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Safety and immunogenicity of heterologous versus homologous prime-boost schedules with an adenoviral vectored and mRNA COVID-19 vaccine (Com-COV): a single-blind, randomised, non-inferiority trial

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Summary

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 (ChAdOx1 nCoV-19, AstraZeneca; hereafter referred to as ChAd) and an mRNA vaccine (BNT162b2, Pfizer–BioNTech; hereafter referred to as BNT) at a 4-week interval are more reactogenic than homologous schedules. Here, we report the safety and immunogenicity of heterologous schedules with the ChAd and BNT vaccines.

Methods Com-COV is a participant-blinded, randomised, non-inferiority trial evaluating vaccine safety, reactogenicity, and immunogenicity. Adults aged 50 years and older with no or well controlled comorbidities and no previous SARS-CoV-2 infection by laboratory confirmation were eligible and were recruited at eight sites across the UK. The majority of eligible participants were enrolled into the general cohort (28-day or 84-day prime-boost intervals), who were randomly assigned (1:1:1:1:1:1:1:1) to receive ChAd/ChAd, ChAd/BNT, BNT/BNT, or BNT/ChAd, administered at either 28-day or 84-day prime-boost intervals. A small subset of eligible participants (n=100) were enrolled into an immunology cohort, who had additional blood tests to evaluate immune responses; these participants were randomly assigned (1:1:1:1) to the four schedules (28-day interval only). Participants were masked to the vaccine received but not to the prime-boost interval. The primary endpoint was the geometric mean ratio (GMR) of serum SARS-CoV-2 anti-spike IgG concentration (measured by ELISA) at 28 days after boost, when comparing ChAd/BNT with ChAd/ChAd, and 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% CI of the GMR of these comparisons was greater than 0·63. The primary analysis was done in the per-protocol population, who were seronegative at baseline. Safety analyses were done among participants receiving at least one dose of a study vaccine. The trial is registered with ISRCTN, 69254139.

Findings Between Feb 11 and Feb 26, 2021, 830 participants were enrolled and randomised, including 463 participants with a 28-day prime-boost interval, for whom results are reported here. The mean age of participants was 57·8 years (SD 4·7), with 212 (46%) female participants and 117 (25%) from ethnic minorities. At day 28 post boost, the geometric mean concentration of SARS-CoV-2 anti-spike IgG in ChAd/BNT recipients (12 906 ELU/mL) was non-inferior to that in ChAd/ChAd recipients (1392 ELU/mL), with a GMR of 9·2 (one-sided 97·5% CI 7·5 to ∞). In participants primed with BNT, we did not show non-inferiority of the heterologous schedule (BNT/ChAd, 7133 ELU/mL) against the homologous schedule (BNT/BNT, 14 080 ELU/mL), with a GMR of 0·51 (one-sided 97·5% CI 0·43 to ∞). Four serious adverse events occurred across all groups, none of which were considered to be related to immunisation.

Interpretation Despite the BNT/ChAd regimen not meeting non-inferiority criteria, the SARS-CoV-2 anti-spike IgG concentrations 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 UK Vaccine Task Force and National Institute for Health Research.

Research in context

Evidence before this study

National regulatory authorities have granted emergency use authorisations for more than 15 vaccines, among which six vaccines have been approved for emergency use by WHO. Although more than 3·8 billion COVID-19 vaccines have been administered as of July 30, 2021, only approximately 28% of the global population has received at least one dose of COVID-19 vaccine, with approximately 1·1% of the population in low-income countries having received a vaccine dose. Heterologous COVID-19 vaccine schedules have the potential to accelerate vaccine roll-out worldwide, especially in low-income and middle-income countries. We searched PubMed for research articles published between database inception and June 22, 2021, using the search terms "(COVID) AND (heterologous) AND (vaccin*) NOT (BCG)" with no language restrictions. In addition to our previously published reactogenicity results, we identified two animal studies using combinations of mRNA, 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. In addition, we identified 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 or cellular responses, and 91·6% vaccine efficacy. Another clinical trial, which randomly assigned participants primed with ChAdOx1 nCoV-19 (AstraZeneca; ChAd) to receive BNT162b2 (Pfizer–BioNTech; BNT) as the boost vaccine or no boost vaccination, reported a robust immune response and an acceptable reactogenicity profile, but with no comparison to a homologous vaccine schedule. A further two preprint articles of cohort studies evaluating ChAd prime and BNT boost schedules showed similar results.

Added value of this study

We report on safety and immunogenicity in 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, and BNT/ChAd). The cellular and humoral responses of the two heterologous vaccine schedules at 28 days after the boost dose are no lower than those of 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

Now that multiple COVID-19 vaccines have been approved for emergency use, the paramount issue in addressing the COVID-19 pandemic is to optimise global vaccine coverage using the currently available vaccines. The results from our study support flexibility in the use of heterologous prime-boost schedules with ChAd and BNT, which could accelerate vaccine roll-out in some settings. Further studies are needed to examine other heterologous schedules, especially those using vaccines that are being deployed in low-income and middle-income countries.

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 (BNT162b2 or tozinameran, Pfizer–BioNTech; hereafter referred to as BNT) and the adenoviral vectored vaccine Vaxzevria (ChAdOx1 nCoV-19, AstraZeneca; hereafter referred to as ChAd), both of which are approved as two-dose homologous schedules in the UK and elsewhere.[2]

As of July 30, 2021, more than 3·8 billion COVID-19 vaccines have been administered worldwide,[3] but many more people remain unimmunised.[4] Heterologous vaccine schedules could ease logistical problems inherent in some national and international vaccine programmes. These schedules could be particularly important in low-income and middle-income countries,[5] as well as in countries that have adopted age-specific restrictions for the use of ChAd.[6–8]

Although 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,[9,10] there are currently no efficacy data using heterologous schedules incorporating COVID-19 vaccines across different platforms. Nevertheless, preclinical studies support evaluation of this approach,[11,12] and results from a randomised study in Spain suggested an increase in binding and neutralising antibody after boosting ChAd-primed participants with BNT, compared with not having a boost dose.[13] 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 a 10-week interval, with cellular responses appearing to be higher in the ChAd/BNT cohort.[14] However, we have previously reported that heterologous schedules incorporating ChAd and BNT are more reactogenic than their homologous schedules.[15]

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

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See **Online** for appendix

vaccine supply chain disruption or changes in guidance for vaccine usage. In addition, mixed schedules might induce an enhanced or more durable humoral or cellular immune response compared with licensed schedules, and might do so against a greater range of SARS-CoV-2 variants.

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

For **REDCap software** see https://www.project-redcap.org

Methods

Study design

Com-COV is a participant-blinded, randomised, phase 2, UK multicentre, non-inferiority study investigating the safety, reactogenicity, and immunogenicity of heterologous prime-boost COVID-19 vaccine schedules. Recruitment occurred at eight National Health Service and academic institutions across the UK. 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.

Four permutations of prime-boost schedules using the ChAd and BNT vaccines are compared, at two different prime-boost intervals (28 days 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 randomly assigned to receive the four vaccine schedules at either a 28-day or 84-day interval. A subset of participants (n=100, selected on the basis of site capacity and participant availability) were enrolled into an immunology cohort, in which participants were randomly assigned only 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 who were randomly assigned to vaccine schedules with a prime-boost interval of 28 days. The full protocol is provided in the appendix (pp 13–94) and online.[16]

Participants

COVID-19 vaccine-naive adults aged 50 years and older, with no or well controlled mild-to-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 (appendix pp 41–42).

