Presidenza del Consiglio dei Ministri

COMITATO TECNICO-SCIENTIFICO
Ex O.C.D.P.C. 3 febbraio 2020, n. 630, come modificata dalla O.C.D.P.C. 17 marzo 2021, n. 751

Verbale n. 25 della riunione tenuta presso il Dipartimento della Protezione Civile il giorno 7 giugno 2021

	Presente	Assente
Franco LOCATELLI (coordinatore)	in videoconferenza	
Silvio BRUSAFERRO (portavoce)		X
Sergio FIORENTINO (segretario)	in videoconferenza	
Sergio ABRIGNANI	in videoconferenza	
Cinzia CAPORALE	in videoconferenza	
Fabio CICILIANO	in videoconferenza	
Donato GRECO	in videoconferenza	
Giuseppe IPPOLITO	in videoconferenza	
Alessia MELEGARO[1]	in videoconferenza	
Giorgio PALÙ	in videoconferenza	
Giovanni REZZA[2]		X

Ordine del giorno, di cui alla nota di convocazione del 4 giugno 2021:

1. Valutazione dei risultati di studi sulla sicurezza e l'immunogenicità della somministrazione di una dose di vaccino Cominarty a soggetti che hanno ricevuto una dose di vaccino Vaxzevria;

2. Valutazione del Protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico fino al 31 dicembre 2022, da adottare con successiva ordinanza del Ministro dell'Istruzione

3. Varie ed eventuali.

[1] Collegata in videoconferenza dalle ore 13,30.
[2] Collegato in videoconferenza dalle ore 13,15.

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*

La seduta inizia alle ore 13,05.

Il Coordinatore propone di invertire l'ordine del giorno, cominciando dall'esame del **punto n. 2**, che ha ad oggetto la valutazione del protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico fino al 31 dicembre 2022, da adottare con successiva ordinanza del Ministro dell'istruzione (v. allegato).

Il parere è richiesto ai sensi dell'art. 59, comma 20, del decreto-legge 25 maggio 2021, n. 73, secondo il quale «*(c)on ordinanza del Ministro dell'istruzione sono definiti appositi protocolli, sottoposti alla previa approvazione del Comitato tecnico-scientifico di cui all'ordinanza del Capo del Dipartimento della protezione civile 3 febbraio 2020, n. 630, e successive modificazioni, relativi alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico fino al 31 dicembre 2022, senza nuovi o maggiori oneri per la finanza pubblica*».

Il Cts, esaminato il documento e considerato che lo stesso riproduce, nella sostanza, altri protocolli già approvati dal Comitato, esprime parere favorevole, con le seguenti osservazioni:

- il riferimento alla distanza interpersonale di un metro va integrato con l'indicazione «*in tutte le direzioni*» (con disposizione "a scacchiera");

- prescrivere la costante sanificazione delle postazioni dei candidati e delle parti comuni;

- prevedere la ripetuta e accurata pulizia dei servizi igienici;

- prescrivere un richiamo stringente dei candidati e del personale interessato all'igienizzazione delle mani, mettendo a disposizione un adeguato numero di distributori di soluzioni idroalcoliche;

- prevedere la costante e diffusa areazione degli ambienti;

- prevedere l'adozione di tutte le misure idonee a evitare assembramenti sia in fase di afflusso, sia in fase di deflusso dei candidati;

- può essere eliminato il riferimento alla «*tosse di recente comparsa*» quale elemento ostativo alla partecipazione, mentre il rifermento alla «*difficoltà respiratoria*» può essere opportunamente integrato con l'indicazione «*di recente comparsa*»;

- coinvolgimento delle autorità sanitarie locali, sia per il dettaglio delle misure da adottare negli specifici contesti, sia per il controllo dell'applicazione di tali misure.

Considerato che, nella previsione normativa, l'efficacia del protocollo si proietta ad epoca remota, sino al 31 dicembre 2022, il CTS precisa la suesposta valutazione è stata effettuata alla stregua dell'attuale scenario epidemiologico e di quello prevedibile nel breve periodo. La valutazione è, quindi, suscettibile di revisione in futuro, essendo onere del Ministero dell'istruzione sottoporre nuovamente la questione al Comitato in caso di significative variazioni del contesto epidemiologico.

***

Il Comitato passa, quindi, a esaminare il **punto n. 1** dell'ordine del giorno, che si incentra sulla valutazione dei tre studi preliminari vertenti sull'impiego di vaccini ad mRNA per completare la vaccinazione di soggetti che hanno ricevuto la prima con vaccino a vettore adenovirale (allegati): «*A Phase 2, Randomised, Multicenter, Adaptive Trial to Evaluate the safety and immunogenicity of one dose of COMIRNATY® in subjects that had received one dose of VAXZEVRIA®*»; «*Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1-nCoV19 and BNT162b2: a prospective cohort study*», a cura di David Hillus e altri e «*Heterologous ChAdOx1 nCoV-19 and BNT162b2 prime-boost vaccination elicits potent neutralizing antibody responses and T cell reactivity*», a cura di Rüdiger Groß e altri.

Il CTS concorda sul fatto che gli studi, originati dalla necessità di far fronte pragmaticamente ai problemi che ha manifestato il vaccino Vaxzevria, seppur condotti su un numero assai limitato di soggetti sembrano confermare che non si sono registrati effetti avversi di rilevante gravità e danno dimostrazione di una efficace risposta immunitaria, non inferiore a quella della vaccinazione con due dosi del vaccino Vaxzevria (vaccinazione omologa). Fermo restando che occorrerà l'approvazione delle agenzie regolatorie e ricordato che l'EMA ha già valutato come considerabile la scelta di completare la vaccinazione con una seconda dose del vaccino Vaxzevria, si può tuttavia, in prima approssimazione, sostenere che, verso l'ipotizzata soluzione, militano diverse considerazioni di natura immunologica, di sicurezza e di strategia sulla eventuale terza dose.

Il CTS conviene che, sull'argomento, è altamente auspicabile che si pervenga, con rapidità, a una soluzione pariteticamente concertata e condivisa con AIFA.

Occorrerà, in ogni caso, valutare l'impatto di una simile scelta sulla logistica e sui tempi della campagna di vaccinazione, sulla quale sarebbe opportuno, quindi, sentire le autorità ad essa preposte.

Nel corso della discussione, alcuni Componenti riferiscono della pubblicazione di diversi studi che segnalano, in conseguenza dell'uso del vaccino Vaxvezria, un aumento degli eventi avversi o, comunque, una incidenza di questi maggiore di quanto fosse stato osservato in passato.

L'affiorare di tali evidenze potrebbe portare ad aggiornare, anche in relazione alla somministrazione della prima dose, le valutazioni condotte dal CTS rispetto all'uso di determinati vaccini, le quali andrebbero, inoltre, attualizzate in considerazione del mutato scenario epidemiologico, atteso che il livello di circolazione del virus e di incidenza dei contagi osservabile all'epoca della decisione assunta nella seduta del 12

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maggio u.s. ne aveva costituito fattore essenziale, in funzione della sua determinante influenza nel calcolo del rapporto rischi/benefici.

All'esito della discussione, il CTS dà, pertanto, mandato al Coordinatore di promuovere a breve una seduta alla quale possano partecipare il Sig. Ministro della Salute, il Commissario straordinario per l'emergenza Covid-19 e il Direttore Generale di AIFA, per ascoltarne le valutazioni e assumere, ove così sarà richiesto al CTS, le conseguenti determinazione sui due temi oggi discussi nell'ambito del primo punto dell'ordine del giorno.

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Alle ore 14,50, in assenza di altri argomenti sui quali concentrare l'attenzione, il Coordinatore dichiara chiusa la seduta.

	Presente	Assente
Franco LOCATELLI (coordinatore)	in videoconferenza	
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Giorgio PALÙ	in videoconferenza	
Giovanni REZZA	in videoconferenza	

Verbale approvato dopo condivisione via e.mail da parte di tutti i Componenti.

IL COORDINATORE
Franco Locatelli

IL SEGRETARIO VERBALIZZANTE
Sergio Fiorentino

A Phase 2, Randomised, Multicenter, Adaptive Trial to Evaluate the safety and immunogenicity of one dose of COMIRNATY® in subjects that had received one dose of VAXZEVRIA®

EudraCT 2021-001978-37
ClinicalTrials.gov number NCT04860739

Preliminary Results.
Confidential information
May 25th

Design

- Independent / academic clinical trial (Institute of Health Carlos III)
- A Phase 2, Comparative, Randomised, Open-label, Adaptive Trial,
- **Primary Immunogenicity Objective:** To assess the humoral immune response against SARS-CoV-2, 14 days after a COMIRNATY boost in individuals that had received a previous, prime dose of VAXZEVRIA 8-12 weeks before

Design

Secondary Immunogenicity Objectives:

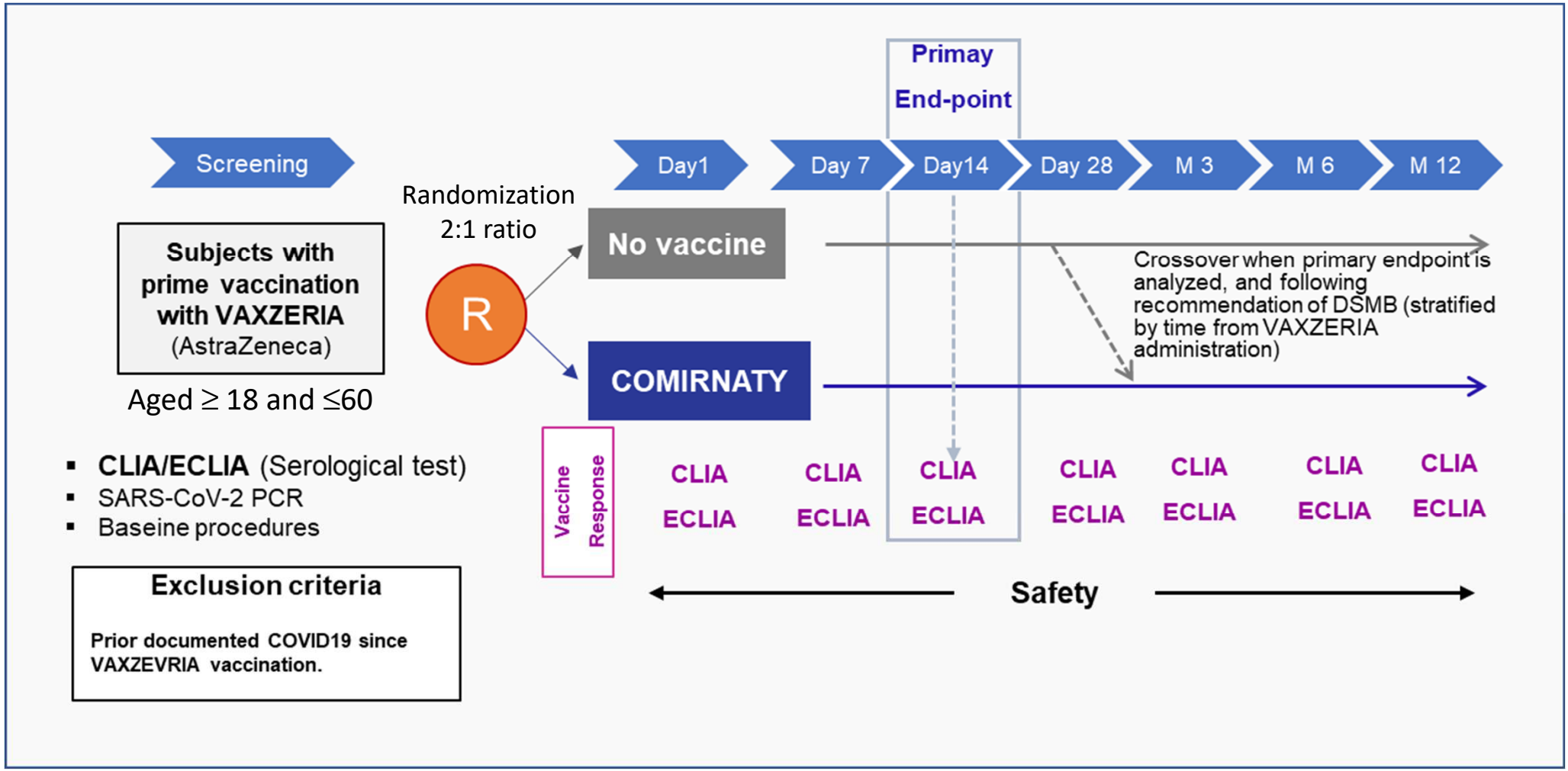
- To assess the **humoral immune response** against SARS-CoV-2 **28 days** after a COMIRNATY boost in subjects that received a prime, single dose of VAXZEVRIA.
- To assess the **long-term (up to 1 year) humoral immune response** against SARS-CoV-2 of a COMIRNATY boost in subjects that received a prime, previous single dose of VAXZEVRIA.
- To assess the **humoral immune response** against **viral variants** of SARS-CoV-2, 14 and 28 days after a COMIRNATY boost in subjects that received a previous, prime single dose of VAXZEVRIA.
- To assess the **cellular immune response** against **viral variants** of SARS-CoV-2, at 14 and 28 days after a COMIRNATY boost in subjects that received a previous, prime single dose of VAXZEVRIA.

Exploratory objective: To evaluate the relationship between the immune response measured as **NAV (Neutralizing Antibodies)** and antibodies against SARS-CoV-2 spike protein measured by immunoassays.

Design

- **Secondary efficacy objectives:** To assess the occurrence of symptomatic molecularly confirmed infection by SARS-CoV-2 and severity of COVID-19 signs and symptoms after the administration of a COMIRNATY boost in subjects that received a prior, prime single dose of VAXZEVRIA.
- **Primary Reactogenicity Objective:** To evaluate the reactogenicity of a COMIRNATY boost in subjects that received a prime, previous single dose of VAXZEVRIA.

Protocol overview



Timeline

March 2021
Signal assessment initiated at PRAC

7 April 2021
EMA: Causal relationship between vaccination with Vaxzevria and TTS

8 April 2021
Vaxzevria is withheld under 50-60 y.o in several MS

15 April 2021
Ethics commitee approval for CombiVacS clinical trial

23 April 2021
EMA/CHMP assessment report

24 April 2021
First subject included in CombiVacS

30 April 2021
Enrolment completed (676 subjects)

18 May 2021
Preliminary data on primary end-point

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Instituto de Salud Carlos III

Instituto de Salud Carlos III **Sponsor** **Coordination** SCReN Spanish Clinical Research Network ISCIII

University Hospitals

SaludMadrid Hospital Universitario La Paz
Hospital Carlos III
Hospital Cantoblanco

SaludMadrid Hospital Clínico San Carlos

CLÍNIC BARCELONA
Hospital Universitari

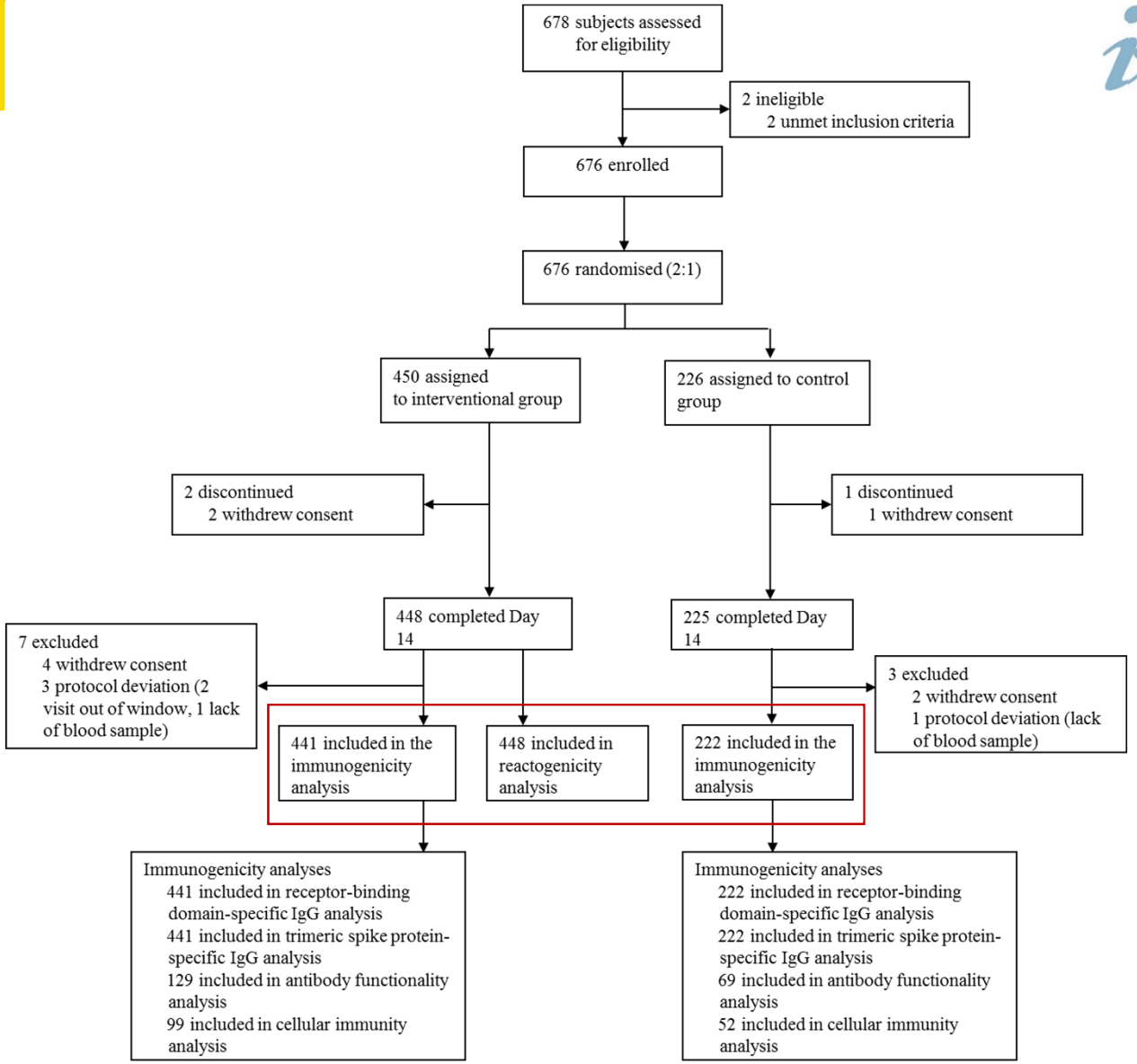
Vall d'Hebron Hospital

Osakidetza
GUO / HUC
GURUTZETAKO UNIBERTSITATE OSPITALEA
HOSPITAL UNIVERSITARIO CRUCES

Central Laboratory

Instituto de Salud Carlos III

NACIONAL CENTER FOR MICROBIOLOGY


678 subjects assessed for eligibility
2 ineligible
2 unmet inclusion criteria
676 enrolled
676 randomised (2:1)
450 assigned to interventional group
226 assigned to control group
2 discontinued
2 withdrew consent
1 discontinued
1 withdrew consent
448 completed Day 14
225 completed Day 14
7 excluded
4 withdrew consent
3 protocol deviation (2 visit out of window, 1 lack of blood sample)
3 excluded
2 withdrew consent
1 protocol deviation (lack of blood sample)
441 included in the immunogenicity analysis
448 included in reactogenicity analysis
222 included in the immunogenicity analysis
Immunogenicity analyses
441 included in receptor-binding domain-specific IgG analysis
441 included in trimeric spike protein-specific IgG analysis
129 included in antibody functionality analysis
99 included in cellular immunity analysis
Immunogenicity analyses
222 included in receptor-binding domain-specific IgG analysis
222 included in trimeric spike protein-specific IgG analysis
69 included in antibody functionality analysis
52 included in cellular immunity analysis

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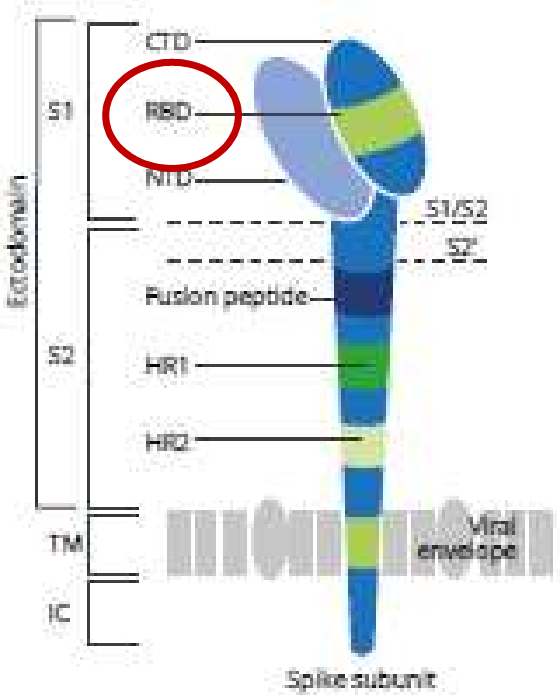
Demographic data

		Control Group (N = 222)	Experimental group (N = 441)
Age	Mean (SD)	44,18 (8,8)	43,98 (8,85)
	Median	45	46
	Min, Max	22 , 55	19 , 56
Age groups	18-49	139 (63%)	287 (65%)
	50-59	82 (37%)	155 (35%)
Gender	Female	123 (56%)	252 (57%)
	Male	98 (44%)	190 (43%)

