Comparative analysis of BNT162b2, mRNA-1273 and ChAdOx1 COVID-19 vaccine induced antibody responses and the 3rd BNT162b2 vaccine induced neutralizing antibodies against Delta and Omicron variants

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Comparative analysis of BNT162b2, mRNA-1273 and ChAdOx1 COVID-19 vaccine induced antibody responses and the 3rd BNT162b2 vaccine induced neutralizing antibodies against Delta and Omicron variants

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Abstract

Two COVID-19 mRNA and two adenovirus vector vaccines have been licensed in Europe and various vaccine combinations and dosing strategies have been exploited to maximize the immunity against COVID-19. Here, we show that among health care workers (n=328) two doses of BNT162b2, mRNA-1273, or ChAdOx1 as also a combination of an adenovirus vector and mRNA vaccines induces equally high levels of anti-SARS-CoV-2 spike antibodies and neutralizing antibodies against B.1 and B.1.617.2 when administrated with a long 12-week dose interval. Two doses of BNT162b2 with a short 3-week interval induce 2-3-fold lower titers of neutralizing antibodies compared to the long interval. Third mRNA vaccine dose for the short-dose interval group increased the antibody levels 4-fold compared to the levels after the second dose. Importantly, sera from all three-times vaccinated neutralized B.1.1.529 (Omicron). The data indicates that a third COVID-19 mRNA vaccine dose efficiently induces cross-protective neutralizing antibodies against multiple variants.

Introduction

The emergence and global spread of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) prompted a rapid development of vaccines to prevent SARS-CoV-2 infection and the coronavirus disease (COVID-19). Currently, the European Medicines Agency (EMA) has authorized four COVID-19 vaccines for use: mRNA-based BNT162b2 (Comirnaty, BioNTech, and Pfizer) and mRNA-1273 (Spikevax, Moderna), and adenoviral vector-based ChAdOx1 (Vaxzevria, AstraZeneca) and COVID-19 Vaccine Janssen (Janssen). All four vaccines utilize SARS-CoV-2 spike protein as the antigen. In Finland, COVID-19 vaccinations started at the end of December 2020 with two doses of BNT162b2 administrated at a 3-week interval. Early 2021 vaccinations expanded to a larger population, and mRNA-1273 and ChAdOx1 vaccines were also included in the

vaccination campaign. The vaccination interval between the first and second doses was prolonged from 3 weeks to 12 weeks. In March 2021, reports on rare cases of increased risk of blood clotting events associated with ChAdOx1[1,2] led into restrictions in the use of the vaccine, and many vaccinees with the first dose of ChAdOx1 received BNT162b2 or mRNA-1273 as the second dose. Recent studies have shown that heterologous 2-dose vaccinations with ChAdOx1 followed by BNT162b2 or mRNA-1273 elicit strong immune responses and higher or similar levels of neutralizing antibodies than homologous 2-dose vaccinations with vector-based or mRNA-based vaccines, respectively[3–6].

However, the antibody levels decline, and the surveillance of vaccine effectiveness and the decline of immune responses highlighted the need for a vaccine booster doses in the first group of vaccinees who received the vaccine with a 3-week interval[7,8]. Initial studies have shown that a third dose of COVID-19 vaccines strongly boost the waning immune responses and on the population level reduce severe COVID-19-associated morbidity and mortality[9–11]. Decisions for the third vaccine dose have been further promoted by the emergence of new SARS-CoV-2 variants-of-concerns (VOCs). Currently, the list of VOCs includes two widely circulating variants: B.1.617.2 (Delta) and B.1.1.529 (Omicron). B.1.617.2 variant has been globally dominant since spring 2021 replacing most of the other circulating SARS-CoV-2 variants[12,13], however, in November 2021, the first cases of B.1.1.529 variant were reported, and only within one month B.1.1.529 variant has spread globally initiating the replacement of the B.1.617.2 variant[12,13]. In many countries the emergence of B.1.1.529 variant and previously observed waning immunity has speeded up the recommendation for a third vaccine dose for all vaccinees.

Knowledge on the duration of vaccine-induced antibody responses by different vaccines and vaccine combinations is essential for making rational decisions regarding the timing, the number, and the combination of vaccine doses. In this work, we followed up for 8 months 328 health-care workers (HCWs) vaccinated with two doses of BNT162b2, mRNA-1273 or ChAdOx1 as well as

with ChAdOx1 combined with BNT162b2 or mRNA-1273. We conducted a comparative analysis of different vaccine combination induced spike protein-specific antibodies and neutralizing antibodies against SARS-CoV-2 variants. Furthermore, we analyzed neutralizing antibodies against B.1 (D614G), B.1.617.2 and B.1.1.529 variants induced by the third mRNA vaccine dose. Our results clearly demonstrate that two vaccine doses of all vaccine combinations elicit SARS-CoV-2 spike-specific antibody responses with high, but subsequently declining, neutralizing titers against B.1 and B.1.617.2 variants. Importantly, the third vaccine dose significantly increased the waning antibody levels and the neutralization titers against the three variants, including the newly emerged B.1.1.529 variant.