Randomisation and masking

Computer-generated randomisation lists were prepared by the study statistician. Participants were block-randomised (block size four; ratio of 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; ratio of 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. In addition to stratification by cohort, randomisation was further stratified by study site. Clinical research nurses who were not involved in safety endpoint evaluation did the randomisation using REDCap version 10.6.13 and prepared and administered the vaccine.

Participants and laboratory staff processing the immunogenicity endpoints were masked to the vaccines received, but not to the 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 masked.

Procedures

Participants who met the inclusion and exclusion criteria via the online screening or the telephone screening (or both) were invited to the baseline visits (day 0). Of these, participants who passed the final eligibility assessment and provided written informed consent were randomly assigned to a study group.

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 0·5 mL intramuscular 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 0·3 mL intramuscular injection into the upper arm.

Vaccines were administered by appropriately trained trial staff at trial sites. Participants were observed for at least 15 min after vaccination. During the baseline visit, participants were given an oral thermometer, tape measure, and diary card (electronic or paper) to record solicited, unsolicited, and medically attended adverse events with instructions. The study sites' physicians reviewed the diary card regularly to record adverse events, adverse events of special interest, and serious adverse events. The timepoints for subsequent visits for immunogenicity blood sampling are shown in the protocol (appendix pp 25–26). 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.

Participants who tested 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.

Serum samples were analysed at Nexelis (Laval, Canada) to determine SARS-CoV-2 anti-spike IgG

concentrations by ELISA (reported as ELISA laboratory units [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 SARS-CoV-2 spike protein.[17] The conversion factors to international standard units can be found in the appendix (p 12). Sera from day 0 were analysed at Porton Down, Public Health England, by electrochemiluminescence immunoassay (Cobas platform, Roche Diagnostics) to determine anti-SARS-CoV-2 nucleocapsid IgG status (reported as negative if below a cutoff index of 1·0). Normalised NT_{50} for live SARS-CoV-2 virus (lineage Victoria/01/2020) was determined by microneutralisation assay (MNA), also at Porton Down, on day 0 and day 56 samples in the ChAd-primed groups only, due to limited laboratory capacity.[17] IFNγ-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.[18] 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 were multiplied by four to express frequencies per million PBMCs.

Outcomes

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

Secondary outcomes included reactogenicity, as measured by solicited local and systemic events for 7 days after immunisation (reported previously for the 28-day prime-boost interval groups[15]) and safety, as measured by unsolicited adverse events for 28 days after immunisation, medically attended adverse events for 3 months after immunisation, and adverse events of special interest and serious adverse events collected throughout the study. Blood biochemistry and haematology assessments were measured at baseline (day 0), on day of boost and 28 days after boost, with an additional day 7 post-boost timepoint (day 35) for the immunology cohort only. The detailed definition of safety outcomes can be found in the protocol (appendix pp 59–64).

Immunological secondary outcomes include SARS-CoV-2 anti-spike binding IgG concentration, cellular responses (measured by IFNγ ELISpot) in peripheral blood, and pseudotype virus neutralisation titres at days 0, 28, and 56. The immunology cohort had additional visits at days 7, 14, 35, and 42 to explore the kinetics of the immune responses further.

	ChAd/ChAd	ChAd/BNT	BNT/BNT	BNT/ChAd
General cohort				
Participants, n	90	90	93	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)
Sex				
Female	38 (42%)	40 (44%)	49 (53%)	41 (46%)
Male	52 (58%)	50 (56%)	44 (47%)	49 (54%)
Ethnicity				
White	70 (78%)	65 (72%)	76 (82%)	66 (73%)
Black	1 (1%)	1 (1%)	0	2 (2%)
Asian	13 (14%)	15 (17%)	7 (8%)	9 (10%)
Mixed	6 (7%)	6 (7%)	8 (9%)	10 (11%)
Other	0	3 (3%)	2 (2%)	3 (3%)
Comorbidities				
Cardiovascular	19 (21%)	16 (18%)	18 (19%)	21 (23%)
Respiratory	16 (18%)	11 (12%)	11 (12%)	11 (12%)
Diabetes	7 (8%)	8 (9%)	0	2 (2%)
Immunology cohort				
Participants, n	25	24	26	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)
Sex				
Female	13 (52%)	9 (38%)	12 (46%)	10 (40%)
Male	12 (48%)	15 (63%)	14 (54%)	15 (60%)
Ethnicity				
White	17 (68%)	17 (71%)	17 (65%)	18 (72%)
Black	0	0	2 (8%)	0
Asian	6 (24%)	4 (17%)	4 (15%)	4 (16%)
Mixed	2 (8%)	3 (13%)	2 (8%)	3 (12%)
Other	0	0	1 (4%)	0
Comorbidities				
Cardiovascular	7 (28%)	6 (25%)	10 (38%)	7 (28%)
Respiratory	5 (20%)	6 (25%)	6 (23%)	5 (20%)
Diabetes	6 (24%)	1 (4%)	2 (8%)	1 (4%)

Data are n (%) unless otherwise indicated. BNT=BNT162b2 vaccine, Pfizer–BioNTech. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca.

Table 1: **Baseline characteristics by cohort and vaccine schedule in the 28-day prime-boost interval study groups**

Statistical analysis

The sample size was calculated assuming the SD of the primary endpoint to be 0·4 at $\log_{10}$ scale and the true geometric mean ratio (GMR) between the homologous and heterologous group to be one. The study needed to recruit 115 participants per group 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.

The primary analysis of SARS-CoV-2 anti-spike IgG was done in participants boosted at day 28 on a per-protocol basis. The analysis population was participants

