Reactogenicity and immunogenicity of BNT162b2 in subjects having received a first dose of ChAdOx1S: initial results of a randomised, adaptive, phase 2 trial (CombiVacS)

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ABSTRACT

Background

There are no immunological data on SARS-CoV-2 heterologous vaccinations schedules in humans. We assessed the immunogenicity and reactogenicity of BNT162b2 (Comirnaty, BioNTech) administered as second dose in participants primed with ChAdOx1-S (Vaxzevria, Astra Zeneca).

Methods

We did a phase 2, open-label, adaptive, randomised, controlled clinical trial on adults under 60 years old, vaccinated with a single dose of ChAdOx1-S between 8 and 12 weeks before screening, and no history of SARS-CoV-2 infection (EudraCT No. 2021-001978-37 and NCT04860739). Participants were randomly assigned (2:1) to receive BNT162b2 (0.3 mL, single intramuscular injection) or observation. The primary outcomes were 7-day reactogenicity and 14-day anti-spike IgG response, measured by immunoassays covering SARS-CoV-2 trimeric spike protein and receptor binding domain (RBD). Antibodies functionality and cellular immune response were assessed using a pseudovirus neutralization assay and IFN-gamma immunoassay, respectively.

Findings

Between April 24 and April 30, 2021, 676 individuals were randomized (n=450 intervention group, n=226 control group) at 5 sites in Spain, and 663 (441 and 222, respectively) completed the study up to day 14 (mean age 44 [SD 9], 56·5% female). In the intervention group, geometric mean titres (GMT) of IgG-RBD increased from 71·46 BAU/mL (95% CI 59·84-85·33) at baseline to 7756·68 (7371·53; 8161·96) at day 14 ($p < 0·0001$). IgG against trimeric spike-protein increased from 98·4 [85.69–112.99] to 3684·87 [3429·87–3958·83]). 100% participants exhibited neutralizing antibodies 14 days after BNT162b2 administration, in comparison to 34.1% at enrolment. A 4-fold increase in cellular immune response was also observed. Reactions were predominantly mild (68·3%) or moderate (29·9%), and consisted more frequently on injection site pain (88·2%), induration (35·5%), headache (44·4%) and myalgia (43·3%). 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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RESEARCH IN CONTEXT

Evidence before this study

Heterologous regimes in Covid-19 has been proposed as an option to best elicit combined antibody and cellular responses resulting in stronger, broader and/or longer-lasting immunity. However, no clinical evidences exist so far.

Added value of this study

This is 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 ChAdOx1S provides a strong immune humoral and cellular response.

Implications of all the available evidence

This study confirms preclinical studies and suggestions anticipating that heterologous vaccination regimen could provide elicit potent combined antibody and cellular responses and pave the way for mix-and-match COVID-19 vaccines development and warrant future studies evaluating this strategy.

INTRODUCTION

The dramatic impact of COVID-19 on healthcare systems and economies over the world has driven an unprecedented research effort globally to find curative and/or prophylactic therapies. As a result, thousands of COVID-19-related clinical trials have been registered and hundreds of vaccine candidates started testing in record time.[1] Indeed, active immunization has become the cornerstone of global healthcare policies against COVID-19. To date, four vaccines have been granted a conditional marketing authorization by the European Commission: mRNA vaccine BNT162b2 (Comirnaty, BioNTech), mRNA vaccine CX-024414 (Moderna), adenovirus vaccine ChAdOx1-S (Vaxzevria, AstraZeneca) and adenovirus vaccine Ad26.Cov2.S (Janssen-Cilag International NV). Both mRNA vaccines and ChAdOx1-S are used based on homologous regimes.[2] As an alternative, the possibility of sequentially administering different SARS-CoV-2 vaccines, known as heterologous schedules, could be an opportunity to make vaccination programs more flexible and reliable in the face of supply fluctuations. In addition, these schemes are also being studied to identify the best option for the administration of third or successive booster doses.

The decisive factor in generating interest in this type of schedules was the appearance of rare but severe thrombotic with thrombocytopenia events in subject vaccinated with ChAdOx1-S. As these uncommon side effects were more frequent in young people, health authorities of several European countries[3] and Canada, among others, modified their national strategies reserving ChAdOx1-S vaccine for older groups of subjects. Consequently, some countries including Sweden, France, Germany, Norway and Denmark advised for administering a second dose with BNT162b2 vaccine in people primed with ChAdOx1-S, even without supporting data regarding reactogenicity or immunogenicity of this schedule. Obviously, heterologous approaches were not novel as they have been previously used in multiple HIV vaccines under development,[2] in the recently authorized Ebola vaccine[4,5] and it is also one of the current strategies to obtain a universal influenza vaccine.[6,7]

Regarding SARS-CoV-2, Spencer et al had recently evidenced immunological advantages using heterologous vaccination regimens in animal models (21) which concurs with the clinical efficacy showed by the heterologous vaccine Gam-COVID-Vac (Sputnik V, Gamaleya National Research Centre for Epidemiology and Microbiology [NRCEM]).[8] Regarding safety, Shaw et al published initial

data from the Com-Cov trial evidencing limited and short-lived reactogenicity when heterologous schedules were used in humans.[3] Unfortunately, no evidence of immunogenicity outcomes in humans with heterologous vaccination strategies are available to date. To answer this fundamental question, we designed a phase 2 randomised controlled trial to evaluate immunogenicity and reactogenicity of second dose of a mRNA COVID19 vaccine BNT162b2 in subjects prime vaccinated with ChAdOx1-S. Here, we present reactogenicity and immunogenicity at 14-day cut-off.

METHODS

Trial design and participants

The study CombiVacS is a phase 2, non-blinded, adaptive, randomized, controlled, multicentre, clinical trial design being done at five centres in Spain (University Hospital de Cruces, Vizcaya; University Hospital Vall d´Hebron, Barcelona; University Hospital Clinic de Barcelona, Barcelona; University Hospital Clínico San Carlos, Madrid; and University Hospital La Paz, Madrid).

An adaptive design was decided to allow flexibility if primary analysis at 14 days confirmed the starting hypothesis, namely immunogenicity after BNT162b2 dose is superior to no vaccination in ChAdOx1-S-primed patients. Participants were healthy, or clinically stable, adults (aged ≥18 and ≤60) who had received a prime ChAdOx1-S vaccination between 8 and 12 weeks before the screening visit. Patients with documented COVID19 or vaccinated with any other vaccine since the prime dose were excluded. A SARS-CoV-2 RT-PCR test was performed at the randomization 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 ≥ 38ºC within 24 hours prior to the planned dose of study vaccine, clinical manifestations compatible with COVID-19 and any condition contraindicating or discouraging BNT162b2 administration, including pregnancy. Full details of the eligibility criteria are described in the trial protocol provided in the appendix 1.