Results

Study population characteristics

This study included a cohort of 328 health care workers (HCWs) who received two doses of COVID-19 vaccine either with a short (2.6-4.0 weeks) interval or a long interval (8.0-16.4 weeks; Table 1, Fig. 1). All participants vaccinated with a short dose interval (n=120) received two doses of BNT162b2 vaccine and a third booster vaccine dose of BNT162b2 (n=47) or mRNA-1273 vaccine (n=73). Participants vaccinated with a long dose interval received two doses of BNT162b2 (n=62), mRNA-1273 (n=72) or ChAdOx1 (n=8) vaccines or a combination of ChAdOx1 and BNT162b2 (n=52) or ChAdOx1 and mRNA-1273 (n=14) vaccines. The cohorts vaccinated with the ChAdOx1 vaccine consisted of fewer participants since Finland restricted the use of ChAdOx1 vaccine in May 2021. Although the six vaccine cohorts were different in size, the cohorts were demographically representative with each other (Table 1). Altogether, 87% were female (mean age 44 years) and 13% were male (mean age 46 years). Twelve participants had a PCR confirmed SARS-CoV-2 infection before the vaccination program.

96 Sequential serum samples were collected from all HCWs before vaccination and three weeks after
97 receiving the first and the second vaccine doses (Fig. 1). From vaccinees with a short dose interval,
98 the follow-up serum samples were collected three, six and eight months after the second dose and
99 three weeks after the third booster vaccine dose. From vaccinees who received at least one dose of
100 BNT162b2 with a long dose interval, the follow-up serum samples were collected three and six
101 months after the second vaccine dose, whereas from vaccinees in other groups only one follow-up
102 serum sample was collected three months after the second vaccine dose (Supplementary Table 1).

103 **COVID-19 vaccine-induced antibody responses with a long vaccine dose interval**

104 To study the levels of antibodies elicited by five combinations of COVID-19 vaccines administered
105 with a long vaccine dose interval, sequential serum samples were collected from HCWs vaccinated
106 with two doses of BNT162b2, mRNA-1273, or ChAdOx1, or with combinations of ChAdOx1 as
107 the first dose and either BNT162b2 or mRNA-1273 as the second dose. SARS-CoV-2 S1-specific
108 IgG antibody levels were measured with EIA. Among the vaccinees who were seronegative before
109 the vaccination, the production of anti-S1 IgG antibodies was induced in higher levels by
110 BNT162b2 and mRNA-1273 vaccines compared with that of ChAdOx1 induced responses (1D3wk,
111 Fig. 2). Three of the HCWs who were seronegative after the first ChAdOx1 vaccine dose were
112 positive when tested with lower serum dilution (Supplementary Fig. 1). Three weeks after the
113 second dose, the antibody levels were significantly increased ($p<0.0001$ or $p<0.001$) in all vaccinee
114 groups, however, the 2x ChAdOx1 group had too few participants for reliable statistical
115 comparisons (2D3wk, Fig. 2). Furthermore, the age of the vaccinees (20-65 years) had little effect
116 on the vaccine induced immune response after two doses (Supplementary Fig. 2). Geometric means
117 of EIA units at 3 weeks after the second dose were 127 for 2x BNT162b2, 158 for 2x mRNA-1273,
118 142 for 2x ChAdOx1, 87 for ChAdOx1 + BNT162b2, and 158 for ChAdOx1 + mRNA-1273,
119 indicating a high overall induction of antibody levels by the second immunization with all five
120 vaccine combinations.

Vaccinees with long vaccine-dose intervals and with prior SARS-CoV-2 infection (n=8; diagnosed 25-393 days before the first serum sample, black dots in Fig. 2) developed high levels of antibodies that exceeded the geometric mean value of all vaccinees after the first vaccine dose. In these vaccinees, the second dose maintained the antibody levels or further increased them. Five of the vaccinees with prior SARS-CoV-2 infection had elevated N-specific antibodies, as did five vaccinees with no diagnosed SARS-CoV-2 infection (Supplementary Fig. 3). Low N-specific antibody levels in some vaccinees with prior SARS-CoV-2 infection may be explained by the long period between infection and sampling.

In all vaccinees, the antibody levels decreased gradually during the follow-up period. However, 3 months after the second vaccine dose 99% (170/172) of HCWs still had detectable levels of anti-S1 IgG antibodies (2D3mo, Fig. 2). Furthermore, two groups (2x BNT162b2 and ChAdOx1 + BNT162b2) were also analyzed 6 months after the second dose. In these vaccinees, although 94% (45/48) still had detectable levels of anti-S1 IgG antibodies, the antibody levels continued to decrease (2D6mo, Fig. 2). Regardless of this decrease, only one possible breakthrough infection was detected in the ChAdOx1 + BNT162b2 group as judged by the development of anti-N IgG antibodies after the second vaccine dose (red line in Supplementary Fig. 3, Table 1). In addition, this vaccinee showed a high increase in S1-specific IgG levels between the 3- and 6-month samples collected after the second vaccine dose (red dots in Fig. 2). These results indicate high antibody responses induced by all vaccine combinations and a very low rate of breakthrough infections after two vaccine doses administered with a long vaccine dose interval during this follow-up period.