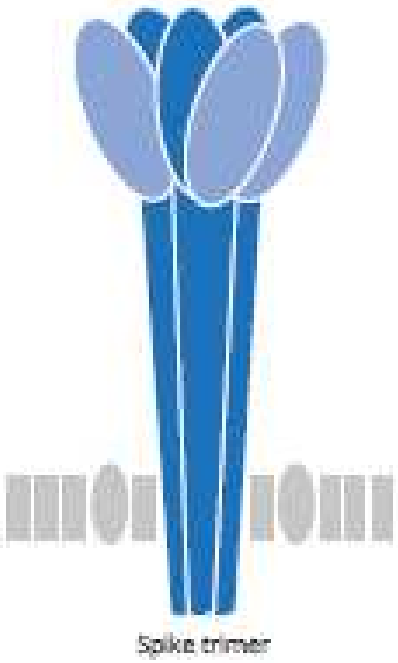
#ESTE VIRUS LO PARAMOS UNIDOS

Serological testing

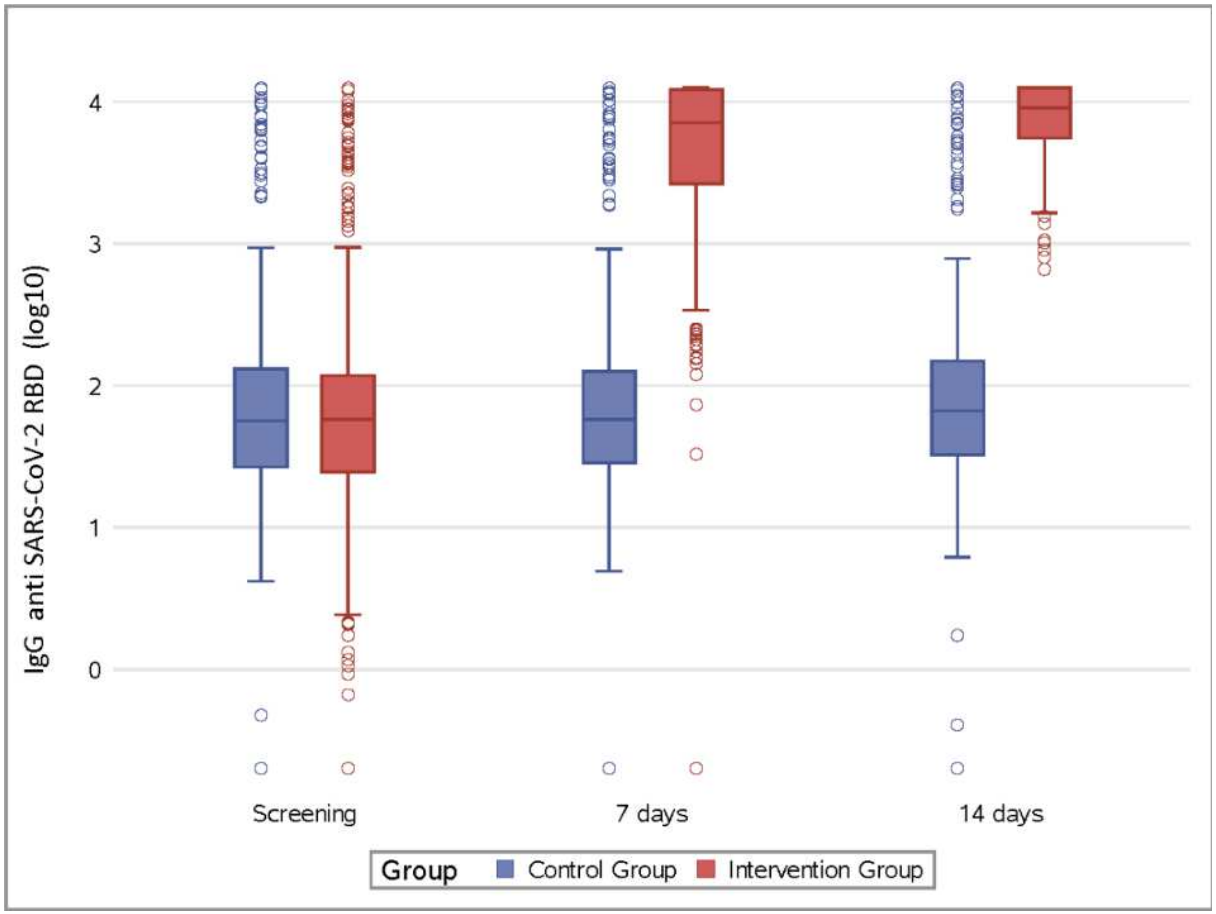
Elecsys® Anti-SARS-CoV-2 (RBD)
(ECLIA)



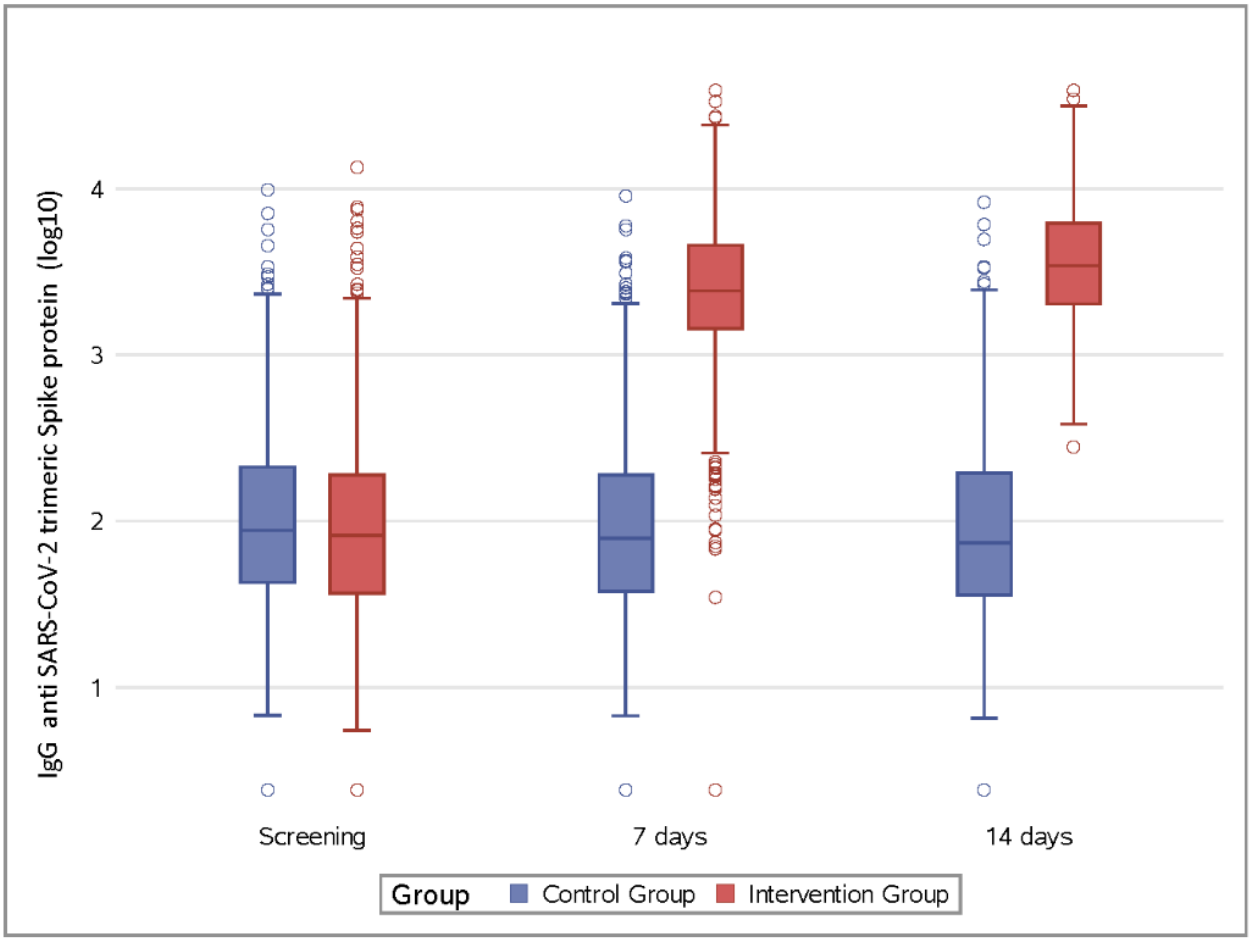
LIAISON® SARS-CoV-2 TrimericS
(CLIA)

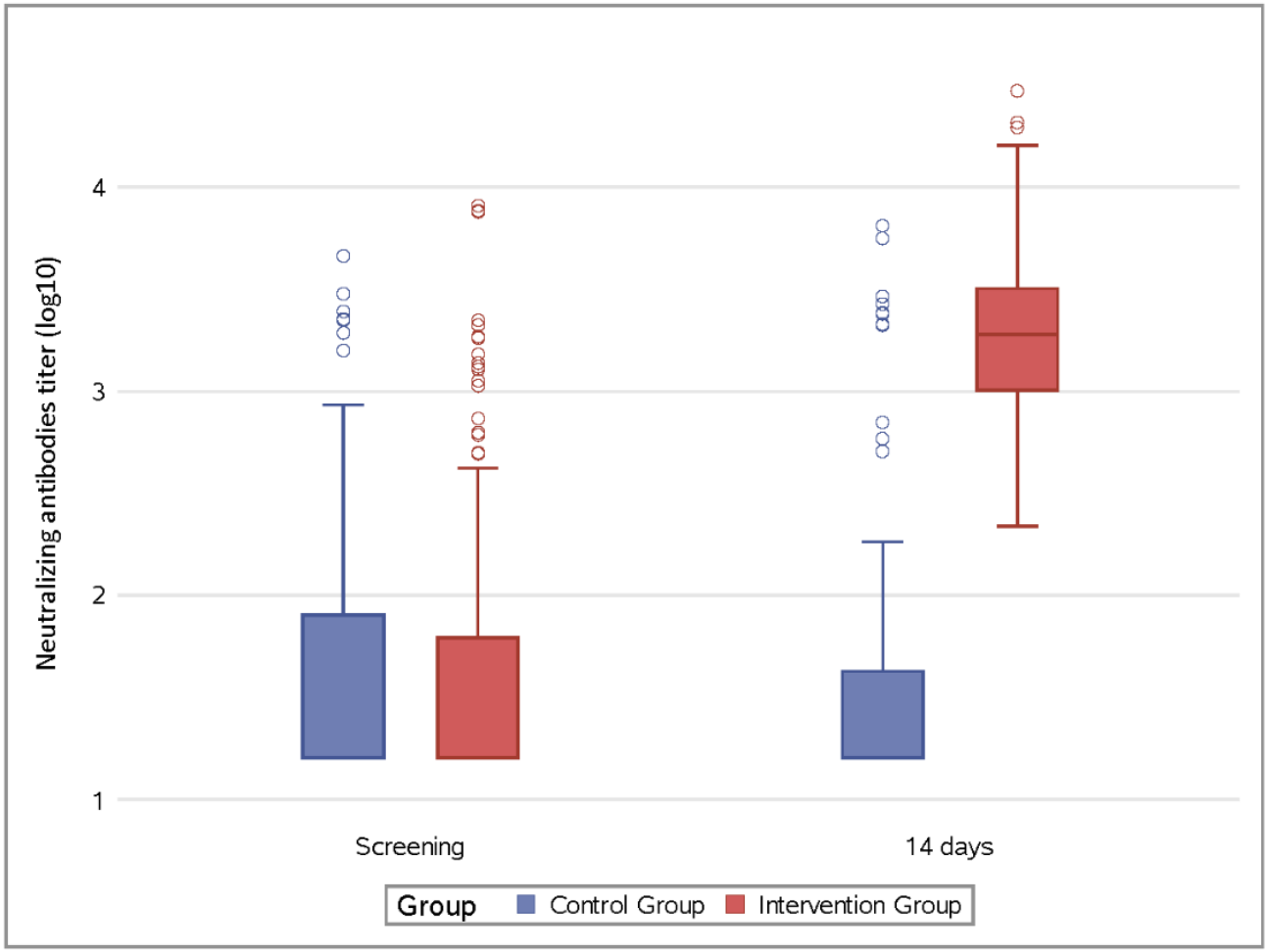


RBD (anti-spike) antibody titers measured on the day of randomization (day 0) and at day 7 and 14.



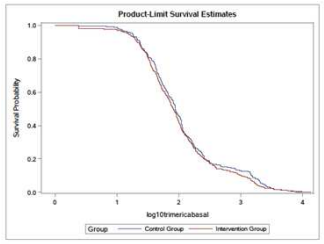
Trimeric S antibody titers against Spike protein measured on intervention and control groups at days 0, 7 and 14.

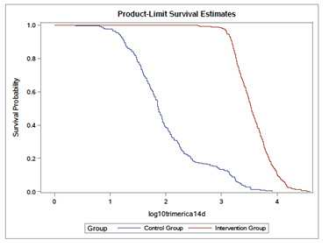



Neutralizing antibodies titer (log10)
4
3
2
1
Screening
14 days
Group
Control Group
Intervention Group

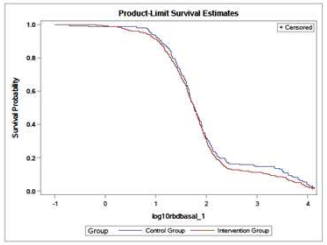
Reverse cumulative distribution curve

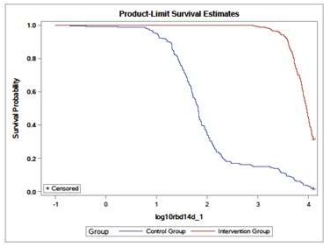
RBD-IgG



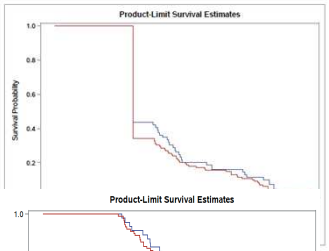


trimericS-IgG

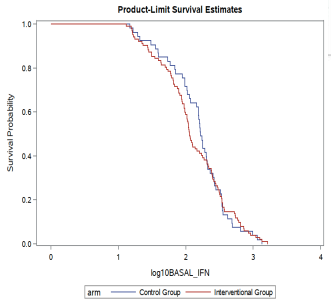


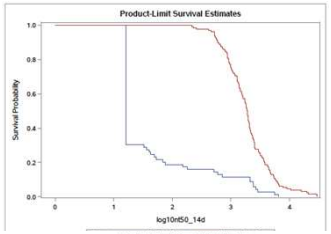


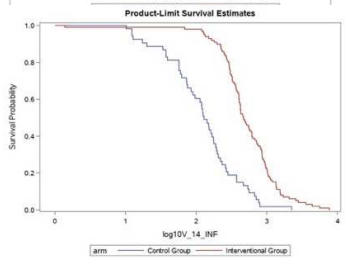
NAv



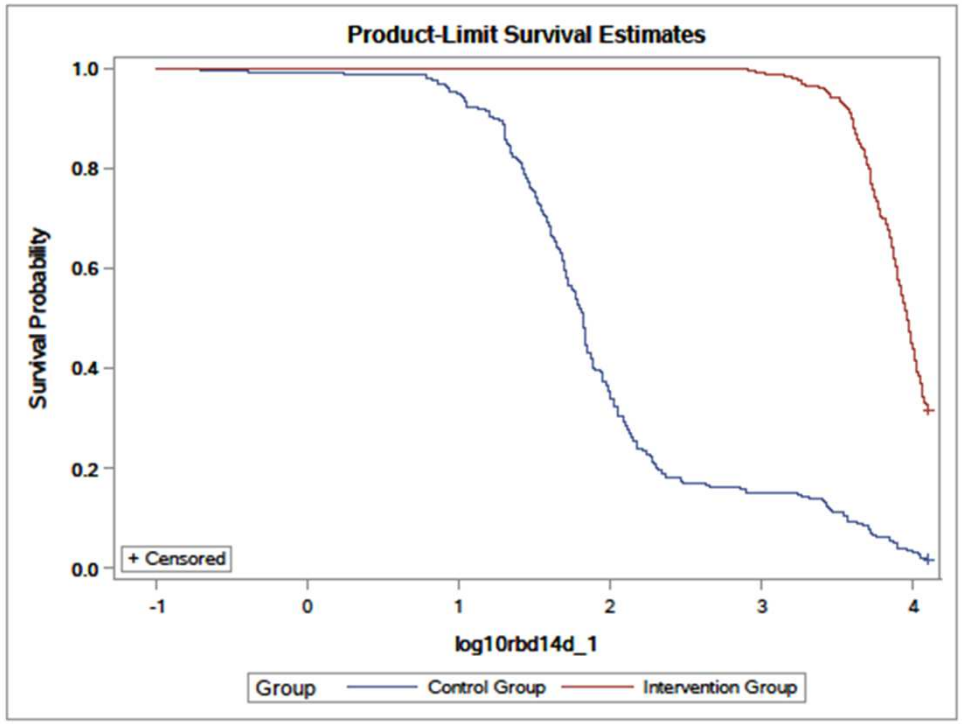
Cellular immune response

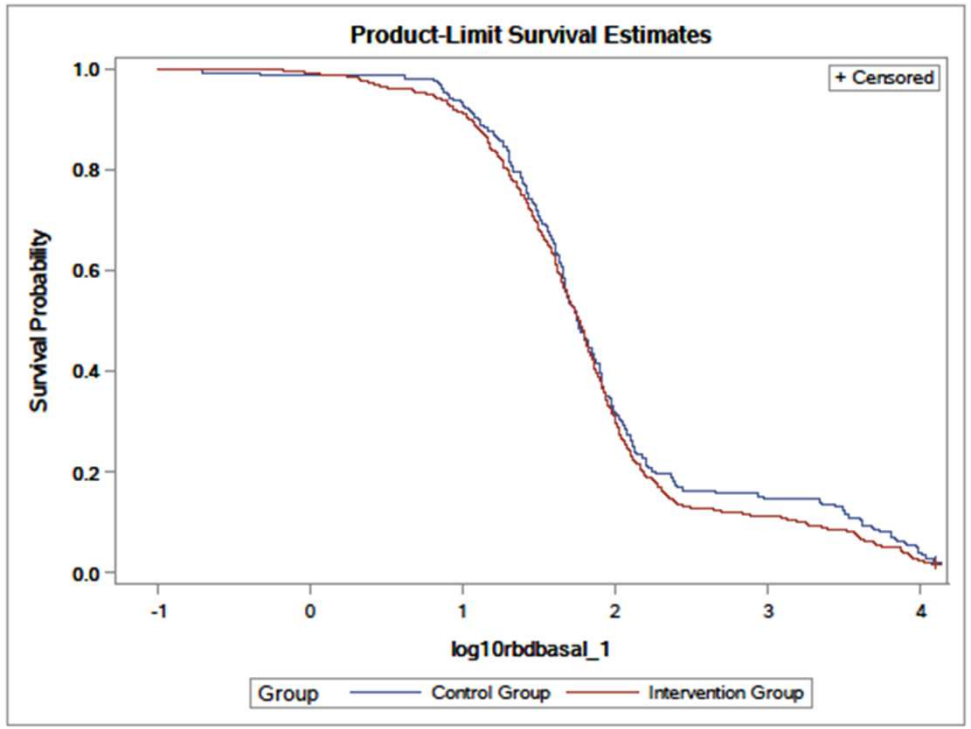






Neutralising activity - Reverse cumulative distribution curve





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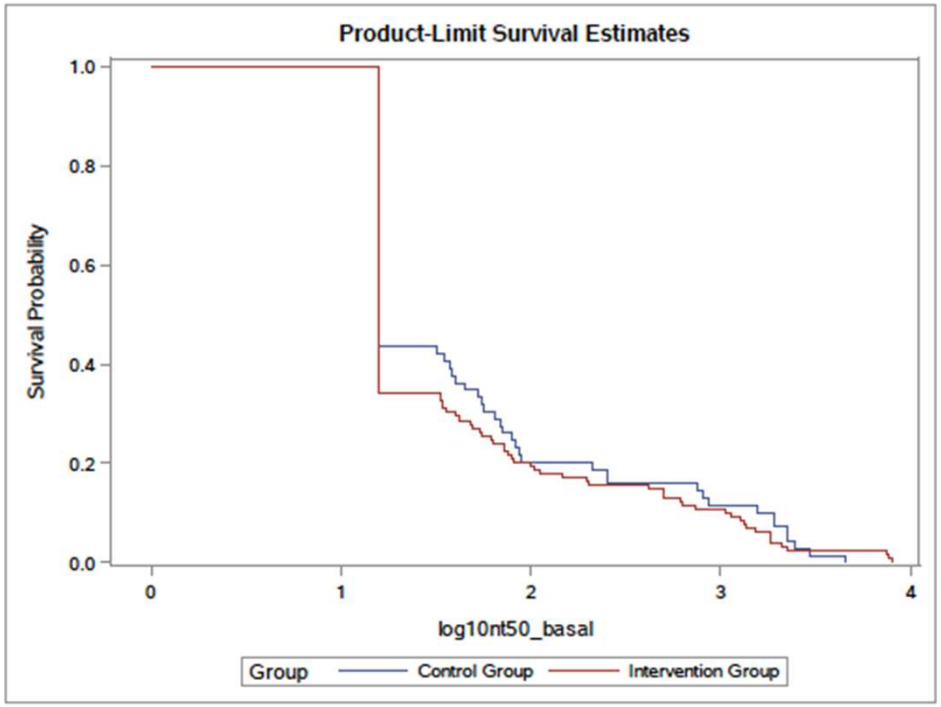
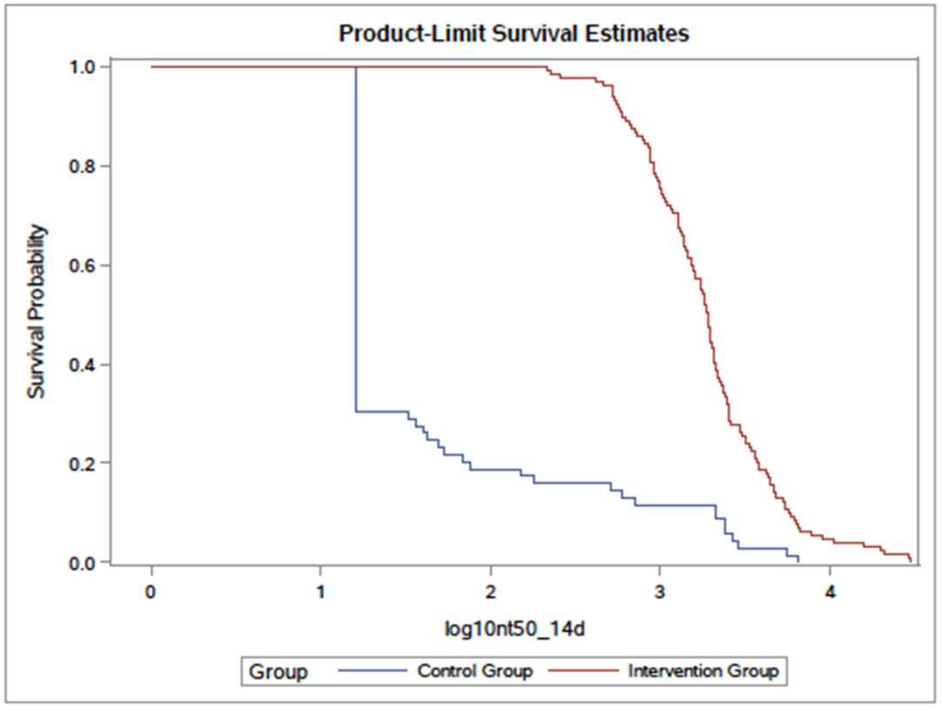
Neutralising activity - Reverse cumulative distribution curve