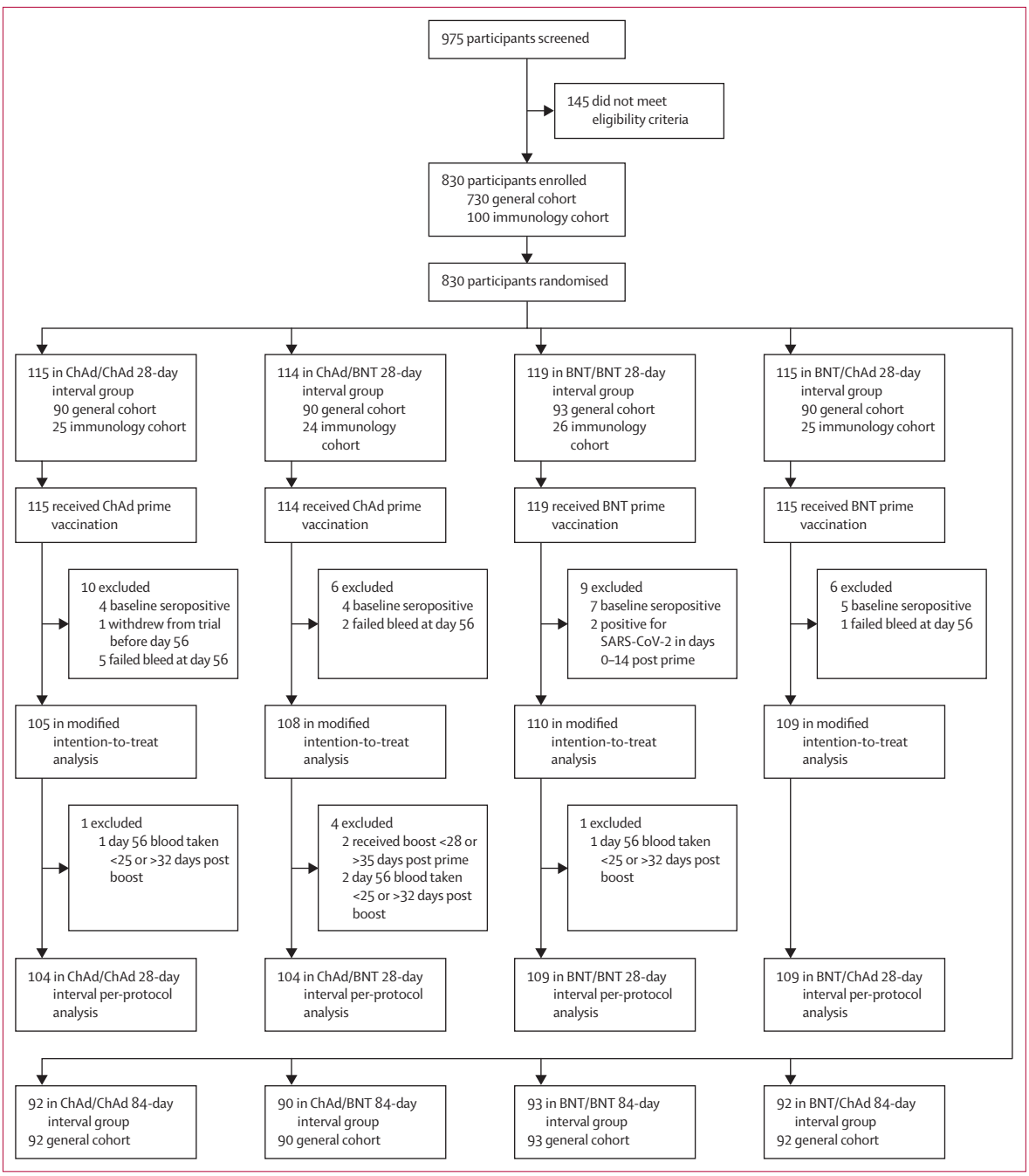

Figure 1: **Trial profile**
BNT=BNT162b2 vaccine, Pfizer–BioNTech. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca.

who were seronegative for SARS-CoV-2 at baseline (defined by anti-nucleocapsid IgG negativity at day 0 and no confirmed SARS-CoV-2 infection within 14 days after prime vaccination), whose primary endpoint data were available, and who had no protocol deviations. The 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 group and that in the homologous group (as the reference), after adjusting for study site and cohort (immunology or 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% CI to adjust for multiple testing because two primary comparisons were made. The criterion for non-inferiority of

	Prime with ChAd			Prime with BNT		
	ChAd/ChAd	ChAd/BNT	GMR*	BNT/BNT	BNT/ChAd	GMR*
Per-protocol analysis						
N	104	104	..	109	109	..
SARS-CoV-2 anti-spike IgG, ELU/mL	1392 (1188 to 1630)	12 906 (11 404 to 14 604)	9·2 (97·5% CI 7·5 to ∞)	14 080 (12 491 to 15 871)	7133 (6415 to 7932)	0·51 (97·5% CI 0·43 to ∞)
Modified intention-to-treat analysis						
N	105	108	..	110	109	..
SARS-CoV-2 anti-spike IgG						
n	105	108	..	110	109	..
Concentration, ELU/mL	1387 (1186 to 1623)	12 995 (11 520 to 14 660)	9·3 (95% CI 7·7 to 11·4)	13 938 (12 358 to 15 719)	7133 (6415 to 7932)	0·51 (95% CI 0·44 to 0·60)
Live virus neutralising antibody						
n	98	104	..	..	..	..
Normalised NT_{50}	201 (171 to 235)	1269 (1107 to 1454)	6·4 (95% CI 5·2 to 7·8)	..	..	..
Pseudotype virus neutralising antibody						
n	101	101	..	102	104	..
NT_{50}	61 (50 to 73)	515 (430 to 617)	8·5 (95% CI 6·5 to 11·0)	574 (475 to 694)	383 (317 to 463)	0·67 (95% CI 0·51 to 0·88)
Cellular response						
n	104	108	..	110	109	..
SFC per million PBMCs	48 (37 to 61)	184 (152 to 223)	3·9 (95% CI 2·9 to 5·3)	80 (63 to 101)	97 (76 to 125)	1·2 (95% CI 0·87 to 1·7)

Data shown are geometric mean (95% CI) for continuous variables. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca. BNT=BNT162b2 vaccine, Pfizer–BioNTech. GMR=geometric mean ratio. ELU=ELISA laboratory units. NT_{50}=50% neutralising antibody titre. SFC=spot-forming units. PBMC=peripheral blood mononuclear cell. *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 intention-to-treat analyses; the non-inferiority margin was 0·63.

Table 2: **Immune responses by vaccine schedule at 28 days post boost dose (56 days post prime) in the 28-day prime-boost interval study groups**

heterologous boost compared with homologous boost was for the lower limit of the one-sided 97·5% CI of the GMR to be greater than 0·63; this cutoff was chosen on a pragmatic basis to approach the WHO criterion of 0·67 for licensing new vaccines when using GMR as the primary endpoint, while still allowing rapid study delivery.[19]

According to recommended practice for non-inferiority trials,[20] we also present the two-sided 95% CI of the adjusted GMRs among the modified intention-to-treat population, which followed the per-protocol population definition but included participants whose visit timelines fell outside protocol windows, to allow a conservative estimation for superiority comparison, as secondary analyses. The heterologous group was considered superior to the homologous group if the lower limit of the two-sided 95% CI was greater than one, and the homologous group was considered superior to the heterologous group if the upper limit of the two-sided 95% CI was less than one. The geometric means of secondary immunological outcomes were reported in the modified intention-to-treat population. The proportions of participants with responses higher than the lower limit of detection or higher than the lower limit of quantification 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 groups using Fisher's exact test. Censored data reported to be below the lower limit of detection or lower limit of quantification 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. If a normal distribution could not be rendered after transformation, the Mann-Whitney *U* test was used. Correlations between different immunological outcomes were evaluated by Pearson correlation coefficients.

As an exploratory analysis, subgroup analyses were done for primary and secondary immunogenicity outcomes by age (50–59 years and ≥60 years), sex (male and female) and baseline comorbidity (presence or absence of cardiovascular disease, respiratory disease, or diabetes). p values for interaction were reported using the 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 a study vaccine 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. All statistical analyses were done using R version 3.6.2.

An independent data safety monitoring board reviewed safety data, and local trial-site physicians provided