BNT162b2 vaccine-induced antibody responses with a short vaccine dose interval and the effect of a third dose

HCWs receiving 2x BNT162b2 vaccine with a short vaccine dose interval have been analyzed in our previous studies for the antibody levels at 6 weeks[14] (n=180) and 6 months[7] (n=52) after the second dose. Here we analyzed the antibody levels of 120 HCWs randomly selected from the

above-mentioned cohort and extended the follow up to 9 months after the second vaccine dose. As shown also in our previous publications, the first vaccine dose induced the production of anti-SARS-CoV-2 S1 IgG antibodies and the second dose significantly increased the antibody levels ($p<0.0001$) in naïve vaccinees (Fig. 3a). In contrast, vaccinees with a previous PCR-confirmed SARS-CoV-2 infection (n=4, black dots and lines in Fig. 3a) mounted a high antibody response already after the first vaccine dose and the second vaccine dose further increased the antibody levels but only weakly. In all vaccinees, the antibody levels decreased gradually during the follow-up period and 7 to 9 months after the second vaccine dose 87% (95/105) of vaccinees had detectable levels of anti-S1 IgG antibodies. One vaccinee (0.8%) had a PCR-confirmed mild SARS-CoV-2 infection 47 days after the second vaccine dose (red dots and red line in Fig. 3a), however, this infection did not affect the S1-or N-specific antibody levels (red line in Supplementary Fig. 3). None of the initially naïve participants developed anti-N IgG antibodies after the second vaccine dose, while two of the vaccinees with a recent PCR-confirmed SARS-CoV-2 infection (diagnosed 16-30 days before the first serum sample) had N-specific antibodies (black dots in Supplementary Fig. 3). Thus, similar to the results of the long vaccine dose interval, the findings indicate a gradual decrease in S1-specific antibody levels and a very low rate of breakthrough infections after two vaccine doses with a short vaccine dose interval.

HCWs vaccinated with a short vaccine dose interval (n=120) received a third booster vaccine dose 7.6–9.3 months after the second vaccine dose (47 received BNT162b2 and 73 received mRNA-1273 vaccine). Three weeks after the third dose, the levels of SARS-CoV-2 S1-specific IgG antibodies were significantly higher than the levels seen after the second vaccine dose (geometric mean 147 and 180 EIA units three weeks after the second and the third dose, respectively) ($p<0.0001$) (Fig. 3a). The third dose induced slightly weaker immune response in older vaccinees (55-65 years) compared to 35–54-year-old vaccinees, and interestingly, lowest antibody levels were seen with the youngest vaccinees (20-34 years, $p<0.0001$ compared to 34-44 and 45-54;

Supplementary Fig. 2). Both mRNA-based vaccines elicited similar booster effect since no difference was detected in antibody levels between the HCWs who received BNT162b2 or mRNA-1273 as a third dose (Fig. 3b).

Third vaccine dose of BNT162b2 induce high levels of neutralizing antibodies

In addition to anti-spike antibody levels, the capability of sera to neutralize SARS-CoV-2 variants was investigated. For this, we analyzed *in-vitro* with microneutralization test (MNT) the neutralization titers against currently circulating SARS-CoV-2 variants B.1.617.2 (Delta) and B.1.1.529 (Omicron), and the original B.1 variant using isolated virus strains. Fig. 4c illustrates the amino acid changes between B.1, B.1.617.2 and B.1.1.529 variants. Fifty-nine HCW vaccinees (no prior PCR-confirmed SARS-CoV-2 infection) who received two BNT162b2 vaccines with a short dose interval were randomly selected. Neutralizing antibodies were analyzed against variants B.1, B.1.617.2 and B.1.1.529 in the serum samples collected at 3 weeks, 3 months, 6 months and 8 months after the second vaccine dose and 3 weeks after the third vaccine dose (Fig. 4a).

Three weeks after the second vaccine dose, all vaccinees (100%, 59/59) neutralized the B.1 variant and 98% (58/59) and 24% (14/59) of vaccinees neutralized B.1.617.2 and B.1.1.529 variants, respectively. By 3 and 6 months after the second vaccine dose, the mean neutralization titers decreased by 2-fold against B.1 and B.1.617.2, while the titers against B.1.1.529 variant were virtually below the detection limit, except for the titers of the vaccinee who had a PCR-confirmed mild SARS-CoV-2 infection (red dots in Fig. 4a). At the time of administration of the third dose (7-9 months after the second dose), 90% (53/59), 56% (33/59), and only 5% (3/59) of HCWs had neutralizing antibodies against B.1, B.1.617.2 and B.1.1.529 variants, respectively. Strikingly, the third dose of BNT162b2 vaccine induced strong responses against all three SARS-CoV-2 variants: three weeks after the third vaccine dose 100% of vaccinees' sera neutralized all three variants (B.1, B.1.617.2 and B.1.1.529) (Fig. 4b). The mean titers of neutralizing antibodies against B.1 at 3 weeks after the second vaccine dose were 2.5- and 23-fold higher as compared to B.1.617.2, and

196 B.1.1.529, respectively. At 3 weeks after the third vaccine dose the differences between B.1 vs.
197 B.1.617.2, and B.1.1.529 were 2- and 6-fold lower, respectively (Fig. 4b, Supplementary Fig. 4).
198 Remarkably, regardless of the accumulating amino acid changes in the S1 of variants (Fig. 4c), sera
199 of three-times mRNA-vaccinated HCWs showed strong neutralizing capability against all three
200 variants.

