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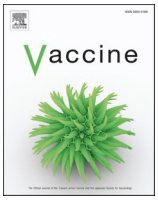

Humoral immune response against SARS-CoV-2 after adapted COVID-19 vaccine schedules in healthy adults: The IMCOVAS randomized clinical trial

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ABSTRACT

Background: To overcome supply issues of COVID-19 vaccines, this partially single blind, multi–centric, vaccine trial aimed to evaluate humoral immunogenicity using lower vaccine doses, intradermal vaccination, and heterologous vaccine schedules. Also, the immunity after a booster vaccination was assessed.

Methodology: 566 COVID-19-naïve healthy adults were randomized to 1 of 8 treatment arms consisting of combinations of BNT162b2, mRNA-1273, and ChAdOx1-S. Anti-Receptor-Binding Domain immunoglobulin G (RBD IgG) titers, neutralizing antibody titres, and avidity of the anti-RBD IgGs was assessed up to 1 year after study start.

Results: Prolonging the interval between vaccinations from 28 to 84 days and the use of a heterologous BNT162b2 + mRNA-1273 vaccination schedule led to a non-inferior immune response, compared to the reference schedule. A low dose of mRNA-1273 was sufficient to induce non-inferior immunity. Non-inferiority could not be demonstrated for intradermal vaccination. For all adapted vaccination schedules, anti-RBD IgG titres measured after a first booster vaccination were non-inferior to their reference schedule.

Conclusion: This study suggests that reference vaccine schedules can be adapted without jeopardizing the development of an adequate immune response. Immunity after a booster vaccination did not depend on the dose or brand of the booster vaccine, which is relevant for future booster campaigns.

The trial is registered in the European Union Clinical Trials Register (number 2021–001993-52) and on clinicaltrials.gov (NCT06189040).

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1. Background

In late 2019, SARS-CoV-2, causing Coronavirus Disease 2019 (COVID-19), emerged in Wuhan, China [1]. By March 2020, the SARS-CoV-2 outbreak was declared as a pandemic by the World Health Organization (WHO) with 118,000 cases of COVID-19 in 114 countries and 4,291 COVID-19-related deaths up until then [2]. About 1 year later, several vaccines, including BNT162b2 (Comirnaty®; Pfizer–BioNTech), mRNA-1273 vaccine (Spikevax®; Moderna), ChAdOx1-S [recombinant] (Vaxzevria®; AstraZeneca), and Ad26.COV2.S (Johnson & Johnson COVID-19 vaccine®; Johnson & Johnson) were authorized by the European Medicines Agency (EMA) for use in the European Union [3], significantly reducing severe disease and hospitalization risks [4].

Nevertheless, vaccine availability was difficult and the world was faced with challenges like production infrastructure, distribution logistics, and healthcare capacity for the administration of the vaccines [5,6]. To overcome issues in the vaccine supply and improve the vaccine availability, it is of major interest to research several strategies to adequately adapt standard vaccine schedules, including dose adjustments, varying intervals between subsequent vaccine doses, alternative administration routes, and heterologous schedules combining vaccines of different brands [6–15].

In order to study the immunogenicity of different vaccine schedules, the multi-centric Immunogenicity after COVID-19 Vaccines in Adapted Schedules (IMCOVAS) randomized clinical trial was designed and conducted during the pandemic's early phase, examining different antigen doses, intervals, administration routes, and the impact of booster vaccinations on humoral immunogenicity in various primary vaccine schedules. The main objective of this trial was to demonstrate non-inferiority of the humoral immune response against SARS-CoV-2 in COVID-19-naïve participants following different adapted vaccine schedules. These were compared with the standard vaccine schedule at 28 days post–second study vaccine dose administration. Non-inferiority of one or more adapted vaccine schedules could provide evidence-based flexibility to vaccination campaigns, making them less dependent on the availability of sufficient doses of specific brands of vaccines. Therefore, the results of this trial could help optimize immunization programs, increase feasibility of vaccination programs, and possibly accelerate them.

2. Methods

2.1. Trial design

This was a partially single-blind, randomized, investigator-driven, interventional trial conducted between May 2021 and July 2022 in 4 Belgian study centers. Ethical approval was received by the Belgian competent authorities (Federal Agency for Medicinal and Health Products [FAMHP]) and centralized ethics committee before the start of any trial activities. The trial was conducted according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use Good Clinical Practice guidelines and the Declaration of Helsinki, and was completed per protocol. The trial is registered in the European Union Clinical Trials Register (number 2021-001993-52) and on clinicaltrials.gov (NCT06189040).

2.2. Trial population

Healthy adults aged 18–55 years, without prior COVID-19 infection or vaccination, were recruited via local advertising between May and June 2021. Of note, recruitment occurred in parallel with Belgium's national COVID-19 vaccination campaign. Key exclusion criteria included recent severe diseases, history of vaccine-related anaphylaxis, immunodeficiency disorders, recent immunosuppressant or immune-modifying drugs use, and pregnancy or lactation. A complete list of inclusion and exclusion criteria can be found in the Supplementary Methods. All participants provided written informed consent at enrolment.

2.3. Vaccines, treatment arms, and randomization

Vaccines investigated in this trial included BNT162b2, mRNA-1273 vaccine, and ChAdOx1-S, all of which had a conditional marketing authorization in the European Union at the time of trial initiation. BNT162b2 was administered intramuscularly in a dose of 30 μg (standard dose; further referred to as 'B' when administered with a standard interval of 28 days between subsequent doses and as 'BL' when administered with a 'long interval' of 84 days) or 20 μg (low dose; 'b'), or intradermally in a dose of 6 μg (intradermal dose; 'BI'). The mRNA-1273 vaccine was administered intramuscularly in a dose of 100 μg (standard dose; 'M') or 50 μg (low dose; 'm'). ChAdOx1-S was administered in a dose of at least $2.5 \bullet 10^8$ infectious units (standard dose; 'C').

The trial consisted of 8 treatment arms in total (Fig. 1). The different treatment arms allowed for the comparison of standard dose BNT162b2 and mRNA-1273 vaccine schedules with a standard 28–day interval (B + B and M + M, respectively [further referred to as standard or reference schedules, and used to compare adapted vaccine schedules against]) with schedules using lower doses (b + b and m + m), heterologous vaccine schedules (B + C and B + M), a vaccine schedule using the intradermal administration route (BI + BI), and a vaccine schedule with an 84-day interval between subsequent doses (BL + BL).

After eligibility confirmation, allocation to 1 of the 8 vaccine schedules happened via a randomization system (REDCap, version 8.10.4). Because of safety concerns regarding ChAdOx1-S in younger adults, the randomization scheme took into account that only adults aged 41–55 years could be randomized to the treatment arm involving this vaccine (i.e. B + C).

2.4. Trial procedures

Participants were followed for 364 days post-first vaccine dose, which was administered on Day 0. Serum samples for humoral immunogenicity were collected at Days 0, 28, 56 or 84 (treatment arms with a 28–day or 84-day interval between subsequent doses, respectively), 112, 182, and 364. An overview of the humoral immunogenicity assays performed at the different timepoints is given in Supplementary Figure 1.

Solicited and unsolicited adverse events were collected for 5 and 14 days after the administration of the study vaccine, respectively. Adverse Events of Special Interest (AESIs), including COVID-19 infections and potential immune mediated diseases (pIMDs), medically attended adverse events (MAAEs), and serious adverse events (SAEs) were collected throughout the trial (See Supplementary Tables 1-4 for an overview of solicited adverse events, AESIs, examples of pIMDs, and severity grading of adverse events, respectively).