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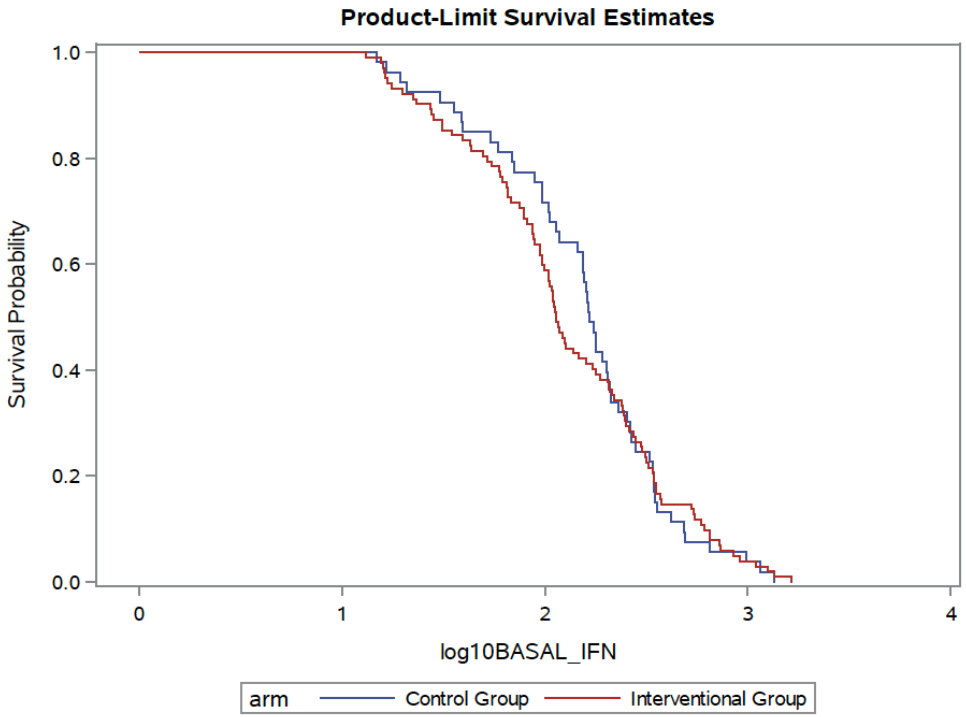
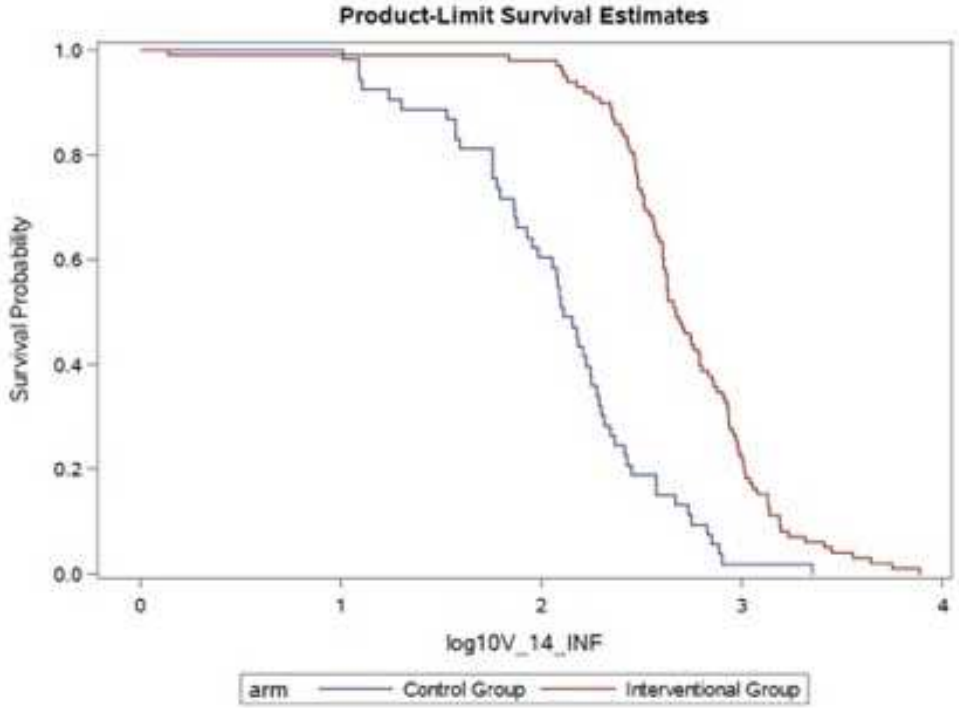
Cellular immunogenicity - Reverse cumulative distribution curve





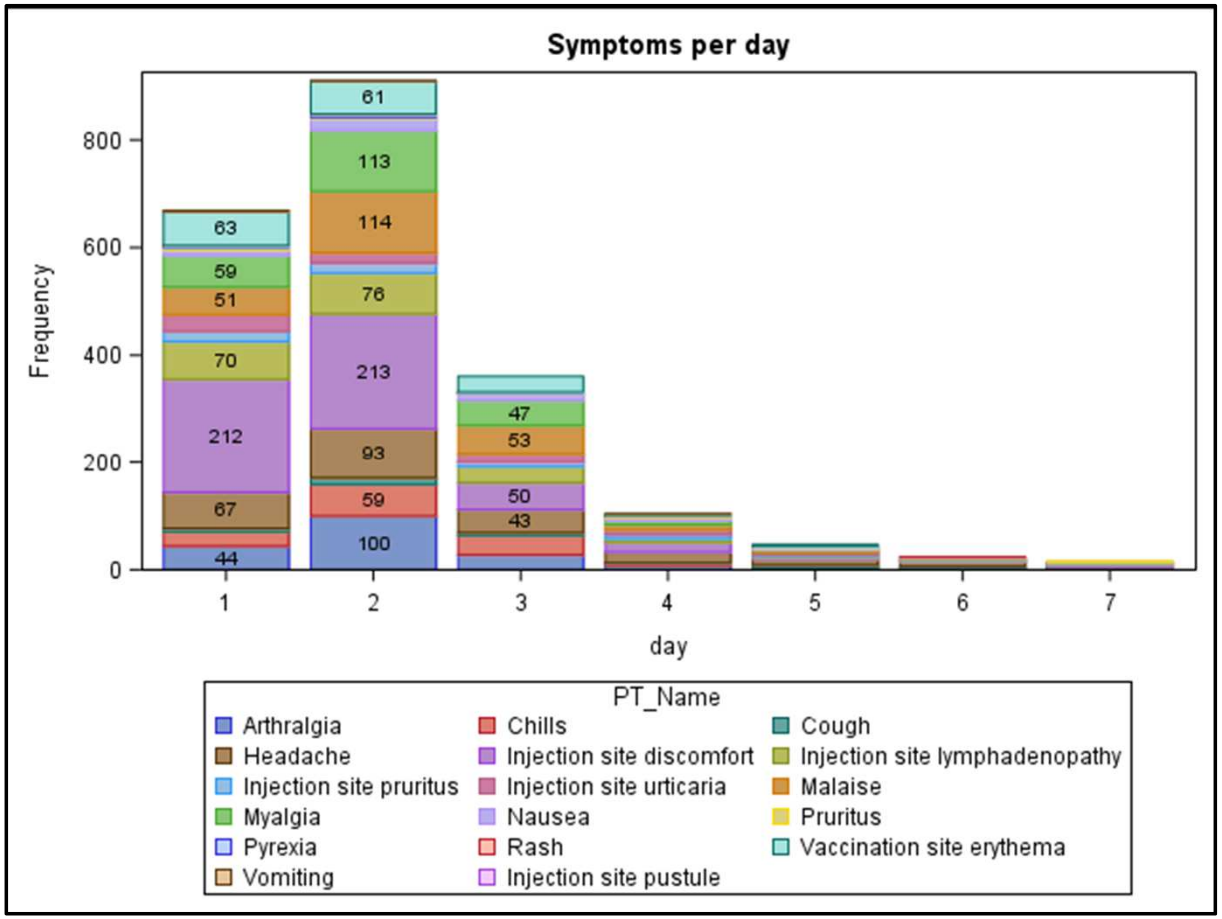
Reactogenicity

Table S6. Local and systemic reactions in first 7 days after BNT162b2 vaccination

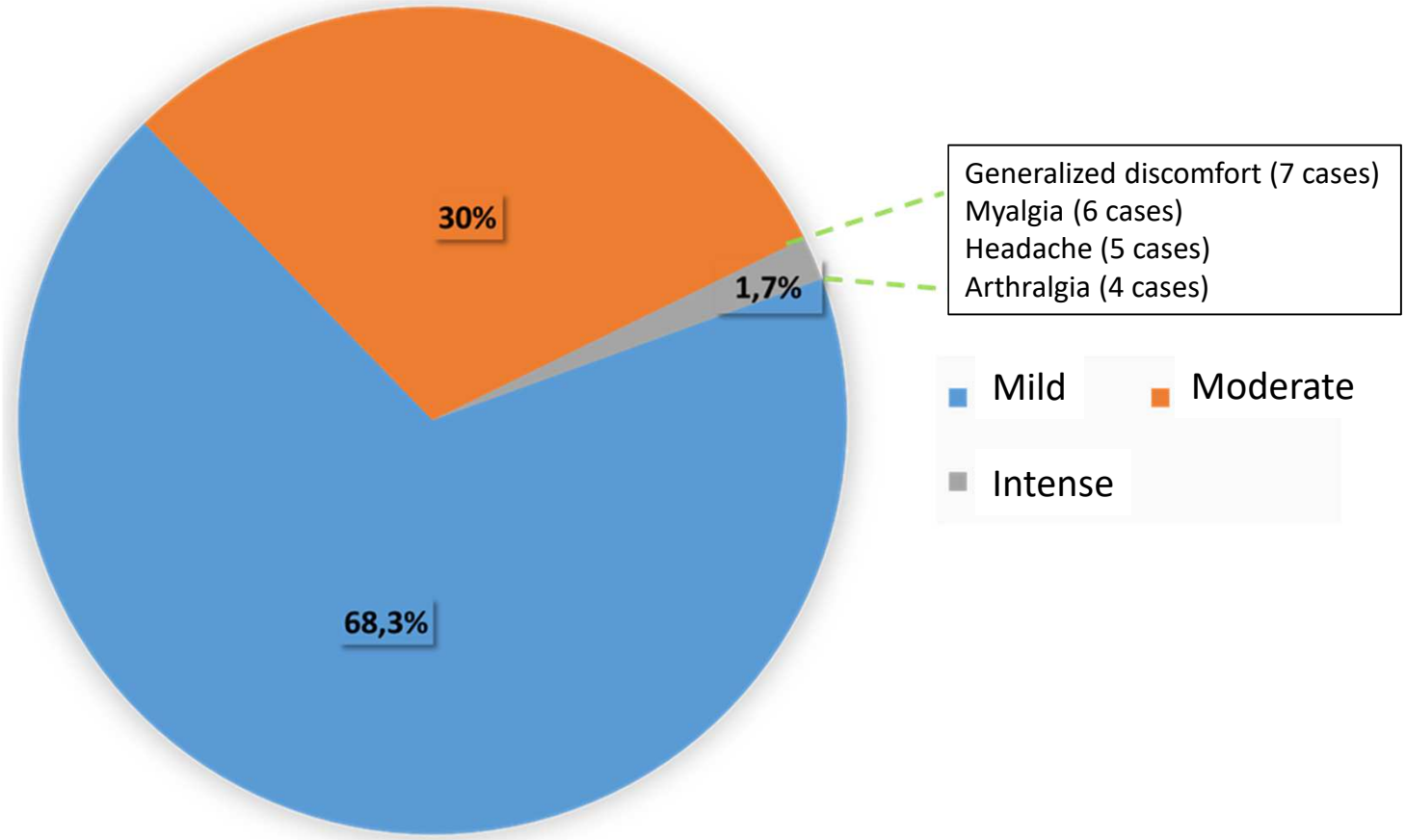
	Interventional Group (n=448)
Chills, n (%)	114 (25.4)
Cough, n (%)	33 (7.4)
Headache, n (%)	199 (44.4)
Injection site discomfort, n (%)	395 (88.2)
Injection site lymphadenopathy, n (%)	159 (35.5)
Injection site urticaria, n (%)	67 (15.0)
Vaccination site erythema, n (%)	139 (31.0)
Myalgia, n (%)	194 (43.3)
Arthralgia, n (%)	155 (34.6)
Injection site pruritus, n (%)	49 (10.9)
Malaise, n (%)	187 (41.7)
Pyrexia, n (%)	11 (2.5)
Nausea, n (%)	49 (10.9)
Pruritus, n (%)	9 (2.0)
Vomiting, n (%)	4 (0.9)
Rash, n (%)	6 (1.3)
Injection site pustule, n (%)	1 (0.2)

Reactogenicity

Symptoms of any severity communicated by subjects in electronic diary (days 1 to 7) after vaccine administration.



Reactogenicity



No SAEs

Summary

- 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.
- In the absence of an experimental arm with vaxzevria as second dose, comparisons with homologous schedules should be done with caution.
- However, the humoral and cellular immune responses observed in this trial seems to be even more intense than the observed with homologous vaccination schedules in previous studies.

Safety, reactogenicity, and immunogenicity of homologous and heterologous prime-boost immunisation with ChAdOx1-nCoV19 and BNT162b2: a prospective cohort study

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Abstract

Objective: to assess reactogenicity and immunogenicity of heterologous prime-boost immunisations of ChAdOx1-nCoV19 (Vaxzevria, ChAdOx) followed by BNT162b2 (Comirnaty, BNT) compared to homologous BNT/BNT immunisation.

Design: prospective, observational cohort study.

Setting: unicenter study in a cohort of health care workers at a tertiary care center in Berlin, Germany.

Participants: 340 health care workers immunised between 27 December 2020 and 21 May 2021 at Charité - Universitätsmedizin Berlin, Germany

Main outcome measures: the main outcomes were reactogenicity assessed on days one, three, five and seven post prime and boost vaccination, and immunogenicity measured by serum SARS-CoV-2 full spike-, spike S1-, and spike RBD-IgG, virus neutralisation capacity, anti-S1-IgG avidity, and T cell reactivity measured by Interferon gamma release assay at 3-4 weeks post prime and boost immunisation.

Results: Heterologous ChAdOx/BNT booster vaccination was overall well-tolerated and reactogenicity was largely comparable to homologous BNT/BNT vaccination. Systemic reactions were most frequent after prime immunisation with ChAdOx (86%, 95CI: 79-91), and less frequent after homologous BNT/BNT (65%, 95CI: 56-72), or heterologous ChAdOx/BNT booster vaccination (48%, 95CI: 36-59). Serum antibody responses and T cell reactivity were strongly increased after both homologous and heterologous boost, and immunogenicity was overall robust, and comparable between both regimens in this cohort, with slightly increased S1-IgG avidity and T cell responses following heterologous booster immunisation.

Conclusions: Evidence of rare thrombotic events associated with ChAdOx has led to recommendation of a heterologous booster with mRNA vaccines for certain age groups in several European countries, despite a lack of robust safety and immunogenicity data for this vaccine regimen. This interim analysis provides evidence that the currently recommended heterologous ChAdOx/BNT immunisation regimen with 10-12 week vaccine intervals is well tolerated and slightly more immunogenic compared to homologous BNT/BNT vaccination with three week vaccine intervals. Heterologous prime-boost immunisation for COVID-19 may be generally applicable to optimise logistics and improve immunogenicity and to mitigate potential intermittent supply shortages for individual vaccines.

Introduction

In light of intermittent supply shortages of individual vaccines and evidence of rare, but severe adverse events following vaccination with vector-based vaccines such as ChAdOx1-nCoV19 COVID-19 vaccine (Vaxzevria, AstraZeneca, ChAdOx) [1–4], heterologous prime-boost regimens for COVID-19 vaccines have gained significant interest [5]. Heterologous booster vaccination with an mRNA vaccine following initial immunisation with ChAdOx is now recommended in specific age groups in several countries, including Germany [6], despite limited or absent data on reactogenicity, safety and immunogenicity of this prime-boost regimen in humans.

On January 29, 2021, the German standing committee on vaccination (STIKO) recommended that ChAdOx should only be administered to persons between 18-64 years of age. Consequently, mainly younger persons, including healthcare workers, received ChAdOx while mRNA vaccines (BNT162b2 (BNT) and Moderna mRNA-1273) were prioritized for use in the elderly. In response to reports about rare blood clotting events, including cerebral venous sinus thrombosis associated with ChAdOx vaccination, especially in younger women [2–4], several European countries restricted their recommendations for ChAdOx vaccination to individuals above a certain age limit (e.g. above 60 years in Germany, and 55 years in France) [7]. Heterologous boost immunisation with an mRNA vaccine (BNT or mRNA-1273) was recommended for persons who had already received a first immunisation with ChAdOx, but who are younger than the revised age limit for ChAdOx [7]. In Phase 2/3 trials, both BNT and ChAdOx demonstrated significant reactogenicity, most commonly pain at the injection site, fatigue, headache, chills, and fever, with only a minor fraction of study participants reporting severe reactions [8,9]. A recent interim analysis of reactogenicity data in the Com-COV trial, investigating various heterologous prime-boost regimens of licensed COVID-19 vaccines, reported no serious side effects, but clearly increased reactogenicity following heterologous boost with BNT 28 days after initial vaccination with ChAdOx [10]. In this interim analysis, up to 80% of persons receiving a heterologous prime-boost with ChAdOx/BNT reported fatigue and other systemic reactions, an up to 40-fold increase compared to the respective homologous boost vaccinations [10]. Both BNT and AZ have been shown to elicit robust immune responses with a significant increase following homologous boost vaccination in clinical trials and real world studies [8,9,11–13]. Heterologous prime-boost immunisation has been shown to elicit increased immunogenicity for other vaccines [5,14,15], and early animal experiments suggest increased immunogenicity of boost vaccination with an mRNA vaccine following initial immunization with adeno-vector based COVID-19 vaccines [16]. However, data on immunogenicity of heterologous prime-boost vaccination for COVID-19 in humans is still lacking.

Heterologous ChAdOx/mRNA vaccination has already commenced across Europe, despite a lack of robust immunogenicity and safety data for this combination. No data on immunogenicity and reactogenicity of heterologous versus homologous BNT/ChAdOx vaccination at 10-12 week intervals, as recommended in many countries, is available to date. Here, we report reactogenicity and immunogenicity data of homologous BNT/BNT and heterologous ChAdOx/BNT prime-boost immunisations in a prospective observational cohort study of 340 healthcare workers in Berlin, Germany. We found comparable reactogenicity and robust immunogenicity of homologous and heterologous vaccine regimens.

Methods

Methodology and study design and assessment of immunogenicity have also been described in detail previously [17].

Study design

Health care workers receiving routine COVID-19 vaccination were enrolled in the EICOV and COVIM prospective, observational cohort studies conducted at Charité - Universitätsmedizin Berlin, Germany, after written informed consent was obtained. EICOV was approved by the ethics committee (IRB) of Charité - Universitätsmedizin Berlin (EA4/245/20) and COVIM (EudraCT-No. 2021-001512-28) was approved by the Federal Institute for Vaccines and Biomedicines (Paul Ehrlich Institute) and by the Ethics committee of the state of Berlin. Both studies were carried out in accordance with the guidelines of Good Clinical Practice (ICH 1996) and the Declaration of Helsinki.