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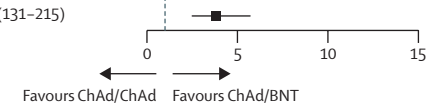
	ChAd/ChAd, geometric mean (95% CI)	ChAd/BNT, geometric mean (95% CI)	GMR (95% CI)	p value for interaction
SARS-CoV-2 anti-spike IgG, ELU/mL				
Age, years				0·93
50–59	1407 (1151–1721)	13578 (11804–15620)	9·5 (7·4–12·0)	
≥60	1348 (1053–1726)	12129 (9746–15095)	9·3 (6·6–13·0)	
Sex				0·34
Male	1230 (999–1516)	12312 (10421–14547)	10·0 (7·7–13·0)	
Female	1609 (1275–2031)	13976 (11772–16594)	8·4 (6·3–11·0)	
Comorbidity				0·57
Yes	1413 (1115–1791)	12055 (9159–15867)	8·5 (5·8–12·0)	
No	1371 (1111–1690)	13452 (11892–15216)	9·8 (7·7–12·0)	
Live virus neutralising antibody, normalised NT_{50}				
Age, years				0·3
50–59	192 (156–235)	1394 (1205–1614)	6·8 (5·4–8·7)	
≥60	221 (173–282)	1097 (846–1423)	5·3 (3·6–7·8)	
Sex				0·14
Male	170 (143–203)	1211 (1002–1465)	7·3 (5·6–9·4)	
Female	247 (187–326)	1351 (1114–1639)	5·4 (3·9–7·4)	
Comorbidity				0·72
Yes	187 (146–241)	1278 (977–1672)	6·5 (4·4–9·5)	
No	210 (171–259)	1265 (1079–1482)	6·2 (4·8–7·9)	
Pseudotype virus neutralising antibody, NT_{50}				
Age, years				0·48
50–59	57 (45–71)	525 (410–672)	9·1 (6·5–13·0)	
≥60	68 (48–98)	502 (387–651)	7·5 (4·6–12·0)	
Sex				0·77
Male	55 (41–74)	466 (356–608)	9·3 (6·3–14·0)	
Female	68 (54–85)	594 (481–734)	8·7 (6·3–12·0)	
Comorbidity				0·12
Yes	64 (47–87)	399 (267–598)	6·3 (3·8–10·0)	
No	58 (46–75)	583 (489–696)	10·0 (7·4–14·0)	
Cellular response, SFC per million PBMCs				
Age, years				0·097
50–59	43 (31–59)	202 (155–263)	4·8 (3·1–7·2)	
≥60	61 (42–87)	159 (121–207)	2·7 (1·7–4·3)	
Sex				0·0081
Male	36 (25–51)	197 (159–244)	5·8 (3·9–8·7)	
Female	68 (49–93)	167 (118–238)	2·6 (1·6–4·1)	
Comorbidity				0·73
Yes	54 (38–77)	224 (169–296)	4·1 (2·5–6·6)	
No	44 (32–62)	168 (131–215)	3·8 (2·5–5·7)	



(Figure 2 continues on next page)

oversight of all adverse events in real time. The trial is registered with ISRCTN, 69254139.

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

Between Feb 11 and Feb 26, 2021, 975 participants were screened at eight study sites across England, among whom 830 were enrolled in the study and randomised. 463 participants were randomly assigned to the four groups with a 28-day prime-boost interval reported in this study, including 100 participants enrolled into the immunology cohort. The mean age of participants was 57·8 years (SD 4·7), with 212 (46%) female participants and 117 (25%) from ethnic minorities. Baseline characteristics were well balanced across the four groups in both the general and immunology cohorts (table 1). At baseline, 20 (4%) participants were positive for anti-nucleocapsid IgG (cutoff index ≥1·0), evenly distributed

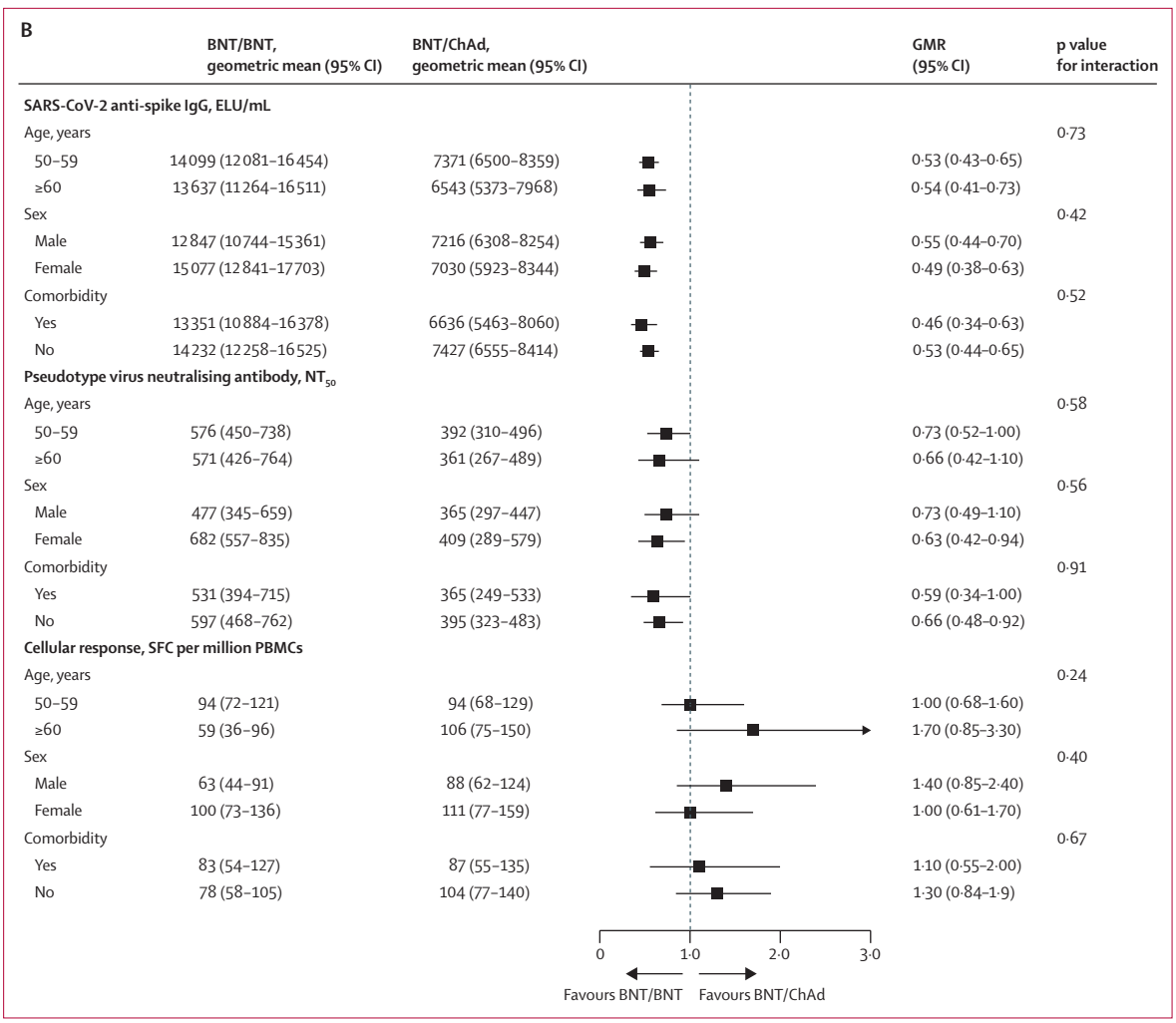


Figure 2: **Subgroup analyses for immune responses by vaccine schedule at 28 days post boost dose in the 28-day boost study groups**
GMRs were adjusted for randomisation stratification variables, including study site and cohort. Two-sided 95% CIs are presented. BNT=BNT162b2 vaccine, Pfizer–BioNTech. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca. ELU=ELISA laboratory units. GMR=geometric mean ratio. NT_{50}=50% neutralising antibody titre. PBMC=peripheral blood mononuclear cell. SFC=spot-forming units.

across groups. 432 participants were included in the modified intention-to-treat analysis and 426 were included in the per-protocol analysis (figure 1).