201 **COVID-19 vaccine-induced antibody responses after 2-dose vaccination with different vaccine**
202 **combinations**

203 To analyze differences in antibody levels elicited by six different vaccine combinations and by
204 different vaccine dose intervals, antibody levels in sera from HCWs vaccinated with 2x BNT162b2
205 with short and long vaccine dose intervals (n=59, n=30, respectively), 2x mRNA-1273 (n=30), 2x
206 ChAdOx1 (n=7), ChAdOx1 + BNT162b2 (n=30) and ChAdOx1 + mRNA-1273 (n=13), collected 3
207 weeks and 3 months after the second doses were compared (EIA units in Fig. 5). In all vaccine
208 combination groups, the antibody levels were elevated 3 weeks after vaccination and decreased by 3
209 months after the vaccination. The rate of decline from 3 weeks to 3 months was similar between all
210 vaccine combinations. The vaccine groups including mRNA-1273 as the second dose presented the
211 highest geometric mean antibody levels 3 weeks and 3 months after vaccination (156-158 and 82-
212 96, respectively), while the vaccine groups including the BNT162b2 vaccine showed the lowest
213 geometric mean antibody levels after vaccination (87-127 and 45-66, respectively).

214 A representative number of serum samples from the vaccinees in six vaccine combination groups
215 (2x BNT162b2 with short dose interval n=60; 2x BNT162b2 with long vaccine dose interval n=30;
216 2x mRNA-1273, n=30; 2x ChAdOx1, n=9; ChAdOx1 + BNT162b2, n=30; ChAdOx1 + mRNA-
217 1273, n=11) was analyzed at 3 weeks and 3 months after the second vaccination for the
218 neutralization capacity against B.1 and B.1.617.2 variants (ID_{50} in Fig. 5). The vaccine group of 2x
219 BNT162b2 with a short vaccine dose interval showed 2-3x lower GMT against the B.1 three weeks

after second vaccine dose compared to the groups vaccinated with long vaccine dose interval: 2x BNT162b2 long ($p<0.0001$), 2x mRNA-1273 ($p<0.0001$), ChAdOx1 + mRNA-1273 ($p<0.0001$) and ChAdOx1 + BNT162b2 ($p<0.0021$). Three months after vaccination, the differences in GMTs between groups against B.1 diminished, and only the group with 2x mRNA-1273 vaccine had higher GMTs than the group vaccinated with 2x BNT162b2 with a short vaccine dose interval ($p<0.0001$).

Neutralization titers against the B.1.617.2 variant were the highest in the group vaccinated with 2x mRNA-1273 (GMT 339 at three weeks and 133 at three months after vaccination) (Fig. 5). At three weeks after vaccination, the GMTs in two vaccine groups with a long vaccine dose interval, 2x mRNA-1273 and 2x BNT162b2 (long), were significantly higher than in the 2x BNT162b2 short dose interval group ($p<0.0001$ and $p<0.014$). However, by three months after vaccination the GMTs against B.1.617.2 decreased to relatively similar levels for all groups. Only the GMT of 2x mRNA-1273 group remained slightly higher than 2x BNT162b2 short ($p<0.015$) and ChAdOx1 + BNT162b2 ($p<0.045$). Three months after the second vaccination, vaccinees without detectable neutralizing antibodies against B.1.617.2 variant were detected in all vaccine groups which obtained BNT162b2 as the second dose (n=1 in ChAdOx1 + BNT162b2, n=2 in 2x BNT162b2 long vaccine interval, and n=2 in 2x BNT162b2 short vaccine interval). One vaccinee (in group ChAdOx1 + BNT162b2) showed no neutralizing activity against B.1 or B.1.617.2 variants three weeks and three months after vaccination. Altogether, our results show that the studied vaccine combinations elicit high levels of SARS-CoV-2 antibodies.

Discussion

SARS-CoV-2 has spread throughout the world and caused millions of deaths, and tremendous social and economic damage. The fast development of efficient COVID-19 vaccines has helped in containment of the pandemic the past year, and data is accumulating on how to utilize the licenced

244 vaccines in the best possible way. New variants escaping infection or vaccine-induced immunity are
245 of great concern, and this year the world has witnessed the appearance of B.1.617.2 variant
246 followed by the currently spreading B.1.1.529 variant, which shows a strong transmission ability
247 leading to an upsurge of SARS-CoV-2 infections in numerous countries.