Study participants either received two doses of BNT three weeks apart or an initial dose of ChAdOx followed by a heterologous boost with BNT 10-12 weeks later, in accordance with the recommendations of the German standing committee on vaccination (STIKO). Baseline data on demographics were collected by questionnaire (eCRF) at enrollment. Blood samples for detection of SARS-CoV-2 specific antibodies and T cell response were collected immediately prior to the first vaccination, and three to four weeks after the first and the second vaccination.

Assessment of reactogenicity and safety

Participants were asked to fill out electronic questionnaires on reactogenicity, adverse events, medication, and medical visits on days 1, 3, 5, and 7 after the first and second vaccination. In addition, the use of antipyretic medication (NSAID, acetaminophen) before and after vaccination was recorded. We assessed local and systemic reactions to the different vaccines using a modified Food and Drug administration (FDA) toxicity scale [18]. Following the initial assessments, all participants were asked to self-report any systemic symptoms and intake of pain medication through an electronic questionnaire every two weeks. Here, we report on the results of questionnaires collected the first seven days following first and second vaccination.

Assessment of immunogenicity

Participants with PCR-confirmed infection or detectable anti-nucleocapsid protein (NP) IgG at any time point in the study were excluded from further analysis. A subset of all study participants was selected for immunogenicity analysis based on multivariate matching for sex and age between vaccine groups. Presence of SARS-CoV-2 specific antibodies was investigated using a microarray-based immunoassay including spike (full spike,S1, RBD) and nucleocapsid (N) as antigens in order to discriminate between vaccine-induced antibody response and convalescent SARS-CoV-2 infection (SeraSpot®Anti-SARS-CoV-2 IgG, Seramun Diagnostica GmbH, Heidesee, Germany, [17]). Functional neutralization capacity was investigated using a surrogate SARS-CoV-2 neutralization test (sVNT, cPass, medac GmbH, Wedel, Germany), following the manufacturer's instructions [17,19]. Maturation of IgG avidity was characterized by a modified anti-SARS-CoV-2 S1 IgG ELISA (anti-SARS-CoV-2 S1 IgG ELISA Kit; Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany) [17] in 30 randomly selected samples each from the homologous and heterologous boost cohorts who were seroreactive three weeks after prime vaccination. SARS-CoV-2 spike specific T cell

responses were measured by an interferon-γ release assay (IGRA; Euroimmun [17]) of S1 peptide stimulated T cells in heparinized whole blood. IFN-γ was measured by ELISA and an arbitrary unit was displayed subtracting the blank optical density (OD) 450/620nm from S1-peptide-pool stimulated samples.

Statistics

Data are presented as median and interquartile range, unless stated otherwise. Statistical analysis was performed using GraphPad PRISM statistics version 27.0 (IBM Deutschland, Ehningen, Germany). Group comparisons were performed in a univariate analysis using Fisher's exact test or nonparametric Kruskal-Wallis test with Dunn's multiple comparisons test. All 95% confidence intervals were calculated according to the Wilson and Brown method [20]. P-values <0.05 were considered statistically significant.

Results

From December 27, 2020, to March 30, 2021, a total of 340 healthcare workers were enrolled at Charité - Universitätsmedizin Berlin, Germany. Twenty-six participants had either a positive PCR result for SARS-CoV-2 during the study or a detectable anti-spike IgG or anti-N IgG antibody response at baseline or follow up (N), and were therefore excluded from further analysis. Eight participants opted for a homologous ChAdOx/ChAdOx booster immunisation and were only included for reactogenicity analysis of prime immunisation. Baseline characteristics of the study population, an overview of the vaccine groups and respective study sub-cohorts for reactogenicity and immunogenicity analyses are given in **Table 1**.

Vaccine group	**BNT/BNT[1] homologous boost**		**ChAdOx[2]/BNT heterologous boost**	
Prime to boost interval , median days (IQR)	21 (21-21)		71 (70-73)	
Prime and boost vaccination	1st BNT, n=179	1st BNT / 2nd BNT n=189	1st ChAdOx n=151	1st ChAdOx / 2nd BNT n=110
Reactogenicity data, n	178	159	148	99
Age, median years (IQR)	34 (29-44)	34 (29-43)	35 (28-47)	37 (29-51)
Female, n (%)	98 (55.0%)	87 (54.7%)	101 (68.2%)	77 (77.8%)
Serology data measured, n	94	101	57	61
Δvaccination to sampling, median days (IQR)	21 (21-21)	28 (27-30.5)	26 (22-28)	21 (I21-21)
Age, median years (IQR)	35 (30.75-48)	35 (30.5-47.5)	38 (31-52.5)	38 (30.5-51.5)
Female, n (%)	66 (70.2%)	73 (72.3%)	46 (80.7%)	47 (77.1%)

Table 1: **Baseline characteristics and schedule of BNT/BNT homologous prime and boost and ChAdOx/BNT heterologous prime and boost study participants.**
[1]BNT: BNT162b2 mRNA COVID-19 vaccine, [2]ChAdOx: ChAdOx1-nCoV19 COVID-19 vaccine, IQR: interquartile range

Reactogenicity

All vaccinations were associated with a relatively high frequency of local reactions, most commonly pain and tenderness. Local reactions were usually mild or moderate (**Fig. 1A, B, Table S1**). No major differences were observed in the frequency or severity of local reactions after either of the prime or boost immunisations, with the exception of a slightly higher frequency of local reactions after heterologous ChAdOx/BNT booster vaccination in comparison to homologous BNT/BNT booster vaccination (**Fig. 1A, B, Table S1**). In contrast, notable differences were reported for systemic reactions. These were most frequently reported following prime immunisation with ChAdOx (86.49%, 95%CI: 80.05-91.08), and after homologous BNT/BNT booster immunisation (64.78%, 95%CI: 57.09-71.78), whereas only 51.52% (95%CI: 41.80-61.12) of participants after the heterologous BNT booster (ChAdOx/BNT) vaccination, and 38.76% (95%CI: 31.92-46.09) of participants after the first immunisation with BNT reported systemic reactions (**Fig. 1C**). Severe systemic symptoms, including fatigue, myalgia, headache, feverishness or chills, and fever >38°C, were reported more frequently following ChAdOx prime immunisation and homologous BNT/BNT booster immunisation compared to heterologous ChAdOx/BNT booster vaccination (**Fig. 1D, Table S1**).

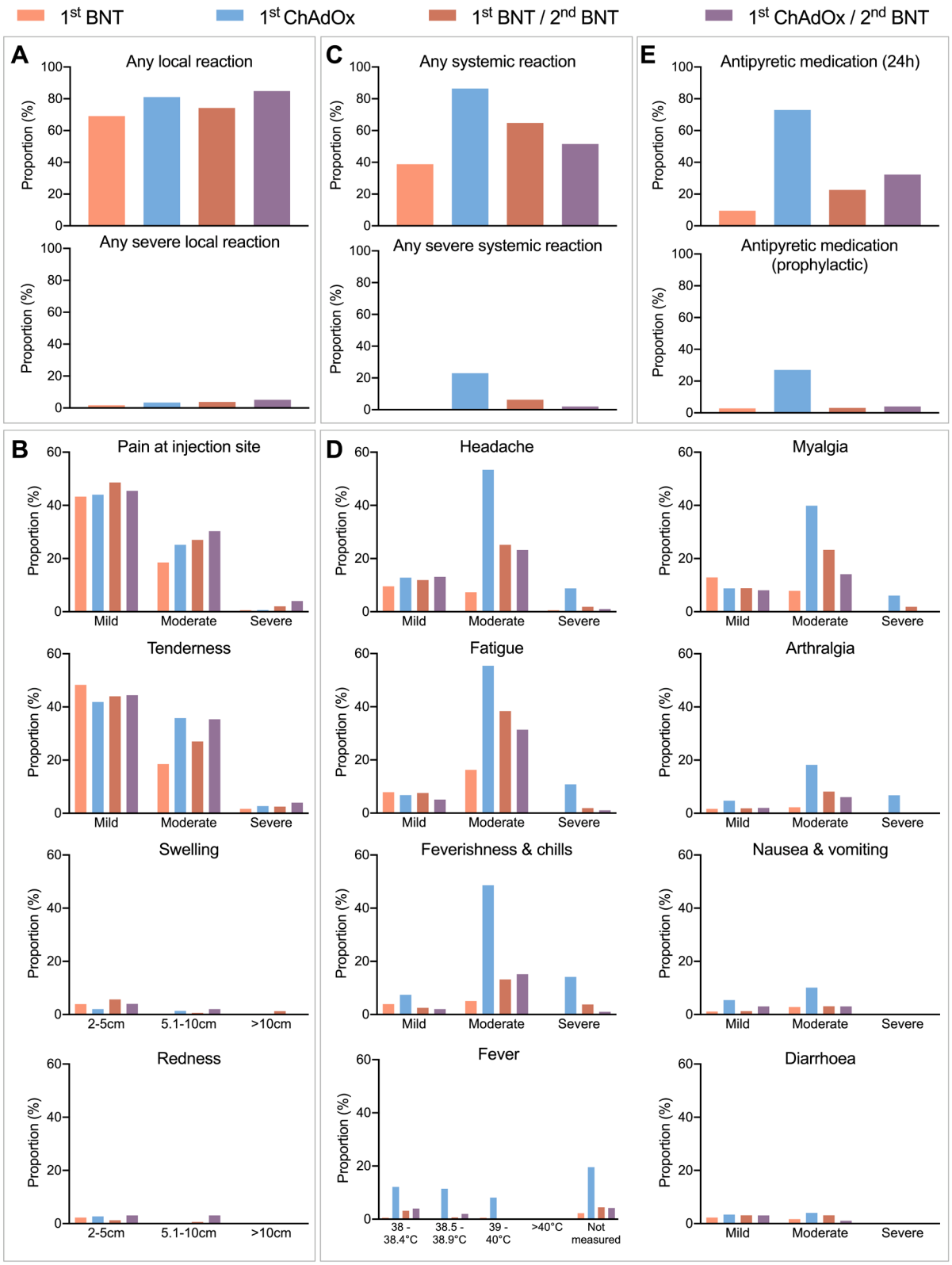

Fig. 1. Local and systemic reactogenicity of BNT or ChAdOx prime immunisations and homologous or heterologous boosting until day seven after vaccination. (**A, B**) Proportion of participants reporting any local reaction (A), and indicated local reactions grouped by severity (B). (**C, D**) Proportion of participants reporting any systemic reaction (C), and indicated systemic symptoms grouped by severity (D). (**E**) Proportion of participants reporting intake of antipyretic medication within 24 hours after vaccination (top) and prophylactic intake of antipyretic medication (bottom). BNT: BNT162b2 / Comirnaty, ChAdOx: ChAdOx1-nCoV19 / Vaxzevria. Definition of severity according to modified Food and Drug administration (FDA) criteria [18]: mild: does not interfere with daily activities, moderate: interferes with daily activities, severe: daily activities no longer feasible.

No potentially life-threatening reactions were reported after any of the vaccine regimens in this study. Intake of antipyretic medication was reported most frequently in conjunction with the first ChAdOx immunisation (**Fig. 1E**). Within 24 hours after the first vaccination with ChAdOx, 72.97% (95%CI: 65.30-79.48) of participants reported antipyretic medication, which was markedly lower following heterologous ChAdOx/BNT boost (32.32%, 95%CI: 23.92-42.05), homologous BNT/BNT boost (22.64%, 95%CI: 16.83-29.75), and after prime immunisation with BNT (9.55%, 95%CI: 6.05-14.76) (**Fig. 1E**). The proportion of participants who reported prophylactic antipyretic medication was highest in the ChAdOx prime immunisation group (27.03%, 95%CI: 20.52-34.70), and distinctly lower in all other groups (1.BNT: 2.81% (95%CI: 1.21-6.41), 1.ChAdOx/2.BNT: 4.04% (95%CI: 1.58-9.93), 1.BNT/2.BNT: 3.14% (95%CI: 1.35-7.15). Thus, prophylactic intake of antipyretics cannot explain differences in adverse reactions between ChAdOx/BNT boost vaccination compared to ChAdOx prime vaccination.

The majority of vaccine reactions were reported on day one and three after vaccination and receded by day seven (**Fig. 2**).

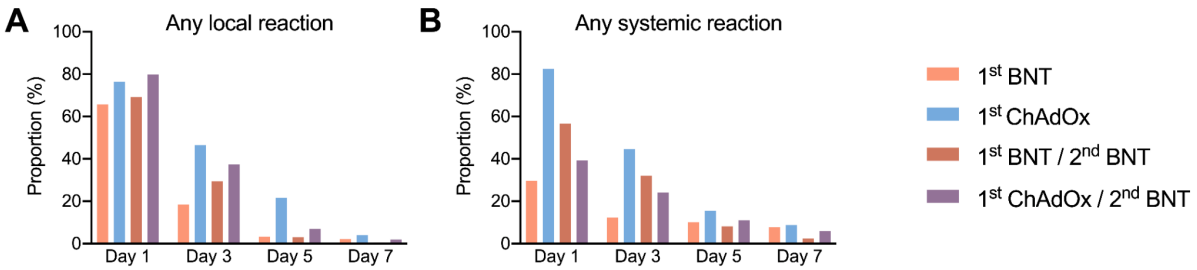

Figure 2: Reactogenicity of BNT or ChAdOx prime immunisation and homologous or heterologous booster vaccination reported until day seven after vaccination. (**A**) Local reactions (any severity) reported by day, over the course of seven days. (**B**) Systemic reactions (any severity) reported by day, over the course of seven days.

Immunogenicity

Three weeks after prime immunisation with BNT, 63/94 (67.02%, 95%CI: 57.01-75.69) participants were reactive for anti-SARS-CoV-2-S1 (S1) IgG compared to only 16/57 (28.07%, 95%CI:18.08-40.43, p<0.0001) participants after ChAdOx prime immunisation (**Fig. 3A**). The proportion of S1 reactivity increased to 100/101 (99.01%, 95%CI: 94.60-99.95) three weeks after homologous BNT/BNT boost immunisation, and to 61/61 (100.00%, 95%CI:94.08-100.00) three weeks after heterologous ChAdOx/BNT boost immunisation (**Fig. 3A**). Compared to BNT immunised participants, ChAdOx immunised participants had significantly lower anti-S1 IgG levels three weeks after prime immunisation (median 2.08 S/Co, IQR:1.45-3.04 vs 0.52 S/Co, IQR: 0.28-1.00, p=0.02, **Fig. 3A**). Levels of anti-RBD IgG (median 1.28 S/Co, IQR: 0.57-2.16 vs 2.84 S/Co, IQR: 2.02-4.06, p=0.14) and anti-full spike IgG (median 1.23 S/Co, IQR: 0.61-1.73 vs 2.08 S/Co, IQR: 1.45-3.04, p=0.62) were slightly lower, but not significantly reduced when correcting for multiple testing following prime immunisation with ChAdOx compared to BNT (**Fig. 3B, Fig. S1**). Three weeks after boost immunisation, antibody responses in homologous BNT/BNT immunised participants was comparable to heterologous ChAdOx/BNT immunised participants (anti-S1 IgG median: 4.52 S/Co, IQR:3.92-5.10 vs 5.37 S/Co, IQR: 4.82-5.86, p=0.31) (**Fig. 3A,B, Fig. S1**).

In addition to antibody levels, we measured serum antibody avidity. High avidity serum antibodies, defined as an antibody avidity index >60%, were not detected after prime immunisation with either BNT or ChAdOx (**Fig. 3C**). Three weeks after boost immunisation 27/30 (90.00%, 95%CI: 74.38-96.54) participants in the homologous BNT/BNT group and 30/30 (100.00, 95%CI: 94.08-100.00) in the heterologous ChAdOx/BNT immunised group exhibited high anti-S1 IgG avidity indices (**Fig. 3C**). Hence, maturation of IgG avidity following boost vaccination was observed with both regimens. The median relative avidity index was slightly higher after heterologous ChAdOx/BNT boost (93.50%, IQR: 91.10-95.41) compared to homologous BNT/BNT boost (73.86%, 95%CI: 62.99-81.55, p=0.04, **Fig. 3C**), which may also be due to the longer dosing interval in the heterologous boost group.

Neutralising antibodies were detected in 89/94 (94.68%, 95%CI: 88.15-97.01) participants receiving BNT and in 48/57 (84.21%, 95%CI:72.64-91.46) participants receiving ChAdOx prime vaccination (**Fig. 3D**). At week three after boost immunisation with BNT, neutralising antibody response rate had increased in both cohorts to 100/101 (99.01%, 95%CI: 94.60-99.95) after BNT/BNT boost and 61/61 (100.00%, 95%CI: 94.08-100.00) after heterologous ChAdOx/BNT boost (**Fig. 3D**). Surrogate virus neutralisation test (sVNT) titers were comparable after homologous and heterologous prime-boost immunisation (**Fig. 3D**).

Serological responses are most widely used to assess immunogenicity of vaccination, but T cell responses are another important marker of anti-SARS-CoV-2 immunity. The spike S1-specific T cell response was measured in 47 ChAdOx prime immunised, 60 ChAZOx/BNT boost immunised and 66 BNT/BNT boost immunised subjects by IFN-γ release (IGRA). Three weeks after ChAdOx prime immunisation, participants showed robust T cell responses (**Fig. 3E**). Notably, T cell reactivity was significantly higher after heterologous ChAdOx/BNT boost immunisation compared to homologous BNT/BNT boosting (1.67 AU, IQR: 1.29-2.45, vs. 2.25 AU, IQR: 1.57-2.73, p=0.0255) (**Fig. 3E**).

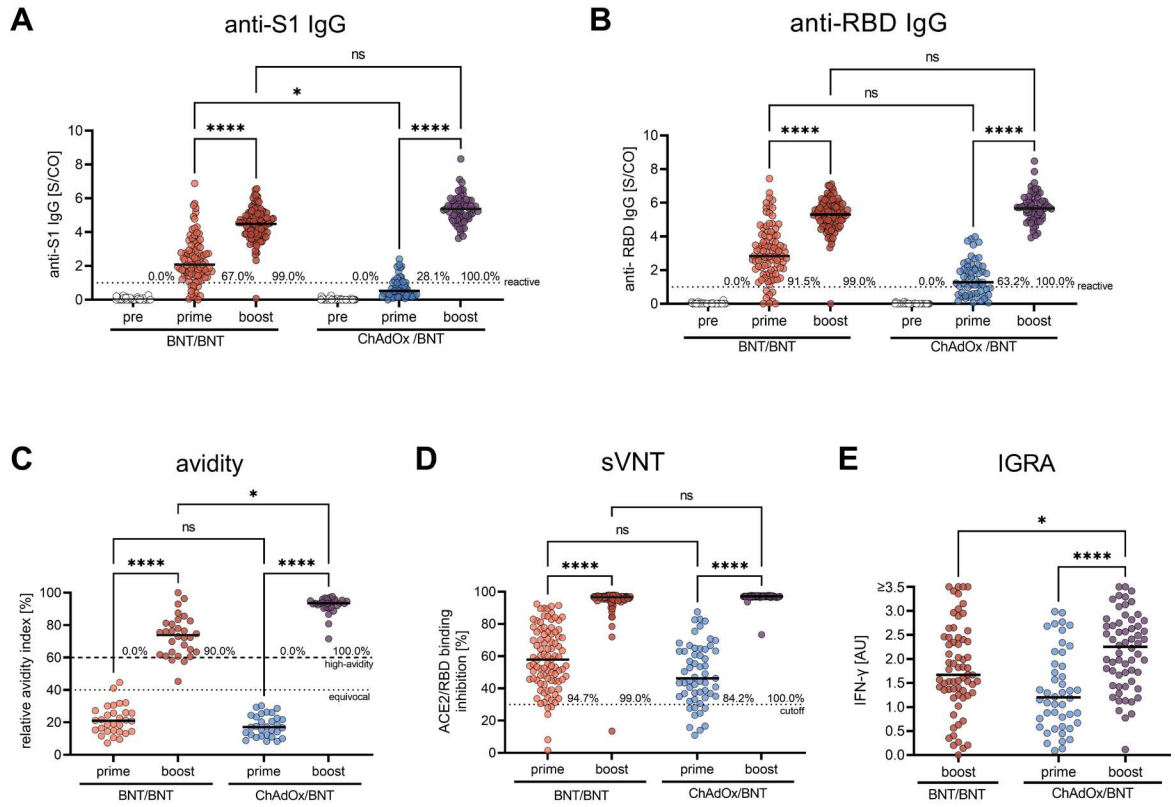

Fig. 3. SARS-CoV-2 specific antibody- and T cell response after BNT or ChAdOx prime immunisations and homologous or heterologous booster vaccination. (**A**) Anti-S1 IgG and (**B**) anti-RBD IgG measured by SeraSpot Anti-SARS-CoV-2 IgG assay, (**C**) anti-S1 IgG avidity, and (**D**) neutralizing capacity measured by sVNT in serum of subjects who had received prime immunisation with BNT or ChAdOx, and homologous BNT/BNT or heterologous ChAdOx/BNT boost. (**E**) T cell reactivity in whole blood samples measured by IGRA. BNT: BNT162b2 / Comirnaty, ChAdOx: ChAdOx1-nCoV19 / Vaxzevria. IgG: Immunoglobulin G, S/CO: signal-to-cutoff ratio, sVNT: surrogate virus neutralization assay, ACE2: angiotensin-converting enzyme 2, RBD: SARS-CoV-2 receptor-binding domain, S1: SARS-CoV-2 Spike protein S1 domain, AU: arbitrary unit. Sampling time points: pre: pre-immune sample prior to first immunisation, prime: three weeks after first vaccination, boost: three to four weeks after boost vaccination. Dotted lines indicate the manufacturer's pre-specified threshold, for anti-RBD IgG >1 S/Co, for sVNT >30% and for avidity 40-60%: borderline avidity, >60%; high avidity. Lines indicate the median. * = p<0.05; ** = p<0.01; **** = p<0.000; ns: not significant.