Among participants primed with ChAd, the geometric mean concentration of SARS-CoV-2 anti-spike IgG at 28 days post boost vaccination was 1392 ELU/mL (95% CI 1188 to 1630) in the homologous vaccine schedule group (ChAd/ChAd) and 12906 ELU/mL (11404 to 14604) in the heterologous group (ChAd/BNT), with a GMR of 9·2 (one-sided 97·5% CI 7·5 to ∞) between heterologous and homologous groups in the per-protocol analysis (table 2). The lower limit of the one-sided 97·5% CI of the GMR was greater than the margin of 0·63, indicating non-inferiority of ChAd/BNT compared with ChAd/ChAd. Similar SARS-CoV-2 anti-spike IgG concentrations were observed in the modified intention-to-treat analysis, with a GMR of 9·3 (two-sided 95% CI 7·7 to 11·4). The GMR of MNA NT_{50} between the heterologous and homologous groups was 6·4 (two-sided 95% CI 5·2 to 7·8) and the GMR of PNA NT_{50} was 8·5 (6·5 to 11·0) in the modified intention-to-treat analysis. Cellular responses by T-cell ELISpot were greater for ChAd/BNT than ChAd/ChAd, with a GMR of 3·9 (2·9 to 5·3; table 2). These results indicate that the ChAd/BNT schedule was statistically superior to the ChAd/ChAd schedule in terms of the SARS-CoV-2 anti-spike IgG, MNA NT_{50}, PNA NT_{50}, and cellular responses.

Among participants primed with BNT, the geometric mean concentration of SARS-CoV-2 anti-spike IgG at 28 days post boost vaccination was 14080 ELU/mL (95% CI 12491 to 15871) in the homologous group (BNT/BNT) and 7133 ELU/mL (6415 to 7932) in the heterologous group (BNT/ChAd), with a GMR of 0·51 (one-sided 97·5% CI 0·43 to ∞) in the per-protocol analysis (table 2). The study therefore failed to show non-inferiority of BNT/ChAd compared with BNT/BNT.

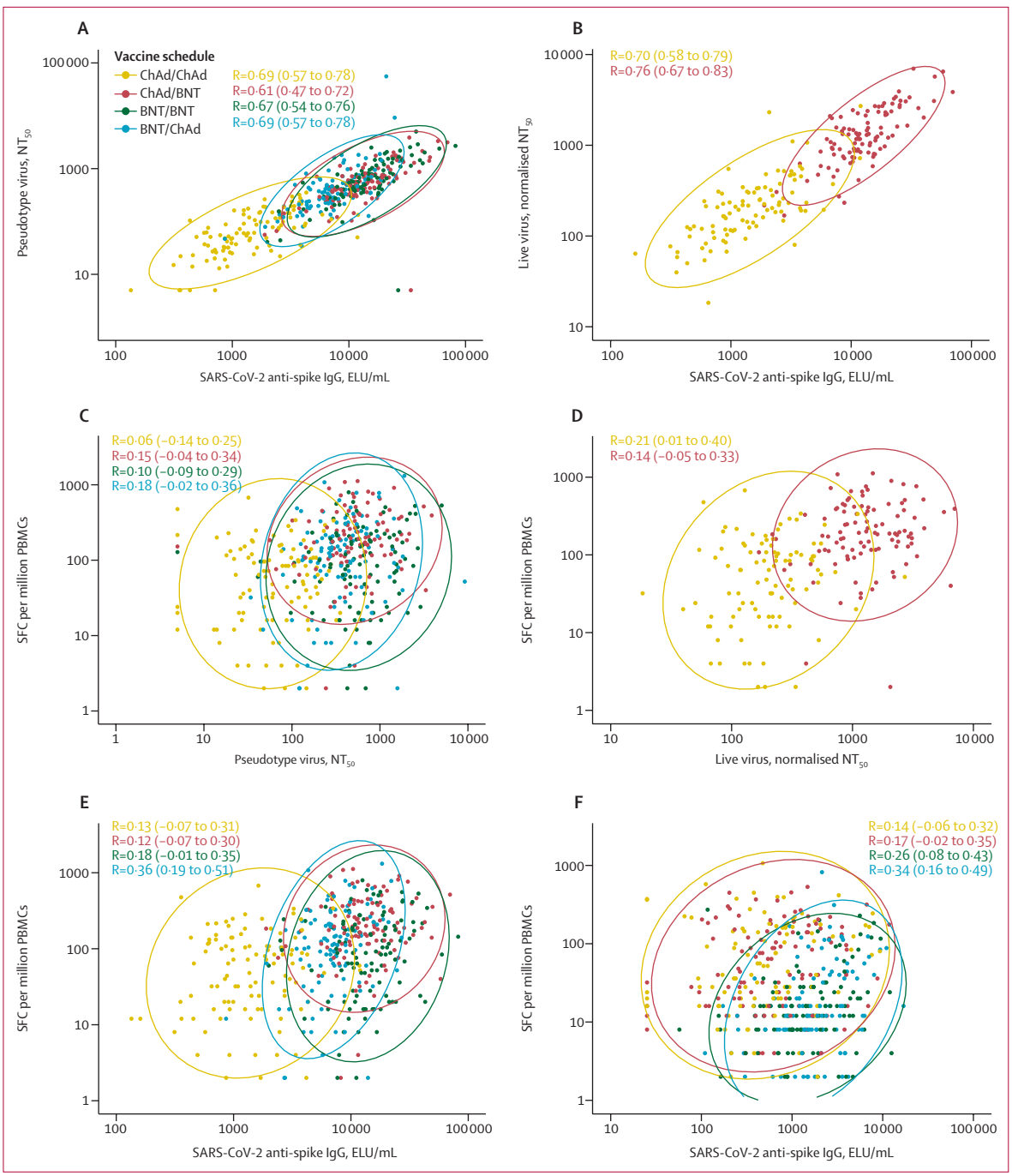


Figure 3: **Correlations between immune responses by vaccine schedule**
Correlations at 28 days post boost dose were analysed between SARS-CoV-2 anti-spike IgG and pseudotype virus neutralising antibodies (A), between SARS-CoV-2 anti-spike IgG and live virus neutralising antibodies (B), between pseudotype virus neutralising antibodies and cellular response by IFNγ ELISpot (C), between live virus neutralising antibodies and cellular response by IFNγ ELISpot (D), and between SARS-CoV-2 anti-spike IgG and cellular response by IFNγ ELISpot (E). The correlation between SARS-CoV-2 anti-spike IgG and cellular response by IFNγ ELISpot was also analysed at 28 days post prime dose (F). Ellipses show the 95% CIs for different vaccine schedules, assuming multivariate normal distributions. Pearson correlation coefficients (95% CIs) are presented for each vaccine schedule. BNT=BNT162b2 vaccine, Pfizer–BioNTech. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca. ELU=ELISA laboratory units. NT_{50}=50% neutralising antibody titre. PBMC=peripheral blood mononuclear cell. SFC=spot-forming units.