By the end of 2021, participants who received both vaccine doses within the IMCOVAS trial, did not receive additional primary COVID-19 vaccine doses outside the trial, and received a third COVID-19 vaccine dose via the Belgian governmental campaign, were invited for an additional *ad hoc* visit 28 days after the administration of BNT162b2 (30 μg) or mRNA-1273 (50 μg or 100 μg) as their booster vaccine to assess the immune response.

2.5. Humoral immunogenicity assays

SARS-CoV-2 anti-Receptor Binding Domain (RBD) IgG levels (Wuhan strain) were quantified by means of an Enzyme-Linked ImmunoSorbent Assay (ELISA) (Wantai SARS-CoV-2 IgG ELISA [Quantitative]; CE-marked; WS-1396; Beijing Wantai Biological Pharmacy Enterprise Co., Ltd, China) and results were expressed as Binding Antibody Units/mL (BAU/mL), as described elsewhere [7]. The lower limit of quantification of the assay was 5 BAU/mL. Anti-RBD IgG levels were measured at all

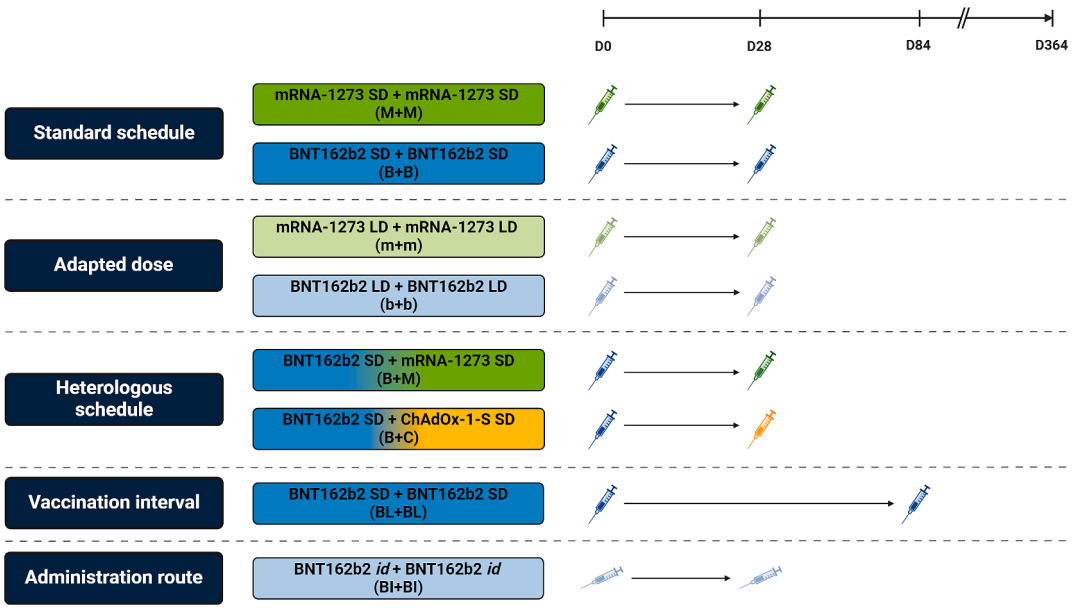

Fig. 1. Overview of the treatment arms within the IMCOVAS trial. Four ways of modifying the BNT16b2 and mRNA-1273 vaccine schedules were considered, potentially allowing for more flexibility in COVID-19 immunization schedules: i) lowering the vaccine dose; ii) combining vaccines of different brands; iii) prolonging the interval between subsequent vaccinations; and iv) using the intradermal administration route. Note that participants were encouraged to receive a third vaccination, offered by the Belgian government, outside of the trial. Abbreviations for the different treatment arms are shown between brackets. D, day; id, intradermal; LD, low dose; SD, standard dose. Created with BioRender.com.

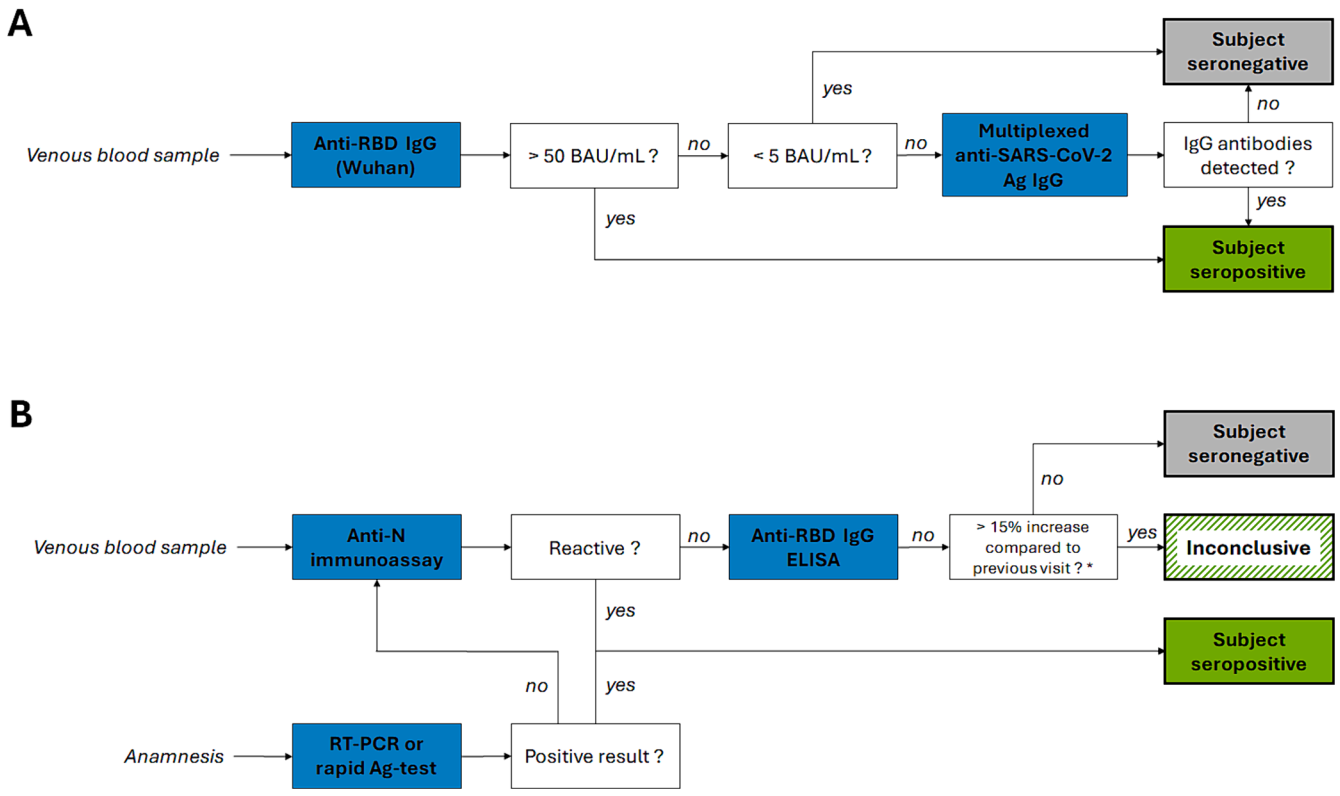

Fig. 2. Identification of infections with SARS-CoV-2. The assessment of infections differed between the baseline visit (A) and the subsequent follow-up visits (B). Blue boxes indicate the use of assays to detect COVID-19 infections using a nasopharyngeal swab (rapid Ag-test or RT-PCR) or blood (all other assays). * Note that the 15% increase in anti-RBD IgG levels can only be used to assess the presence of a breakthrough infection if the subject did not get vaccinated the previous visit. Ag, antigen; BAU, binding antibody units; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; N, nucleocapsid antigen; RBD, receptor-binding domain; RT-PCR, reverse transcription polymerase chain reaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

timepoints.