All the participants provided written informed consent before enrolment. The trial complies with the principles of the Declaration of Helsinki and Good Clinical Practice. This study was approved by the Spanish Agency of Medicines and Healthcare Products (AEMPS) and by the Ethics Committee at University Hospital La Paz.

Randomisation and masking

Participants were randomly assigned (2:1) to receive one intramuscular injection of BNT162b2 (interventional group) or maintain observation (control group). Subjects assigned to the interventional group were vaccinated by healthcare personnel who were aware of trial-group assignments but were not otherwise involved with other trial procedures or data collection. If the main immunogenicity objective is met, and always under the perspective of acceptable reactogenicity, participants included in the control group would be offered to receive BNT162b2 as a second dose at day 28. Alternatively, ChAdOx1-S may be used as a second dose in the control group if requested by the participant or established by local health authorities. The randomization list was centrally generated with the SAS software for Windows (version 9.4; SAS Institute Inc., Cary, NC, USA); systematic randomisation stratified by study site, gender and age (18-49 years, and 50-59 years) was used to achieve balanced randomization in the two treatment groups. The randomization list was imported into the secure Research Electronic Data Capture platform (REDCap version 8.7.4; Vanderbilt University, Nashville, TN, USA) used for the study electronic case report form (eCRF).

Procedures

The BNT162b2 vaccine used in this trial is available in Europe after a conditional marketing authorization was granted by the European Medicines Agency (EMA) in December 2020. BNT162b2 was administered at the approved dose of 0.3 mL as a single intramuscular injection. All participants were RT-PCR tested for SARS-CoV-2 infection, clinically assessed and had blood samples drawn for safety as well as immunology at day 0 (randomisation, 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. Participants will also be followed-up at days 28, 90 (month 3), 180 (month 6) and 360 (month 12). Participants in the interventional group were observed on site for at least 15 minutes after BNT162b2 vaccination for safety monitoring. Any adverse events occurred up to the end of the observation period were recorded. Participants in both groups were asked to record any adverse events using an electronic diary throughout the follow-up period. Participant uploaded events were

accessible 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 these cases, the investigator contacted the participant to assess seriousness. At the present cut-off, participants were inquired 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 according to a 4-grade scale: grade 1 (mild), grade 2 (moderate), grade 3 (severe), and grade 4 (life-threatening). Causality of unsolicited adverse events was defined as related or not related to study treatment based on reasonable possibility, temporal relationship and alternate aetiology criteria, and was assessed in reported unsolicited adverse events. Full description of safety definitions and a list of solicited adverse events are provided in the trial protocol supplied as appendix 1.

Antigen-specific humoral immune response was analysed using two commercial immunoassays and one pseudovirus neutralization assay. The Elecsys® Anti-SARS-CoV-2 S assay (Roche Diagnostics GmbH, Mannheim, Germany) is an electrochemiluminescence immunoassay (ECLIA) detecting IgG antibodies to the SARS-CoV-2 spike protein receptor binding domain (RBD) on the cobas e411 module.[9] According to the manufacturer, the measuring range spanned from 0.4 U/mL to 250 U/ml (up to 2,500 U/ml with on-board 1:10 dilution and up on 12,500 with on-board 1:50 dilution). Values higher than 0.8 BAU/mL were considered positive. Correlation between U/ml and BAU (International OMS standard) is U=0.972 BAU. The LIAISON® SARS-CoV-2 TrimericS IgG assay (DiaSorin Inc., Stillwater, USA) is a chemiluminescence immunoassay (CLIA), detecting IgG antibodies anti-trimeric spike glycoprotein of SARS-CoV-2 in human serum or plasma samples on the LIAISON® XL.[10] Measuring range spanned from 4·81 BAU/mL to 2,080·00 BAU/mL. According to the manufacturer, values > 2,080·00 BAU/mL were diluted 1:20 and values higher than 33·8 BAU/mL were considered positive. To measure neutralizing antibodies titres, dilutions of participants' plasma samples were pre-incubated with pseudoviruses generated by co-transfection of pNL4-3ΔenvRen and an expression vector for the viral spike (pcDNA3.1-S-CoV2Δ19-G614) and added at a concentration of 10ng p24Gag/well to Vero E6 cells in 96-well plates. At 48 hours post-infection, viral infectivity was assessed by measuring luciferase activity (Renilla Luciferase Assay, Promega) using a 96-well plate luminometer "LB 960 Centro XS³" (Berthold). The titre of

neutralizing antibodies was calculated as 50% inhibitory dose (neutralizing titre 50, NT50), expressed as reciprocal of four-fold serial dilution of heat-inactivated sera (range 1:32 – 131·072) resulting in a 50% reduction of pseudovirus infection compared to 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 neutralization was assessed using a non-related pseudovirus expressing the Vesicular Stomatitis Virus envelope. Cellular immune response was measured by quantification of IFN-gamma present in plasma upon overnight stimulation of whole blood with pools of SARS-CoV-2 peptides (S; 2 µg/ml) or DMSO control in whole blood culture. This methodological approach requires only 1 ml of blood, which facilitates longitudinal tests in a large cohort of individuals, allowing the rapid quantification of SARS-CoV-2-specific T cells in vaccine recipients.[11,12] Cytokines were quantified using Ella (ProteinSimple, San Jose, California). Neutralizing antibodies were planned to be analyzed in 200 participants randomly selected from the full sample included, while cellular immune response was analysed in participants from two study sites (University Hospital Clínico San Carlos, and University Hospital La Paz). Full details on the pseudovirus neutralising assay and cellular immunity quantification are provided in the appendix 1 (pp 14).

Outcomes

The primary outcomes were reactogenicity and immunological 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 neutralizing antibodies titres measured by virus neutralization assay at day 14. 1-year safety was also planned to be assessed. Two exploratory outcomes were included: a) the relationship between neutralizing antibodies and antibodies against SARS-CoV-2 spike protein measured by immunoassay, and b) cellular response to vaccination defined as inflammatory IFN-gamma cytokine production against SARS-CoV-2 spike peptide pools at day 14. Another secondary and exploratory immunogenicity and efficacy outcomes – planned at 28, 90, 180 or 360 days – are not applicable to the present analysis but are also detailed in the protocol provided in the appendix 1.

