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Immunogenicity and reactogenicity of BNT162b2 booster in ChAdOx1-S-primed participants (CombiVacS): a multicentre, open-label, randomised, controlled, phase 2 trial

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Summary

Background To date, no immunological data on COVID-19 heterologous vaccination schedules in humans have been reported. We assessed the immunogenicity and reactogenicity of BNT162b2 (Comirnaty, BioNTech, Mainz, Germany) administered as second dose in participants primed with ChAdOx1-S (Vaxzevria, AstraZeneca, Oxford, UK).

Methods We did a phase 2, open-label, randomised, controlled trial on adults aged 18–60 years, vaccinated with a single dose of ChAdOx1-S 8–12 weeks before screening, and no history of SARS-CoV-2 infection. Participants were randomly assigned (2:1) to receive either BNT162b2 (0·3 mL) via a single intramuscular injection (intervention group) or continue observation (control group). The primary outcome was 14-day immunogenicity, measured by immunoassays for SARS-CoV-2 trimeric spike protein and receptor binding domain (RBD). Antibody functionality was assessed using a pseudovirus neutralisation assay, and cellular immune response using an interferon-γ immunoassay. The safety outcome was 7-day reactogenicity, measured as solicited local and systemic adverse events. The primary analysis included all participants who received at least one dose of BNT162b2 and who had at least one efficacy evaluation after baseline. The safety analysis included all participants who received BNT162b2. This study is registered with EudraCT (2021-001978-37) and ClinicalTrials.gov (NCT04860739), and is ongoing.

Findings Between April 24 and 30, 2021, 676 individuals were enrolled and randomly assigned to either the intervention group (n=450) or control group (n=226) at five university hospitals in Spain (mean age 44 years [SD 9]; 382 [57%] women and 294 [43%] men). 663 (98%) participants (n=441 intervention, n=222 control) completed the study up to day 14. In the intervention group, geometric mean titres of RBD antibodies increased from 71·46 BAU/mL (95% CI 59·84–85·33) at baseline to 7756·68 BAU/mL (7371·53–8161·96) at day 14 (p<0·0001). IgG against trimeric spike protein increased from 98·40 BAU/mL (95% CI 85·69–112·99) to 3684·87 BAU/mL (3429·87–3958·83). The interventional:control ratio was 77·69 (95% CI 59·57–101·32) for RBD protein and 36·41 (29·31–45·23) for trimeric spike protein IgG. Reactions were mild (n=1210 [68%]) or moderate (n=530 [30%]), with injection site pain (n=395 [88%]), induration (n=159 [35%]), headache (n=199 [44%]), and myalgia (n=194 [43%]) the most commonly reported adverse events. No serious adverse events were reported.

Interpretation BNT162b2 given as a second dose in individuals prime vaccinated with ChAdOx1-S induced a robust immune response, with an acceptable and manageable reactogenicity profile.

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For the French translation of the abstract see Online for appendix 1

For the Spanish translation of the abstract see Online for appendix 2

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Introduction

Active immunisation is the cornerstone of global health-care policies against COVID-19. To date, four COVID-19 vaccines have been granted conditional marketing authorisation by the European Commission, namely the mRNA vaccines BNT162b2 (Comirnaty, BioNTech, Mainz, Germany) and CX-024414 (Moderna, Cambridge, MA, USA), and the adenovirus vaccines ChAdOx1-S (Vaxzevria, AstraZeneca, Oxford, UK) and Ad26.Cov2.S (Janssen-Cilag International NV, Beerse, Belgium).

To date, the administration of both mRNA vaccines and ChAdOx1-S has followed a homologous schedule (ie, sequential administration of the same vaccine).[1] The ability to sequentially administer different COVID-19 vaccines—ie, a heterologous schedule—could be an opportunity to make vaccination programmes more flexible and reliable in response to fluctuations in supply. Additionally, these schemes are being studied for successive booster doses.

Interest in a heterologous schedule for COVID-19 vaccines came from the appearance of rare, but severe, thrombotic events with thrombocytopenia in people vaccinated with ChAdOx1-S.[2] These uncommon side-effects were more frequent in young people, resulting in the

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See Online for appendix 3

Research in context

Evidence before this study

Heterologous regimens in COVID-19 have been proposed as an option to elicit combined antibody and cellular responses resulting in stronger, broader, or longer-lasting immunity. However, no clinical evidence has been reported to date. We searched PubMed on April 15, 2021, for any article published from database inception until the date of search, without language restrictions, using the terms "heterologous" OR "heterologous vaccination" AND "vaccination" OR "vaccine" AND "COVID-19" OR "SARS-CoV-2"; however, no publications reporting reactogenicity and immune response after the use in humans of heterologous vaccination with ChAdOx1-S and BNT162b2 in COVID-19 were returned.

Added value of this study

This is, to our knowledge, the first study evaluating the immune and cellular response to a heterologous vaccination strategy against SARS-CoV-2. Administration of a dose of BNT162b2 vaccine after a first dose of ChAdOx1-S provides a strong immune humoral and cellular response.