In a subset of 30 participants per treatment arm that reached 28 days post-second vaccination without a COVID-19 infection, humoral immunogenicity was assessed in more detail. This subset is further referred to as the humoral immunogenicity subset. In this subset, neutralizing antibody levels against SARS-CoV-2 original lineage B (Wuhan-Hu-1), Delta variant (B.1.617.2), and BA.1 and BA.5 Omicron variant (B.1.1.529) were measured 28 days after the second vaccination, at the *ad hoc* visit, and at Day 364, using a live-virus neutralization test (See Supplementary Methods for detailed laboratory methods), as described previously [8,9]. In the same subset, avidity of anti-RBD IgG was quantified via bio–layer interferometry with an Octet HTX instrument (Sartorius) using AR2G biosensors, as published elsewhere [10]. The technology allows for the measurement of real-time kinetic parameters for the association and dissociation phases of an antibody-antigen interaction. Avidity is expressed as the reciprocal of the dissociation rate, which is proportional to the stability of the antibody-antigen complexes. Avidity of anti-RBD IgG was quantified for all treatment arms in samples collected at Days 28, 56 or 84, and 112.

See Supplementary Figure 1 for an overview of the humoral immunogenicity assays per timepoint.

2.6. Detection of SARS-CoV-2 infection

Seropositivity at baseline was identified using the anti-RBD IgG ELISA. Measurements below 5 and above 50 BAU/mL were considered seronegative and seropositive, respectively. In case of inconclusive measurements, seropositive and seronegative participants were differentiated by performing an in-house multiplex immunoassay quantifying IgG antibodies to RBD, S1, S2, and N SARS-CoV-2 antigens (Wuhan strain), as described elsewhere (Fig. 2A) [8].

Throughout the trial, SARS-CoV-2 infections were identified using a combination approach (Fig. 2B) including self-reported SARS-CoV-2 infections confirmed by an antigen–based rapid diagnostic test or by a reverse transcription polymerase chain reaction (RT-PCR) test, anti-N IgG seropositivity (Elecsys Anti-SARS-CoV-2 immunoassay [Cobas, Roche diagnostics]), and a $\geq$ 15 % increase of anti-RBD IgG between subsequent visits not encompassing a vaccination. Infections that occurred as of 28 days after receiving the second vaccine, were referred to as breakthrough infections (BTIs).

2.7. Endpoints

The primary endpoint was the assessment of the geometric mean titer (GMT) of antibodies binding to the RBD of SARS-CoV-2 S protein of the Wuhan-Hu-1 virus strain 28 days after the administration of the second study vaccine.

The secondary endpoints included a comparison of the safety and reactogenicity, as well as immunogenicity between the different treatment arms. Key immunogenicity endpoints included the GMT of antibodies binding to the RBD of the SARS-CoV-2 S protein 28 days after the administration of the third vaccine and neutralizing antibody titers against the Wuhan, Delta, and Omicron strains 28 days post-second and −third vaccinations. Avidity of anti-RBD IgG 28 days post-second vaccination was considered a tertiary endpoint.

2.8. Statistical analysis

Based on a contemporary trial, the required sample size was calculated assuming a 0.27 standard deviation (SD) in log10-transformed GMT of anti-RBD IgG antibodies [10]. The true difference in log10–-transformed GMT was assumed to be 0. The non-inferiority margin was set at a −0.2 GMT difference, corresponding to a GMT ratio larger than 0.63, aligning with other trials [11]. To ensure 90 % power at a one-sided 2.5 % family-wise error rate (FWER), 56 participants per treatment arm were required. Considering potential exclusions due to baseline seropositivity for SARS-CoV-2 and dropouts, this sample size was increased to 70 participants per treatment arm.

A non-inferiority analysis was conducted on a modified intention-to-treat (mITT) trial population, including only seronegative participants at baseline who received both vaccines and had available primary endpoint data. For details, refer to the Supplementary Methods.

3. Results

3.1. Trial population

Of the 580 participants assessed for eligibility, 566 were deemed eligible and randomized. Of these, 60 participants were excluded from the analysis of the primary endpoint due to SARS-CoV-2 seropositivity at baseline, becoming seropositive before the primary endpoint, or having a major protocol deviation impacting the primary endpoint, resulting in a mITT population of 506 participants. In total, 50.40 % of the participants were male, the majority (96.05 %) was Caucasian, and the mean age was 33 $\pm$ 10 years (Table 1). Of all randomized participants reaching the primary endpoint, 134 participants (26.48 %) received mRNA-1273 as their primary vaccine and 372 participants (73.52 %) received BNT162b2 as their primary vaccine. The distribution of gender, race, and age did not differ significantly between participants randomized to the M + M and m + m treatment arms ($p = 0.86$, $p = 0.71$, and $p = 0.21$, respectively), and the distribution of gender and race did not differ significantly between participants randomized to the 6 BNT162b2-based treatment arms ($p = 0.86$ and $p = 0.68$, respectively). The median age of participants was significantly higher in the B + C treatment arm, compared to all other BNT162b2-based treatment arms ($p < 0.001$) due to the age limitation for this group following safety concerns for ChAdOx1-S in younger adults. Excluding B + C participants, age did not differ significantly between the BNT162b2-based treatment arms ($p = 0.77$).

3.2. SARS-CoV-2 S RBD binding IgG

The observed GMTs of antibodies binding to the RBD of the SARS-CoV-2 S protein of the Wuhan–Hu–1 strain 28 days after the second vaccination are presented in Table 2. The m + m schedule was non–-inferior to the standard M + M schedule (GMT ratio = 0.96 [95 % CI = 0.79–1.18]) (Fig. 3A).

The long interval schedule showed a non-inferior humoral response, compared to the standard B + B schedule (GMT ratio = 0.90 [95 % CI = 0.66–1.24]). Non-inferiority could not be shown for the b + b schedule (GMT ratio = 0.83 [95 % CI = 0.62–1.12]), nor for the BI + BI schedule (GMT ratio = 0.62 [95 % CI = 0.46–0.83]).

The heterologous B + M schedule resulted in a non-inferior antibody response, compared to the standard B + B schedule (GMT ratio = 1.32 [95 % CI = 0.98–1.78]). In contrast, the development of the humoral response following the heterologous B + C schedule was inferior to the reference schedule (GMT ratio = 0.41 [95 % CI = 0.29–0.57]).

Twenty-eight days after the third vaccination, the observed GMT of antibodies binding to the RBD of the SARS-CoV-2 S protein was assessed in COVID-19-naïve participants. All adapted vaccine schedules remained non-inferior to their reference schedule (Table 2 and Fig. 3B). The humoral immune response did not depend on the administered booster vaccine (BNT162b2 30 μg, mRNA-1273 50 μg, or mRNA-1273 100 μg).