In addition, BNT/ChAd was statistically inferior for both SARS-CoV-2 anti-spike IgG (GMR 0·51, 95% CI 0·44 to 0·60) and PNA NT_{50} (0·67, 0·51 to 0·88) compared with BNT/BNT in modified intention-to-treat analyses. The geometric mean SFC frequency (T-cell ELISpot) was higher in the heterologous group compared with the

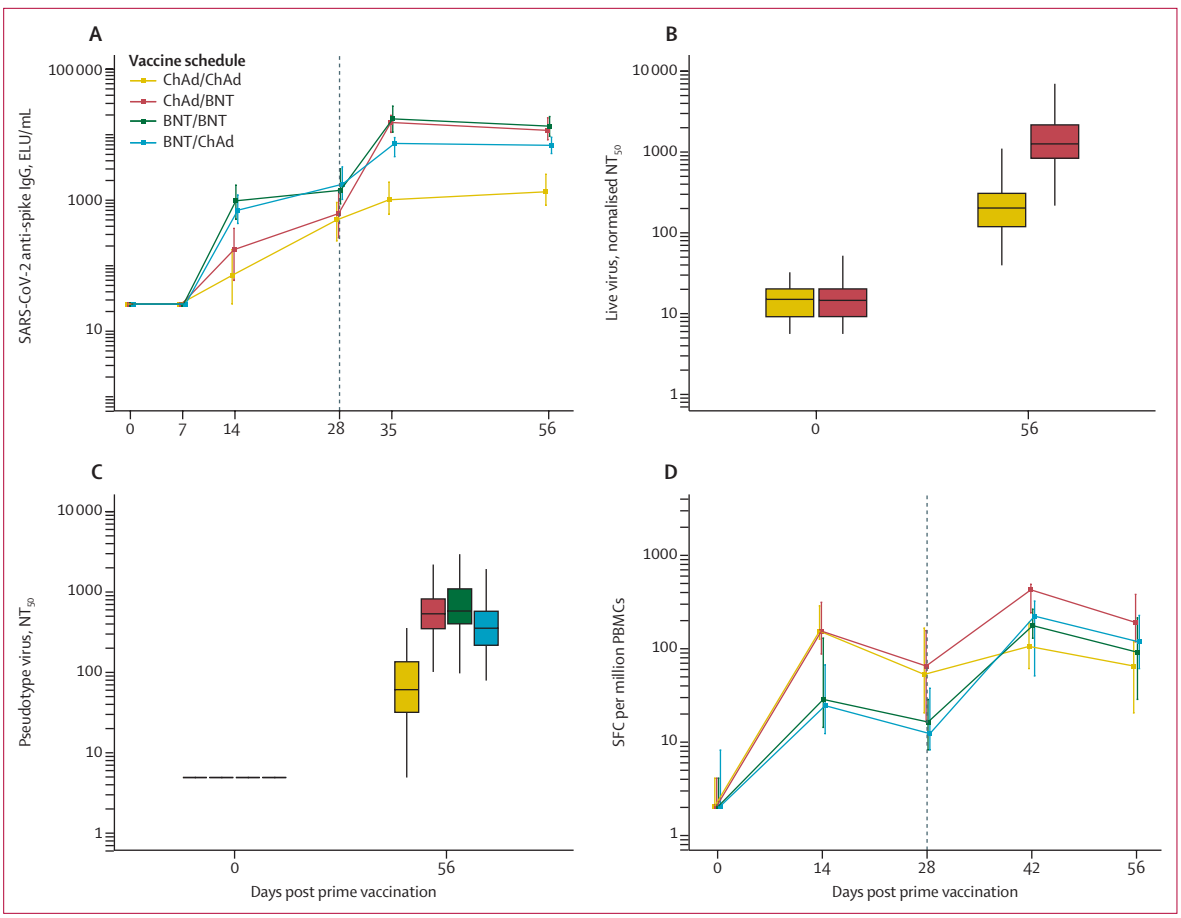


Figure 4: **Kinetics of immunogenicity by vaccine schedule**
Data presented at days 0, 28, and 56 are based on all participants in the modified intention-to-treat population, whereas data at days 7, 14, 35, and 42 are for the modified intention-to-treat population in the immunology cohort only. In parts A and D, data points are medians with IQRs. In parts B and C, the boxplots represent the median and 25th and 75th percentiles; the whiskers extend up to the largest value, not greater than 1·5 times the IQR beyond the box (values greater than this cutoff are not shown). BNT=BNT162b2 vaccine, Pfizer–BioNTech. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca. ELU=ELISA laboratory units. NT_{50}=50% neutralising antibody titre. PBMC=peripheral blood mononuclear cell. SFC=spot-forming units.

homologous group, although the difference was not statistically significant (GMR 1·2, two-sided 95% CI 0·87 to 1·7).

Similar patterns of GMRs were found in all subgroup analyses, with SARS-CoV-2 anti-spike IgG and PNA NT_{50} consistently higher in the ChAd/BNT group compared with the ChAd/ChAd group and higher in the BNT/BNT group compared with the BNT/ChAd group (figure 2). Strong correlations were found between SARS-CoV-2 anti-spike IgG and PNA NT_{50}, and between SARS-CoV-2 anti-spike IgG and MNA NT_{50} at 28 days post boost (Pearson correlation coefficients of 0·6–0·7), whereas the correlations between humoral responses and cellular response were weak (Pearson correlation coefficients <0·4) across all vaccine schedules (figure 3).

Across all four schedules in the immunology cohort, 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, table 3, appendix p 8). 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 occur 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 p 8).

At 14 days post prime, the SARS-CoV-2 anti-spike IgG geometric mean concentration was 129 ELU/mL (95% CI 83–200) in participants primed with ChAd and 843 ELU/mL (658–1081) in participants primed with BNT (p<0·0001). At 28 days post prime, the concentration was 555 ELU/mL (469–657) in participants primed with ChAd and 1597 ELU/mL (1407–1812) in participants primed with BNT (p<0·0001).

By 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 SFC per million PBMCs

	Prime with ChAd			Prime with BNT		
	ChAd/ChAd (n=105)	ChAd/BNT (n=108)	p value*	BNT/BNT (n=110)	BNT/ChAd (n=109)	p value*
SARS-CoV-2 anti-spike IgG						
Day 7†‡						
n	21	19	..	23	23	..
Median, ELU/mL	25 (25–25)	25 (25–25)	..	25 (25–25)	25 (25–25)	0·95
≥50·3 ELU/mL	0 (0%, 0–16)	0 (0%, 0–18)	>0·99	2 (9%, 1–28)	2 (9%, 1–28)	>0·99
Day 14†						
n	21	19	..	23	23	..
Geometric mean, ELU/mL	87 (54–141)	198 (96–408)	0·041	967 (718–1304)	735 (495–1092)	0·39
≥50·3 ELU/mL	14 (67%, 43–85)	16 (84%, 60–97)	0·28	23 (100%, 85–100)	23 (100%, 85–100)	>0·99
Day 28						
n	105	108	..	110	109	..
Geometric mean, ELU/mL	501 (394–638)	613 (485–776)	0·22	1487 (1233–1795)	1715 (1447–2033)	0·28
≥50·3 ELU/mL	100 (95%, 89–98)	104 (96%, 91–99)	0·75	110 (100%, 97–100)	109 (100%, 97–100)	>0·99
Day 35†						
n	22	20	..	22	24	..
Geometric mean, ELU/mL	1151 (825–1605)	15 365 (11 764–20 068)	<0·0001	17011 (12 446–23 248)	6798 (5060–9133)	<0·0001
≥50·3 ELU/mL	22 (100%, 85–100)	20 (100%, 83–100)	>0·99	22 (100%, 85–100)	24 (100%, 86–100)	>0·99
Cellular response						
Day 14†						
n	21	19	..	23	23	..
Geometric mean, SFC per million PBMCs	182 (133–251)	136 (83–223)	0·21	37 (17–64)	32 (20–51)	0·92
≥4 SFC per million PBMCs	21 (100%, 84–100)	19 (100%, 82–100)	>0·99	22 (96%, 78–100)	23 (100%, 85–100)	>0·99
≥24 SFC per million PBMCs	21 (100%, 84–100)	17 (89%, 67–99)	0·22	12 (52%, 31–73)	12 (52%, 31–73)	>0·99
Day 28						
n	103	107	..	109	108	..
Geometric mean, SFC per million PBMCs	53 (41–69)	52 (41–66)	0·98	15 (12–18)	16 (13–20)	0·81
≥4 SFC per million PBMCs	101 (98%, 93–100)	107 (100%, 97–100)	0·24	101 (93%, 86–97)	97 (90%, 83–95)	0·48
≥24 SFC per million PBMCs	74 (72%, 62–80)	75 (70%, 60–79)	0·88	34 (31%, 23–41)	35, (32%, 24–42)	0·88
Day 42†						
n	22	18	..	22	23	..
Geometric mean, SFC per million PBMCs	97 (60–157)	375 (266–528)	0·0022	135 (83–219)	130 (69–243)	0·87
≥4 SFC per million PBMCs	22 (100%, 85–100)	18 (100%, 81–100)	>0·99	22 (100%, 85–100)	22 (96%, 78–100)	>0·99
≥24 SFC per million PBMCs	19 (86%, 65–97)	18 (100%, 81–100)	0·24	19 (86%, 65–97)	20 (87%, 66–97)	>0·99