248 Initially, the vaccinations were administered with a 3-week dose interval, and later this was
249 prolonged to several weeks (up to 12 weeks) depending on the country. In addition, due to a varying
250 availability of vaccines, different combinations of vaccine doses have been used. It has been a
251 matter of great concern and debate, how the timing and the various combinations of vaccines affect
252 the immune responses. We show here that the five combinations of vaccines used in Finland
253 resulted in similar antibody responses, with the mRNA-1273 vaccine eliciting slightly higher
254 antibody responses. Similar results have been obtained by others[3–6], indicating that both
255 heterologous and homologous vaccinations elicit proper immune response in healthy individuals.

256 Interestingly, our data shows somewhat higher neutralizing antibody levels in vaccinees who
257 received mRNA-1273 in comparison to those who received BNT162b2 or ChAdOx1. This result is
258 in line with the vaccine effectiveness studies where mRNA-1273 has been shown to be somewhat
259 more efficient than BNT162b2 in preventing COVID-19 [19,20]. This is well in line with the concept
260 that neutralizing antibodies against SARS-CoV-2 correlate with a protection against symptomatic
261 SARS-CoV-2 infection [21]. As for the timing of dosages, previous studies have shown that vaccine
262 combinations given with long dosing intervals elicit higher or similar antibody responses compared
263 to BNT162b2 with a short dosing interval [19,20]. The longer vaccine dosing interval has also resulted
264 in at least equally high vaccine effectiveness as that seen with a short dosing interval of BNT162b2
265 [19,20]. Similarly, according to our results, sera collected three months after the second vaccination
266 with 3-week and 12-week dosing intervals, shows comparable neutralization capacity. Thus, it is
267 likely that any COVID-19 vaccine can be combined for an effective vaccination, enabling efficient
268 use of existing vaccine stores. This could also facilitate the development of new vaccine candidates,

since it is likely that combinations of any highly immunogenic COVID-19 vaccines can be utilized for effective protection against a severe disease. We would like to point out that our study is an unbiased analysis of licenced vaccines, and the study has not received funding from the vaccine manufacturers.

The currently spreading VOC, B.1.1.529, contains more than 30 amino acid changes in the spike protein, many of which have been associated with increased infectivity and antibody evasion in previous variants of concern[22]. Preliminary studies implicate, that antibodies elicited by infection with the original SARS-CoV-2 variant[23] or by two vaccine doses, have markedly reduced capability to neutralize B.1.1.529 variant[24]. Our data clearly confirms this initial observation that two doses of BNT162b2 vaccine does not provide good long-term immunity against the B.1.1.529 variant. However, the third vaccine dose of BNT162b2 or mRNA-1273 induced strong immune responses against the B.1 variant and high levels of cross- neutralizing antibodies against the B.1.617.2 and B.1.1.529 variants. This observation supports the concept that the present vaccines, as long as administered in sufficient doses (at least 3 doses), induce adequate protective immunity against the B.1.1.529 variant. It is also likely that cell-mediated immunity induced by vaccines contributes to protective efficacy against all VOCs. In subsequent analysis it will be of great interest to measure cell-mediated immunity against the emerging VOCs.

It has been suggested that ca. 10-20 substitutions in the spike protein are sufficient to create a variant that escapes neutralizing antibodies elicited by vaccinations. However, individuals with a previous SARS-CoV-2 infection followed by a subsequent vaccination efficiently neutralize these highly mutated spike proteins[25]. It has been concluded that the breadth and the amount of antibodies produced by memory B-cells is higher when anti-SARS-CoV-2 immunity is based on previous infection combined with a vaccination as compared to vaccination or infection alone[26,27]. Vaccinations alone seem to result in high levels of antibodies with a more limited breadth in neutralizing capacity, indicating the need for booster vaccinations to induce high antibody levels for

294 providing maximal protective efficacy against the infection. Our data are well in line with this,
295 showing that the third vaccine dose significantly increases the antibody levels and the neutralizing
296 capacity, particularly against B.1.1.529 (Omicron) variant, strongly indicating the benefits of a third
297 vaccine dose in containment of the SARS-CoV-2 pandemic.

298

299 ## Materials and Methods

300 ### Study population

301 Health care workers (HCWs) who completed a full two-dose vaccine regimen with a long 12-week
302 (8.0-16.4 weeks) dose interval (n=208) were included in the study. HCWs who completed a full
303 three-dose vaccine regimen with a short 3-week (2.6-4.0 weeks) dose interval between the first two
304 doses (n=120) were selected from a larger cohort [14]. Vaccinees with three vaccine doses were
305 followed up to 3 weeks after the third dose, and vaccinees in the long vaccine dose group were
306 followed for a maximum of six months after the second vaccine dose. Serum samples were
307 collected at regular time points (Supplementary Table 1). Participants filled symptom
308 questionnaires before every sample collection and, if symptomatic, were encouraged to take a
309 COVID-19 RT-qPCR test which was arranged as part of local infection control practice. The
310 demographics of the vaccinated HCWs are presented in Table 1. The timeline of vaccinations and
311 sample collection is presented in Figure 1.