3.3. Neutralizing antibodies

Twenty-eight days after the second vaccination, the GMTs of the neutralizing antibody titers against the Wuhan-Hu-1 strain were assessed (50 % neutralization titers [NT50] are presented in Table 2 and Fig. 3C). Compared to the standard schedule B + B, a lower BNT162b2 vaccine dose and a longer interval between subsequent vaccinations

Table 1
Demographic characteristics of the mITT population.

		Standard schedule		Adapted dose		Heterologous schedule		Vaccination interval	Administration route	
		BNT162b2 SD + BNT162b2 SD (B + B, n = 57)	mRNA-1273 SD + mRNA-1273 SD (M + M, n = 66)	BNT162b2 LD + BNT162b2 LD (b + b, n = 69)	mRNA-1273 LD + mRNA-1273 LD (m + m, n = 68)	BNT162b2 SD + mRNA-1273 SD (B + M, n = 68)	BNT162b2 SD + ChAdOx1-S SD (B + C, n = 59)	BNT162b2 SD + BNT162b2 SD long-interval (BL + BL, n = 52)	BNT162b2 *id* + BNT162b2 *id* (BI + BI, n = 67)	Total (n = 506)
Gender	**Male**	29 (50.88 %)	31 (46.97 %)	38 (55.07 %)	33 (48.53 %)	38 (55.88 %)	26 (44.07 %)	24 (46.15 %)	36 (53.73 %)	255 (50.40 %)
	Female	28 (49.12 %)	35 (53.03 %)	31 (44.93 %)	35 (51.47 %)	30 (44.12 %)	33 (55.93 %)	28 (53.85 %)	31 (46.27 %)	251 (49.60 %)
Age		31 ± 9	30 ± 8	30 ± 9	32 ± 8	32 ± 10	46 ± 4	32 ± 10	30 ± 9	33 ± 10
Race	**Caucasian**	55 (96.49 %)	63 (95.45 %)	66 (95.65 %)	63 (92.65 %)	65 (95.59 %)	56 (94.92 %)	52 (100.00 %)	66 (98.51 %)	486 (96.05 %)
	Other	2 (3.51 %)	3 (4.55 %)	3 (4.35 %)	4 (5.88 %)	3 (4.41 %)	2 (3.39 %)	0 (0.00 %)	1 (1.49 %)	18 (3.56 %)
	Unknown	0 (0.00 %)	0 (0.00 %)	0 (0.00 %)	1 (1.47 %)	0 (0.00 %)	1 (1.69 %)	0 (0.00 %)	0 (0.00 %)	2 (0.40 %)

id = intradermal; LD = low dose; mITT = modified intention-to-treat: SD = standard dose.
Results are shown as mean ± standard deviation or as number (percentage of total).

established a non-inferior production of neutralizing antibodies (GMT ratio = 0.84 [95 % CI = 0.63–1.12] and GMT ratio = 2.26 [95 % CI = 1.66–3.06], respectively). Non-inferiority to the reference schedule was also demonstrated for the B + M schedule (GMT ratio = 1.27 [95 % CI = 0.95–f1.69]), but could not be demonstrated for the heterologous B + C schedule (GMT ratio = 0.60 [95 % CI = 0.43–0.84]). Additionally, the level of neutralizing antibodies 28 days after the second vaccination was non-inferior for the m + m schedule, compared to the standard M + M schedule (GMT ratio = 0.97 [95 % CI = 0.81–1.16]).

Twenty-eight days after the third COVID-19 vaccination, 100.00 % and 98.69 % of COVID-19-naïve participants showed vaccine-induced immunity detectable through neutralizing antibody titers against the Wuhan and Delta strains, respectively (n = 139 participants). Non-inferiority of the neutralizing antibody titers could only be confirmed for the heterologous B + C schedule (Fig. 3D). However, this trial was not powered to confirm non-inferiority in the small subset of participants included in this analysis and results should therefore be interpreted with caution.

A strong association between anti-RBD IgG levels and neutralizing antibodies (NT50) against the Wuhan or Delta strains was observed in most treatment arms at the primary endpoint (Fig. 4). The conversion factor for neutralizing antibodies against the Wuhan strain over the anti-RBD IgG levels was higher for the long interval BL + BL schedule, compared to the standard schedule B + B (0.90 ± 0.05 *versus* 0.79 ± 0.04, respectively). Likewise, the conversion factor for neutralizing antibodies against the Delta strain over the anti-RBD IgG levels was increased in the BL + BL schedule (BL + BL: 0.68 ± 0.04 *versus* B + B: 0.59 ± 0.04). All other BNT16b2-based schedules and the mRNA-1273 vaccine-based schedule had conversion factors similar to that of the reference schedule.

3.4. Anti-RBD IgG avidity

Anti-RBD IgG avidity was measured 28, 56 or 84, and 112 days after the first vaccine dose (Table 3). Twenty-eight days after the second vaccination, anti-RBD IgG avidity was found to be significantly lower for the BI + BI and B + C schedules, compared to the standard schedule B + B ($p = 0.00071$ and $p < 0.00001$, respectively). In contrast, anti-RBD IgG avidity was significantly higher in the BL + BL schedule ($p = 0.02284$). Furthermore, anti-RBD IgG avidity was higher for the m + m schedule than the standard M + M schedule ($p < 0.00001$).

In the standard interval treatment arms, avidity waned significantly in the 2 months following the initial avidity increase 28 days post-second vaccination. The standard B + B schedule had a stronger increase in avidity in the 28 days following the second vaccination than the standard M + M schedule ($p < 0.0001$). However, the subsequent waning of avidity was less pronounced in the standard M + M schedule ($p < 0.0001$).

3.5. Protection against infection with SARS-CoV-2

Of the 506 participants included in the primary endpoint analyses, 446 remained in the trial until the final visit (Visit 6, Day 364), received the third COVID-19 vaccine after September 2021, and did not receive additional COVID-19 vaccines outside the trial. Of these 446 participants, 231 (51.79 %) reported a total of 235 SARS-CoV-2 (re-)infections during the trial (Table 4). Only 5 SARS-CoV-2 infections were deemed severe (2.13 %), while 49 participants (20.85 %) required medical attention for their SARS-CoV-2 (re-)infection. None of the infections required hospitalization.

In addition to reported adverse events, BTIs occurring in between visits could be detected in the serum. Combining the reported and detected infections, a total of 330 participants (74.00 %) experienced a BTI during the trial period (Table 4). The majority of BTIs occurred after the Day 182 visit (81.21 %). The lowest and highest frequencies of BTIs were observed for the participants in the B + C treatment arm (31/51, 60.78 %) and the BL + BL treatment arm (39/46, 84.78 %), respectively.

3.6. Safety and reactogenicity

After the first and second study vaccination, a total of 3,612 solicited adverse events were reported by the 506 participants of the mITT population, of which 1,642 adverse events (45.46 %) were local and 1,970 adverse events (54.54 %) were systemic adverse events. The most frequently reported solicited adverse events included injection site pain or tenderness (98.02 % of the participants), fatigue (73.32 %), and headache (61.07 %). Most solicited events were deemed mild (63.93 %) or moderate (30.12 %) in severity, and only 7 (0.19 %) required medical

Table 2
Humoral immunogenicity markers measured 28 days after the second and third (booster) vaccination.