Implications of all the available evidence

This study confirms preclinical studies and suggestions anticipating that a heterologous vaccination regimen could elicit potent combined antibody and cellular responses, which might lead to mix-and-match COVID-19 vaccine programmes. Trials directly comparing full homologous and heterologous vaccination strategies are warranted to confirm safety and vaccine effectiveness of heterologous strategies.

health authorities of several European countries[3] and Canada, among others, modifying their national immunisation strategies and reserving the ChAdOx1-S vaccine for older people. Consequently, some countries, including Sweden, France, Germany, Norway, and Denmark, advised that BNT162b2 should be administered as the booster dose in people primed with ChAdOx1-S. This advice came without supporting data regarding reactogenicity or immunogenicity of this schedule. Heterologous prime-boost strategies based on the sequential administration of two gene expression systems has been widely used for protection against different infectious diseases.[1] Spencer and colleagues[4] had shown a combination of increased SARS-CoV-2 IgG-specific titres with neutralisation ability and a robust T-helper-1-type response using a heterologous regimen based on either ChAdOx1-S or BNT162b2 as prime or booster doses in animal models,[5] which is in agreement with the clinical efficacy (91%) shown by the heterologous Ad26 and Ad5 vaccine Gam-COVID-Vac (Sputnik V, Gamaleya National Research Centre for Epidemiology and Microbiology, Moscow, Russia).[6] Shaw and colleagues[3] published initial data from the Com-COV trial showing limited, short-lived reactogenicity when heterologous schedules were used in humans.

In many countries, at the beginning of April, 2021, many people vaccinated with a first dose of ChAdOx1-S could not complete the vaccination scheme because health authorities had suspended administration of a second dose until risks were re-evaluated. This withdrawal left open the possibility of public health authorities using heterologous vaccination schedules, without data about immunogenicity outcomes in humans. We designed a randomised, controlled, phase 2 trial to evaluate immunogenicity and reactogenicity of a second dose of the mRNA vaccine BNT162b2 in people prime vaccinated with ChAdOx1-S. Here, we present reactogenicity and immunogenicity 14 days after vaccination.

Methods

Study design and participants

CombiVacS is a phase 2, multicentre, open-label, randomised, controlled trial done at five university hospitals in Spain (Hospital Universitario de Cruces, Vizcaya; Hospital Universitari Vall d'Hebron, Barcelona; Hospital Clínic de Barcelona, Barcelona; Hospital Clínico San Carlos, Madrid; and Hospital Universitario La Paz, Madrid). The trial complies with the principles of the Declaration of Helsinki and Good Clinical Practice, and was approved by the Spanish Agency of Medicines and Healthcare Products and by the ethics committee at University Hospital La Paz. The study protocol and the statistical analysis plan are provided in appendix 3 (p 28).

Our hypothesis was that immunogenicity after BNT162b2 would be superior to no vaccination in ChAdOx1-S-primed participants. Participants were healthy, or clinically stable, adults (aged 18–60 years) who had received a prime ChAdOx1-S vaccination between 8 weeks and 12 weeks (50–84 days) before the screening visit. Participants with documented RT-PCR-confirmed COVID-19, or who had been vaccinated with any other vaccine since the prime dose were excluded. A SARS-CoV-2 RT-PCR test was done at the randomisation visit, and blood samples were collected to determine baseline SARS-CoV-2 serological status. Additional key exclusion criteria were the presence of clinically significant acute illness or temperature of at least 38°C within the 24 h before the planned dose of study vaccine, clinical manifestations compatible with COVID-19, and any condition contraindicating or discouraging BNT162b2 administration, including pregnancy. Full eligibility criteria are given in the protocol (appendix 3 p 28).

Study information was disseminated using social networking, and interested candidates contacted a study site directly, at which time a personal interview was booked for study personnel to explain the study and check selection criteria. All participants provided written informed consent before enrolment.

Randomisation and masking

Participants were randomly assigned (2:1) to receive one intramuscular injection of BNT162b2 (intervention group) or maintain observation (control group). Participants assigned to the intervention group were vaccinated by health-care personnel who were aware of group allocation, but were not otherwise involved in trial procedures or data collection. If the main immunogenicity objective was met, and always under the perspective of acceptable reactogenicity, participants included in the control group would be offered BNT162b2 as a second dose at day 28. Alternatively, ChAdOx1-S might be used as a second dose in the control group if requested by the participant or established by local health authorities. The randomisation list was centrally generated using SAS, version 9.4; systematic randomisation stratified by study site, sex, and age (18–49 years and 50–59 years) was used to achieve balanced randomisation in the two study groups. The randomisation list was imported into the secure Research Electronic Data Capture platform (REDCap, version 8.7.4) used for the study electronic case report form.

Procedures

BNT162b2 was administered at the approved dose of 0·3 mL as a single intramuscular injection. All participants were clinically assessed and had blood samples drawn for safety and immunology at day 0 (randomisation and BNT162b2 dose administration). Follow-up visits on days 7 and 14 were scheduled to measure vital signs, review any adverse events, update medical and medication records, and collect blood samples. The trial is ongoing and further follow-up data will be reported in future publications.

Participants in the intervention group were observed on site for at least 15 min after BNT162b2 vaccination for safety monitoring. Any adverse events occurred up to the end of the observation period were recorded. All participants were asked to record any adverse events using an electronic diary throughout the 14-day follow-up period. Participant-recorded events were accessible to the study team online through the electronic diary, which emailed an automatic alert to the investigator when the adverse event was reported as severe by the participant. In all severe cases, the investigator contacted the participant to assess seriousness according to the adverse events severity scale. At 14 days, participants were asked about both solicited and unsolicited adverse events up to day 7, as well as unsolicited adverse events up to day 14. Intensity of adverse events was graded as mild (grade 1), moderate (grade 2), severe (grade 3), or life-threatening (grade 4). Causality of unsolicited adverse events was defined as related or unrelated to study treatment based on reasonable possibility, temporal relationship, and alternate cause criteria. Causality was also assessed in reported unsolicited adverse events. Safety definitions and a list of solicited adverse events are provided in appendix 3 (p 28).

