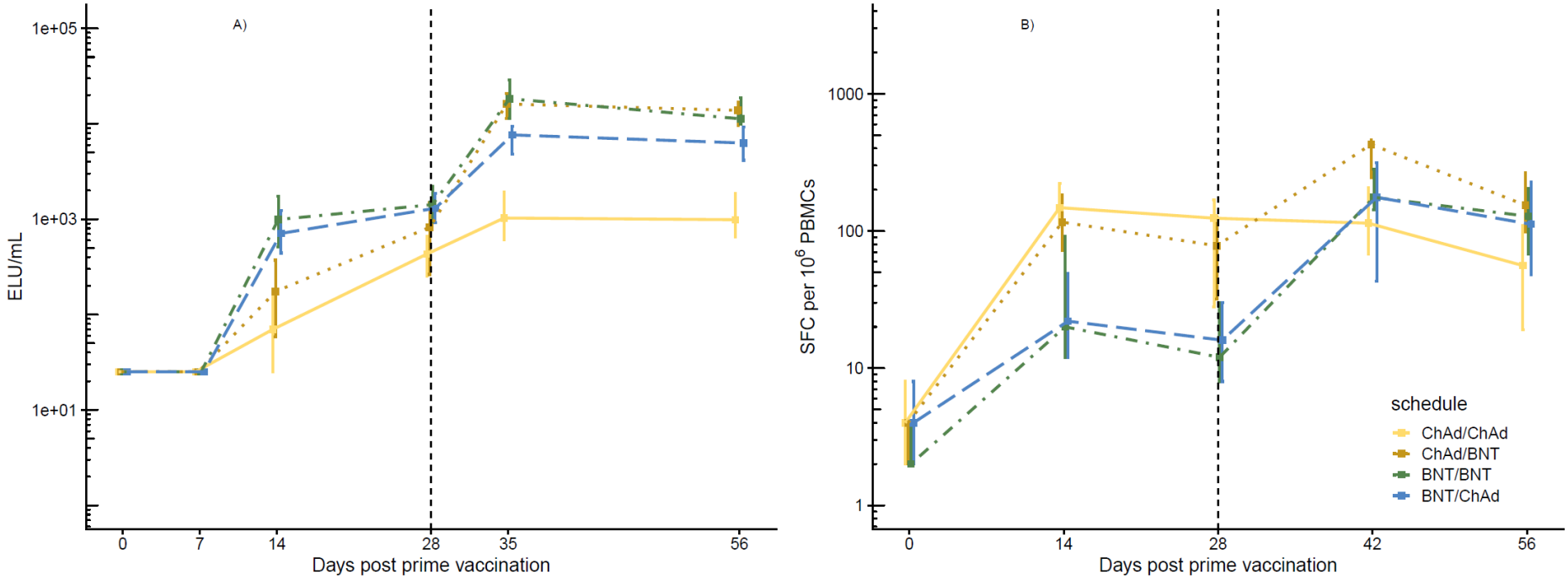
Supplementary Table 5. Serious adverse events in all study arms until data lock

Days since prime	Days since boost	Study arm	MedDRA parent term	MedDRA system organ class	Duration (days)	Causality assessment*	Serious adverse event type
7	N/A	ChAd/ChAd-28	Septic arthritis staphylococcal, Staphylococcal bacteraemia	Infections and infestations	49	Unlikely	Hospitalisation
107	23	ChAd/ChAd-84	Orchitis	Infections and infestations	5	Unlikely	Hospitalisation
85	N/A^	ChAd/ChAd-84	Fallopian tube abscess	Infections and infestations	33[#]	Not related	Hospitalisation
88	0	BNT/BNT-84	Bladder obstruction, Acute kidney injury	Renal and urinary disorders	17[#]	Not related	Hospitalisation

* See protocol for causality assessment guidance

^Boosted at D94

[#]Ongoing at time of data-lock



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686 **Supplementary Figure 1. Kinetics of immunogenicity by vaccine schedules in the immunology cohort A) SARS-CoV-2 anti-spike IgG and B) Cellular**

687 **response by IFN-γ ELISpot.**

688 Data points are medians with IQRs.

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