312

313 **SARS-CoV-2 S1- and N-protein based immunoassays**

314 SARS-CoV-2 S1 and N protein-specific antibodies were analyzed with an in-house enzyme
315 immunoassay (EIA)[15]. Briefly, purified recombinant SARS-CoV-2 antigens were coated on 96-well
316 plates (2.0 µg/ml of N and 3.5 µg/ml of S1). IgG levels of serum samples (diluted 1:1000) were
317 determined with absorbance measurement at 450nm wavelength. EIA results were confirmed with
318 1:300 serum dilution (Supplementary Fig. 1 and 3). Optical density (OD) values were converted to
319 EIA units using linear interpolation between OD-values of a positive (=100 EIA units) and a
320 negative control (=0 EIA units) serum specimen. Thresholds to determine seropositivity were
321 calculated as described earlier[14].

322 **SARS-CoV-2 variants**

323 SARS-CoV-2 isolates FIN25-20 (B.1, D614G variant, MW717675.1 and EPI_ISL_412971)[16],
324 FIN37-21 (B.1.617.2, Delta variant, MZ945494 and EPI_ISL_2557176)[17] and FIN55-21
325 (B.1.1.529, Omicron variant, pending for GISAID ID) were isolated from SARS-CoV-2 PCR-
326 positive nasopharyngeal samples by incubation with VeroE6 (FIN25-20) or VeroE6-TMPRSS2-
327 H10 cells[18] (FIN37-21 and FIN55-21) and further passaged in VeroE6-TMPRSS2-H10 cells in
328 DMEM supplemented with 2% FBS, 2 mM L-glutamine, and penicillin-streptomycin. Titration of
329 virus stocks was done using $TCID_{50}$ assay and isolates were sequenced by next-generation
330 sequencing as described previously[14].

331 **Microneutralization test**

332 The neutralization capacity of the serum samples was measured by microneutralization test (MNT)
333 as described previously[7]. Briefly, two-fold dilution series starting from 1:10 dilution was prepared
334 on 96-well plate for each serum into 50µl of DMEM supplemented with 2% fetal bovine serum
335 (FBS), 2 mM L-glutamine, and penicillin-streptomycin. Serum dilutions were incubated with 50
336 $TCID_{50}$ virus in total volume of 100µl for 1h at +37°C (final serum dilution 1:20) before addition of

50 000 VeroE6-TMPRSS2-H10 cells into the virus-serum dilution mixture to a final volume of 150µl. The cells were incubated at +37°C, 5% CO_2, for 4 days, fixed with 4% formaldehyde, stained with crystal violet and visualized for cell death. Reciprocal of serum dilution inhibiting 50% of cell death was determined as the neutralization titer. A serum was considered positive for neutralizing antibodies if it inhibited 50% of cell death at a dilution of 1:20 or above. Serum with known neutralizing titer was used as a control on each plate.

Ethical statement

Study participants were recruited among healthcare personnel of Turku University Hospital (TUH, Turku, Finland) (Southwest Finland health district ethical permission ETMK 19/1801/2020, EudraCT 2021-004419-14) and Helsinki University Hospital (HUH, Helsinki, Finland) (Helsinki-Uusimaa health district ethical permission HUS/1238/2020, EudraCT 2021-004016-26) prior to receiving COVID-19 vaccines as part of hospital occupational health care. At enrollment, written informed consent was collected from all participants.

Statistical analysis, and illustration of changes in spike structure

Data was analyzed in GraphPad Prism (version 8). Paired samples were tested with Wilcoxon signed-rank test. Differences between vaccine groups were tested with unpaired t-test or Kruskal Wallis test followed with Dunn's multiple comparisons test. All tests were two-sided and p-values <0.05 were considered statistically significant. Changes in SARS-CoV-2 spike structure (PDB: 6VXX) were illustrated with UCSF Chimera 1.15.

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Author contributions

M.B., P.J., P.K., L.K., J.L., A.K., and I.J. designed the experiments; M.B., P.J., P.K., R.L., A.R. and L.K. did microneutralization tests and analyzed the data; M.B., P.J., and P.K. did EIA tests and analyzed the data; L.L., E.V., M.S. and P.Ö. isolated and characterized the virus isolates; H.K.H., S.H.P., P.A.T., E.O., S.M., T.S., O.V., L.I., J.L. and A.K. recruited vaccinees and patients and collected their sera and data; A.P., M.A.R., R.A.N., P.J. and O.R. produced antigens for EIA; M.B., P.J. and P.K. analyzed all data sets; M.B., P.J., P.K., L.K. and I.J. wrote the manuscript and all co-authors contributed to the edition of the text.

Competing interests

The authors declare no competing interests.

441 **Table 1.** Demographics of COVID-19 vaccinated HCWs (n=328).