Discussion

This observational cohort study involving 340 health care workers provides real-world data on reactogenicity and immunogenicity of homologous BNT/BNT immunisation compared to heterologous ChAdOx/BNT vaccination against COVID-19. Overall, both regimens were well-tolerated. We observed no major difference in reactogenicity between both prime-boost regimens. Overall, local reactions were frequently observed for all vaccines. Systemic reactions, including severe reactions, were most frequent following prime immunisation with ChAdOx, whereas reactogenicity of BNT/BNT and ChAdOx/BNT was comparable, with slightly decreased systemic reactions of the heterologous booster. We observed robust immunogenicity of both homologous and heterologous prime-boost regimens. Increased S1-reactive T cell responses as measured by IGRA, were increased three weeks after heterologous ChAdOx/BNT boost compared to BNT/BNT boost vaccination. Thus, heterologous ChAdOx/BNT immunisation with a vaccine interval of 10-12 weeks is well tolerated and highly immunogenic, comparable to homologous BNT/BNT vaccination.

Strengths and limitations of this study

This is the first report of immunogenicity of heterologous ChAdOx/BNT compared to homologous BNT/BNT prime-boost vaccination. This is also the first report of real-world reactogenicity of ChAdOx/BNT vaccination with a 10-12 week vaccine interval, compared to BNT/BNT vaccination with a 3-week vaccine interval. Another strength is the longitudinal follow-up of up to 15 weeks after first immunisation. Data of this nature is urgently needed due to ongoing heterologous vaccinations in several countries.

Our study also has several potential limitations, as it is not a randomized controlled trial. Due to the current recommendations for heterologous ChAdOx/mRNA vaccination in persons <60 years of age, we were not able to recruit a matched cohort of homologous ChAdOx/ChAdOx vaccinated health care workers, since most of the study participants opted for the recommended heterologous booster. Hence, we cannot determine the exact effect of the heterologous BNT booster vaccine compared to ChAdOx homologous boosting alone. This is an interim analysis as the study is still recruiting and a comparison with homologous ChAdOx/ChAdOx vaccination may be possible with the next analysis. Here, we compared reactogenicity and immunogenicity of homologous BNT/BNT and heterologous ChAdOx/BNT vaccination. In addition to the different combinations of prime and boost vaccines, the interval between first and second vaccine was significantly different in the homologous (21 days) and heterologous vaccination group (71 days) (**Table 1**). Thus, it is unclear to which extent the observed differences may also be attributable to the extended vaccine interval in the heterologous vaccination group. The observed increased anti-S1 IgG avidity, for instance, is likely to be caused by the extended vaccination interval, since antibody affinity maturation increases over time.

Comparison to other studies

We observed comparable reactogenicity of homologous BNT/BNT vaccination and heterologous ChAdOx/BNT vaccination, both of which were well-tolerated in our cohort with a 10-12 week dosing interval. This is in contrast with interim results of the Com-COV trial, which reported increased systemic vaccine reactions following heterologous ChAdOx/BNT vaccination, compared to homologous ChAdOx/ChAdOx and BNT/BNT regimens in a

comparable sample size [10]. There are several differences in the study design (RCT vs. observational study), study population demographics, and vaccine interval that may explain this discrepancy. The median age in Com-CoV was 57 years (46% females), and 34 years (29-45 years, 60.18% females) in the present study (**Table 1**). The interval between first and second vaccination with either BNT or ChAdOx was 28 days in the Com-COV study, compared to 71 days reported here. We hypothesize that extending the vaccine interval to 10-12 weeks may limit the reactogenicity of heterologous ChAdOx/BNT vaccination.

Phase 1/2 studies have previously reported robust immunogenicity of homologous BNT and ChAdOx immunisations [21,22]. In contrast, immunogenicity of heterologous ChAdOx/BNT immunisation has not been previously reported. Our data indicate that both homologous and heterologous regimens induced high titers of high-affinity antibody responses and high T cell reactivity in healthy individuals. Whereas a slightly higher humoral response was noted after prime immunization with BNT compared to ChAdOx, we found no significant difference in antibody levels, or -neutralisation capacity at three weeks post homologous or heterologous booster vaccination, indicating that BNT booster immunisation induces strong humoral immune responses, even following weaker initial responses after ChAdOx prime immunisation. This is in line with previous studies reporting increased antibody responses in COVID-19 convalescents following a single dose of BNT, compared to seronegative persons receiving two doses of BNT [23]. Both vaccine regimens induced robust T cell responses, but, we observed slightly increased T cell reactivity after heterologous ChAdOx/BNT immunisation compared to the homologous BNT/BNT regimen, indicating that heterologous vaccination may increase immunogenicity.

Policy implications

Heterologous prime-boost vaccination is currently recommended for individuals with ChAdOx prime immunisation in several countries, following reports of rare but serious adverse events associated with ChAdOx, particularly in younger women [7]. A heterologous boost with an mRNA vaccine (BNT or mRNA1273) with a vaccine interval of 12 weeks is currently recommended in Germany for persons under the age of 60 who have previously received one dose of ChAdOx [6]. Our study provides real-world evidence for the safety and immunogenicity of this vaccine regimen. Heterologous vaccination schedules might also alleviate logistical challenges and mitigate intermittent supply shortages of individual vaccines. In light of increasing occurrence of new virus variants carrying immune escape mutations, it will be important to determine whether heterologous vaccination regimens might enhance protection against infection and severe COVID-19. Further controlled studies are required to answer this question.

Conclusions

In summary, this study provides evidence that heterologous ChAdOx/BNT immunisation with 10-12 week intervals, currently recommended in several countries, is well tolerated and equally immunogenic as homologous BNT/BNT vaccination, with evidence of enhanced T cell responses. Our data support further studies into the applicability of heterologous prime-boost vaccination strategies for COVID-19.

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Conflict of interest

VMC is named together with Euroimmun GmbH on a patent application filed recently regarding the diagnosis of SARS-CoV-2 by antibody testing.

Supplementary Material

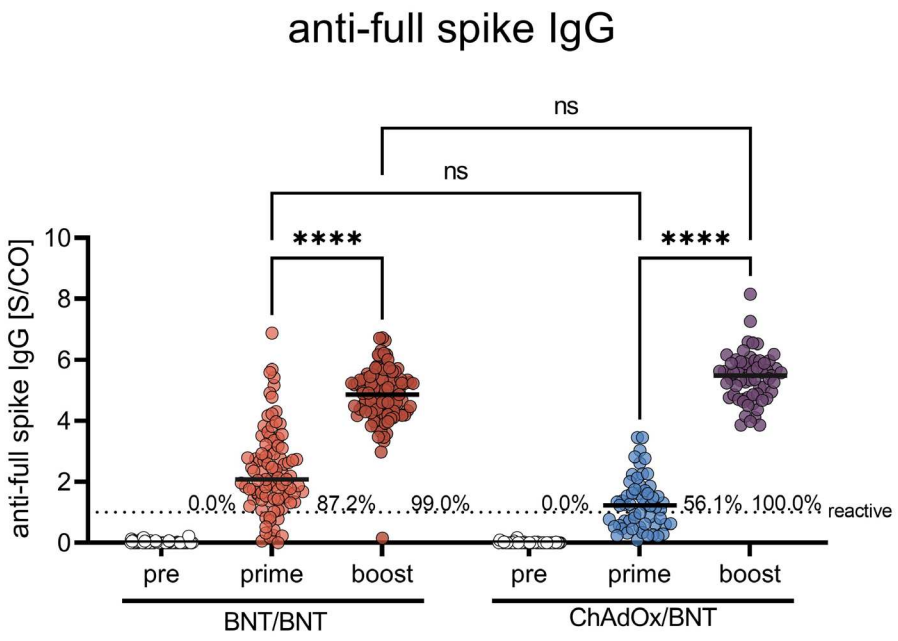

Figure S1: Serum anti-full spike IgG response after BNT or ChAdOx prime immunisations and homologous or heterologous booster vaccination.
Anti-full spike- IgG in serum measured by SeraSpot Anti-SARS-CoV-2 IgG assay. BNT: BNT162b2 / Comirnaty; ChAdOx: ChAdOx1-nCoV19 / Vaxzevria; IgG: Immunoglobulin G; S/CO: signal-to-cutoff ratio. Sampling time points: pre: pre-immune sample prior to first immunisation; prime: three weeks after first vaccination; boost: three to four weeks after boost vaccination. The dotted line indicates the manufacturer's pre-specified threshold (>1 S/Co). Lines indicate the median. **** = p<0.0001; ns: not significant.

Local reaction	1st BNT	1st BNT / 2nd BNT	1st ChAdOx	1st ChAdOx / 2nd BNT
Local reaction (any severity)	69,10 (61,97 - 75,43)	74,21 (66,9 - 80,39)	81,08 (74,02 - 86,57)	84,85 (76,5 - 90,6)
Local reaction (only severe)	1.68 (0.45 - 4.83)	3.77 (1.74 - 7.99)	3.38 (1.45 - 7.66)	5.05 (2.18 - 11.28)
Pain at injection site (any severity)	62.36 (55.05 - 69.15)	69.81 (62.28 - 76.41)	77.70 (70.34 - 83.66)	79.80 (70.85 - 86.52)
Tenderness (any severity)	68.54 (61.39 - 74.91)	73.58 (66.23 - 79.82)	80.41 (73.28 - 86.00)	83.84 (75.35 - 89.80)
Swelling (any severity)	3.93 (1.92 - 7.89)	7.55 (4.37 - 12.73)	3.38 (1.45 - 7.66)	6.06 (2.81 - 12.60)
Redness (any severity)	2.25 (0.88 - 5.63)	1.89 (0.51 - 5.40)	2.70 (1.06 - 6.74)	6.06 (2.81 - 12.60)
Systemic reaction				
Systemic reaction (any severity)	38.76 (31.92 - 46.09)	64.78 (57.09 - 71.78)	86.49 (80.05 - 91.08)	51.52 (41.80 - 61.12)
Systemic reaction (only severe)	0.56 (0.03 - 3.11)	6.29 (3.45 - 11.19)	22.97 (16.93 - 30.38)	2.02 (0.36 - 7.07)
Headache (any severity)	17.42 (12.55 - 23.66)	38.99 (31.76 - 46.75)	75.00 (67.45 - 81.28)	37.37 (28.48 - 47.21)
Fatigue (any severity)	24.16 (18.46 - 30.95)	47.80 (40.18 - 55.52)	72.97 (65.30 - 79.48)	37.37 (28.48 - 47.21)
Feverishness & chills (any severity)	8.99 (5.61 - 14.1)	19.50 (14.09 - 26.34)	70.27 (62.47 - 77.05)	18.18 (11.82 - 26.92)
Myalgia (any severity)	20.79 (15.47 - 27.33)	33.96 (27.06 - 41.62)	54.73 (46.69 - 62.53)	22.22 (15.16 - 31.36)
Arthralgia (any severity)	3.93 (1.92 - 7.89)	10.06 (6.29 - 15.72)	29.73 (22.95 - 37.53)	8.08 (4.15 - 15.14)
Nausea & vomiting (any severity)	3.93 (1.92 - 7.89)	4.40 (2.15 - 8.81)	15.54 (10.58 - 22.24)	6.06 (2.81 - 12.60)
Diarrhoea (any severity)	3.93 (1.92 - 7.89)	6.29 (3.45 - 11.19)	7.43 (4.20 - 12.82)	4.04 (1.58 - 9.93)
Antipyretic medication				
Intake within first 24 hours	9.55 (6.05 - 14.76)	22.64 (16.83 - 29.75)	72.97 (65.30 - 79.48)	32.32 (23.92 - 42.05)
Prophylactic intake	2.81 (1.21 - 6.41)	3.14 (1.35 - 7.15)	27.03 (20.52 - 34.70)	4.04 (1.58 - 9.93)

Table S1: Local and systemic reactogenicity of BNT or ChAdOx prime immunisations and homologous or heterologous boosting until day 7 after vaccination.
Proportion of participants reporting local and systemic reactions and intake of antipyretic medication per group (95% CI). 95% confidence intervals were calculated according to the Wilson and Brown method.

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Heterologous ChAdOx1 nCoV-19 and BNT162b2 prime-boost vaccination elicits potent neutralizing antibody responses and T cell reactivity

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Abstract:

Background

Heterologous prime-boost schedules with vector- and mRNA-based COVID-19 vaccines are already administered, but immunological responses and elicited protection have not been reported.

Methods

We here analyzed a cohort of 26 individuals aged 25-46 (median 30.5) years that received a ChAdOx1 nCoV-19 prime followed by a BNT162b2 boost after an 8-week interval for reactogenicity, antibody responses and T cell reactivity.

Results

Self-reported solicited symptoms after ChAdOx1 nCoV-19 prime were in line with previous reports and less severe after the BNT162b2 boost. Antibody titers increased significantly over time resulting in strong neutralization titers 2 weeks after the BNT162b2 boost. Neutralizing activity against the prevalent strain B.1.1.7 was 3.9-fold higher than in individuals receiving homologous BNT162b2 vaccination, only 2-fold reduced for variant of concern B.1.351, and similar for variant B.1.617. In addition, $CD4^+$ and $CD8^+$ T cells reacted to SARS-CoV-2 spike peptide stimulus 2 weeks after the full vaccination.

Conclusions

The heterologous ChAdOx1 nCoV-19 / BNT162b2 prime-boost vaccination regimen is not associated with serious adverse events and results in a potent humoral immune response and elicits T cell reactivity. Variants of concern B.1.1.7, B.1.351 and B.1.617 are potently neutralized by sera of all participants. These results suggest that this heterologous vaccination regimen is at least as immunogenic and protective as homologous vaccinations.

Introduction:

The first cases of the coronavirus disease 2019 (COVID-19) were reported to the World Health Organization on December 31st 2019[1], and within 93 days the causative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) had infected over 1 million people worldwide [2]. Only 250 days later, the first person received a COVID-19 vaccine outside a clinical trial, and vaccinations are now considered a key strategy for ending the pandemic[3]. Approved vaccines include the adenovirus-based ChAdOx1 nCoV-19 (Vaxzevria, AstraZeneca) and mRNA-based BNT162b2 (Comirnaty, BioNTech/Pfizer), which induce humoral and cellular immunological responses [4–7], showed high efficacy in clinical trials [8,9] and a high degree of protection from COVID-19 in real-world settings[10,11]. However, the occurrence of rare thrombotic events after ChAdOx1 nCoV-19 vaccinations, especially in individuals younger than 60 years, associated with the generation of auto-platelet factor 4 antibodies halted the administration of the second dose of ChAdOx1 nCoV-19 for this group [12–14]. As a consequence, several public health agencies now recommend that boost vaccination for these individuals is carried out in a heterologous regimen with an mRNA vaccine [15]. Recent studies indicate that such a heterologous schedule is associated with more severe [16] or similar [17] solicited symptoms, and preclinical data suggests immunogenicity to be similar to or higher than in animals receiving homologous mRNA-based prime-boost vaccination[18]. However, evidence for immunogenicity of such a regimen in humans and for optimal timing between prime and boost is lacking. In addition, it is currently unclear to which degree a heterologous vaccination regimen confers protection against the variants of concern[19].

Here, we studied a cohort of 26 individuals (16 female, 10 male; median age 30.5, range 25-46) (Table I) who received ChAdOx1 nCoV-19 prime and, due to changing recommendations in Germany, [15] a BNT162b2 boost vaccination with a 56 day interval and evaluated solicited adverse reactions, humoral and cellular immune responses.

Table I: Study participants

	Total	**m**	**f**
Participants	26	10	16
Age median	30.5 (25-46)	32 (26-44)	30.5 (25-46)
Prior SARS-CoV-2 infection	1	0	1
Platelet factor 4 autoantibodies (determined in [20])	0	0	0

Materials and Methods:

Collection of serum and PBMC samples

Blood samples from individuals were obtained after recruitment of participants and written informed consent as approved by the ethics committee of Ulm university (99/21). Participants received a heterologous vaccination regimen because after their ChAdOx1 nCoV-19 prime vaccination, the German Standing Committee on Vaccination (STIKO) had changed the recommendation for individuals < 60 years of age to receive an mRNA vaccine as boost vaccination to avoid risk of thrombotic complications [12,15]. At days -2/0 before vaccination, days 15-16, 30-37, 53-57 after ChAdOx1 nCoV-19 vaccination, and days 6-11 and 14-19 after heterologous BNT162b2 boost (days 64-65 and 72-73 after ChAdOx1 nCoV-19, respectively), blood was drawn into S-Monovette® Serum Gel (Sarstedt) or S-Monovette® K3 EDTA tubes. Sera from individuals vaccinated twice with BNT162b2 were obtained 13-15 days after the second dose under approval by the ethics committee of Ulm university (31/21); these sera were previously described and re-analyzed for this study[21]. Serum Gel collection tubes were centrifuged at 1,500 × g at 20°C for 15 min, aliquoted stored at -20°C until further use. Peripheral blood mononuclear cells (PBMCs) were obtained from EDTA tubes using density gradient centrifugation by Pancoll human (Pan Biotech, Germany), and erythrocytes removed by ACK lysis buffer (Lonza, Walkersville, MD, U.S.A). Mononuclear cells were counted for viability using a Countess II Automated Cell Counter (Thermo Fisher) with trypan blue stain and were cryopreserved in aliquots of up to $1x10^7$ cells in 10% DMSO in heat-inactivated FCS.

Vaccine reactogenicity

Solicited adverse reactions (SAR) were self-reported by the participants via questionnaire following prime and boost vaccination. Participants were asked to list symptoms, their duration (< 1 h, few hours, one day or more than one day) and severity (mild (grade 1), moderate (grade 2), severe (grade 3). Grading criteria were adapted from the US Department of Health and Human Services CTCEA (Common Terminology Criteria for Adverse Events, v4.03) [22], with grade 1-2 being considered for some symptoms, grade 1-3 for most. For calculation of cumulative SAR (cSAR) scores, the grades of all symptoms listed were summed up, with an additional score point added for symptoms experienced for more than one day (0-4).

Determination of antibody titers

IgG and IgA levels in serum were determined by anti-SARS-CoV-2 assay (Euroimmun), an ELISA which detects antibodies against the SARS-CoV-2 S1 spike domain. The assay was performed according to the manufacturer's instructions. Briefly, serum samples were diluted 10-fold in sample buffer and pipetted

into rSARS-CoV-2 spike precoated strips of eight single wells of a 96-well microtiter. After incubation for 60 min at 37°C, wells were washed three times, peroxidase-labeled anti-IgG or anti-IgA added and incubated. After 30 min, three additional washing steps were performed before substrate was added and incubated for 15-30 min in the dark. Thereafter, stop solution was added, and optical density (OD) values measured on a POLARstar Omega plate reader (BMG LABTECH, Ortenberg, Germany) at 450 nm corrected for 620 nm. Finally, OD ratios were calculated based on the sample and calibrator OD values, where a ratio <0.8 was considered to be negative and >1.1 to be positive. To quantify antibody responses, IgG and IgM were measured as units per ml (U/ml) that correlates with the WHO standard unit for the SARS-CoV-2 binding antibody units per ml (BAU/ml). To this end, serum was analyzed using the commercial electrochemiluminescence Elecsys Anti-SARS-CoV-2 S immunoassay (Roche, Mannheim, Germany) by a cobas® e801 immunoassay analyzer according to the manufacturer's instructions (Roche).

Surrogate SARS-CoV-2 neutralization test

Prevention of SARS-CoV-2 spike RBD interaction with ACE2 by sera was evaluated by SARS-CoV-2 Surrogate Virus Neutralization Test Kit (GenScript) according to the manufacturer's instructions. To this end, sera were incubated with a peroxidase-conjugated RBD fragment and the mixture added to a human ACE-2 coated plate, and unbound RBD washed away. Thereafter, substrate was added and the reaction stopped by stopping reagent. ODs at 450 nm were measured at a microplate reader. The inhibition score compared to the negative control was calculated as percentages. Scores <20% were considered negative and scores >20% positive.

Cell culture

Vero E6 (African green monkey, female, kidney; CRL-1586, ATCC, RRID:CVCL_0574) cells were grown in Dulbecco's modified Eagle's medium (DMEM, Gibco) which was supplemented with 2.5% heat-inactivated fetal calf serum (FCS), 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1x non-essential amino acids. HEK293T (human, female, kidney; ACC-635, DSMZ, RRID: CVCL_0063) cells were grown in DMEM with supplementation of 10% FCS, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 mM L-glutamine. All cells were grown at 37°C in a 5% CO_2 humidified incubator.