Antigen-specific humoral immune response was analysed using two commercial immunoassays and one pseudovirus neutralisation assay. The Elecsys Anti-SARS-CoV-2 S assay (Roche Diagnostics, Mannheim, Germany) is an electrochemiluminescence immunoassay used to detect antibodies (including IgG) to the SARS-CoV-2 spike protein receptor-binding domain (RBD) on the Cobas e411 module (Roche Diagnostics, Mannheim, Germany),[7] with a measuring range from 0·4 U/mL to 250 U/mL (up to 2500 U/mL with onboard 1:10 dilution, and up to 12 500 U/mL with onboard 1:50 dilution). Values higher than 0·8 BAU/mL were considered positive. Correlation between U/mL and BAU/mL was, U is 0·972 BAU. The LIAISON SARS-CoV-2 TrimericS IgG assay (DiaSorin, Stillwater, MN, USA) is a chemiluminescence immunoassay used to detect IgG antibodies to the anti-trimeric spike glycoprotein of SARS-CoV-2 in human serum or plasma samples on the LIAISON XL (DiaSorin, Saluggia, Italy),[8] with a measuring range from 4·81 BAU/mL to 2080·00 BAU/mL. As per the manufacturer's instructions, values more than 2080·00 BAU/mL were diluted 1:20 and values higher than 33·8 BAU/mL were considered positive.[9] To measure neutralising antibody titres, diluted plasma samples were preincubated with pseudoviruses generated by cotransfection of the plasmid pNL4-3ΔenvRen and an expression vector for the viral spike (pcDNA3·1-S-CoV2Δ19-G614) and added at a concentration of 10 ng p24Gag per well to Vero E6 cells in 96-well plates. At 48 h post infection, viral infectivity was assessed by measuring luciferase activity (Renilla Luciferase Assay, Promega, Madison, WI, USA) using a 96-well plate luminometer LB 960 Centro XS³ (Berthold Technologies, Oak Ridge, TN, USA). The titre of neutralising antibodies was calculated as 50% inhibitory dose (neutralising titre 50, NT_{50}), expressed as the reciprocal of four-fold serial dilution of heat-inactivated sera (range 1:32–1:131·072), resulting in a 50% reduction of pseudovirus infection compared with control without serum. Samples below the detection threshold (1:32 serum dilution) were given 1:16 value. Positive and negative controls were included in the assay and non-specific neutralisation was assessed using a non-related pseudovirus expressing the vesicular stomatitis virus envelope. Cellular immune response was measured by quantification of interferon-γ (IFN-γ) present in plasma on overnight stimulation of whole blood with pools of SARS-CoV-2 spike peptides (2 μg/mL) or dimethyl sulfoxide control in whole blood culture, requiring only 1 mL of blood.[10,11] Cytokines were quantified using the next-generation ELISA tool, Ella (ProteinSimple,

San Jose, CA, USA). Neutralising antibodies were analysed in a subset of 198 participants randomly selected and stratified by centre, while cellular immune response was analysed in participants from the two study sites in Madrid. Full details on the pseudovirus neutralising assay and cellular immunity quantification are provided in appendix 3 (pp 20–21).

Outcomes

The primary outcome was the assessment of the humoral immune response to vaccination as per antibodies against SARS-CoV-2 spike protein titres measured by immunoassay 14 days after the BNT162b2 dose. A secondary immunogenicity outcome measure was neutralising antibody titres measured by virus neutralisation assay at day 14. 1-year safety was also planned to be assessed. The exploratory outcomes were the relationship between neutralising antibodies and antibodies against SARS-CoV-2 spike protein measured by immunoassay, and cellular response to vaccination (defined as inflammatory IFN-γ cytokine production against SARS-CoV-2 spike peptide pools at day 14). Other secondary and exploratory immunogenicity and efficacy outcomes—planned at day 28, 90, 180, or 360—are not applicable to the present analysis, but are detailed in the protocol (appendix 3 p 28).

Statistical analysis

The immunogenicity analysis population included all the participants who were randomly assigned, completed all visits, and for whom serological samples were available both on day 14 and at the baseline visit. 663 individuals were included in the immunogenicity analysis for RBD-specific and trimeric spike protein-specific IgG analysis to explore main objective of the trial. Secondary objectives to explore functionality of SARS-CoV-2-specific antibodies included 198 individuals randomly selected from both groups and, after a protocol amendment, 151 individuals from Madrid sites for cellular immunity analysis. Data were presented as geometric mean and 95% CI or, for categorical variables, number, and percentage, unless otherwise stated. For serological measurements, difference at each time—basal, 7 days, and 14 days—was evaluated using ratio of geometric means. Antibody titres against SARS-CoV-2 spike protein at 14 days was the response variable, and treatment effect was evaluated comparing the interventional group titre and control group titre. Additional post-treatment ANCOVA adjusting for pretreatment was done, with baseline immunity value, age, and sex as covariables. Additionally, reverse cumulative distribution curve (RCDC) was plotted. A subgroup analysis by sex and age groups was done at baseline and 14 days for the primary and secondary endpoints. Missing values were not imputed (appendix 3 p 19). Laboratory parameters with values below detection limit were replaced by a value equal to the lowest limit divided by two. All analyses were carried out using the statistical software SAS, version 9·4. The reactogenicity analysis population included all the participants who had received at least one dose of BNT162b2 in the interventional group regardless of the availability of data for primary endpoint analysis. Reactogenicity analyses were presented as numbers and percentages of participants who had suffered local and systemic adverse events during 7 consecutive days after each vaccination. Sample size calculation for a log-transformed outcome measure[12] was done to assess the humoral immune response against SARS-CoV-2 14 days after the dose of BNT162b2 in participants that received a previous single dose of ChAdOx1-S, as compared with no dosing. A sample size of 600 participants (n=400 in the interventional group) was required to identify a 35% increase in antibody titres in participants who received BNT162b2 (denoted G[Y1]) compared with participants who did not receive it (denoted G[Y2]) at 14 days, assuming a coefficient of variation equal to 1·2 or 1·0 and similar between groups, at least 80% power and a one-sided 1% significance level (H1 G[Y1]/G[Y2]>1). The sample size was increased by 15% due to possible non-participation.