	2x BNT162b2 short dose interval	2x BNT162b2 long dose interval	2x mRNA-1273 long dose interval	2x ChAdOx1 long dose interval	ChAdOx + BNT162b2 long dose interval	ChAdOx1 + mRNA-1273 long dose interval
N	120	62	72	8	52	14
Female (%)	100 (83.3%)	54 (87.1%)	67 (93.1%)	8 (100.0%)	44 (84.6%)	13 (92.9%)
Male (%)	20 (16.7%)	8 (12.9%)	5 (6.9%)	0 (0.0%)	8 (15.4%)	1 (7.1%)
Previous PCR confirmed SARS-CoV-2 infections	4	3	3	0	2	0
Age in years						
Mean	44	47	44	43	48	43
Median	44	50	43	39	50	44
Range	25–65	22–64	25–66	28–62	24–67	23–62
Mean time between vaccine doses (range)						
Between 1st and 2nd dose in weeks	3.0 (2.6-4.0)	11.7 (8.0-15.0)	12.1 (11.9-16.4)	12.6 (12.0-16.3)	12.2 (10.9-16.0)	12 (12.0-12.0)
Between 2nd and 3rd dose in months	8.3 (7.6-9.3)	-	-	-	-	-
Breakthrough infections after two vaccine doses						
Based on PCR test	1	0	0	0	0	0
Based on antibody test	0	0	0	0	1	0

442

Figures

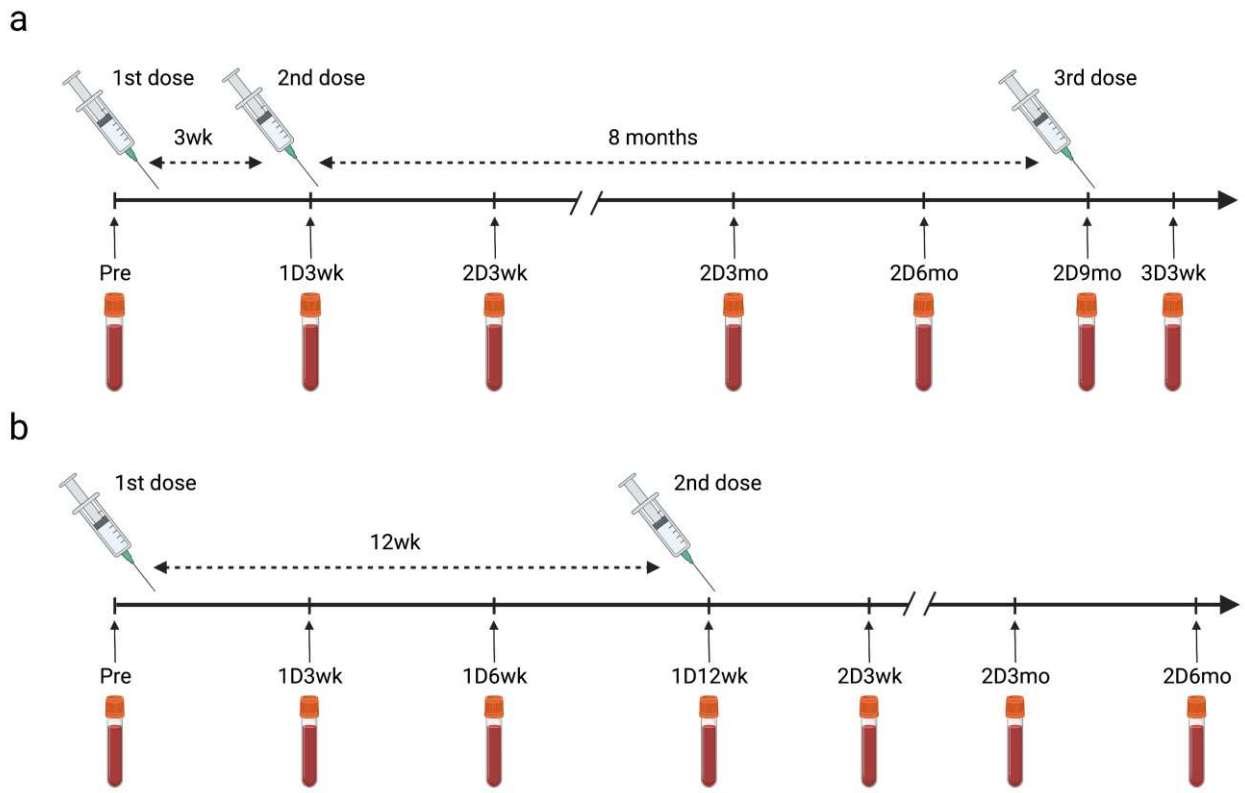
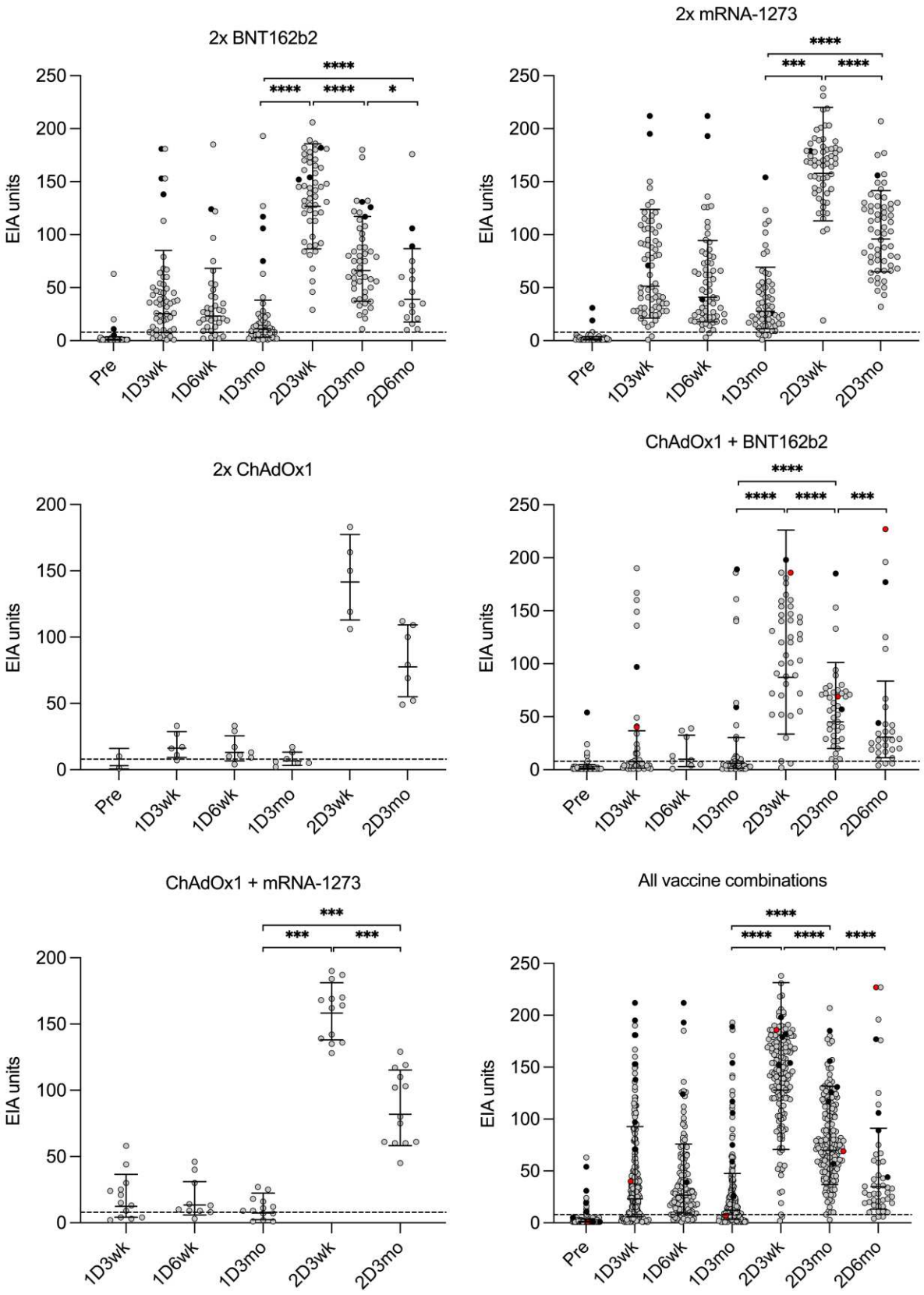