Statistical Analysis

The immunogenicity analysis population included all the participants who were randomized, completed all visits and for whom serological samples were available both on day 14 and at the baseline visit. Data was presented as geometric mean and 95% confidence interval (95% CI) or, for categorical variables, number and percentage, unless otherwise stated. Antibodies titres against SARS-CoV2 spike protein at 14 days was the response variable and treatment effect was evaluated comparing those titres between interventional versus control group. Additional post-treatment ANCOVA adjusting for pre-treatment was performed, with baseline immunity value, age, and sex as co-variable. The primary and secondary laboratory objectives were described using geometric means and difference at each time, basal, 7 (only for serologic determinations) and 14 days, was evaluated with ratio of geometric means. Additionally, reverse cumulative distribution curve was plotted. A subgroup analysis by sex, and age groups was performed at each time, baseline and 14 days, for the primary and secondary endpoints. Laboratory parameter with value below detection limit were replaced by a value equal to the lowest limit divided by 2. All analyses were carried out using the statistical software SAS, version 9.4 of the SAS system for Windows (SAS Institute Inc., Cary, NC, USA). All analyses were carried out using the statistical software SAS, version 9.4 of the SAS system for Windows (SAS Institute Inc., Cary, NC, USA). The reactogenicity analysis population included all the participants who had received at least one dose of BNT162b2 in the interventional group regardless 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[13] was performed to assess the humoral immune response against SARS-CoV-2 14 days after dose of BNT162b2 in subjects that received a prior single dose of ChAdOx1-S, as compared with no dosing. A sample size of 600 participants (400 in the interventional group) was required to identify a 35% of increase in antibodies titres in subjects receiving the dose of BNT162b2, G(Y1), in relation with those not receiving it, G(Y2) at 14 days, assuming a coefficient of variation equal to 1.2 or 1.0 and similar between arms, at least 80% power and a one-sided 1% significance level (H1: G(Y1)/G(Y2) >1). A low value alpha, 0·01, was used for the one-sided hypothesis to avoid a type I error, especially when the evaluation will be replicated at

other specific times. The sample size was increased by 15% due to possible no-participation. This study is registered at EudraCT (No. 2021-001978-37) and ClinicalTrials.gov (NCT04860739).

Role of the funding source

The funder – Institute of Health Carlos III, or ISCIII – designed the trial in cooperation with the Spanish Clinical Trials Platform (SCReN), a public network of clinical trials unit at the Spanish National Health System funded by the ISCIII through PTC20/00018 and PT17/0017 Trial coordination, patient recruitment and data analysis has been performed by SCReN. All immunological procedures were performed at ISCIII. All authors review and approve the original draft. All authors had full access to the full data in the study and accept responsibility to submit for publication.

RESULTS

Between April 24 and April 30, 2021, 676 patients were enrolled into the study and randomly assigned to receive BNT162b2 vaccine (n=450) or no vaccine (n=226) but 2 and 1 individuals withdrew consent before vaccination and were discontinued in experimental and control group, respectively. A total of 663 participants were included in the immunogenicity analyses, after 7 participants from the vaccine group and 3 from the control group were excluded (figure 1). 448 participants who received the second dose were included in the reactogenicity population, including 1 from the control group who was erroneously vaccinated. One individual was excluded due to lost to follow-up after receiving the BNT162b2 dose.

Demographics and baseline characteristics (table 1) were balanced between the two study groups, 382 (56·5%) participants were female, 437 (64·6%) participants were within 18-49 age group and the mean age was 43·98 (SD 8·85). Time elapsed since ChAdOx1-S administration was between 8 and 9 weeks for 411 participants (60·8%) and between 10 and 12 weeks for 263 participants (38·9%).

In the interventional group, geometric mean titres (GMT) of IgG 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) vs. the control group (99·84 BAU/mL, 95% CI 76·93;129·59). Immunogenic response in the interventional group was observed as soon as day 7 (4353·51 BAU/mL, 95% CI

3851·58-4920·85 [interventional group] vs. 90·05 BAU/mL, 95% CI 69·16-117·27 [control group]; p < 0·0001) (figure 2a; appendix 1 pp 2). When antibodies against SARS-CoV-2 spike protein were measured by a CLIA technique covering the trimeric spike protein, 14-day immunogenic response in the interventional group was also confirmed as statistically significant (3684·87 BAU/mL, 95% CI 3429·87-3958·83 [interventional group] vs. 101·2 BAU/mL, 95% CI 82·45-124·22 [control group]; p < 0·0001), which meant a 37-fold increase from baseline. Likewise, titres of antibodies at day 7 were significantly higher in the interventional group (2246·25 BAU/mL, 95% CI 2010.4-2509.78 [interventional group] vs. 102·25 BAU/mL, 95% CI 83·52-125·18 [control group]; p < 0·0001) (figure 2b; appendix 1 pp 2). Reverse cumulative distribution curves for RBD- and trimeric- S protein antibodies are shown in appendix 1 (pp 3-4). Titres of antibodies measured by both techniques showed strong positive correlation (R^2=0·85; p<0·001) (appendix 1 pp 5). Subgroup analysis evidenced that immunological response was numerically lower in males but no differences were evidenced by age subgroups (Appendix 1 pp 6-7).

The functional capability of the antibodies induced in the interventional group were analysed in 198 participants randomly selected (129 from the interventional group and 69 from the control group). In the interventional group, 74·4% participants showed no or very low neutralizing activity at day 0, whereas 100% exhibited neutralizing antibodies at day 14, showing high (NT50 >1:300/<1:1000) or very high (NT50 >1:1000) activity in 99·7% of them (appendix 1 pp 8). At day 14, GMT of neutralizing antibodies increased 45-fold from 41·84 (95% CI 31·28-55·96) to 1905·69 (95%CI1625·65; 2233·98) in the interventional group, compared to 41·81 (95% CI 27·18;64·32) present at day 14 in the control group (p<0·0001). GMT of neutralizing antibodies in controls was not significantly different from baseline (GMT 50·84, 95%CI 33.56-76.99) (figure 3a; appendix 1 pp 9). Reverse cumulative distribution curves for neutralizing antibodies are shown in appendix 1 (pp 10). Neutralizing antibody responses had a strong positive correlation with RBD antibody titres (R^2=0·82; p<0·001) (figure 3b).

Dynamic changes of functional spike-specific T cell response were analysed in 151 participants (n=99 [interventional group] and n=52 [control group]). Results revealed substantial levels of IFN-gamma production at day 0 (geometric mean 129·63 pg/mL, 95% CI 103·51-162·35 [interventional

group]; and 151·63 pg/mL, 95% CI 114·09-201·53 [control group]), consistent with a prior immunization with a single dose of ChAdOx1-S. On day 14, the production of IFN-gamma had significantly increased in the interventional group (geometric mean 521·22 pg/mL, 95% CI 422·44;643·09; p<0·0001) in comparison with the control group (122·67 pg/mL, 95% CI 88·55;169·95; p<0·0001) that remain unchanged. Reverse cumulative distribution curves for immunological response are shown in appendix 1 (pp 11).