The primary analysis included all participants who received at least one dose of BNT162b2 and who had at least one efficacy evaluation after baseline. The safety analysis included all participants who received BNT162b2.

An independent data monitoring committee consisting of independent scientists not otherwise involved in the study was appointed and reviewed, and will continue to review the data regularly during the study for safety and scientific integrity. The committee will make recommendations to the funder regarding the stopping of an intervention for harm or for futility (appendix 3 pp 25, 50).

This study is registered with EudraCT (2021-001978-37) and ClinicalTrials.gov (NCT04860739).

Role of the funding source

The funder, Institute of Health Carlos III, designed the trial in cooperation with the Spanish Clinical Trials Platform. Trial coordination, participant recruitment, and data analysis were done by the Spanish Clinical Research Network. All immunological procedures were done at Instituto de Salud Carlos III.

Results

Between April 24 and 30, 2021, 676 participants were enrolled and randomly assigned to receive BNT162b2 vaccine (intervention group n=450) or no vaccine (control group n=226). Two individuals in the intervention group and one individual in the control group withdrew consent before vaccination. 663 participants (intervention group n=441, control group n=222) were included in the immunogenicity analyses, after seven participants from the interventional group and three from the control group

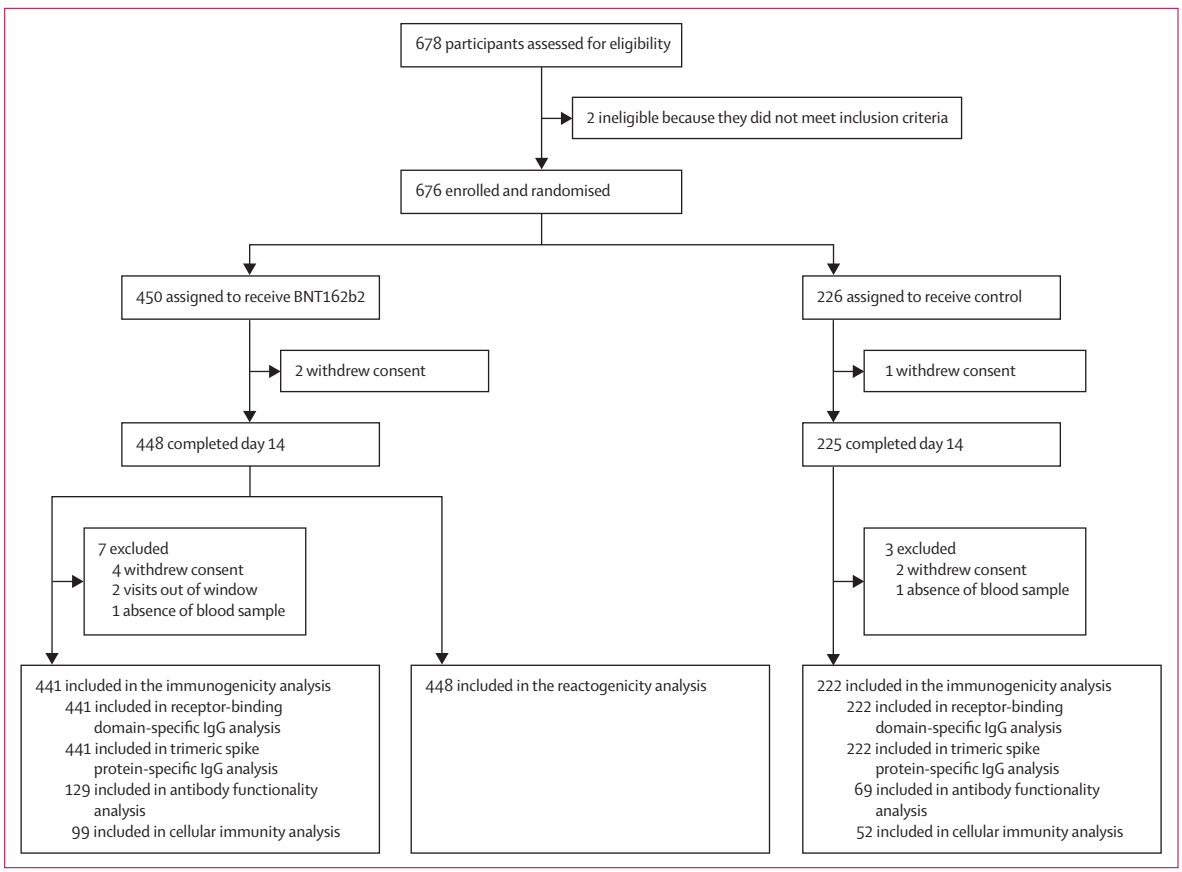

Figure 1: **Trial profile**
663 individuals were included in the immunogenicity analysis for receptor-binding domain-specific and trimeric spike protein-specific IgG analysis to explore the primary objective of the trial. Secondary objectives to explore functionality of SARS-CoV-2-specific antibodies included 198 individuals randomly selected from both groups and, after a protocol amendment, 151 individuals from Madrid sites for cellular immunity analysis.

were excluded (figure 1). 448 participants who received the second dose were included in the reactogenicity population, including one from the control group who was erroneously vaccinated. One individual was lost to follow-up after receiving the BNT162b2 dose and was excluded.

Demographics and baseline characteristics (table; appendix 3 p 2) were balanced between groups. No race or ethnicity data were collected. 382 (57%) participants were women and 294 (43%) were men. 437 (65%) participants were aged 18–49 years, and the mean age of both groups was 43·98 years (SD 8·85). Time elapsed since ChAdOx1-S administration was between 8 weeks and 9 weeks for 411 (61%) participants and between 10 weeks and 12 weeks for 263 (39%) participants.