Preparation of pseudotyped particles

Expression plasmids for vesicular stomatitis virus (VSV, serotype Indiana) glycoprotein (VSV-G) and SARS-2-S variants B.1.1.7, B.1.351 and B.1.617 (codon-optimized; with a C-terminal truncation of 18 amino acid residues) have been described elsewhere [19,21]. Transfection of cells was carried out by Transit LT-1 (Mirus). Rhabdoviral pseudotype particles were prepared as previously described [23]. A replication-

deficient VSV vector in which the genetic information for VSV-G was replaced by genes encoding two reporter proteins, enhanced green fluorescent protein and firefly luciferase (FLuc), VSV∗ΔG-FLuc [24] (kindly provided by Gert Zimmer, Institute of Virology and Immunology, Mittelhäusern, Switzerland) (Berger Rentsch and Zimmer, 2011) was used for pseudotyping. One day after transfection of HEK293T cells to express the viral glycoprotein, they were inoculated with VSV∗ΔG-FLuc and incubated for 1-2 h at 37°C. Then the inoculum was removed, cells were washed with PBS and fresh medium added. After 16-18 h, the supernatant was collected and centrifuged (2,000 × g, 10 min, room temperature) to clear cellular debris. Cell culture medium containing anti-VSV-G antibody (I1-hybridoma cells; ATCC no. CRL-2700) was then added to block residual VSV-G-containing particles. Samples were then aliquoted and stored at -80°C.

Pseudovirus neutralisation assay

For pseudovirus neutralisation experiments, VeroE6 were seeded in 96-well plates one day prior (6,000 cells/well). Heat-inactivated (56°C, 30 min) sera were serially titrated in PBS, pseudovirus stocks added (1:1, v/v) and the mixtures incubated for 30 min at 37°C before being added to cells. After an incubation period of 16-18 h, transduction efficiency was analyzed. For this, the supernatant was removed, and cells were lysed by incubation with Cell Culture Lysis Reagent (Promega) at room temperature. Lysates were then transferred into white 96-well plates and FLuc activity was measured using a commercially available substrate (Luciferase Assay System, Promega) and a plate luminometer (Orion II Microplate Luminometer, Berthold). For analysis of raw values (RLU/s), background signal of an uninfected plate was subtracted and values normalized to pseudovirus treated with PBS only. Results are given as serum dilution resulting in 50% virus neutralization (NT50) on cells, calculated by nonlinear regression ([Inhibitor] vs. normalized response -- Variable slope) in GraphPad Prism Version 9.1.1.

Determination of $CD4^+$ and $CD8^+$ T SARS-CoV-2 spike -specific cell responses by intracellular cytokine staining (ICS)

Cryopreserved PBMCs of study participants were thawed and rested overnight at 37°C with 1 µl/ml of DNAse (DNase I recombinant, RNase-free (10,000 U) Roche), in RPMI medium supplemented to contain a final concentration of 10% FCS (Corning Life Sciences/Media Tech Inc, Manassas, VA), 10 mM HEPES, 1x MEM nonessential amino acids (Corning Life Sciences/Media Tech Inc, Manassas, VA), 1 mM Sodium Pyruvate (Lonza, Walkersville, MD, U.S.A), 1mM Penicillin/Streptomycin (Pan Biotech, Germany) and 1x 2-Mercaptoethanol (GIBCO, Invitrogen, Carlsbad, CA, U.S.A). Stimulation of PBMCs for detection of cytokine production by T cells was adapted from Kasturi *et al.*, 2020 [25]. Briefly, $1x10^6$ PBMCs were cultured in 200 µl final volume in 96-well U bottom plate in the presence of anti-CD28 (1 µg/ml) and anti-CD49d (1 µg/ml) [Biolegend] under the following conditions: a) negative control with DMSO, b) SARS-CoV-2 spike peptide pool (1-315 peptides from Wuhan SARS-CoV-2 spike, JPT Germany) at a final concentration of 2 µg/ml, c) PMA/Ionomycin. Cells were cultured for 2 hours before adding Brefeldin A at 10 µg/ml (Sigma-Aldrich, St Louis, MO) for an additional 5 hours. Cells were then washed with PBS, and stained for dead cells (Live/ Dead Fixable; Aqua from Thermo Fisher) in PBS at room temperature for

10 minutes. Without washing, cells were incubated with surface antibody cocktail (prepared in 1:1 of FACS buffer and brilliant staining buffer) for 30 minutes at room temperature with BV510-anti-human CD14 (clone M5E2), BV510-anti-human CD19 (clone HIB19), AF700 anti-human CD3 (clone OKT3), BV605 CD4 (clone OKT4), PerCP-Cy5.5 CD8 (clone RPA-T8) from Biolegend. Next, cells were fixed using Cytofix/Cytoperm buffer (BD Biosciences, CA) for 20 minutes at room temperature, and then kept in FACS buffer at 4°C overnight. 1x Perm/Wash (BD Biosciences, CA) was used for cells permeabilization for 10 minutes at room temperature and followed by intracellular staining for 30 minutes at room temperature with AF647 anti-human IFNγ (clone 4S.B3) and AF488 anti- human IL-2 (clone MQ1-17H12) from Biolegend, and PE/Cy7 anti- human TNFα (clone Mab11) from Thermo Fisher Scientific. Up to 100,000 live $CD3^+$ T cells were acquired on a LSRFortessa flow cytometer (BD Biosciences), equipped with FACS Diva software. Analysis of the acquired data was performed using FlowJo software (version 10.7.1). The background from each participant was removed by subtracting the % of $spike^+$ cells to the % of $DMSO^+$ cells. An arbitrary value below 0.01% of $CD4^+/CD8^+$ T cells was considered negative.

Statistical analysis

The SARS-CoV-2 convalescent individual was excluded in all statistical analyses. Non-parametric Spearman rank correlation was used to check for possible associations at single blood sample measurements. A paired t-test was used to compare the adverse event scores calculated for each participant after both vaccinations. For this, the individual mean differences were checked for normal distribution by means of QQ-plots and histograms. A comparison of participants receiving heterologous vaccination with controls who received homologous BNT162b2 vaccinations after the last blood sample measurements was done by the Mann-Whitney-U test because of skewed distributions for neutralization scores as well as IgM/IgG measurements. Longitudinal antibody measurements were analyzed by means of a mixed linear regression model including a random intercept in order to account for the repeated measures structure of the underlying data. The mixed linear model approach enabled to simultaneously account for possible confounding due to participants' sex and for the presence of missing data [26]. Therefore, no formal imputation of missing interim values was required. A two-sided alpha error of 5% was applied to analyses. Analysis of repetitive measurements of sera provided by a cohort of 26 participants can be considered statistically valid. All analyses were done by GraphPad Prism version 9.1.1 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com, R (version 4.0.1) and SAS (version 9.4).

Results:

Reactogenicity following prime and boost vaccination was evaluated by all study participants by self-reporting of solicited local and systemic symptoms according to a standardized questionnaire. Symptom severity (mild, moderate, severe) and duration (<1 h, few h, ~1 day, > 1 day) is reported for each individual participant (Figure S1A) and percentage of participants (Figure 1A,B).

Both, prime and boost vaccination, induced mild to moderate solicited adverse reactions in most participants with 88.4% (23/26) reporting at least one mild or moderate symptom following prime; 23/26 (88.4%) and 21/26 (80.8%) reporting at least one mild or moderate symptom following boost vaccination (Figure 1A,B). Most common symptoms after prime vaccination with ChAdOx1 nCoV-19 were pain at the injection site (92.3%), fatigue (80.8%), headache (73.1%), chills (61.5%), myalgia (61.5%) and fever (61.5%). Following boost vaccination with BNT162b2, most participants again reported pain at the injection site (84.6%) and fatigue (84.6%), but chills (19.2%), myalgia (38.5) and fever (19.2%) were less common. 23% of participants (6/26) reported at least one severe symptom following prime, 15.4% (4/26) after boost. Fatigue (7.7%) and headache (15.4% for prime, 3.8% for boost) were amongst symptoms reported as severe for both doses, while myalgia was reported as severe by 11.5% of participants following prime, but none after boost.

Comparing cumulative solicited adverse reaction (cSAR) scores, reactogenicity following prime with ChAdOx1 nCoV-19 was significantly ($p = 0.005$) higher than following boost with BNT162b2 (cSAR score median 11 and 6 respectively, Figure 1C). Individually, most participants (19/26, 73.07%) had milder reaction to boost compared to prime. 6/26 (23.07%) of participants described more severe reactions to boost vaccination (Figure S1B). A trend towards higher cSAR scores reported by female participants was seen for both boost and prime vaccinations (Figure 1D,E). No correlation was observed between participant age and reactogenicity (Figure S1C,D). Reactogenicity towards prime and boost vaccination was weakly but significantly correlated (Figure 1F, $p = 0.039$).

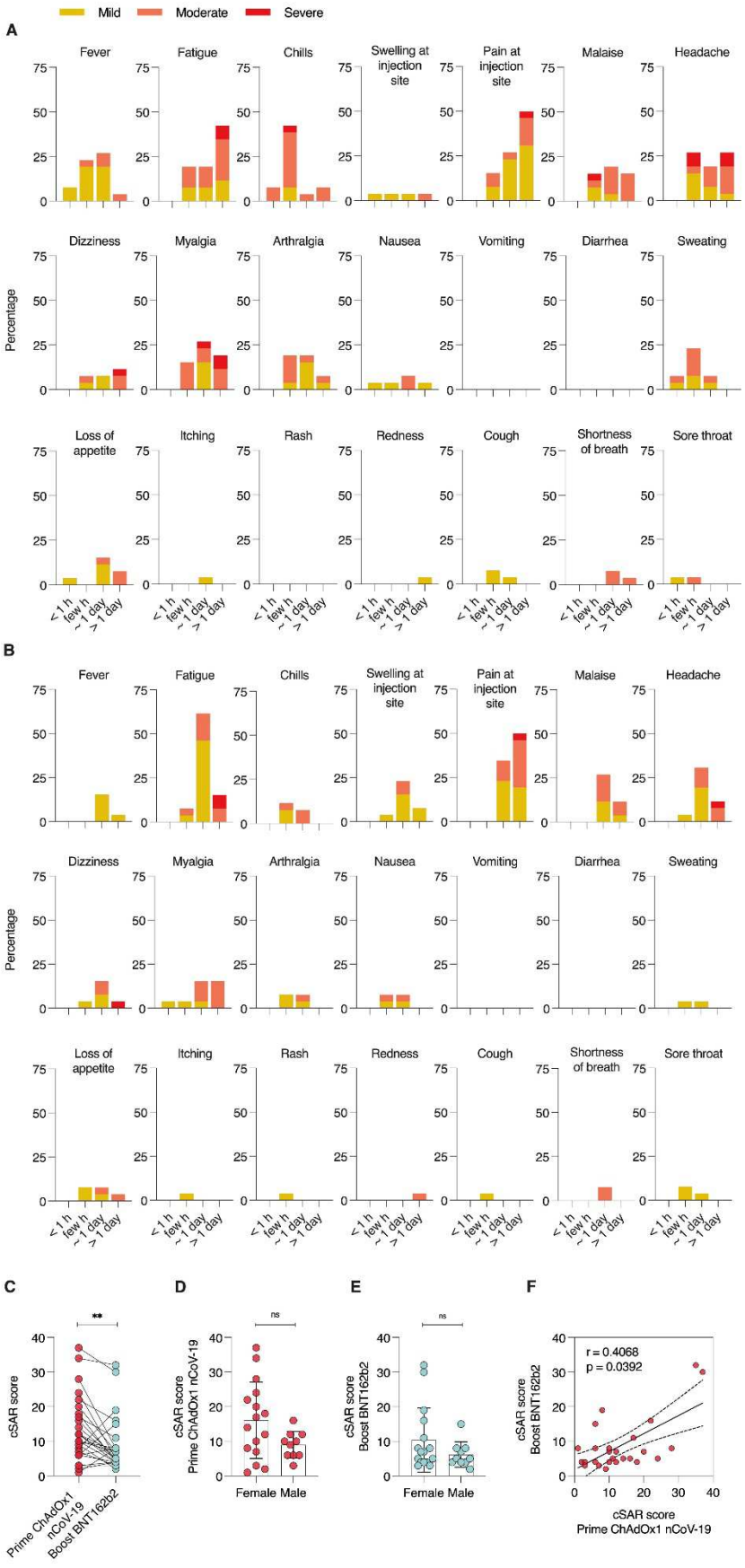

Fig. 1. Solicited adverse reactions following ChAdOx1 nCoV-19 prime and BNT162b2 boost vaccination. Percentages of participants with individual symptoms following prime (A) or boost (B) vaccination. Severity is graded on a scale of 1-2 (for some symptoms) or 1-3 (for most), according to Common Terminology Criteria for Adverse Events (US Department of of Health and Human Services, Version 4.03) [22]. (C) Cumulative solicited adverse reaction (cSAR) scores of all participants following prime and boost vaccination. For calculation of cSAR scores, symptom gradings are summed and an additional score point is added for symptoms lasting more than 24 h. Analysis of cSAR scores by (D, E) participant gender, and (F) comparison between cSAR scores following prime and boost vaccination. The SARS-CoV-2 convalescent individual was excluded in all statistical analyses. Paired t-test; ns not significant; ** $p < 0.01$

We collected sera from participants 2 days (-2) or on the same day (0) before vaccination, and at days 15 – 16, 30 – 37, and 53 – 57 after ChAdOx1 nCoV-19 prime, and days 6 - 11 and 14 – 19 after BNT162b2 boost (64 – 65 or 72 – 73 after prime, respectively) to determine antibody responses (Figure 2). Already 15-16 days after prime, 19/25 (76%) participants showed detectable anti-SARS-CoV-2-spike-IgG levels and 17/25 (65%) detectable IgA levels (Figure 2A,B). IgG levels peaked after 30 - 37 days and were detectable in 24/25 (96%) participants. Until days 53 – 57, IgG levels slightly decreased, consistent with previous results after single ChAdOx1 nCoV-19 dose [4,5]. IgA values were highest already at days 15-16 and became undetectable in 24 (92%) participants at days 53 - 57. Notably, only 6 - 11 days after the BNT162b2 boost, IgG was detectable in all (100%) and IgA in 23 (92%) of 25 participants. Until day 14-19 after boost (72-73 post ChAdOx1 nCoV-19), IgG and IgA were detectable in all participants. This corresponds to an at least 3.7-fold increase in median IgG levels from pre-boost to 2 weeks post-boost. We next quantified cumulative anti-SARS-CoV-2-spike-IgM and IgG concentrations and detected median antibody levels of 3.39 (range 0-2,126) units per ml (U/ml) 15-16 days after prime vaccination in 22/25 (88%) participants (Figure 2C). From days 30 – 37 on, IgM and IgG were detected in all participants and medians continuously increased to 28 (1.86-1,436) and 63.9 (4.27-1,005) U/ml after days 30 - 37 or 53 - 57, respectively. After BNT162b2 boost, titers increased 134-fold to 8,614 (126 – 24,831) at days 6 – 11 and 135-fold to 8,815 (1,206 – 19,046) 14 - 19 days after the second dose. Strikingly, the resulting titers were 8.1-fold higher than those determined for sera obtained after 13-15 days of a homologous BNT162b2 boost (individuals with median age 41 (25-55); median titers 1,086; range 498-3,660). Cumulative IgM/G titers correlated with IgG titers at each timepoint analysed post prime (Figure S2, Table S1).

Sera were also evaluated for their potential to inhibit SARS-CoV-2-spike-receptor binding domain/ACE2 interaction using a surrogate virus neutralisation test (sVNT) (Figure 2D). 15-16 days after ChAdOx1 nCoV-19 administration 13/25 (52%) participant sera showed ACE2 neutralizing activity, correlating significantly with IgG and IgM/G titers (Figure S2, Table S1). Median neutralization activity of the positive sera was 46% (range 32-97%). Until days 53-57, the number of participants with neutralizing sera increased to 19/26 (73%) and the median ACE2 neutralization to 62% (range 32-95%), again in correlation with IgG and IgM/G values (Figure S2, Table S1). After BNT162b2 boost, all participants showed potent neutralization with a median of 97% (range 32-98%) after 6-11, and 98% (range 89-98%) after 14-16 days suggesting a strong and functional antibody response after heterologous vaccination in all participants.

The potency of neutralizing activity was further quantified using vesicular stomatitis virus (VSV)-based pseudoviruses carrying the SARS-CoV-2 spike protein of the most prevalent SARS-CoV-2 B.1.1.7 variant. This system faithfully recapitulates SARS-CoV-2 entry into cells and its inhibition[21,27,28]. 15-16 days after ChAdOx1 nCoV-19 prime, neutralizing titers ranging from 36-906 were detectable in 8/25 (32%) participants (Figure 2E). The number of participants with detectable neutralization increased to maximum in 12/25 (48%) individuals at days 30-37 with a median neutralization titer of 74 (range 20-

552) in responders, which slightly decreased until days 53-58. Two weeks after the BNT162b2 boost, neutralizing titers were detected in all participants with a median titer of 2,744 (range 209-8,985). Of note, while for some individuals the titers further increased from week 1 to week 2 after BNT162b2 boost, other individuals plateaued at titers > 1,000 (Figure S3). At all time points, neutralizing activity correlated with IgG or IgM/G titers (Figure S2, Table S1). Remarkably, the median titer of these individuals was 3.9-fold higher than the median titer of 14 individuals vaccinated with BNT162b2 in a homologous regimen (709; range 305-1,806) suggesting a stronger humoral protection after a heterologous vaccination. Of note, a SARS-CoV-2 convalescent individual (triangle symbol) showed a strong response after the first dose in all assays, high IgG, IgA or IgM/G values, most effective ACE2- neutralization and a high neutralization titer of 906 15 – 16 days after prime that decreased over the days to 201 at day 53-57 (Figure 2A-E).

Additionally, we evaluated the neutralizing activities of sera obtained 2 weeks post full vaccination against the variants of concern (VOCs) B.1.351 and B.1.617. Pseudovirus entry driven by B.1.351 spike was neutralized with 2-fold lower potency ($p < 0.05$) compared to B.1.1.7 spike. However, it was still entirely blocked at higher doses with a median titer of 1,297 (range 252 - 6,523). Neutralization of the B.1.617 spike was not reduced compared to B.1.1.7 spike (median titer of 1,309; range 150 – 13,252) (Figure 2F). Sera of individuals vaccinated with homologous BNT162b2 showed lower neutralizing titers against all spike variants tested (Figure 2F), suggesting stronger humoral protection after a heterologous vaccination also against VOCs.

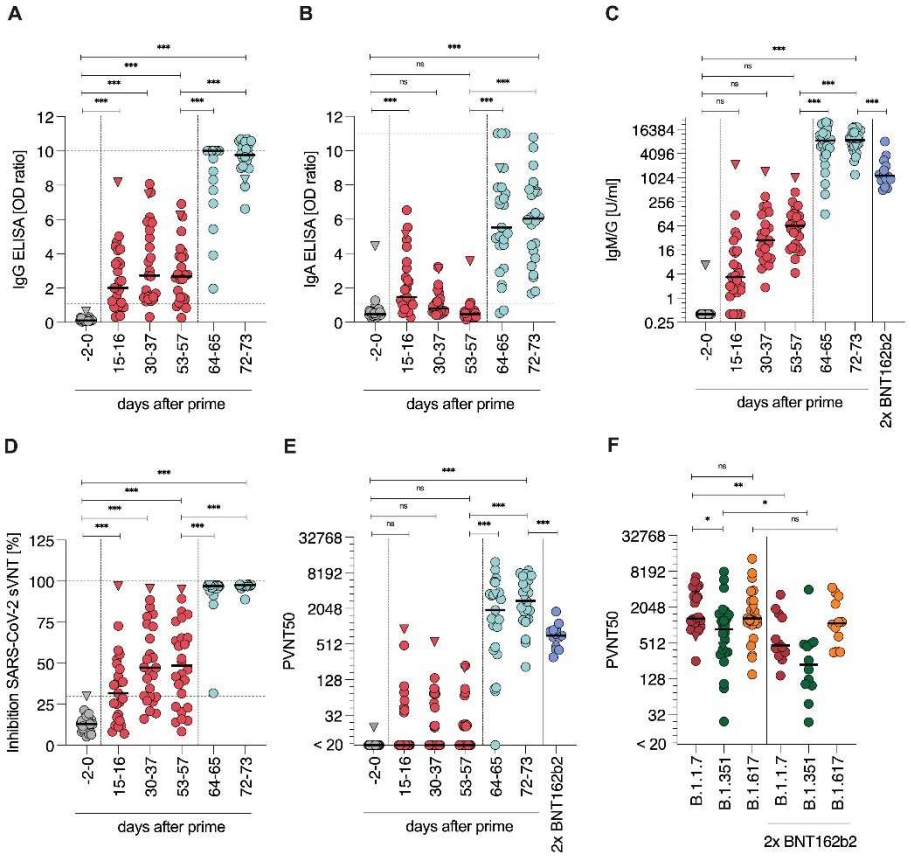

Fig. 2. Humoral response. Quantification of anti-SARS-CoV-2 S1 spike domain (A) IgG and (B) IgA titers. (C) Quantification of anti-SARS-CoV-2 spike IgG and IgM responses as units per ml (U/ml) by immunoassay. (D)

SARS-CoV-2 surrogate virus ACE2 neutralization test. (E) VSV-based B.1.1.7 SARS-CoV-2 spike pseudovirus neutralization assay. (F) VSV-based B.1.1.7, B.1.351 and B.1.617-SARS-CoV-2 spike pseudovirus neutralization assay. Titers expressed as serum dilution resulting in 50% pseudovirus neutralization (PVNT50). Triangle indicates SARS-CoV-2 convalescent individual, who was excluded from all statistical analyses. Grey symbols indicate datapoints pre-vaccination, red datapoints indicate datapoints after prime and light-blue after boost vaccination. Dark-blue indicates samples of participants with homologous BNT162b2 prime-boost regimen. Dashed horizontal lines indicate upper and lower limit of detection/cutoff, respectively. Dashed vertical lines indicate prime and boost vaccination. Longitudinal antibody measurements were analyzed by means of a mixed linear regression model. Mann-Whitney-U test compares ChAdOx1 nCoV-19 and BNT162b2 titers *** $p < 0.0001$, ** $p < 0.001$, * $p < 0.05$, ns not significant

To evaluate cellular immunity, we isolated peripheral blood mononuclear cells from blood samples provided by 19/26 participants on days 14-19 post BNT162b2 boost (72-73 days post prime), considered as full vaccination according to the vaccination schedule. Cells were exposed to a SARS-CoV-2 spike-spanning peptide-pool and analyzed for intracellular cytokines TNFα, IFNγ, and IL2 to determine spike-specific $CD4^+$ and $CD8^+$ T cell responses (Figure 3, S4). Upon antigen stimulation, $CD4^+$ T cells secreting IFNγ (median 0.055, range 0.018-0.168), IL2 (median 0.055, range 0-0.134) or TNFα (median 0.057; range 0.01 – 0.193) were detected in all participants suggesting they developed a robust spike-specific T helper 1 (TH1) $CD4^+$ T cell response. In addition, in 89% (17/19) of participants a substantial population of spike-specific $CD8^+$ T cells, with a predominant $IFN\gamma^+$ (median 0.092, range 0-0.665) and $TNF\alpha^+$ (median 0.055, range 0 – 0.375) phenotype was detected. We observed lower levels of $CD8^+$ $IL2^+$ (median 0.01, range 0-0.052) T cells which is in agreement with responses after homologous BNT162b2 vaccination [6]. Interestingly, unstimulated $CD8^+$ T cells of the convalescent individual were already reactive before SARS-CoV-2 spike peptide stimulation. Overall, these findings show a robust humoral and cellular immune response after heterologous vaccination.