Reactogenicity analysis was based on solicited adverse events in 448 individuals from the intervention group evidencing headache (194; 44·4%), myalgia (194; 43·3%) and malaise (187; 43·3%) as the most commonly reported systemic reactions. Other systemic adverse reactions, including fever (2·5%) were less common and shown in appendix 1 (pp 12). As expected, injection site pain (395; 88·2%), induration (159; 35·5%) and erythema (139; 31%) were the most commonly reported local reactions. Other local adverse reactions were less common and shown in appendix 1 (pp 12). In general, local and systemic reactions were most frequently reported by female participants. No differences in frequency were observed by age groups (appendix 1 pp 13). Solicited adverse events in the 7 days following vaccination in the interventional group were predominantly mild (68·3%) and moderate (29·9%), and self-limited. Importantly, only 1.75% of the adverse events were self-reported as severe. Within this category, the most frequent symptoms were malaise (22·5%), myalgia (19·3%) and headache (16·1%). All these subjects 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 the first report evidencing that a SARS-CoV-2 heterologous vaccination schedule induces a strong immune response in humans and is associated to an acceptable and manageable reactogenicity profile. Our approach is based on BNT162b2 given as a second dose 8-12 weeks after a first dose of ChAdOx1-S and the potent immune response was confirmed using four different tests.

Although our conclusions should be restricted to this scenario keeping in mind the absence of a homologous vaccination arm, comparison with previously reported immunogenicity data may help to

put in context the results of the study. This indirect comparison suggests that the intensity of the immune response with the heterologous vaccination schedule used in this study is higher than those previously reported by other authors using homologous schedules. According to previous data coming from the Oxford COVID Vaccine Trial Group, after a second dose of ChAdOx1-S humoral response is associated with a 10-fold increase of anti SARS-CoV-2 spike protein IgG standardised ELISA titres.[14,15] On the other hand, in phase I/II BNT162b2 trials[16] RBD-binding antibodies also increased 10-fold after the second dose of BNT162b2 vaccine in comparison with first dose (from 1,536 U/ml to 16,166 U/ml) whereas neutralizing antibody titres raised from 29 to 437 (15-fold). In phase I/II CX-024414 trials,[17] in the 100 μg group, antibodies against the RBD raised 6-fold two weeks after the second vaccine dose (from 93,281 to 558,905). In our study heterologous second vaccination with BNT162b2 induced a 108/37-fold increase in IgG against RBD and trimeric spike protein, respectively. Although these effects could come from the different techniques to measure SARS-CoV-2 IgG employed in these studies, the strong positive correlation observed between the two IgG CLIA/ECLIA methods and the pseudovirus neutralization assay employed in the present work ensure the robustness of the measures and suggest a potential advantage of the heterologous over the homologous vaccination strategies. In this regard, it is very important to note that in our study immunogenicity response explored by spike protein-binding antibodies titres was in a similar incremental ratio between baseline and day 14 (37- and 108-fold) to the immunological response evidenced by neutralizing antibodies titres (40-fold). The proportionality between the increase in anti-RBD, anti-trimericS and neutralizing antibodies from our study agrees with the published data for BNT162b2[16] but are quite different to that reported in the public assessment report of ChAdOx1-S wherein the bright increase in anti-spike titres after a homologous boost was associated with a very modest increase in neutralizing antibodies titres.[18] Therefore, the sequential use of ChAdOx1-S and BNT162b2 may be the explanation to our findings. Besides, the time elapsed between the first and second dose probably have played a relevant role, since our participants received the second dose of vaccine a minimum of 50 days after the first dose. In this regard, two studies[14,15] and a pooled analysis of four randomised trials from the Oxford COVID Vaccine Trial Group[19] evidenced that the longer interval before the ChAdOx1-S second

dose administration, the higher SARS-CoV-2 IgG spike specific response. This effect was more evident in individuals younger than 55 years old using ChAdOx1-S but also described in people aged over 80 years vaccinated under an extended interval between two doses of BNT162b2.[20] Consequently, our study design could have maximized the effect of the interval between the two doses.

We also found that neutralizing activity as determined using a pseudovirus assay was strongly increased after BNT162b2 immunization. In fact, deployment a neutralizing capacity after our heterologous regimen was not due to a minority of subjects as 14 days after intervention NT50 was above 1.000 in 75·2% of subjects and overall 97·7% of all subjects increased NT50 value above 1:300. Because our study did not include an arm immunized with a second ChAdOx1-S dose it is not possible to compare both strategies. However, neutralization assays using pseudoviruses are quite similar across our study and ChAdOx1-S trials,[14,15,19] allowing some comparisons. In this regard, in ChAdOx1-S trials neutralization titres 28 days after vaccination with first dose were between 40 and 162 (expressed as median), and increased 3- to 6-fold (NT50 between 237 and 451) after a second dose of ChAdOx1-S. In our study, patients were included between 8 and 12 weeks after first ChAdOx1-S dose and basal levels were in the 40-50 (expressed as geometric mean) range in both control and intervention groups, which is very similar to basal data 56 days after priming with ChAdOx1-S.[12] After BNT162b2 immunization NT50 raised to 1,950 (45-fold increase) confirming a strong immunogenicity and the induction of strong humoral responses and neutralization titres with the heterologous vaccination regimen proposed. Of note, a recent study has reported that neutralization level is highly predictive of immune protection and suggest that neutralization titre will be an important predictor of vaccine efficacy in the future as new vaccines emerge.[21]

In addition, 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. This enhancer effect is very interesting since second doses of ChAdOx1-S in homologous schedules have failed to demonstrate an improvement in the cellular response obtained after an initial dose,[14,15,22] suggesting that cellular response is maintained during time irrespective of vaccination interval, age

and gender following a two-dose homologous vaccination strategy with ChAdOx1. On the contrary, the enhancer effect of the second dose on the cellular immune response has been described in the limited data available with homologous mRNA vaccine schedules.[23–25]

Regarding reactogenicity, solicited adverse events profile in CombiVacS is similar to those showed after homologous vaccination with ChAdOx1-S[14] or BNT162b2;[26] and those recently communicated in a cohort of healthcare workers in Germany.[27] However, our findings differ from those reported by Shaw and the Com-COV Study Group.[3] Shaw and colleagues[3] describes an increase in systemic reactogenicity after the boost dose reported by participants in heterologous vaccine schedules in comparison to homologous vaccine schedules, particularly in a self-reported feeling of feverishness. In contrast, although participants in our study were younger (mean 44 years old), results showed a lower frequency of reactogenicity events, which was unexpected and may be explained, at least in part, by different administration interval between both studies (28 day in Shaw and colleagues vs. 8-12 weeks in ours). Notwithstanding this, comparisons must be cautious due to differences between both studies. Apart from this limitation, the lack of an active control arm does not allow us direct comparisons with reactogenicity elicited by homologous ChAdOx1-S/ ChAdOx1-S vaccination.