In the intervention group, geometric mean titres (GMTs) of antibodies specific to the SARS-CoV-2 RBD at day 14 were significantly (p<0·0001) higher in the interventional group (7756·68 BAU/mL, 95% CI 7371·53–8161·96) compared with the control group (99·84 BAU/mL, 76·93–129·59; interventional:control ratio 77·69, 95% CI 59·57–101·32). Immunogenic response in the intervention group was observed at day 7

	Intervention group (n=450)	Control group (n=226)	Overall (n=676)
Sex			
Male	193 (43%)	101 (45%)	294 (43%)
Female	257 (57%)	125 (55%)	382 (57%)
Age, years	43·93 (8·88)	44·10 (8·82)	43·98 (8·85)
Age group			
18–49 years	293/450 (65%)	144/226 (64%)	437/676 (65%)
Male	123/293 (42%)	65/144 (45%)	188/437 (43%)
Female	170/293 (58%)	79/144 (55%)	249/437 (57%)
50–59 years	157/450 (35%)	82/226 (36%)	239/676 (35%)
Male	70/157 (45%)	36/82 (44%)	106/239 (44%)
Female	87/157 (55%)	46/82 (56%)	133/239 (56%)
Time since ChAdOx1-S vaccination*, weeks			
8–9	273/449 (61%)	138/225 (61%)	411/674 (61%)
10–12	176/449 (39%)	87/225 (39%)	263/674 (39%)

Data are n (%), mean (SD), or n/N (%). *Two participants were excluded: one because 7 weeks had elapsed since ChAdOx1-S vaccine, and another due to dropout on day 0.

Table: **Baseline characteristics**

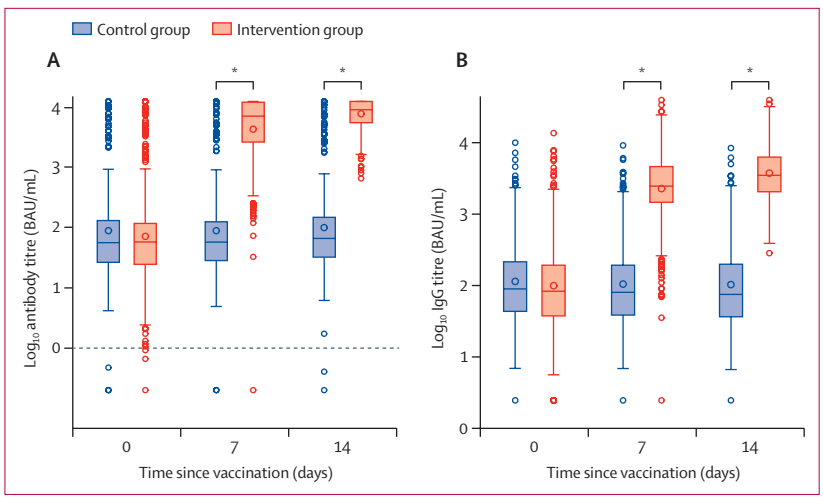

Figure 2: **Antibody titres**
Receptor-binding domain (anti-spike protein) antibody titres (A), and trimeric spike protein antibody titres (B), measured in both intervention and control groups on days 0, 7, and 14. *p<0·0001.

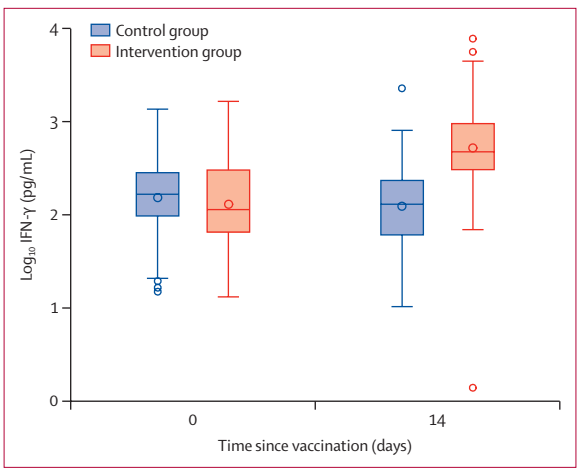

Figure 4: **IFN-γ concentrations measured in both intervention and control groups on days 0 and 14**
IFN=interferon. *p<0·0001.

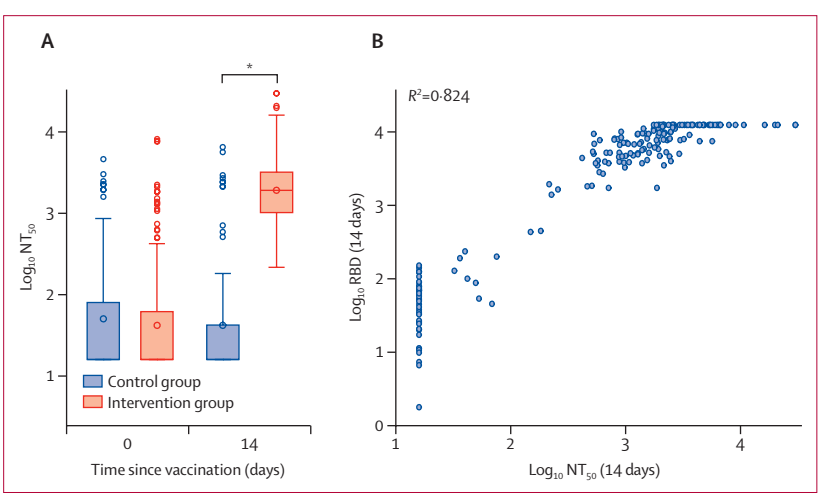

Figure 3: **Neutralisation responses**
(A) Neutralising antibodies measured in both intervention and control groups on days 0 and 14. (B) Correlation between NT_{50} and RBD (anti-spike protein) antibody titres. NT_{50}=titres that achieved 50% neutralisation. RBD=receptor-binding domain. *p<0·0001.