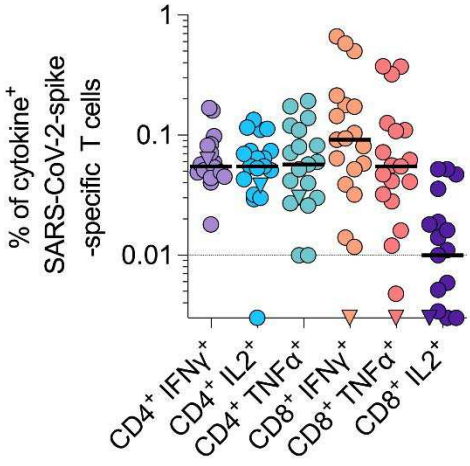

Fig. 3. SARS-CoV-2 spike-specific $CD4^+$ and $CD8^+$ T cell responses. PBMCs of study participants were stimulated with a SARS-CoV-2 spike peptide-pool and cytokine secretion determined by flow cytometry. $Cytokine^+$ T cells were background corrected for unstimulated cells and values lower than 0.01% were considered negative. Triangle symbol indicates SARS-CoV-2 convalescent individual, where cytokine release was already high in absence of stimulation.

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Discussion

Based on the regulatory approvals for ChAdOx1 nCoV-19 and mRNA vaccines, the interval between prime and boost vaccinations ranges between 4 -12 weeks [29–31]. For ChAdOx1 nCoV-19, a 12 week interval has been shown to result in stronger immune responses [32], most likely because the immunity against the vector wanes. Accordingly, e.g. in Germany heterologous vaccinations are currently typically performed after 12 weeks. Existing vector immunity, however, is irrelevant in the context of a mRNA boost vaccination, on which basis our cohort received the boost after 8 weeks. This heterologous ChAdOx1 nCoV-19/BNT162b2 vaccination elicited strong IgM/G and IgA responses, neutralizing activities and T cell responses in all previously uninfected participants, while solicited adverse reactions to vaccination were as expected for a prime ChAdOx1 nCoV-19 vaccination and reduced following heterologous BNT162b2 boost.

A previous study showed that a heterologous vaccination schedule with 4-week interval results in stronger reactogenicity after boost [16], whereas a preprinted study with a 12-week interval did not confirm this effect [17]. We did not directly compare different vaccination schemes. Thus, we cannot draw definitive conclusions on differences, which might also depend on cohort age [5]. With an 8-week interval, we observed an overall milder reactogenicity following heterologous boost with BNT162b2 than after initial prime vaccination with ChAdOx1 nCoV-19 and no serious adverse events, arguing for the safety of this regimen in young adults.

Our immunological data suggest that a heterologous vector-based/mRNA prime-boost schedule is highly effective in preventing COVID-19, as neutralizing antibody levels correlate with immune protection from symptomatic SARS-CoV-2 infection [33] and $CD8^+$ T cell responses have been associated with a mild disease course [34,35]. Endpoint antibody titers determined 2 weeks post boost were significantly higher than those detected upon homologous BNT162b2 vaccinations (Figure 2 C,E). This is in line with findings in preclinical models [18], but might also be influenced by cohort age. Factors contributing to this high degree of immunogenicity might be the circumvention of vector immunity. The BNT162b2 encoded spike sequence contains a two-proline mutation not present in ChAdOx1 nCoV-19, which fix spike in a pre-fusion confirmation [9]. It is tempting to speculate that altered spike confirmations may be beneficial for effective neutralizing responses.

Neutralizing activity towards VOC B.1.351, previously reported to show partial evasion of vaccination-induced antibodies [21,36,37], was only slightly decreased following heterologous vaccination. Neutralization of emerging VOC B.1.617 was not reduced compared to B.1.1.7. Whether these immunological findings translate into effective general protection from VOCs in real-life setting remains to be determined. However, the substantial neutralization capacity against two highly transmissible virus variants is encouraging.

Secretory IgA responses at the mucosal site of SARS-CoV-2 entry are of particular interest with regard to prevention of virus transmission and (re-)infection [38]. We detected a general increase in serum IgA levels with strong variation between participants, suggesting mucosal protection shortly after vaccination. However, IgA levels decreased over time after prime vaccination, and future studies, especially assessing IgA and secretory IgA levels and persistence at mucosal entry sites after boost are warranted.

In all participants SARS-CoV-2 specific $CD8^+$ or $CD4^+$ T cells were detected 2 weeks after full vaccination. These effects were similar to those reported after a single ChAdOx1 nCoV-19 dose and after homologous BNT162b2 vaccination [4,6]. This suggests that T cell responses are similarly effective after heterologous vaccination.

In line with previous results, in an individual participant who was previously tested SARS-CoV-2 positive, a single prime vaccination dose already elicited strong antibody responses [39,40]. In this case, the observed neutralizing titers 2 weeks after prime were as high as the median titer of those receiving the homologous BNT162b2 vaccination, However, titers (IgM/G) further increased 8-fold after boost, suggesting that prime-boost might provide more potent and longer lasting protection.

In conclusion, heterologous vaccination schedule of ChAdOx1 nCov-19 prime, followed by BNT162b2 boost after 8 weeks for participants with a median age of 30 years was safe and effective. This provides flexibility for future vaccination strategies and will be useful for vaccine schedules during shortages. Whether heterologous vaccination is superior to homologous regimens and should be considered as a strategy to elicit particularly strong immune responses e.g. against VOCs or for highly exposed individuals remains to be determined. Similarly, whether other vector- or mRNA-based vaccine combinations or those based on other technologies are similarly effective needs to be addressed in future studies.

Limitations

The study cohort of 26 participants is not large, but the repetitive measurements suffice for a comprehensive analysis of serological responses. With a median age of 30.5 (range 25 - 46) years, the results do not provide information on the elderly. However, our study offers insight into how the younger age group reacts to a heterologous vaccination regimen. This is of high significance, because individuals younger than 60 have regularly been primed with ChAdOx1 nCov-19 and are now offered heterologous boost vaccination. Our study group received the second vaccination after 8 weeks, which is within the range of recommendation of 4-12 weeks.

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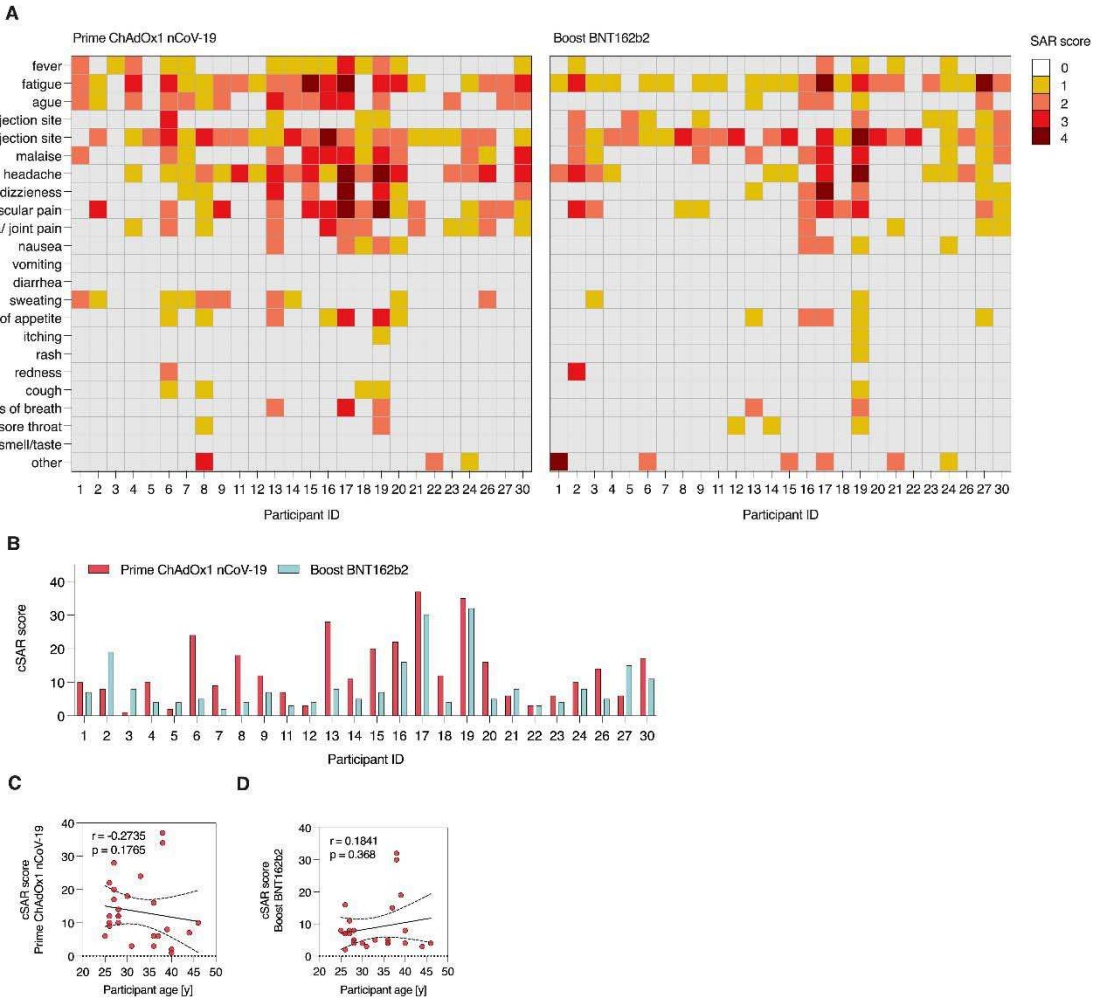

Figure S1: Extended analysis of solicited adverse reactions following ChAdOx1 nCoV-19 prime and BNT162b2 boost vaccination. (A) Heatmap showing SAR scores per participant and symptom used for calculation of cSAR scores. Severity is graded on a scale of 1-2 (for some symptoms) or 1-3 (for most), according to Common Terminology Criteria for Adverse Events (US Department of Health and Human Services, Version 4). For calculation of cSAR score (A, B), symptom gradings are summed and an additional score point is added for symptoms lasting more than 24 h (final scores 1-4 per symptom). Correlation analysis for cSAR scores with participant age for prime (C) and boost (D) vaccination.

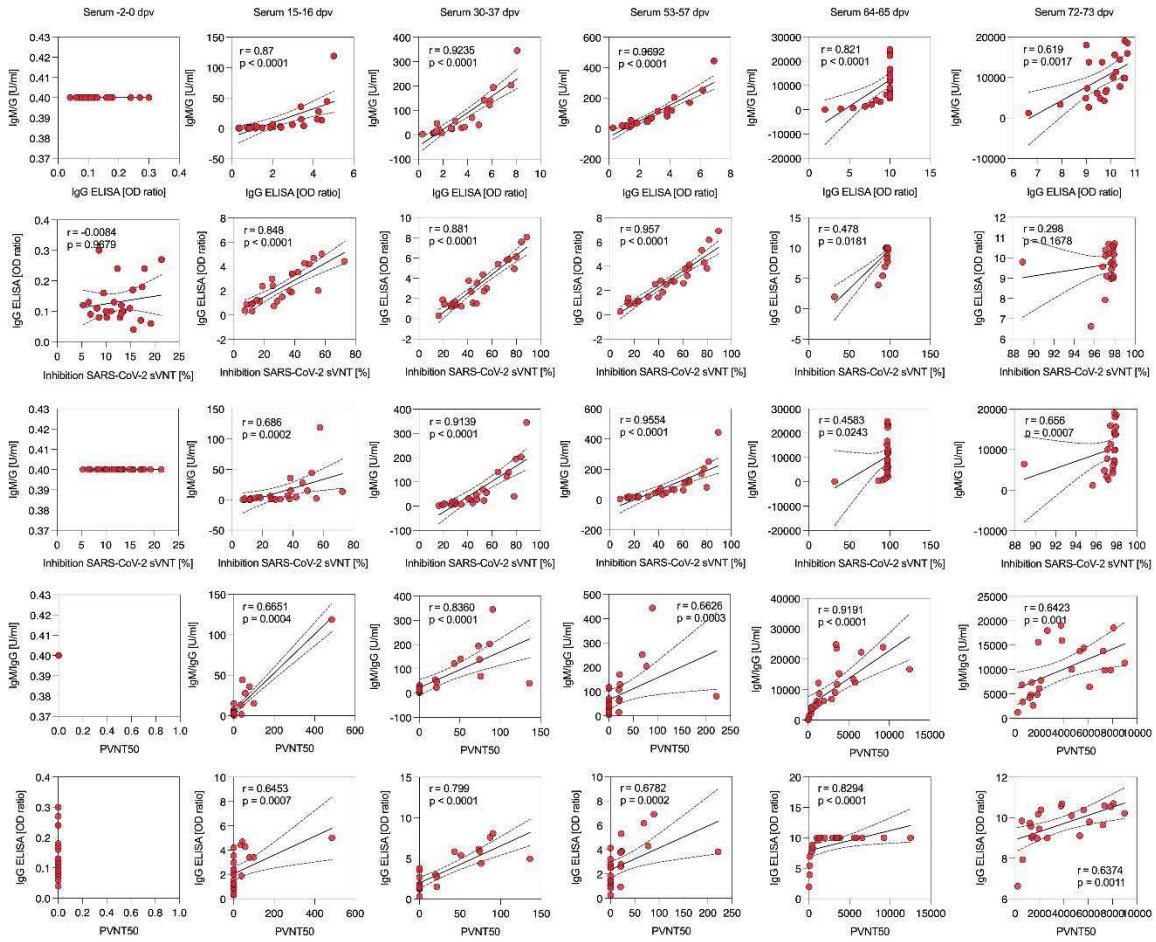

Figure S2. Correlation analysis of humoral response metrics. Data on humoral response (IgG, IgA, IgM/G, PVNT50, Inhibition SARS-CoV-2-sVNT) were analysed for correlation for each timepoint. Spearman correlation, two-tailed p values, dashed lines indicate 95% confidence interval. The SARS-CoV-2 convalescent individual was excluded from the analysis.

561 **Table S1. Summary of correlation analysis of humoral response metrics.** Spearman r values of Figure S2.

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	-2 - 0	15 – 16	30 - 37	53 - 57	64 - 65	72 - 73
IgG:IgM/G	/	0.87	0.9235	0.9692	0.821	0.619
sVNT:IgG	-0.0084	0.848	0.881	0.957	0.478	0.296
sVNT:IgM/G	/	0.686	0.9139	0.9554	0.4583	0.656
PVNT50:IgM/G	/	0.6651	0.836	0.6626	0.9191	0.6423
PVNT50:IgG	/	0.6453	0.799	0.6782	0.8294	0.6374

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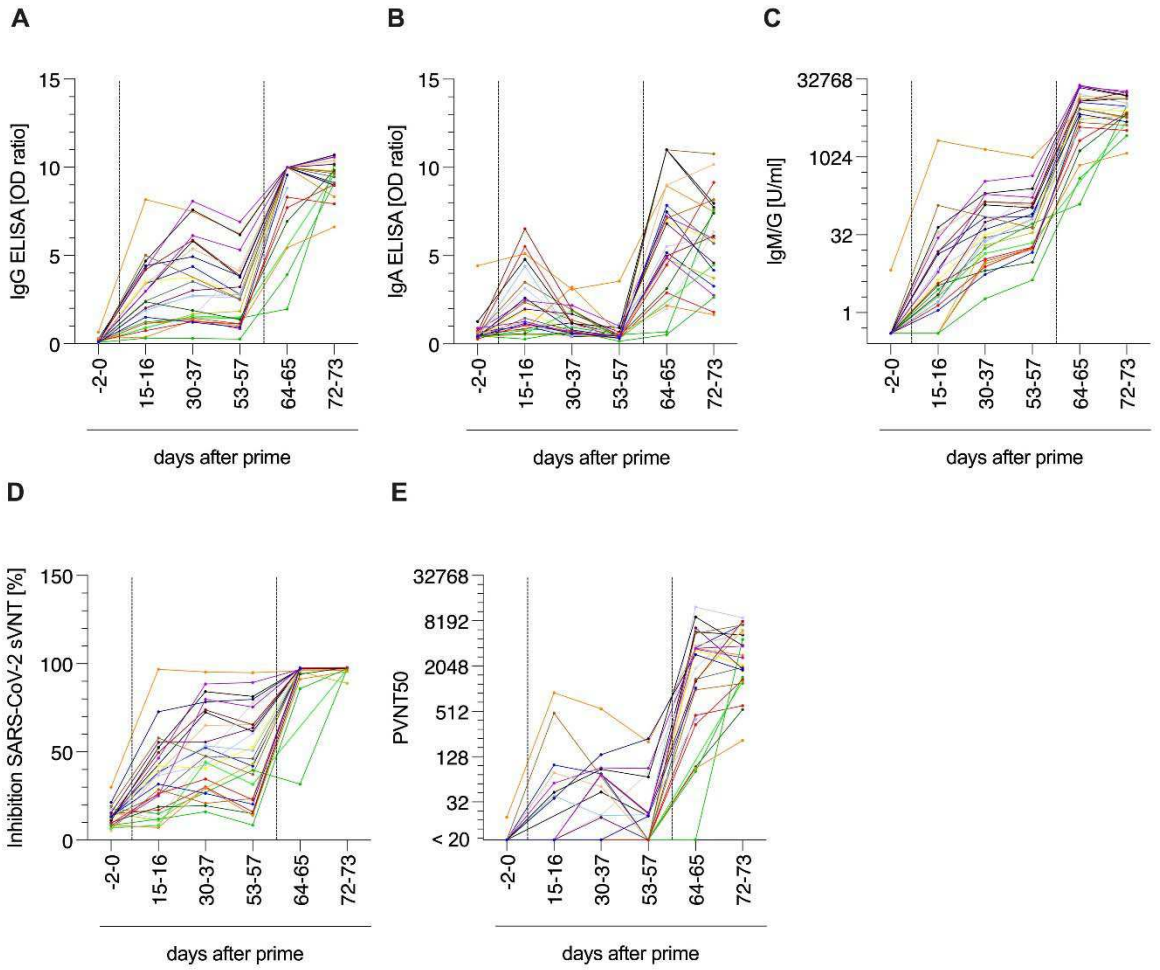

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566 **Figure S3. Time course of humoral responses.** Time course of anti-SARS-CoV-2 S1 spike domain (A) IgG and (B) IgA
567 titers. (C) Time course of anti-SARS-CoV-2 spike IgG and IgM responses as units per ml (U/ml) by immunoassay. (D)
568 Time course of SARS-CoV-2 surrogate virus ACE2-RBD interaction neutralization as assessed by surrogate virus
569 neutralisation test (sVNT). (E) VSV-based B.1.1.7 SARS-CoV-2 spike pseudovirus neutralization assay. Titers
570 expressed as serum dilution resulting in 50% pseudovirus neutralization (PVNT50).