Data shown are geometric mean (95% CI) for continuous variables, and n (%, 95% CI) for binary variables, unless otherwise indicated; 50·3 ELU/mL was the LLOQ for SARS-CoV-2 anti-spike IgG; 4 SFC per million PBMCs was the LLOD and 24 SFC per million PBMCs was the LLOQ for cellular response. ChAd=ChAdOx1 nCoV-19 vaccine, AstraZeneca. BNT=BNT162b2 vaccine, Pfizer–BioNTech. ELU=ELISA laboratory units. SFC=spot-forming units. PBMC=peripheral blood mononuclear cell. LLOQ=lower limit of quantification. LLOD=lower limit of detection. *For continuous variables, p values were reported using linear regression model adjusting for age, sex, study site, and cohort (where applicable); Fisher's exact test was used to report p values for binary variables. †Immunology cohort only. ‡Data shown are median (IQR) due to a high proportion of censored data; p values were reported using Mann-Whitney *U* test.

***Table 3*: Immune responses by vaccine schedule at weekly timepoints post prime dose in the 28-day prime-boost interval study groups**

(95% CI 119–211) for ChAd versus 32 SFC per million PBMCs (22–47) for BNT, and at 28 days was 53 SFC per million PBMCs (44–63) for ChAd versus 15 SFC per million PBMCs (13–18) for BNT.

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 after 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 after a homologous second dose of ChAd (figure 4, appendix p 8), a significant increase in cellular response was seen after a homologous boost with BNT, such that those

receiving BNT/BNT had a significantly higher number of SARS-CoV-2-specific T cells per million PBMCs than ChAd/ChAd (p=0·0028) at 28 days post boost with a 28-day interval (figure 4).

Solicited adverse events in the week after immunisation have been reported previously.[15] In summary, we observed an increase in systemic reactogenicity after boost in participants receiving heterologous schedules compared with homologous schedules with the same prime vaccine. In participants randomly assigned to 28-day interval groups, there were 316 adverse events in 178 participants up to 28 days after boost immunisation (appendix p 2). No significant difference was observed between the vaccine schedules in the proportion of participants with at least one adverse event (p=0·89). Adverse events of grade 3 or higher are described in the appendix (pp 3–4).

Among all participants up to June 6, 2021 (date of data lock), there were seven adverse events of special interest, of which four were COVID-19 diagnoses (appendix pp 5–6). The non-COVID-19 adverse events of special interest were not considered to be related to immunisation. Three of the cases of COVID-19 diagnosis were within 7 days of prime immunisation; one was 54 days after prime immunisation, and the individual had not received their planned 28-day boost due to travel (appendix p 6). There were four serious adverse events across all groups in the study up to the data lock, none of which were considered to be related to immunisation (appendix p 7).

Discussion

Our findings show that all four 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.[21] Nevertheless, 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 greater than those induced by BNT/BNT immunisation. Cellular immune responses in the BNT vaccine-containing schedules were similarly all at least as high as the 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 schedule 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% efficacious against severe disease.[22] This schedule is known to be more immunogenic when administered at an 8–12-week schedule.[21] When deployed in this manner, efficacy against hospitalisation has been shown to be 86% (53–96) due to infection with the alpha variant (B.1.1.7) and 92% (75–97) with the delta variant (B.1.617.2),[23] and efficacy against symptomatic infection has been shown to be 66% (54–75) with the alpha variant and 60% (95% CI 29–77) with the delta variant. Given the established associations between humoral responses and vaccine efficacy,[21] our findings indicate that 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 2–3 months after priming with ChAd and showed a 37-fold increase in SARS-CoV-2 anti-spike IgG at 14 days post boost, higher than the 22-fold increase at 7 days and 19-fold increase at 28 days post boost in this study.[13] Potential explanations for these differences include the longer prime-boost interval, the different sampling timepoints, and a younger population in the Spanish study.[13] Fold increases in the cellular response were, however, similar (four-fold in the Spanish study versus 3·7-fold in this study). Early results from a prospective cohort study in Germany, which compared health-care 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.[14] Another German cohort study of 26 participants aged 25–46 years receiving a ChAd/BNT schedule with an 8-week prime-boost interval reported a robust humoral immune response, with a suggestion of better retention of neutralising activity against beta (B.1.351) and delta variants than that observed in a non-randomised cohort receiving BNT/BNT.[24]

Together with the finding 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 schedule options. However, in contrast with recent non-randomised and non-blinded studies, we did observe increased reactogenicity in the 28-day ChAd/BNT schedule,[15] compared with ChAd/ChAd. This discrepancy could 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-to-moderate symptoms were transient, they do need to be taken into consideration when deploying the ChAd/BNT schedule, especially in individuals younger than the participants enrolled in this study, given the reported trend towards increased reactogenicity with decreasing age.[25,26] Additional considerations for deployment of mixed schedules include potential logistical challenges within the health-care infrastructure, as well as the complex public communications surrounding this strategy.

Numerous other randomised heterologous prime-boost COVID-19 vaccine studies are now underway or planned,[27] including Com-COV2, which incorporates vaccines manufactured by Moderna and Novavax.[16] Crucially, several of these studies include vaccines manufactured by CanSinoBIO, Gamaleya Research Institute of Epidemiology and Microbiology, and Sinovac that are extensively used in low-income and middle-income countries, which are potentially more likely to rely on mixed schedules. These data on heterologous vaccination will also inform third-dose booster immunisation programmes, currently being considered in preparation for the northern hemisphere 2021–22 winter[28] and being studied in the ongoing COV-Boost study.

For more on the **COV-Boost study** see https://www.covboost.org.uk

This study has several limitations. First, 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,[21,29,30] the extent to which variations in these measures above a specific, unknown, threshold affect protection against severe disease is unclear. 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 might be expected), and this will be evaluated at study visits up to 1-year post enrolment. An additional limitation is the generalisability of these results to a younger population given the age (50–70 years) of participants in this trial. Previous randomised trials 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,[25,31,32] and there is no reason to expect this pattern would be different for the heterologous schedules, but it has not been extensively shown. 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.[33] A longer prime-boost interval is reported to result in a higher post-boost SARS-CoV-2 anti-spike IgG response for ChAd/ChAd[21] and for BNT/BNT,[34] 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 that the heterologous and homologous schedules of ChAd and BNT can induce robust immune responses with a 4-week prime boost interval. These results support 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 for vaccines being deployed in low-income and middle-income countries.

Contributors

MDS and JSN-V-T conceived the trial and MDS is the chief investigator. MDS, ASVS, RHS, and XL contributed to the protocol and design of the study. ASVS, ELP and RHS led the implementation of the study. XL and MG did the statistical analysis and have verified the underlying data. ASVS, 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. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

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. 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 on Immunisation. He is an investigator 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 GlaxoSmithKline, VPI Pharmaceuticals, 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 or provides 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. 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. He 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 the ChAd 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. All other authors declare no competing interests.