(intervention 4353·51 BAU/mL, 95% CI 3851·58–4920·85 *vs* control 90·05 BAU/mL, 69·16–117·27; p<0·0001; figure 2; appendix 3 p 3). When antibodies against SARS-CoV-2 spike protein were measured by a chemiluminescence immunoassay technique covering the trimeric spike protein, 14-day immunogenic response in the intervention group was statistically significant (intervention 3684·87 BAU/mL, 3429·87–3958·83 *vs* control 101·2 BAU/mL, 82·45–124·22; interventional:control ratio 36·41, 95% CI 29·31–45·23; p<0·0001), which was a 37-fold increase from baseline. Likewise, titres of antibodies at day 7 were significantly higher in the intervention group (2246·25 BAU/mL, 95% CI 2010·4–2509·78) than the control group (102·25 BAU/mL, 83·52–125·18; p<0·0001; figure 2; appendix 3 p 3). In the intervention group, at baseline, GMTs of RBD antibodies and IgG against trimeric spike protein were 71·46 BAU/mL (95% CI 59·84–85·33) and 98·40 BAU/mL (85·69–112·99), respectively. Results were similar when analysed by interval between first and second dose (appendix 3 p 4). RCDCs for RBD and trimeric spike protein antibodies are shown in appendix 3 (pp 5–6). Titres of antibodies measured by both techniques showed strong positive correlation (R^2 0·85; p<0·0001; appendix 3 p 7). Subgroup analysis showed that immunological response at day 14 was significantly lower in men in both RBD (p=0·0162) and trimeric spike protein antibodies (p<0·0001), and no differences was observed in age subgroups (appendix 3 pp 8–9).

The functional capability of the antibodies induced in the intervention group were analysed in 198 randomly selected participants (n=129 intervention group, n=69 control group). In the intervention group, 96 (74%) participants showed no or very low neutralising activity at day 0, independently of interval since prime dose (appendix 3 p 10). In comparison, 129 (100%) participants exhibited neutralising antibodies at day 14, showing high (NT_{50}>1:300 and <1:1000) or very high (NT_{50}>1:1000) activity in 126 (98%) of 129 participants (appendix 3 p 11). At day 14, the GMT of neutralising antibodies increased 45-times, from 41·84 (95% CI 31·28–55·96) to 1905·69 (1625·65–2233·98) in the intervention group, compared with 41·81 (27·18–64·32) at day 14 in the control group (p<0·0001). Moreover, this increase was observed in all participants independently of baseline NT_{50} levels (appendix 3 p 12). GMT of neutralising antibodies in the control group was not significantly different from baseline (GMT 50·84, 95% CI 33·56–76·99; figure 3; appendix 3 p 13). RCDCs for neutralising antibodies are shown in appendix 3 (p 14) for both study groups, and by baseline NT_{50} (p 15). Neutralising antibody

responses had a strong positive correlation with RBD antibody titres (R^2 0·82; p<0·0001; figure 3).

Dynamic changes of functional spike-specific T-cell response were analysed in 151 (22%) of 676 participants (n=99 intervention group, n=52 control group), showing significant levels of IFN-γ production at day 0 (GMT 129·63 pg/mL, 95% CI 103·51–162·35 intervention group *vs* 151·63 pg/mL, 114·09–201·53 control group), consistent with a previous immunisation with a single dose of ChAdOx1-S. On day 14, the production of IFN-γ had significantly increased in the intervention group (GMT 521·22 pg/mL, 422·44–643·09; p<0·0001) compared with the control group (122·67 pg/mL, 88·55–169·95; p<0·0001), in which IFN-γ production remained unchanged (figure 4). RCDCs for immunological response are shown in appendix 3 (p 16).

Reactogenicity analysis was based on solicited adverse events in 448 individuals from the intervention group, with headache (n=199 [44%]), myalgia (n=194 [43%]), and malaise (n=187 [42%]), the most commonly reported systemic reactions. Other systemic adverse reactions, including fever (n=11 [2%]), were less common (appendix 3 p 17). Injection site pain (n=395 [88%]), induration (n=159 [35%]), and erythema (n=139 [31%]) were the most commonly reported local reactions. Other local adverse reactions were less common (appendix 3 p 17). Local and systemic reactions were most frequently reported by female participants (appendix 3 p 18). No differences in adverse event frequency were observed by age groups (appendix 3 p 18). Of 1771 solicited adverse events reported in the 7 days after vaccination in the intervention group, most were mild (n=1210 [68%]) or moderate (n=530 [30%]), and self-limited. In 31 participants, the most frequent severe adverse events were malaise (n=7 [23%]), myalgia (n=6 [19%]), and headache (n=5 [16%]). All these participants were contacted and subsequently evaluated by investigators, who did not report any serious adverse events. The severity of solicited local and systemic reactions was highest on day 2 after vaccination (figure 5).