571

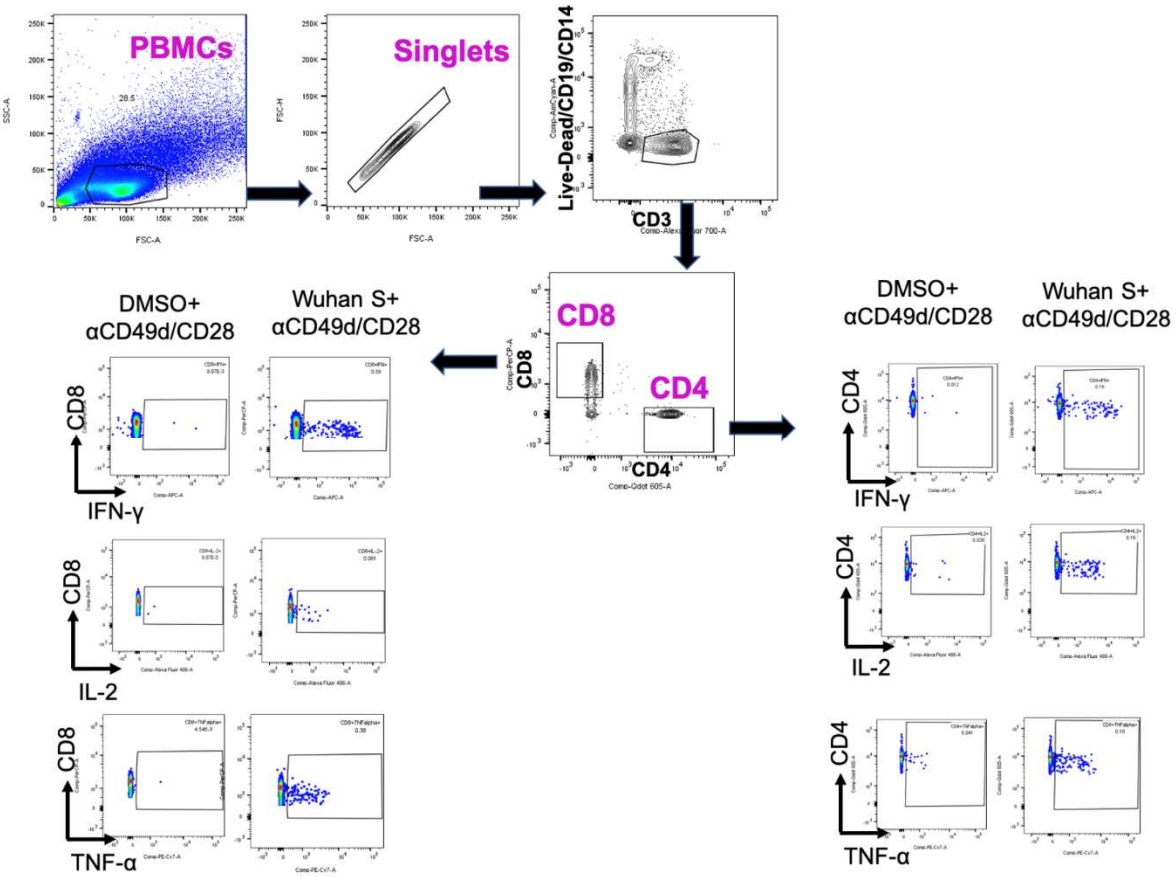

Figure S4. Gating strategy for analysis of T cell reactivity. SARS-CoV-2 spike peptide stimulated and unstimulated (DMSO) PBMCs were initially gated on the basis of light scatter (SSC-A versus FSC-A) and for singlets (FSC-H versus FSC-A). Dead cells, monocytes, and B cells were excluded using a dump channel and by gating on $CD3^+$ T cells. Total $CD8^+$ and $CD4^+$ cells were then selected, and individual cytokine gating was performed.

Ministero dell'Istruzione

Ufficio di Gabinetto

Al Gabinetto del Ministro della salute
c.a. del Capo di Gabinetto
Pres. Goffredo Zaccardi

E, p.c.

Al Coordinatore del
Comitato Tecnico Scientifico
Prof. Franco Locatelli

In attuazione dell'articolo 59, comma 20, del decreto-legge 25 maggio 2021, n. 73, si sottopone alla valutazione del Comitato Tecnico Scientifico il Protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico fino al 31 dicembre 2022, da adottare con successiva ordinanza del Ministro dell'Istruzione.

Si resta a disposizione per ogni eventuale chiarimento e si segnala l'urgenza, in considerazione dell'imminente svolgimento delle prove concorsuali.

Si ringrazia per la consueta collaborazione.

D'ORDINE DEL MINISTRO
IL CAPO DI GABINETTO
Cons. Luigi Fiorentino

Firmato digitalmente da
FIORENTINO LUIGI
C=IT
O=MINISTERO ISTRUZIONE
UNIVERSITA' E RICERCA

Ministero dell'Istruzione

Adozione del protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico in attuazione dell'articolo 59, comma 20, del decreto-legge 25 maggio 2021, n.73.

IL MINISTRO

VISTO il decreto legislativo 16 aprile 1994, n. 297, recante «*Approvazione del testo unico delle disposizioni legislative vigenti in materia di istruzione, relative alle scuole di ogni ordine e grado*»;

VISTO il decreto legislativo 30 marzo 2001, n. 165 recante «*Norme generali sull'ordinamento del lavoro alle dipendenze delle amministrazioni pubbliche*»;

VISTO il decreto del Presidente della Repubblica 28 dicembre 2000, n. 445, recante *«Testo unico delle disposizioni legislative e regolamentari in materia di documentazione amministrativa»*;

VISTO il decreto legislativo 13 aprile 2017, n. 59, recante *«Riordino, adeguamento e semplificazione del sistema di formazione iniziale e di acceso nei ruoli di docente, nella scuola secondaria per renderlo funzionale alla valorizzazione sociale e culturale della professione, a norma dell'articolo 1, commi 180 e 181, lett. b), della legge 13 luglio 2015, n.107»;*

VISTO il decreto-legge 12 luglio 2018, n. 87, recante *«Disposizioni urgenti per la dignità dei lavoratori e delle imprese»*, convertito, con modificazioni, dalla legge 9 agosto 2018, n. 96;

VISTO il decreto-legge 29 ottobre 2019, n. 126, recante «*Misure di straordinaria necessità ed urgenza in materia di reclutamento del personale scolastico e degli enti di ricerca e di abilitazione dei docenti»*, convertito con modificazioni dalla legge 20 dicembre 2019 n. 159;

VISTO il decreto-legge 1° aprile 2021, n. 44, in corso di conversione, recante «*Misure urgenti per il contenimento dell'epidemia da COVID-19, in materia di vaccinazioni anti SARS-CoV-2, di giustizia e di concorsi pubblici»*;

VISTO il decreto-legge 25 maggio 2021, n. 73 recante *«Misure urgenti connesse all'emergenza da COVID-19 per le imprese, il lavoro, i giovani, la salute e i servizi territoriali»* ed in particolare l'articolo 59, rubricato «*Misure straordinarie per la tempestiva nomina dei docenti di posto comune e di sostegno e semplificazione delle procedure concorsuali del personale docente»*;

VISTO il decreto dipartimentale 21 aprile 2020, n. 497, recante «*Procedura straordinaria, per esami, finalizzata all'accesso ai percorsi di abilitazione all'insegnamento nella scuola secondaria di primo e secondo grado su posto comune*» come modificato e integrato dal decreto dipartimentale 1° luglio 2020, n. 748;

VISTO il decreto dipartimentale 21 aprile 2020 n. 498, recante *«Concorso ordinario, per titoli ed esami, finalizzato al reclutamento del personale docente per i posti comuni e di sostegno della scuola dell'infanzia e primaria»;*

VISTO il decreto dipartimentale 21 aprile 2020, n. 499, recante *«Concorso ordinario, per titoli ed esami, finalizzato al reclutamento del personale docente per posti comuni e di sostegno nella scuola secondaria di primo e secondo grado*», come modificato e integrato dai decreti dipartimentali 3

giugno 2020, n. 649 e 1° luglio 2020, n. 749;

CONSIDERATA la necessità che le procedure concorsuali del personale scolastico abbiano a svolgersi nel pieno rispetto di ogni adeguata misura volta al contenimento del possibile contagio e alla tutela della salute degli utenti e degli operatori, e che l'organizzazione e la gestione delle prove selettive delle procedure concorsuali finalizzate al reclutamento siano tali da consentirne lo svolgimento in condizioni di sicurezza e contenimento del rischio connesso alla pandemia da Covid - 19;

VISTE le disposizioni e misure tecniche come definite dal *«Protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico in attuazione dell'art. 59, comma 20, del decreto-legge 25 maggio 2021, n. 73»*;

VISTA l'approvazione da parte del Comitato Tecnico Scientifico nella seduta del XXXX;

SENTITE le Organizzazioni sindacali rappresentative del comparto "Istruzione e Ricerca";

ORDINA

Articolo 1

(Protocollo per lo svolgimento in sicurezza dei concorsi pubblici per il personale scolastico)

1. Al fine di prevenire il rischio epidemiologico, contrastare la pandemia da Covid-19 e tutelare la salute, è adottato il "*Protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico in attuazione dell'art. 59, comma 20, del decreto-legge 25 maggio 2021, n.73*" allegato alla presente ordinanza.

2. Salvo sopravvenute esigenze di tutela della salute pubblica o di modifica della disciplina normativa, il protocollo di cui al precedente comma si applica alle procedure concorsuali per il personale scolastico fino al 31 dicembre 2022, senza nuovi o maggiori oneri per la finanza pubblica.

La presente ordinanza è inviata agli organi di controllo.

Il MINISTRO
Prof. Patrizio Bianchi

Ministero dell'Istruzione

Protocollo relativo alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico in attuazione dell'art. 59, comma 20 del decreto-legge 25 maggio 2021, n.73

1. Ambito di applicazione

1. L'articolo 59, comma 20, del decreto legge 25.5.2021, n. 73 prevede che "*Con ordinanza del Ministro dell'istruzione sono definiti appositi protocolli, sottoposti alla previa approvazione del Comitato tecnico-scientifico di cui all'ordinanza del Capo del Dipartimento della protezione civile 3 febbraio 2020, n. 630, e successive modificazioni, relativi alle modalità di svolgimento in sicurezza dei concorsi per il personale scolastico fino al 31 dicembre 2022, senza nuovi o maggiori oneri per la finanza pubblica*."
2. La disposizione fa riferimento allo svolgimento della fase "in presenza" delle prove concorsuali. Restano prive di limitazioni le procedure per le quali la valutazione dei candidati sia effettuata esclusivamente su basi curriculari ovvero in modalità telematica, nonché la possibilità per le commissioni di procedere alla correzione delle prove scritte in presenza oppure con collegamento da remoto, fatte salve le cautele generali proprie del contenimento della diffusione epidemiologica.
3. Il presente protocollo, stabilisce le linee guida dirette a prevenire e a contenere il diffondersi del contagio dal virus COVID-19 in occasione dello svolgimento delle prove selettive delle procedure concorsuali finalizzate al reclutamento del personale scolastico, in modo da realizzare un adeguato bilanciamento tra la salvaguardia delle esigenze organizzative connesse al loro svolgimento e la necessità di garantire condizioni di tutela della salute dei candidati, della commissione esaminatrice, del comitato di vigilanza, del personale individuato con compiti di sorveglianza ed assistenza interna per lo svolgimento delle prove e, in generale, di tutte le altre figure presenti nelle aree concorsuali. Salva diversa previsione normativa, le prove selettive in presenza potranno avere una durata sino ad un massimo di 120 minuti.

2. *Requisiti dell'area e delle aule concorsuali*

1. L'area concorsuale deve disporre di un locale autonomo ove accogliere e isolare gli eventuali soggetti sintomatici.
2. Il numero massimo dei candidati presenti contemporaneamente nell'aula, sede di esame, dovrà essere determinato in rapporto alla capienza degli spazi individuati.
3. Dovranno essere garantite misure di distanziamento tra canditati, personale di supporto, membri della commissione e del comitato di vigilanza, responsabili tecnici d'aula ed in generale tra tutte le figure presenti nelle aree concorsuali, da definire anche in ragione delle caratteristiche dei locali utilizzati per lo svolgimento delle prove, attraverso la previsione di una fascia di protezione individuale che permetta il rispetto del distanziamento interpersonale di almeno un metro.

3. *Organizzazione dell'accesso, seduta e dell'uscita dei candidati*

1. I candidati potranno, accedere all'interno dell'area concorsuale solo uno per volta. Ad essi è fatto obbligo di:
   a. igienizzarsi le mani con il gel contenuto negli appositi dosatori all'ingresso;
   b. indossare obbligatoriamente, a pena di esclusione dalla procedura concorsuale per tutto il tempo di permanenza all'interno dell'area concorsuale, dal momento dell'accesso sino all'uscita facciali filtranti FFP2 che coprano correttamente le vie aeree (bocca e naso) messi a disposizione ai candidati. Non deve essere consentito in ogni caso nell'area concorsuale l'uso di mascherine chirurgiche, facciali filtranti e mascherine di comunità in possesso del candidato.
   c. presentarsi da soli e senza alcun tipo di bagaglio (salvo motivate situazioni eccezionali). In tal caso il candidato utilizzerà un sacco contenitore in cui deporre il bagaglio, da appoggiare, chiuso, lontano dalle postazioni, secondo le istruzioni ricevute in aula);
   d. non presentarsi presso la sede concorsuale se affetti da uno o più dei seguenti sintomi riconducibili al virus COVID- 19:
      - temperatura superiore a 37,5°C e brividi;
      - tosse di recente comparsa;
      - difficoltà respiratoria;
      - perdita improvvisa dell'olfatto (anosmia) o diminuzione dell'olfatto (iposmia), perdita del gusto (ageusia) o alterazione del gusto (disgeusia);
      - mal di gola;
   e. non presentarsi presso la sede concorsuale se sottoposto alla misura della quarantena o isolamento domiciliare fiduciario e/o al divieto di allontanamento dalla propria dimora/abitazione come misura di prevenzione della diffusione del contagio da COVID-19;
   f. presentare all'atto dell'ingresso nell'area concorsuale un referto relativo ad un test antigenico rapido o molecolare, effettuato mediante tampone oro/rino-faringeo presso una struttura pubblica o privata accreditata/autorizzata in data non antecedente a 48 ore dalla data di svolgimento delle prove. La prescrizione non si applica a coloro che abbiano già completato il percorso vaccinale per il COVID 19 e che presentino relativo certificato vaccinale;
   g. sottoporsi alla rilevazione della temperatura corporea, prioritariamente mediante termoscanner oppure nel caso in cui tale strumento non sia disponibile potranno essere utilizzati termometri manuali che permettano la misurazione automatica. Qualora la temperatura corporea rilevata risulti superiore ai 37, 5 C°, il candidato non potrà accedere all'area concorsuale. Il personale addetto alla vigilanza dovrà provvedere all'allontanamento del soggetto, accompagnandolo in un'apposita area dedicata all'isolamento del caso sospetto e dovrà tempestivamente avvertire le autorità sanitarie competenti e i numeri di emergenza per il Covid 19 forniti dalla regione o dal Ministero della salute, nonché le forze dell'ordine in caso di rifiuto. Il candidato è tenuto, comunque, a informare tempestivamente e responsabilmente i commissari del concorso ed il comitato di vigilanza della presenza di qualsiasi sintomo influenzale, anche durante l'espletamento prova scritta, avendo cura di rimanere ad adeguata distanza dalle persone presenti.
2. Gli obblighi di cui alle lettere d) ed e) devono essere oggetto di un'apposita autodichiarazione da prodursi ai sensi degli artt. 46 e 47 del DPR 445/2000. I candidati dovranno compilare l'apposito modulo, scaricabile dal sito web del Ministero nella sezione dedicata alla specifica procedura concorsuale, che dovrà essere esibito presso le apposite postazioni al personale addetto all'identificazione dei candidati.

3. Qualora una o più delle sopraindicate condizioni non dovesse essere soddisfatta, ovvero in caso di rifiuto a produrre l'autodichiarazione, non potrà essere consentito al candidato l'ingresso all'interno dell'area concorsuale.
4. I candidati ammessi, saranno invitati dal personale di assistenza a raggiungere, opportunamente distanziati e in base alla segnaletica orizzontale e verticale, le postazioni di identificazione. Dovrà essere garantita l'identificazione prioritaria delle donne in stato di gravidanza, dei candidati con disabilità e dei candidati richiedenti tempi aggiuntivi. Presso le postazioni di identificazione dovranno essere resi disponibili appositi dispenser di gel idroalcolico. Gli operatori dovranno invitare i candidati a procedere all'igienizzazione delle mani prima e dopo le operazioni di identificazione e/o consegna e/o ricezione di materiale concorsuale. Per le operazioni di identificazione dovranno essere rese disponibili penne monouso per i candidati.
5. Successivamente i candidati, verranno invitati ad accedere all'aula concorsuale, e ad occupare una postazione informatizzata, rispettando la distanza prevista, preventivamente determinata attraverso segnaposti adesivi. I candidati, una volta raggiunta la postazione loro assegnata dovranno:
   - rimanere seduti per tutto il periodo che precede la prova, durante e al termine dello svolgimento della stessa finché non saranno autorizzati all'uscita;
   - durante l'orario d'esame sarà permesso l'allontanamento dalla propria postazione esclusivamente per recarsi ai servizi igienici o per altri motivi indifferibili.
   - indossare obbligatoriamente il facciale filtrante FFP2 messo a diposizione dalla amministrazione organizzatrice;
   - non consumare alimenti ad eccezione delle bevande di cui i candidati si devono munire preventivamente.
6. Il personale incaricato dovrà verificare il rispetto delle misure di sicurezza durante tutte le fasi della procedura, di svolgimento della prova e per tutto il tempo in cui i candidati permangano all'interno dell'area concorsuale, nonché nelle operazioni di entrata ed uscita dalla struttura. Dovrà assicurare che non si creino assembramenti durante le operazioni connesse all'espletamento della prova. Inoltre il personale di assistenza e di supporto dovrà provvedere a regolamentare il flusso di accesso e d'uscita dall'area concorsuale, assicurando il rispetto della distanza di almeno 1 metro e apposite misure per le donne in stato di gravidanza, per i candidati diversamente abili, per gli immunodepressi e per quelli che necessitano tempi aggiuntivi sulla base della normativa vigente. È obbligatorio il rispetto del distanziamento interpersonale, al quale si potrà derogare esclusivamente per motivi di soccorso e sicurezza.

4. Misure di sicurezza per la commissione, per il comitato di vigilanza e per il restante personale presente nell'aula concorsuale

1. I componenti delle commissioni, del comitato di vigilanza, il personale individuato con compiti di sorveglianza ed assistenza interna per lo svolgimento delle prove ed in generale tutte le altre figure presenti nelle aree concorsuali, hanno l'obbligo di:
   a. igienizzarsi le mani con apposito gel disinfettante contenuto nei dosatori all'ingresso prima di accedere all'interno dell'area concorsuale;
   b. indossare, prima di accedere e per tutto il tempo di permanenza all'interno dell'area concorsuale, sino all'uscita dalla struttura, facciali filtranti FFP2 privi di valvola di espirazione forniti dall'amministrazione organizzatrice;

c. compilare il modulo di autodichiarazione ai sensi degli artt. 46 e 47 del DPR 445/20003 scaricabile dal sito web del Ministero secondo le modalità descritte al paragrafo 3;
d. circolare solo nell'aree e nei percorsi indicati ed evitare di avvicinarsi ai candidati a distanza inferiore ad 1 metro.

5. Adempimenti dei responsabili dell'aree concorsuali

1. I responsabili delle aree concorsuali provvederanno:
   - a munirsi per la misurazione della temperatura corporea di termoscanner oppure di un termometro manuale;
   - a mettere a disposizione facciali filtranti FFP2 per i candidati, per la commissione, per il personale di vigilanza e per tutte le figure presenti all'interno dell'area concorsuale;
   - a fornire penne monouso per i candidati;
   - a predisporre la segnaletica di carattere prescrittivo, informativo e direzionale;
   - ad individuare ed allestire un apposito locale dedicato all'accoglienza e all'isolamento dei soggetti che presentano sintomi riconducibili al virus COVID-19;
   - a collocare all'ingresso dell'area concorsuale e in più punti delle aree (es., aule, servizi igienici, etc.) un adeguato numero di dispenser di gel igienizzante;
   - a garantire adeguata aerazione di tutti i locali, mantenendo costantemente (o il più possibile) aperti gli infissi esterni, anche dei servizi igienici o assicurando un adeguato ricambio d'aria attraverso strumenti meccanici;
   - alla pulizia e disinfezione giornaliera dell'area concorsuale;
   - a sottoporre a regolare pulizia e igienizzazione delle aule, gli arredi, le maniglie, le postazioni di lavoro dei candidati e gli strumenti utilizzati (sedie, banchi, computer, mouse e tastiera), sia prima dello svolgimento della prova, tra una sessione e l'altra e al termine delle stesse;
   - alla pulizia e igienizzazione dei servizi igienici con idonei prodotti;
   - a dotare i servizi igienici di dispenser con gel igienizzante per le mani, salviette e pattumiere;
   - a regolare l'accesso ai servizi igienici, al fine di evitare sovraffollamento all'interno dei locali, che dovranno essere costantemente puliti e sanificati dopo ogni singolo utilizzo.

6. Piano operativo specifico della procedura concorsuale

Le amministrazioni organizzatrici delle prove concorsuali dovranno predisporre un piano operativo, che dovrà essere pubblicato sul sito web dell'Ufficio scolastico regionale dedicato alla specifica procedura concorsuale entro 3 giorni precedenti lo svolgimento della prova, contenente una descrizione dettagliata delle varie fasi della procedura, tenendo conto di quanto evidenziato nel presente protocollo e delle ulteriori misure di sicurezza previste dalla normativa vigente.