		Standard schedule		Adapted dose		Heterologous schedule		Vaccination interval	Administration route
		BNT162b2 SD + BNT162b2 SD (B + B)	mRNA-1273 SD + mRNA-1273 SD (M + M)	BNT162b2 LD + BNT162b2 LD (b + b)	mRNA-1273 LD + mRNA-1273 LD (m + m)	BNT162b2 SD + mRNA-1273 SD (B + M)	BNT162b2 SD + ChAdOx1-S SD (B + C)	BNT162b2 SD + BNT162b2 SD long-interval (BL + BL)	BNT162b2 *id* + BNT162b2 *id* (BI + BI)
Post-second vaccination	**Anti-RBD IgG (BAU/mL)**	4,158 ± 2,814 (n = 57)	5,803 ± 3,522 (n = 66)	3,656 ± 2,534 (n = 69)	5,313 ± 3,142 (n = 68)	5,517 ± 3,381 (n = 68)	1,194 ± 790 (n = 59)	3,803 ± 2,659 (n = 51)	2,755 ± 2,134 (n = 67)
	NT50 (IU/mL)	783 ± 528 (n = 57)	1,141 ± 713 (n = 66)	692 ± 493 (n = 69)	1,024 ± 538 (n = 68)	961 ± 596 (n = 66)	297 ± 179 (n = 59)	1,604 ± 897 (n = 51)	566 ± 467 (n = 67)
Post-third vaccination	**Anti-RBD IgG (BAU/mL)**	4,003 ± 2,580 (n = 30)	4,593 ± 2,706 (n = 35)	3,971 ± 3,176 (n = 35)	3,645 ± 2,426 (n = 42)	4,459 ± 2,921 (n = 31)	3,122 ± 2,343 (n = 34)	3,632 ± 2,098 (n = 29)	4,399 ± 2,641 (n = 35)
	NT50 (IU/mL)	2,595 ± 1845 (n = 17)	3,313 ± 2,660 (n = 19)	2,648 ± 1,866 (n = 15)	2,133 ± 1,478 (n = 22)	2,310 ± 1,764 (n = 19)	2,763 ± 1,985 (n = 16)	2,306 ± 1,429 (n = 15)	2,408 ± 1,521 (n = 16)

BAU = Binding Antibody Units; id = intradermal; IgG, immunoglobulin G; IU = International Units; LD = low dose; NT50 = 50 % neutralization titers; RBD = Receptor Binding Domain; SD = standard dose.
Markers concern immunity against the Wuhan-Hu-1 strain. Results are shown as mean ± standard deviation.

attention. Participants allocated to the BI + BI treatment arm experienced more local solicited adverse events (n = 365) compared to the other treatment arms (n = 140 up to 242 local solicited adverse events per arm). The most commonly reported solicited systemic adverse event, fatigue, was experienced by 88.24 % of participants immunized receiving the B + M schedule but only by 48.08 % of participants vaccinated receiving the BL + BL schedule.

In total, 666 unsolicited events were reported after the administration of study vaccines. Common adverse events included headache (7.81 % of the reported unsolicited events), pruritus at the injection site (7.36 %), and movement impairment at the injection site (5.56 %). Most unsolicited adverse events were considered related to the administration of a COVID-19 vaccine by the investigators (68.92 %). Events were mostly mild (67.27 %) or moderate (29.28 %) in severity and required medical attention in 52 cases (7.81 %). Throughout the trial, 26 SAEs were reported, all of which required hospitalization. None of the SAEs were considered related to the administered vaccines.

4. Discussion

This large, multi-centric interventional trial studied the effect of various vaccine schedules on humoral immunogenicity.

The heterologous B + M and the homologous m + m and BL + BL vaccine schedules were all found to be non-inferior to their reference schedules in terms of both anti-RBD IgG and neutralizing antibody development 28 days after a second vaccination (Fig. 5). Non-inferiority of the homologous BNT162b2 schedule with a 12-week interval compared to a 4-week interval was also reported by Shaw et al in 2022 [13]. These findings suggest that replacing the second vaccination of a BNT162b2 schedule by the mRNA-1273 vaccine, reducing the dose of the mRNA-1273 vaccine, or extending the interval between subsequent BNT162b2 vaccines, does not hamper the development of an adequate humoral immune response. Therefore, these adapted COVID-19 vaccine schedules may be considered as an acceptable strategy to mitigate challenges in vaccine-availability and offer flexibility in the vaccine supply chain.

Conversely, the homologous b + b and BI + BI, and the heterologous B + C vaccine schedules did not demonstrate non-inferiority in terms of anti-RBD IgG development, and for the BI + BI and B + C schedules also in terms of neutralizing antibody development, 28 days after a second vaccination (Fig. 5). Thus, the use of a lower BNT162b2 vaccine dose, administering the BNT162b2 vaccine in a lower dose intradermally, or replacing the second vaccination of the BNT162b2 vaccine by ChAdOx1–S was not supported in this trial. The latter is in line with previous findings by Liu et al from 2021 [11], but in contrast with findings by Schmidt et al from 2021 showing that a heterologous vaccine schedule of a ChAdOx1-S vaccination followed by a vaccination with one of the mRNA vaccines (BNT162b2 or mRNA-1273) resulted in an equally strong induction of spike-specific IgGs and neutralizing antibodies compared to a homologous mRNA vaccination schedule [14]. It is, however, important to also take into account that in our trial, the participants receiving the B + C schedule were significantly older than those receiving the other vaccine schedules, due to the age restrictions following the safety concerns regarding ChAdOx1-S in younger adults. This could also have contributed to the lower humoral immunogenicity observed in this group [15]. Most likely, also the order of the vaccines in the heterologous schedule affects the maturation of the antibody response, stressing the importance of adequate priming.

Based on the observed GMT of antibodies binding to the RBD of the SARS-CoV-2 S protein, a third (booster) vaccination, given about 7 months after the first vaccination, showed a non-inferior humoral immune response in participants with adapted vaccine schedules, irrespective of the brand or dose of the booster vaccination (30 μg BNT161b2, 50 μg mRNA-1273 vaccine or 100 μg mRNA-1273 vaccine). The same was concluded by Liu et al in 2022 [16]. This suggests that, in order to boost immunity after one is fully vaccinated against SARS-CoV-