Discussion

This is, to our knowledge, the first report to show that a COVID-19 heterologous vaccination schedule induces an immune response in humans and is associated with an acceptable and manageable reactogenicity profile. The early response obtained 7 days after the second dose, and confirmed at day 14, showed a boost effect linked to the heterologous scheme. In particular, there was a robust coherence between the immune response evaluated by titres of specific antibodies against SARS-CoV-2 spike protein and the proportional increase of the functional capacity of neutralisation in the corresponding test. There was strong positive correlation observed between the two immunoassays and the pseudovirus neutralisation assay. Immune cellular response 14 days after the booster vaccine also provides support for the effectiveness of the heterologous approach. The immune response

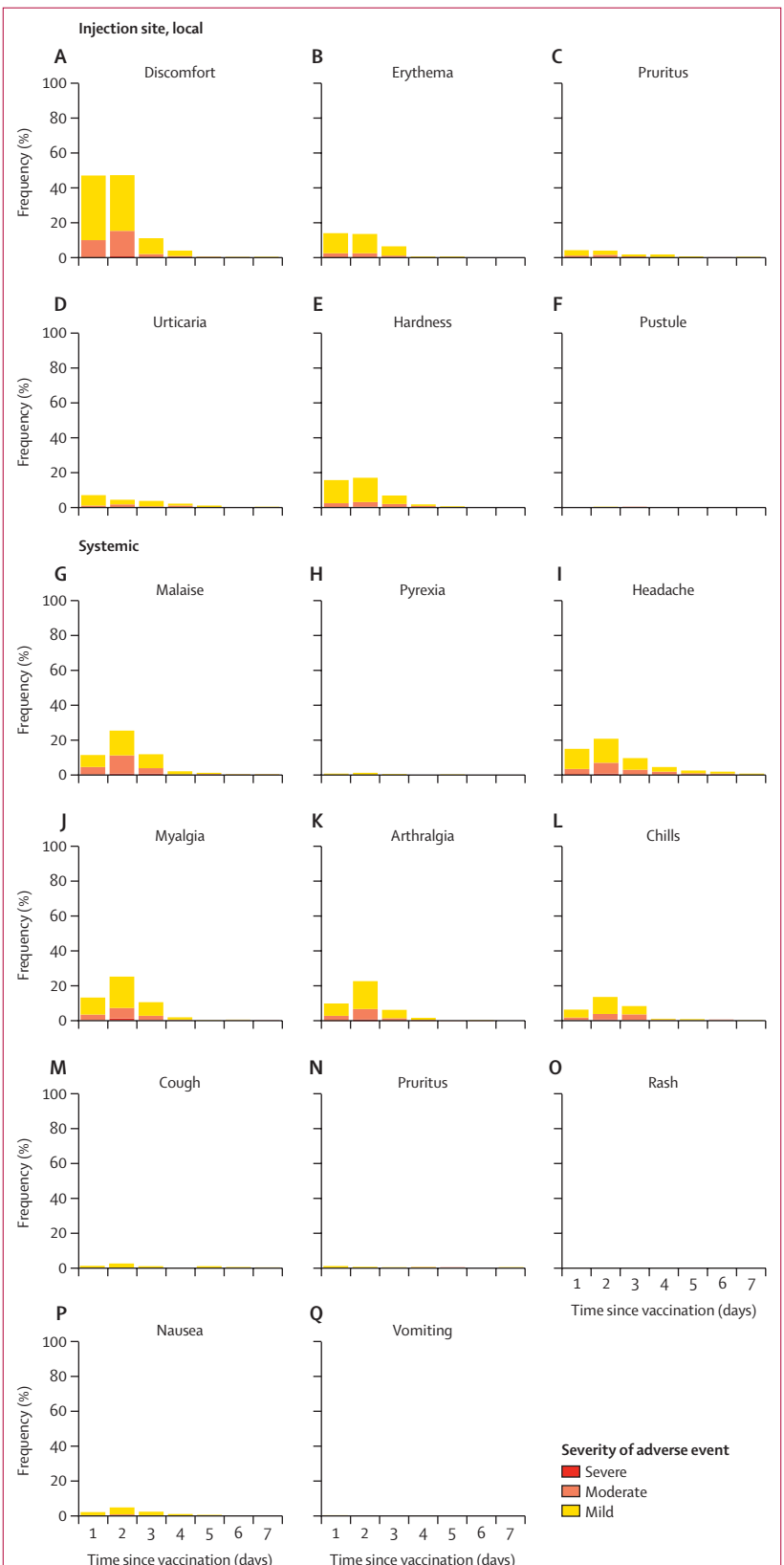

Figure 5: **Solicited local and systemic adverse reactions in first 7 days after vaccination**

with the heterologous vaccination schedule was within the range of those previously reported using homologous schedules. Data from the Oxford COVID Vaccine Trial Group showed that after a second dose of ChAdOx1-S, humoral response was associated with a ten-times increase of anti-SARS-CoV-2 spike protein IgG standardised ELISA titres, as compared with one dose.[13] Additionally, in phase 1/2 BNT162b2 trials,[14] RBD antibodies also increased from 1536 U/mL to 16 166 U/mL after the second dose of BNT162b2, and neutralising antibody titres increased from 29 GMT to 437 GMT. In phase 1/2 CX-024414 trials,[15] RBD antibodies increased from 93 231 GMT to 558 905 GMT, 2 weeks after the second vaccine.[15]

In the present study, the time between the doses was also likely to have had a role in immunogenicity and reactivity; the window to receive the second dose ranged from 8 weeks to 12 weeks, which was longer than that used in earlier homologous approaches. Two studies[12,13] and a pooled analysis of four randomised trials from the Oxford COVID Vaccine Trial Group[16] showed that the longer the interval between the first and second doses of ChAdOx1-S, the higher SARS-CoV-2 IgG spike protein-specific response. This effect was more evident in individuals younger than 55 years,[16] but was also described in people older than 80 years who were vaccinated under an extended interval between two doses of BNT162b2.[17] We also found that neutralising activity (determined using a pseudovirus assay) increased after BNT162b2 immunisation in all participants, independently of NT_{50} at baseline. 14 days after immunisation, NT_{50} was above 1:1000 in 75% and above 1:300 in 98% of participants in the intervention group. A study has reported that neutralisation level is highly predictive of immune protection, and perhaps also vaccine efficacy.[4] Baseline NT_{50} was low in our study (42–51). Levels reported in earlier studies range from an NT_{50} of 88 to 140 at day 28,[13,14] showing a sharp decrease (NT_{50} 40–70) at day 56, after the first vaccine dose.[14] Our baseline findings appear to be consistent with these results. Analysing by interval since first dose, we found that baseline NT_{50} was numerically, but not significantly, lower in participants included at weeks 10–12 (NT_{50} 36) compared with participants in weeks 8–9 (NT_{50} 51). Notwithstanding, our data are limited and interpretations from indirect comparisons must be made cautiously.