Finally, in figures 2a, 2b and 3a the presence of individuals with elevated antibody titres at the time of randomization is evident. In the event that we can rule out individual variability as a cause of these titres, we would have to hypothesize the participation of individuals who had been inadvertently infected at some time prior to the start of the trial. In that case, the titres obtained in these individuals would depend directly on a heterologous combination of antigens as they have been exposed to wild-type SARS-CoV-2 and ChAdOx1-S, which would confirm our findings. However, this is a hypothesis to be assayed in the population of our study.

In summary, our study confirms a robust humoral and cellular inmmune response after a second dose of BNT162b2 in individuals previously primed with ChAdOx1-S between 8 and 12 weeks before. Future studies comparing homologous versus heterologous vaccination schedules will help to confirm and better understand the humoral and cellular immune responses observed in this clinical trial.

Contributors

Trial conceptualization was performed by CB, AMB, AJC, JA and JF. AJC, IF and AA developed the study methodology. AMB, MPO, LC, MJB, JGC, MC, AP, MGP, EAA, MAN, FDF, AA, NIA, LBM, CP and JO were study investigators. MTGM, DL and AGC contributed to ensure data accuracy. AMB, AJC, MPO, DL, AGC, JO, JA and JF were responsible for statistical analysis. CB, AMB, MPO, LC, MC, MJB, AP, JO, JA and JF contributed to study supervision. CB was responsible for funding acquisition. CB, AMB, AJC, MPO, JO, JA and JF contributed to write the original draft and all authors contributed to the manuscript review and editing.

Declaration of interests

CB is the Deputy General Manager of the ISCIII. JRA has received fees from Janssen, outside of the submitted work. AMB is principal investigator of clinical trials sponsored by GSK, Daiichi-Sankyo, Janssen and Farmalider, outside of the submitted work. The other authors declare no competing interests

Data sharing

The study protocol and the statistical analysis plan are provided in the appendix 1 (pp16 et seq.). Individual participant data will be made available when the trial is complete, upon requests directed to the corresponding authors; after approval of a proposal, data can be shared through a secure online platform.

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564

Table 1. Baseline characteristics of the randomized population

	Interventional group (n=450)	Control group (n= 226)	Overall (n=676)
Sex			
Male	193 (42·9%)	101 (44·7%)	294 (43·5%)
Female	257 (57·1%)	125 (55·3%)	382 (56·5%)
Age (years)	43·93 (8·88)	44·10 (8·82)	43·98 (8·85)
Age group			
18-49 years	293 (65·1%)	144 (63·7%)	437 (64·6%)
Male	123 (27·3%)	65 (28·8%)	188 (27·8%)
Female	170 (37·8%)	79 (34·9%)	249 (36·8%)
50-59 years	157 (34·9%)	82 (36·3%)	239 (35·3%)
Male	70 (15·5%)	36 (15·9%)	106 (15·7%)
Female	87 (19·3%)	46 (20·3%)	133 (19·7%)
Time since prime ChAdOx1-S vaccination*			
8-9 weeks	273 (60·7%)	138 (61·1%)	411 (60·8%)
10-12 weeks	176 (39·1%)	87 (38·5%)	263 (38·9%)

Data are n (%) and mean (SD). *Two patients excluded: (1) 7 weeks elapsed since ChAdOx1-S vaccine, and (2) dropout on day 0.