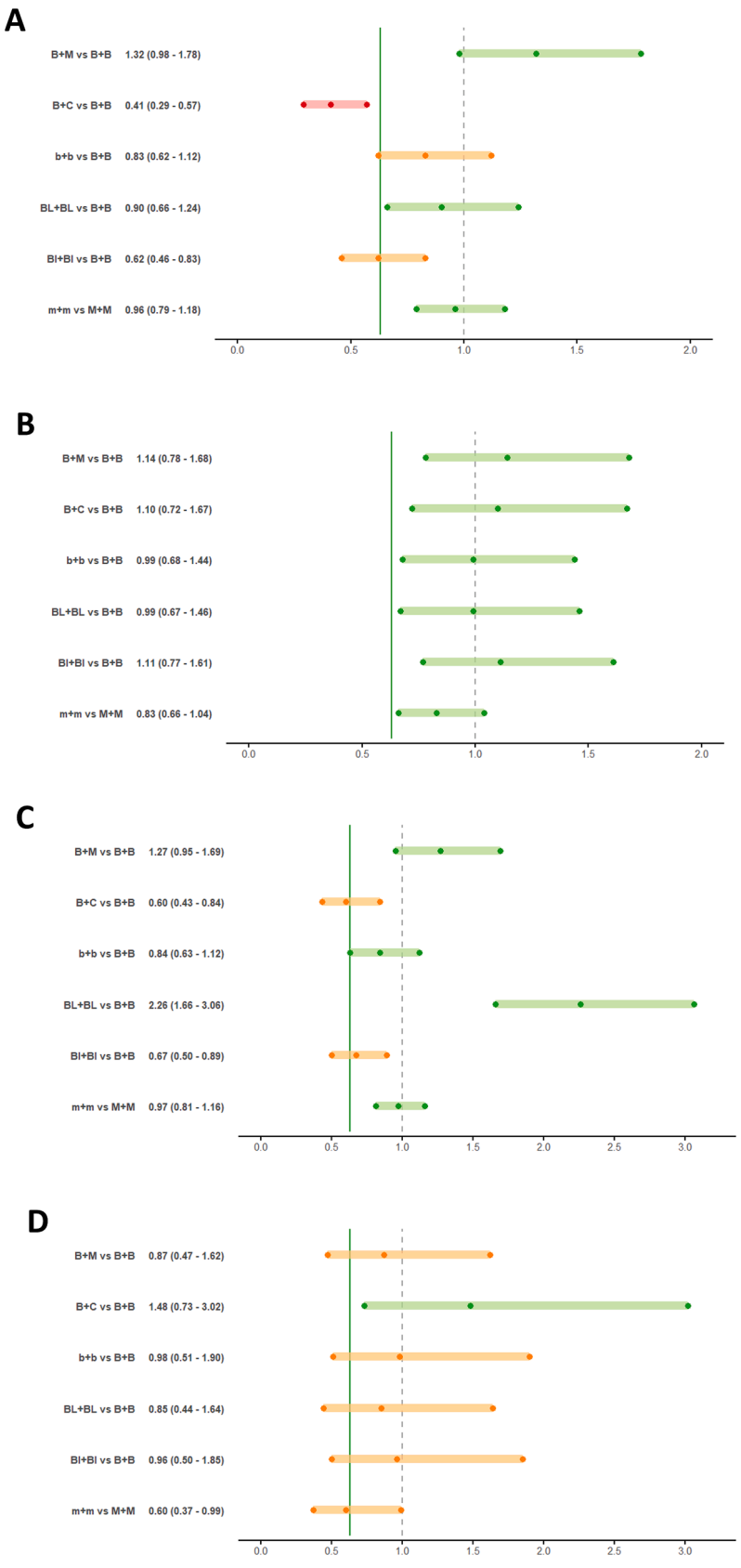

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Fig. 3. A. Anti-RBD IgG antibodies measured 28 days after the second vaccination. B. Anti-RBD IgG antibodies measured 28 days after the third (booster) vaccination. C. Neutralizing antibodies against the Wuhan-Hu-1 strain measured 28 days after the second vaccination. D. Neutralizing antibodies against the Wuhan-Hu-1 strain measured 28 days after the third (booster) vaccination. Adapted vaccine schedules were deemed non-inferior if the 95 % confidence interval of the geometric mean titer ratio was strictly larger than the non-inferiority margin, which was set at 0.63. Green coloring indicates that non-inferiority was observed, whereas red indicates that the schedule is inferior to the reference schedule. Orange coloring indicates that non-inferiority could not be shown nor rejected. IgG, immunoglobulin G; RBD, Receptor Binding Domain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2, it does not matter which COVID-19 vaccine is used.

The fact that the conversion factor for neutralizing antibodies against the Wuhan strain over the anti–RBD IgGs was higher for the long interval BL + BL schedule compared to the standard schedule B + B, suggests the development of a higher quality humoral immune response following a longer interval between subsequent doses. This observation is also supported by a significantly better anti-RBD IgG avidity as assessed in this treatment arm.

The trial evaluated anti-RBD IgG avidity as a measure of humoral immune response quality. Results from early longitudinal studies published by the end of 2021 indicated an increase in IgG avidity up to at least 6 months after SARS-CoV-2 infection, suggesting an ongoing maturation of the immune response over time [17,18]. Likewise, vaccination against SARS-CoV-2 was shown to increase IgG avidity [10,19,20]. Our trial suggests that adapted vaccine schedules influence the maturation of the immune response. Prolonging the interval between subsequent mRNA vaccinations to 16 weeks was previously shown to increase IgG avidity in smaller cohorts [21,22]. It is suggested that this is due to prolonged affinity maturation resulting in higher affinity B cells for plasma cell maturation at the time of the second vaccination [23,24]. In line with these results, the IMCOVAS trial found an increase in IgG avidity when prolonging the interval between vaccinations from 28 days to 84 days (12 weeks) in a larger study population. Remarkably, the heterologous B + C schedule showed lower IgG avidity after full vaccination. Similar to the findings regarding anti-RBD IgG levels and neutralizing antibodies following this heterologous vaccine schedule, this is in contrast with previous findings from 2022 indicating that a heterologous vaccine schedule of a ChAdOx1-S vaccination followed by a BNT162b vaccination induces a higher IgG avidity compared to a homologous BNT162b schedule [25,26]. This shows again that the order of the vaccines in the heterologous schedule, and thus priming, may affect the maturation of the antibody response. Finally, an increased IgG avidity was shown after a lower dose of mRNA-1273 vaccine (50 μg as opposed to the standard dose of 100 μg). To our knowledge, this trial is the first to report such an inverse dose–response relationship. In nonhuman primates, different doses of the mRNA-1273 vaccine (0.3–100 μg) did not elicit any differences in IgG avidity after 2 vaccinations [27]. Our findings in healthy adults warrant further investigations of the mechanisms underlying such differences in IgG avidity maturation. Possibly, a lower dose of the mRNA-1273 vaccine may result in the development of antibodies that fit the SARS-CoV-2 S protein epitopes better. Furthermore, our IgG avidity measurements suggest that it is important to study both the quantity and the quality of the antibody response after vaccination to fully understand the development of an adequate immune response.

The fact that the majority of BTIs occurred after the Day 182 visit (81.21 %) is in alignment with the increased circulation of the Omicron strain in Belgium at the time [12]. Remarkably, the trial noted the lowest rate of BTIs in the B + C treatment arm, despite an inferior humoral immune response. Conversely, the highest BTI rate was reported in the BL + BL treatment arm, which showed a non-inferior immune response. This suggests that humoral immunogenicity as assessed in this trial did not correlate well with clinical protection against infection with SARS-CoV-2 and more information is needed than what is observed in humoral immunogenicity only. Although no formal statistical analysis was performed to compare the BTI rate between the different treatment arms, BTI rates appeared to be comparable across the different treatment arms (64.45 % and 82.76 % following the administration of the standard B + B and M + M schedules, respectively, and ranging from 60.78 % to 84.78 % following the administration of any adapted vaccine schedule). The underlying reason behind this observed effect may be the lack of cellular immunogenicity findings, as this was not assessed in the current trial.

Based on the current findings on humoral immune responses and BTI rates following several adapted COVID-19 vaccine schedules, there does not seem to be a superior alternative vaccine schedule. However, there seems to be room for flexibility to adapt standard vaccine schedules with regard to the dose, the interval between subsequent doses, and combining different vaccination brands.