Additionally, our results indicate that the use of BNT162b2 as a second dose in a heterologous scheme increases the cellular immunity responses obtained after the initial dose of ChAdOx1-S. To date, second doses of ChAdOx1-S in homologous schedules have failed to show an improvement in the cellular response obtained after an initial dose,[12,13,18] suggesting that cellular response is maintained irrespective of vaccination interval, age, and sex after a two-dose homologous vaccination strategy with ChAdOx1-S. The enhancer effect of the second dose on the cellular immune response has been described with homologous mRNA vaccine schedules.[19–21]

The solicited adverse events profile in CombiVacS is similar to that after homologous vaccination with ChAdOx1-S[13] or BNT162b2,[22] and in a cohort of healthcare workers in Germany.[23] However, our findings differ from those reported by Shaw and colleagues.[3] Shaw and colleagues describe an increase in systemic reactogenicity after the booster dose in heterologous vaccine schedules, compared with homologous vaccine schedules—particularly in a self-reported feeling of feverishness.[3] Although participants in our study were younger than in Shaw and colleagues' study, we reported a lower frequency of reactogenicity events, which might be explained—at least in part—by the difference in administration intervals (ie, 28 days[3] *vs* 8–12 weeks). Notwithstanding, comparisons between studies should be cautious due to the differences between the studies—ie, time from the prime dose, evaluation of some adverse events (fever *vs* feverishness) or age differences. The absence of an active control group did not allow for a direct comparison with reactogenicity elicited by homologous ChAdOx1-S vaccination.

Regarding the higher frequency of adverse events reported by women in our study, women have been reported to have a stronger immune response to vaccines than men; conversely, they also present more frequent and severe adverse events.[24,25] Unfortunately, sex-disaggregated data about immunogenicity and reactogenicity have not been reported in COVID-19 vaccines trials to date. Although the data are preliminary, thromboembolitic events associated with ChAdOx1-S,[26] and anaphylaxis associated with mRNA vaccines,[27] are more frequent in females. Our data show a numerically higher incidence of unsolicited adverse events, and also a higher immune response in women than men. The trial is ongoing, and a full analysis of the effect of sex and age on reactogenicity and immunogenicity will be reported on completion of the trial.

Finally, in figures 2 and 3, the presence of individuals with elevated antibody titres at the time of randomisation is evident. If we can eliminate individual variability as the cause of these titres, we hypothesise we enrolled individuals who had been inadvertently infected some time before the start of the trial. In that case, the titres obtained in these individuals would depend directly on a heterologous combination of antigens, because they have been exposed to wild-type SARS-CoV-2 and ChAdOx1-S, which would confirm our findings. However, this hypothesis will be assessed in our study population.

A limitation of the study is the absence of a control group completing the homologous ChAdOx1-S scheme. At the time of the clinical trial design, the use of ChAdOx1-S had been suspended in Spain. Considering that supplies of other vaccine modalities were not yet able to cope with the existing demand, we used BNT162b2 because the supplies we had were adequate enough for a

potential application in real life of the eventual results emanating from the study. Consequently, we evaluated the immune response of one heterologous scheme compared with the suboptimal vaccination situation, by which those persons affected by the suspension could be left if they did not receive a second dose. We do not know whether the immunogenic response observed in our study will result in better efficacy and effectiveness—a fact that should be taken into account when considering our results in decisions regarding vaccination programmes. The adverse events in our study could also be underestimated because of the small sample size and the short period of observation. We estimate that the 4-week delay in the vaccination of the control group did not pose any ethical issue, since the data reported to date suggest that the immune response would not be worse than in the 8–12-week interval.[16,17]

In summary, our study shows a 14-day robust humoral and cellular immune response after a second dose of BNT162b2 in individuals primed with ChAdOx1-S 8–12 weeks earlier. The trial is ongoing; thus, the results of this and future studies comparing homologous and heterologous vaccination schedules will allow direct comparisons and substantiate COVID-19 vaccination decision making.

Contributors

Trial conceptualisation was done by CB-I, AMB, AJC, JA, and JF. AJC, IF, and AAg developed the study methods. AMB, MP-O, LC, MJB, JG-P, MC, AP, MG-P, EA-A, MA, FD-F, AAs, NI-A, LEB-M, CP-I, JO, and JRA were study investigators. MTGM, DL, and AGC ensured data accuracy. AMB, AJC, MP-O, DL, AGC, JO, JA, and JF were responsible for statistical analysis. CB-I, AMB, MP-O, LC, MC, MJB, AP, JO, JA, JF, and JRA supervised the study. CB-I was responsible for funding acquisition. CB-I, AMB, AJC, MP-O, JO, JA, and JF wrote the original draft of the Article. All authors reviewed and edited the manuscript, and approved the manuscript for submission. All authors reviewed and approved the original draft. All authors had full access to the full data in the study and accept responsibility to submit for publication.

Declaration of interests

CB-I is the deputy general manager of the Instituto de Salud Carlos III. JRA has received fees from Janssen, outside of the submitted work. AMB is principal investigator of clinical trials sponsored by GlaxoSmithKline, Daiichi-Sankyo, Janssen, and Farmalider, outside of the submitted work. All other authors declare no competing interests.

Data sharing

Individual participant data will be made available when the trial is complete, on request to the corresponding authors, After approval of a proposal, data will be shared through a secure online platform.

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