Figure 1. Trial profile

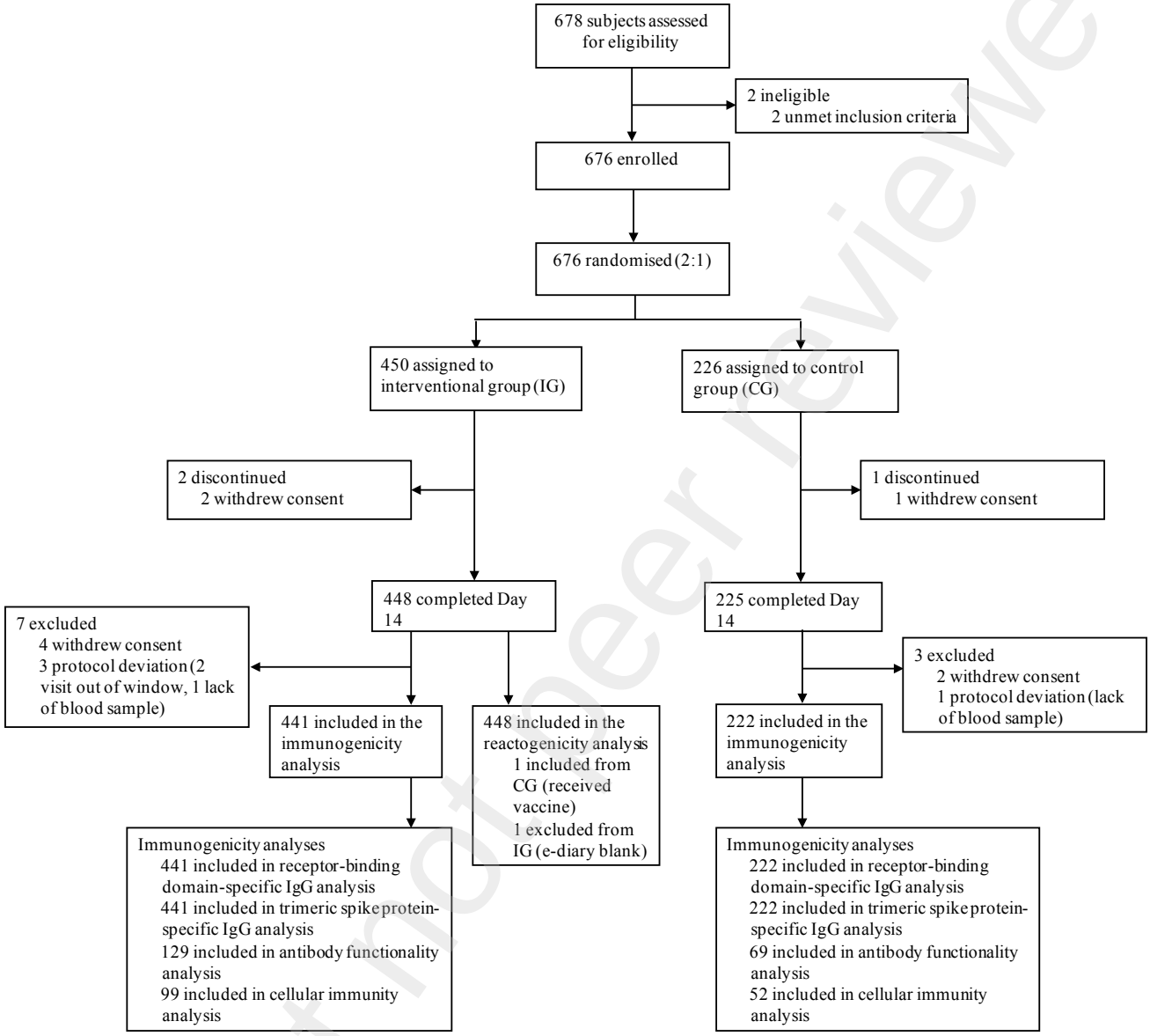
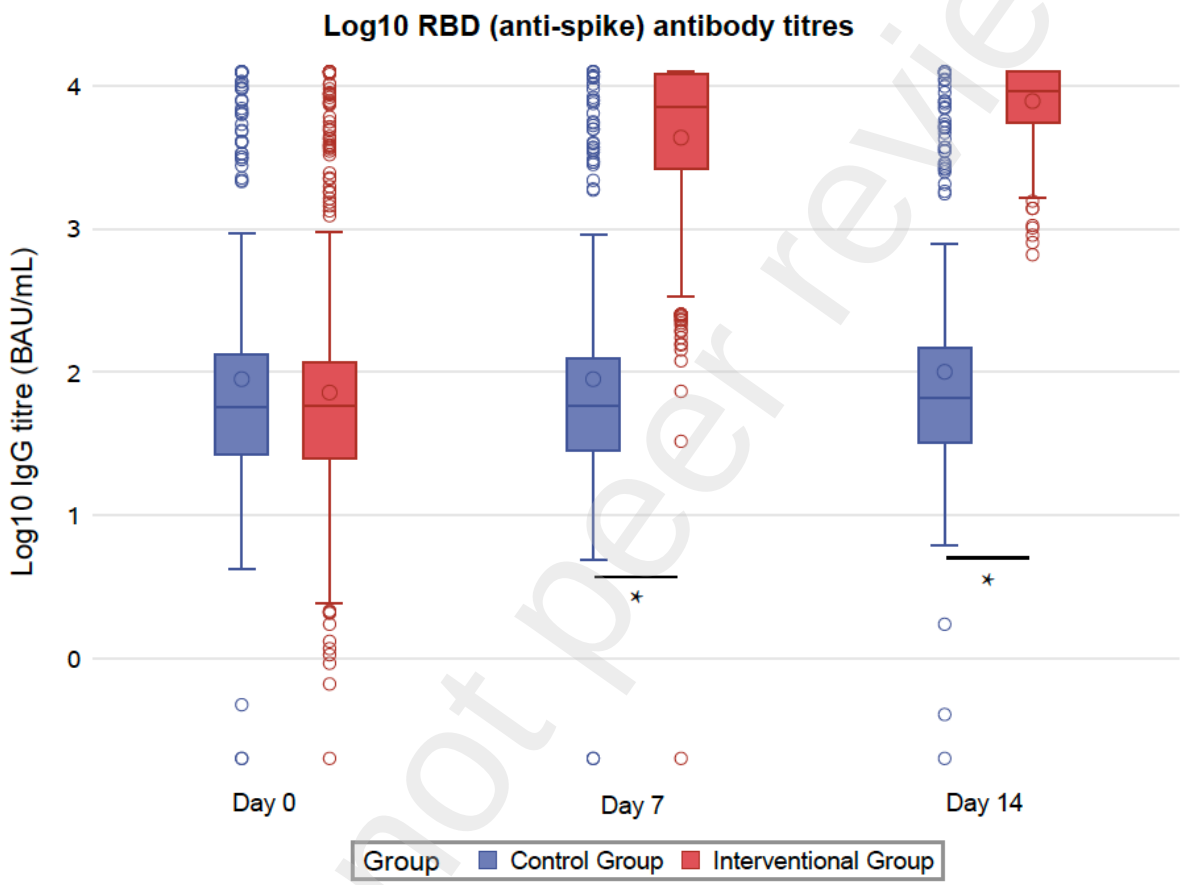


Figure 2. a) RBD (anti-spike) antibody titres, and b) Trimeric S protein antibody titres, measured in both interventional (red) and control (blue) groups on days 0, 7 and 14

* p<0·0001

2a)