Our trial has several limitations. The first concerns its main focus on serological markers rather than clinical protection. However, multiple studies in large cohorts have demonstrated that both binding anti–RBD IgG levels, as well as neutralizing antibody levels correlate with protection against severe COVID–19 [28–30]. Therefore, we believe that the serological findings in this trial are clinically relevant. In line with previous research [29], this trial supports the assessment of binding and neutralizing antibodies to evaluate and compare the efficacy of different COVID-19 vaccine schedules. Future studies assessing non-inferiority of the adapted schedules with regard to vaccine efficacy in preventing COVID-19 infection could focus on the schedules that were shown to develop a non-inferior humoral immune response, i.e. the heterologous B + M schedule or long interval BL + BL schedule. A second limitation concerns the younger age of the study population, due to the fact that the initial Belgian vaccination campaign started with the vaccination of the immunocompromised and elderly, gradually shifting to younger age groups and that it had started before the recruitment of participants in the IMCOVAS trial. Since increased age is an important risk factor for COVID-19 morbidity, it would be relevant to confirm our findings in an older population that might benefit more from vaccination [31]. A final limitation of our trial is its singular focus on humoral immunogenicity. More research is required to assess the differences between vaccine schedules regarding other components of the immune response, such as cellular immunogenicity.

Despite these limitations, this trial is unique in several ways. First, it included a COVID-19-naïve study population, as participants were neither exposed to SARS-CoV-2, nor previously vaccinated against the virus. Throughout the trial, BTIs were monitored and participants exposed to SARS-CoV-2 were removed from the analyses to ensure that the measured effects concerned only vaccine-induced immunity. A second strength concerns the multi-centric design of the trial and the extensive follow-up. Indeed, participants participated up to 1 year in the trial, allowing for the collection of safety and immunogenicity data for a relatively long period. Finally, the evolution of the COVID-19 pandemic made it possible to assess the effect of a third (booster) vaccination in a well-defined population of participants that had been immunized with a variety of primary vaccine schedules.

In conclusion, the findings in this trial show that standard COVID-19 vaccine schedules may be adapted in several ways (including dose reduction, extending the interval between subsequent vaccine doses, and considering a heterologous vaccine schedule) in order to overcome issues in vaccine supply and to improve vaccine availability without jeopardizing the development of an adequate immune response.

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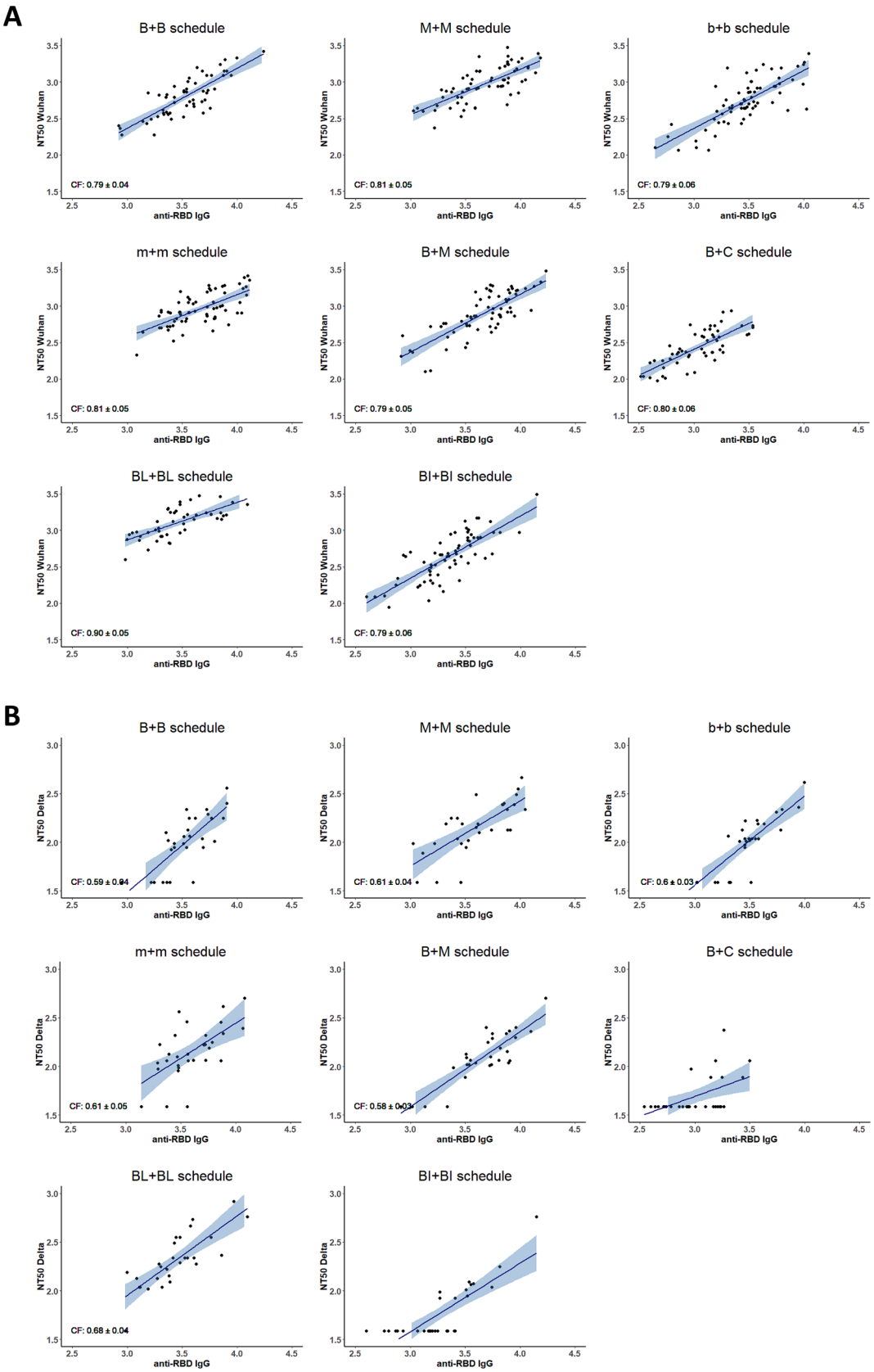

Fig. 4. A. Association between neutralizing antibody titers against the Wuhan-Hu-1 strain and anti-RBD IgG measurements 28 days after the second vaccination. B. Association between neutralizing antibody titers against the Delta strain and anti-RBD IgG measurements 28 days after the second vaccination. Alle values were log10-transformed. RBD, Receptor Binding Domain; IgG, immunoglobulin G; NT50, 50 % neutralization titers; CF, conversion factor.

Table 3
Avidity measurements at different timepoints.