Data sharing

The study protocol is provided in the appendix (pp 13–94). 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.

Acknowledgments

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References

1 WHO. Impact of COVID-19 on people's livelihoods, their health and our food systems. Oct 13, 2020. https://www.who.int/news/item/13-10-2020-impact-of-covid-19-on-people%27s-livelihoods-their-health-and-our-food-systems (accessed June 10, 2021).

2 WHO. Status of COVID-19 vaccines within WHO EUL/PQ evaluation process. 2021. https://extranet.who.int/pqweb/key-resources/documents/status-covid-19-vaccines-within-who-eulpq-evaluation-process (accessed July 30, 2021).

3 WHO. WHO coronavirus (COVID-19) dashboard. https://covid19.who.int (accessed June 5, 2021).

4 Our World in Data. Coronavirus (COVID-19) vaccinations. https://ourworldindata.org/covid-vaccinations (accessed June 20, 2021).

5 COVAX. Workshop report: COVAX clinical development & operations SWAT team workshop on "Booster and mix & match COVID-19 vaccine strategies—planning ahead in an environment of increasing complexity". June 3, 2021. https://media.tghn.org/medialibrary/2021/07/20210603_COVAX_Booster_Mix_Match_Workshop_REPORT_FINAL.pdf (accessed June 10, 2021).

6 Public Health Agency of Sweden. Information on the use of the Astra Zeneca vaccine in the vaccination of people 65 and older. 2021. https://www.folkhalsomyndigheten.se/the-public-health-agency-of-sweden/communicable-disease-control/covid-19/vaccination-against-covid-19/information-on-the-continued-use-of-the-astra-zeneca (accessed May 20, 2021).

7 French Health Authority. Covid-19: quelle stratégie vaccinale pour les moins de 55 ans ayant déjà reçu une dose d'AstraZeneca? April 9, 2021. https://www.has-sante.fr/jcms/p_3260335/en/covid-19-quelle-strategie-vaccinale-pour-les-moins-de-55-ans-ayant-deja-recu-une-dose-d-astrazeneca (accessed May 20, 2021).

8 Danish Health Authority. Denmark continues its vaccine rollout without the COVID-19 vaccine from AstraZeneca. April 14, 2021. https://www.sst.dk/en/english/news/2021/denmark-continues-its-vaccine-rollout-without-the-covid-19-vaccine-from-astrazeneca (accessed May 20, 2021).

9 Logunov DY, Dolzhikova IV, Zubkova OV, et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet* 2020; **396:** 887–97.

10 Logunov DY, Dolzhikova IV, Shcheblyakov DV, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet* 2021; **397:** 671–81.

11 Spencer AJ, McKay PF, Belij-Rammerstorfer S, et al. Heterologous vaccination regimens with self-amplifying RNA and adenoviral COVID vaccines induce robust immune responses in mice. *Nat Commun* 2021; **12:** 2893.

12 He Q, Mao Q, An C, et al. Heterologous prime-boost: breaking the protective immune response bottleneck of COVID-19 vaccine candidates. *Emerg Microbes Infect* 2021; **10:** 629–37.

13 Borobia AM, Carcas AJ, Pérez-Olmeda M, et al. Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial. *Lancet* 2021; **398:** 121–30.

14 Hillus D, Schwarz T, Tober-Lau P, et al. Safety, reactogenicity and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1-nCoV19 and BNT162b2: a prospective cohort study. *medRxiv* 2021; published online June 2. https://doi.org/10.1101/2021.05.19.21257334 (preprint).

15 Shaw RH, Stuart A, Greenland M, Liu X, Nguyen Van-Tam JS, Snape MD. Heterologous prime-boost COVID-19 vaccination: initial reactogenicity data. *Lancet* 2021; **397:** 2043–46.

16 Com-CoV. Study protocol. https://comcovstudy.org.uk/study-protocol (accessed June 17, 2021).

17 Bewley KR, Coombes NS, Gagnon L, et al. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. *Nat Protoc* 2021; **16:** 3114–40.

18 Oxford Immunotec. T-cell Xtend. http://www.oxfordimmunotec.com/international/products-services/t-cell-xtend (accessed June 16, 2021).

19 WHO. Guidelines on clinical evaluation of vaccines: regulatory expectations. 2016. https://www.who.int/biologicals/BS2287_Clinical_guidelines_final_LINE_NOs_20_July_2016.pdf (accessed June 10, 2021).

20 D'Agostino RB Sr, Massaro JM, Sullivan LM. Non-inferiority trials: design concepts and issues—the encounters of academic consultants in statistics. *Stat Med* 2003; **22:** 169–86.

21 Voysey M, Costa Clemens SA, Madhi SA, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet* 2021; **397:** 881–91.

22 AstraZeneca. AZD1222 US phase III primary analysis confirms safety and efficacy. March 25, 2021. https://www.astrazeneca.com/content/astraz/media-centre/press-releases/2021/azd1222-us-phase-iii-primary-analysis-confirms-safety-and-efficacy.html (accessed July 6, 2021).

23 Stowe J, Andrews N, Gower C, et al. Effectiveness of COVID-19 vaccines against hospital admission with the delta (B.1.617.2) variant. 2021. https://media.tghn.org/articles/Effectiveness_of_COVID-19_vaccines_against_hospital_admission_with_the_Delta_B._G6gnnqJ.pdf (accessed July 6, 2021).

24 Groß R, Zanoni M, Seidel A, et al. Heterologous ChAdOx1 nCoV-19 and BNT162b2 prime-boost vaccination elicits potent neutralizing antibody responses and T cell reactivity. *medRxiv* 2021; published online July 15. https://doi.org/10.1101/2021.05.30.21257971 (preprint).

25 Ramasamy MN, Minassian AM, Ewer KJ, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomised, controlled, phase 2/3 trial. *Lancet* 2021; **396:** 1979–93.

26 Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine. *N Engl J Med* 2020; **383:** 2603–15.

27 COVAX. Booster and mix & match COVID-19 vaccine strategies—planning ahead in an environment of increasing complexity. June 3, 2021. https://media.tghn.org/medialibrary/2021/06/20210603_Workshop_MASTER_DECK_FINAL_UPDATED_Bm5iywZ.pdf (accessed June 17, 2021).

28 Mahase E. Covid-19: booster vaccine to be rolled out in autumn as UK secures 60m more Pfizer doses. *BMJ* 2021; **373:** n1116.

29 Khoury DS, Cromer D, Reynaldi A, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med* 2021; **27:** 1205–11.

30 Feng S, Phillips DJ, White T, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. *medRxiv* 2021; published online June 24. https://doi.org/10.1101/2021.06.21.21258528 (preprint).

31 Anderson EJ, Rouphael NG, Widge AT, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. *N Engl J Med* 2020; **383:** 2427–38.

32 Walsh EE, Frenck RW Jr, Falsey AR, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. *N Engl J Med* 2020; **383:** 2439–50.

33 WHO. AstraZeneca ChAdOx1-S/nCoV-19 [recombinant], COVID-19 vaccine. May 10, 2021. https://www.who.int/publications/m/item/chadox1-s-recombinant-covid-19-vaccine (accessed June 9, 2021).

34 Parry H, Bruton R, Stephens C. Brown, Zuo J, Moss P. Extended interval BNT162b2 vaccination enhances peak antibody generation in older people. *medRxiv* 2021; published online May 17. https://doi.org/10.1101/2021.05.15.21257017 (preprint).