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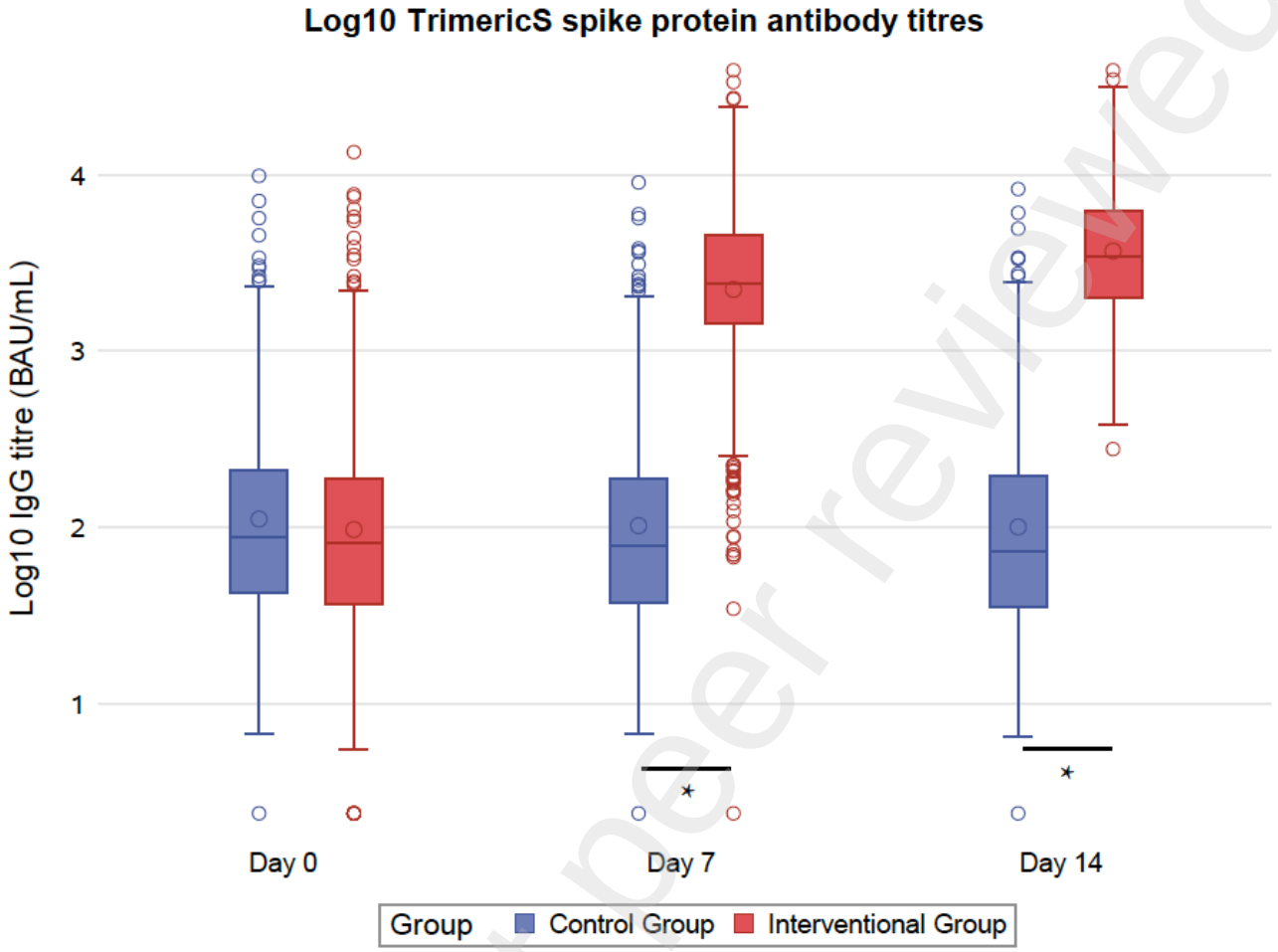
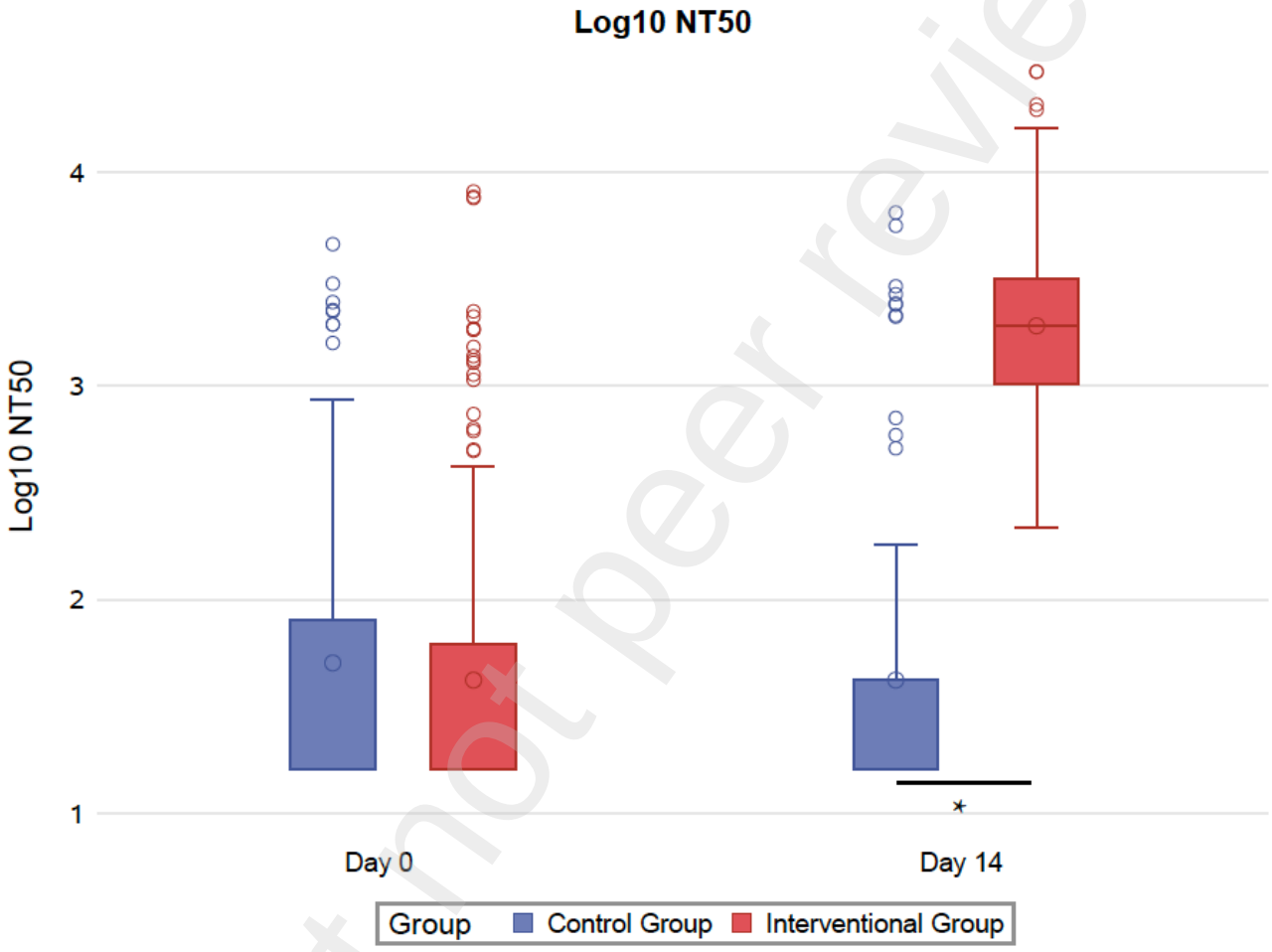


Figure 3. a) Neutralizing antibodies measured in both interventional (red) and control (blue) groups on days 0 and 14. b) Correlation between Focus Reduction Neutralization Test 50 (FRNT50) and RBD (anti-spike) antibody titres

* p<0·0001

3a)