Anti-RBD IgG avidity	Standard schedule		Adapted dose		Heterologous schedule		Vaccination interval	Administration route
	BNT162b2 SD + BNT162b2 SD (B + B)	mRNA-1273 SD + mRNA-1273 SD (M + M)	BNT162b2 LD + BNT162b2 LD (b + b)	mRNA-1273 LD + mRNA-1273 LD (m + m)	BNT162b2 SD + mRNA-1273 SD (B + M)	BNT162b2 SD + ChAdOx1-S SD (B + C)	BNT162b2 SD + BNT162b2 SD long-interval (BL + BL)	BNT162b2 *id* + BNT162b2 *id* (BI + BI)
Day 28 (seconds)	1,621 ± 783 (n = 57)	2,859 ± 1317 (n = 30)	1,879 ± 888 (n = 30)	2,560 ± 867 (n = 30)	1,747 ± 947 (n = 29)	1,798 ± 768 (n = 30)	1,765 ± 929 (n = 52)	1,304 ± 755 (n = 30)
Day 56/84* (seconds)	13,092 ± 6,137 (n = 51)	8,283 ± 2,195 (n = 30)	11,545 ± 4,534 (n = 30)	12,041 ± 3,060 (n = 30)	11,386 ± 5,515 (n = 29)	4,830 ± 2,714 (n = 30)	990 ± 678 (n = 45)	8,614 ± 5,702 (n = 30)
Day 112 (seconds)	5,437 ± 2,637 (n = 54)	6,833 ± 2,790 (n = 30)	5,586 ± 2,724 (n = 30)	8,508 ± 3,383 (n = 30)	6,252 ± 3,448 (n = 29)	2,864 ± 1,985 (n = 29)	15,338 ± 8,157 (n = 51)	4,086 ± 2,670 (n = 29)

id = intradermal; IgG, immunoglobulin G; LD = low dose; RBD = Receptor Binding Domain; SD = standard dose.
*Day 84 for the BL + BL treatment arm, just before the second vaccination. For all other treatment arms, the second assessment was done during the Day 56 visit, four weeks after second vaccination. Results are shown as mean ± standard deviation.

Table 4
COVID-19 Infections as reported by participants and breakthrough infections with SARS-CoV-2 during the trial for participants who remained in the study until the final visit (Visit 6, Day 364), received the third COVID-19 vaccine after September 2021, and had no additional COVID-19 vaccines outside the study.

		Standard schedule		Adapted dose		Heterologous schedule		Vaccination interval	Administration route	
		BNT162b2 SD + BNT162b2 SD (B + B, n = 55)	mRNA-1273 SD + mRNA-1273 SD (M + M, n = 58)	BNT162b2 LD + BNT162b2 LD (b + b, n = 63)	mRNA-1273 LD + mRNA-1273 LD (m + m, n = 60)	BNT162b2 SD + mRNA-1273 SD (B + M, n = 55)	BNT162b2 SD + ChAdOx1-S SD (B + C, n = 51)	BNT162b2 SD + BNT162b2 SD long-interval (BL + BL, n = 46)	BNT162b2 *id* + BNT162b2 *id* (BI + BI, n = 58)	Total (n = 446)
Number of reported SARS-CoV-2 infections		23	38	35	35	30	22	26	26	235
Severity	**mild**	19 (82.61 %)	20 (52.63 %)	20 (57.14 %)	22 (62.86 %)	19 (63.33 %)	10 (45.45 %)	15 (57.69 %)	14 (53.85 %)	139 (59.15 %)
	moderate	3 (13.04 %)	18 (47.37 %)	14 (40.00 %)	12 (34.29 %)	11 (36.67 %)	11 (50.00 %)	11 (42.31 %)	11 (42.31 %)	91 (38.72 %)
	severe	1 (4.35 %)	0 (0.00 %)	1 (2.86 %)	1 (2.85 %)	0 (0.00 %)	1 (4.55 %)	0 (0.00 %)	1 (3.85 %)	5 (2.13 %)
Medically attended SARS-CoV-2 infections	**yes**	4 (17.39 %)	5 (13.15 %)	4 (11.43 %)	7 (20.00 %)	3 (10.00 %)	9 (40.91 %)	5 (19.23 %)	12 (46.15 %)	49 (20.85 %)
Participants with ≥ 1 breakthrough infection with SARS-CoV-2		36 (64.45 %)	48 (82.76 %)	50 (79.37 %)	42 (70.00 %)	45 (81.82 %)	31 (60.78 %)	39 (84.78 %)	39 (67.24 %)	330 (74.00 %)

id = intradermal; LD = low dose; SD = standard dose.
Results are shown as number (percentage of total).

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CRediT authorship contribution statement

Katie Steenackers: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Nikita Hanning:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis. **Liesbeth Bruckers:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Conceptualization. **Isabelle Desombere:** Writing – review & editing, Resources, Data curation. **Arnaud Marchant:** Writing – review & editing, Supervision, Resources, Data curation, Conceptualization. **Kevin K. Ariën:** Writing – review & editing, Supervision, Resources, Data curation, Conceptualization. **Daphnée Georges:** Writing – review & editing, Resources, Data curation. **Patrick Soentjens:** Writing – review & editing, Supervision, Investigation, Data curation. **Valentino D'Onofrio:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Data curation. **Maya Hites:** Writing – review & editing, Supervision, Investigation, Data curation. **Nicole Berens-Riha:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Data curation. **Ilse De Coster:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Pierre Van Damme:** Writing – review & editing, Supervision, Resources, Methodology, Investigation,

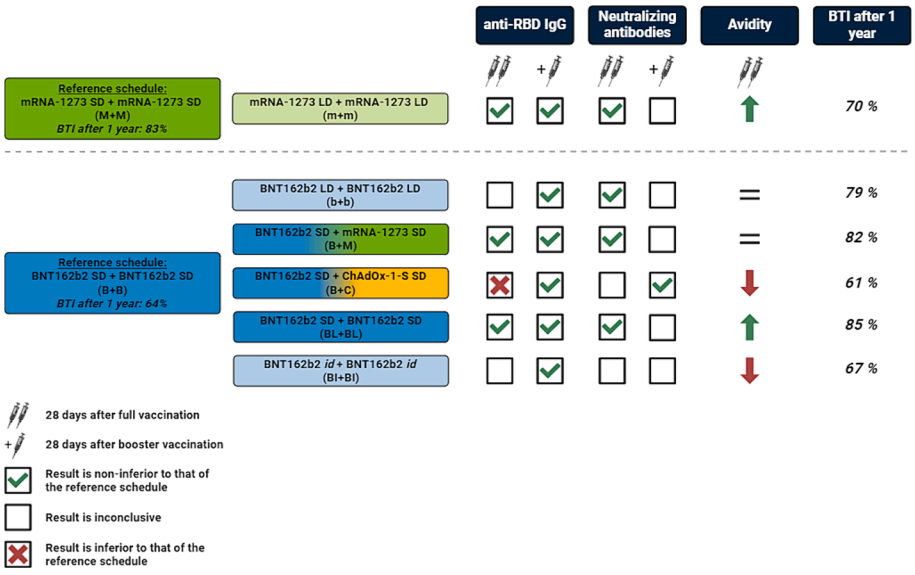

Fig. 5. Summary of the trial results, showing how each vaccine regimen impacted the immunological parameters studied in this trial (anti-RBD IgG, neutralizing antibodies, avidity, and BTI) compared to the reference schedules. Only participants included in the primary endpoint analyses, who remained in the trial until the final visit (Visit 6, Day 364), received the third COVID-19 vaccine after September 2021, and did not receive additional COVID-19 vaccines outside the trial, are taken into consideration for the reporting of BTI's. Abbreviations for the different treatment arms are shown between brackets. BTI, Break-through Infection; id, intradermal; IgG, immunoglobulin G; LD, low dose; SD, standard dose; RBD, Receptor Binding Domain. Created with BioRender.com.

Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [Pierre Van Damme reports financial support was provided by Belgian Health Care Knowledge Centre. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper].

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2024.07.018.

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