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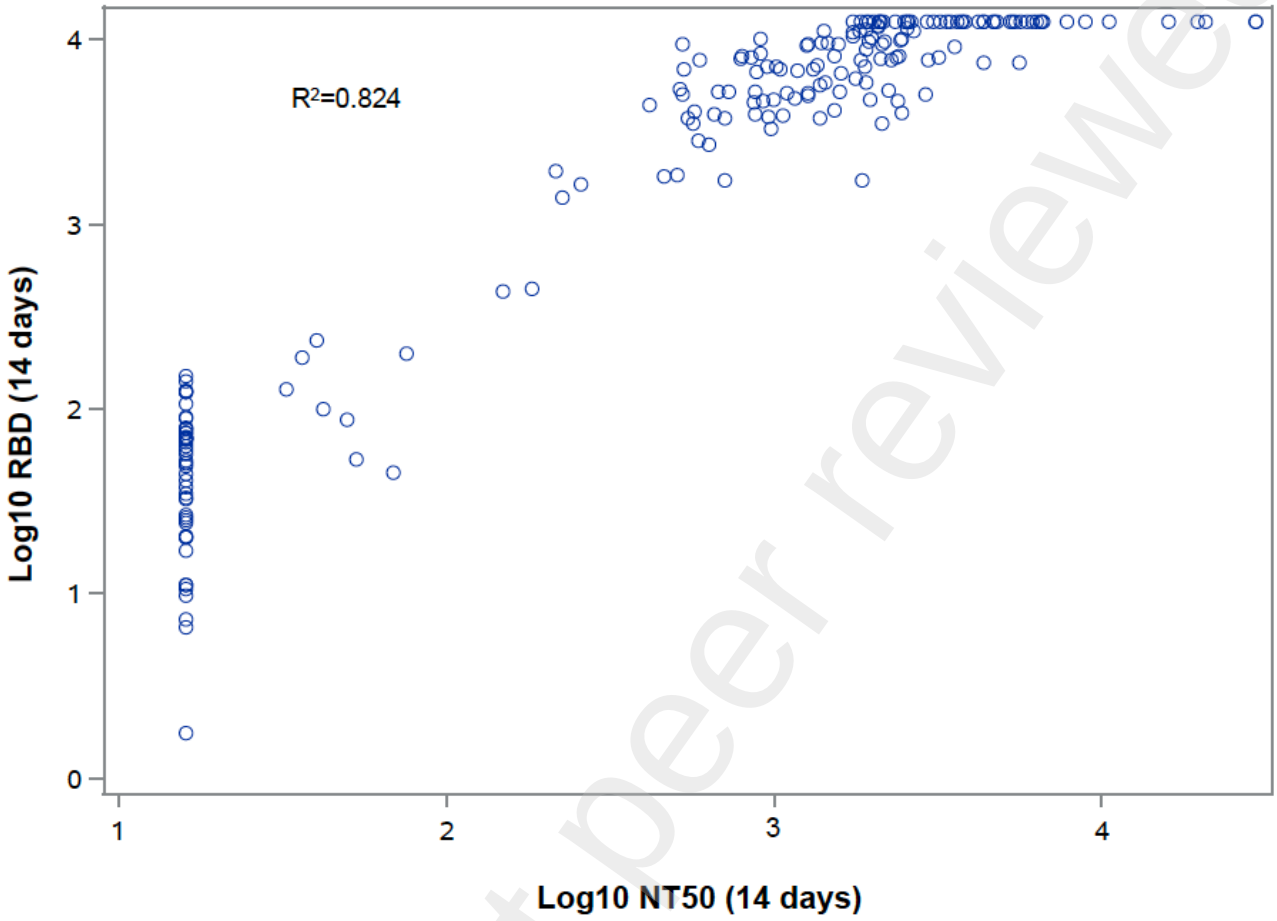

Figure 4. IFN-gamma concentrations measured in both interventional (red) and control (blue) groups on days 0 and 14

* p<0·0001

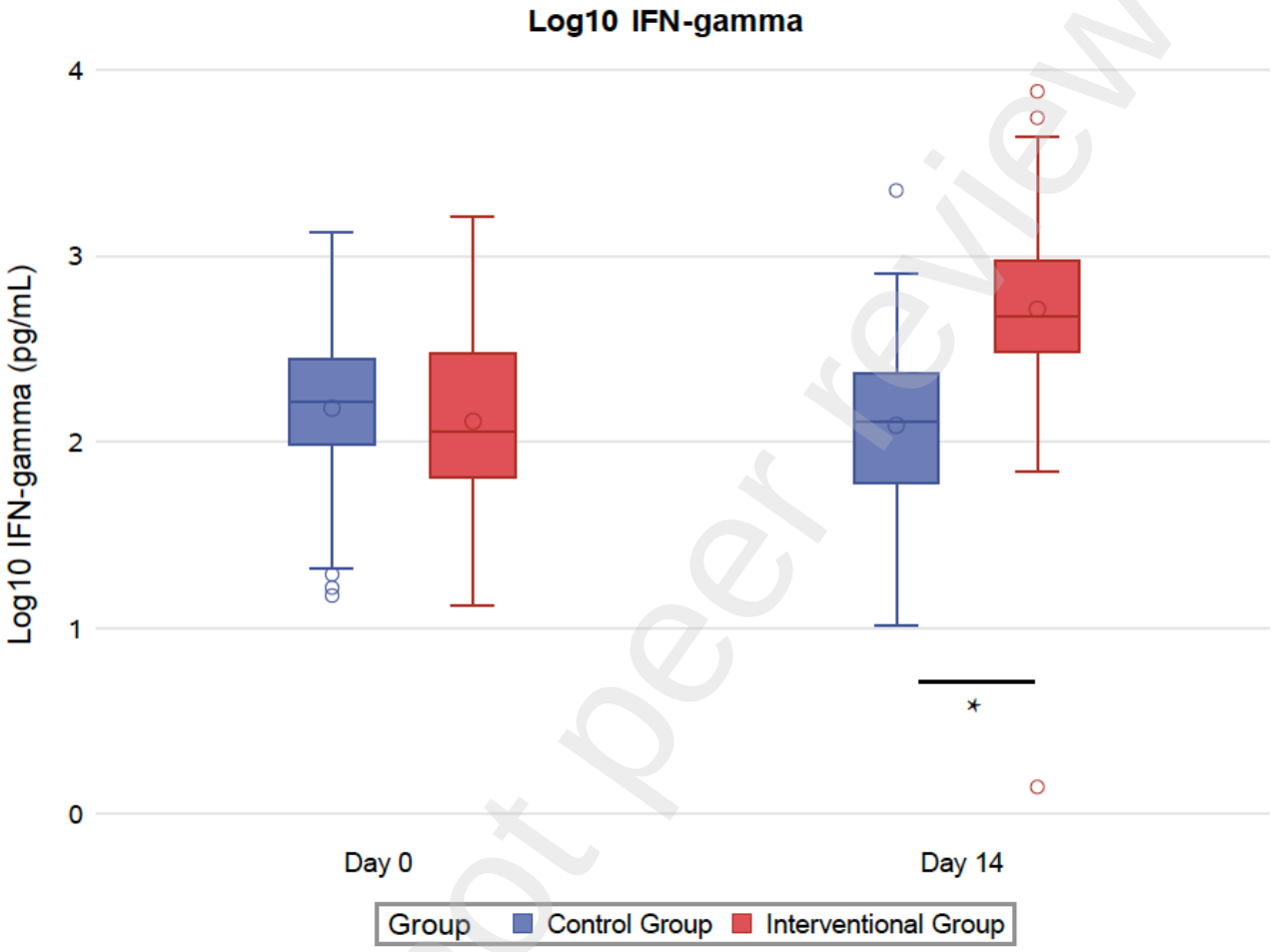

Figure 5. Solicited local and systemic adverse reactions in first 7 days after vaccination as recorded in participant symptom electronic diaries