Figure 1.

Timeline of vaccinations and samplings with 3-week and 12-week dosing intervals.


2x BNT162b2
2x mRNA-1273
2x ChAdOx1
ChAdOx1 + BNT162b2
ChAdOx1 + mRNA-1273
All vaccine combinations
EIA units
Pre
1D3wk
1D6wk
1D3mo
2D3wk
2D3mo
2D6mo

448

Figure 2. Antibody responses of HCWs after receiving one and two doses of COVID-19 vaccines with long interval. SARS-CoV-2 S1-specific IgG antibody levels were measured in all vaccine combination groups (2x BNT162b2, 2x mRNA-1273, 2x ChAdOx1, ChAdOx1 + BNT162b2, ChAdOx1 + mRNA-1273) with EIA from sera collected before vaccination (Pre), three weeks (1D3wk), six weeks (1D6wk) and twelve weeks (1D3mo) after the first vaccine dose (1D), and three weeks (2D3wk), three months (2D3mo), and six months (2D6mo) after the second vaccine dose (2D). Eight HCWs with PCR confirmed SARS-CoV-2 infection prior to vaccinations are represented with black dots and one HCW with a breakthrough infection (>20 EIA unit increase between samples) after the second vaccine dose is represented with red dots. Geometric mean with geometric SD is represented in figures. Statistical differences between samples collected before vaccination vs. three weeks after the first vaccine dose, between samples collected three vs. twelve weeks after the first dose, between samples collected twelve weeks after the first dose vs. three weeks after the second dose, and between samples collected three weeks after both vaccine doses, were analyzed with Wilcoxon signed-rank test. P-values <0.05 were considered statistically significant. ****<0.0001, ns=not significant. Cut-off values are indicated with dashed lines.

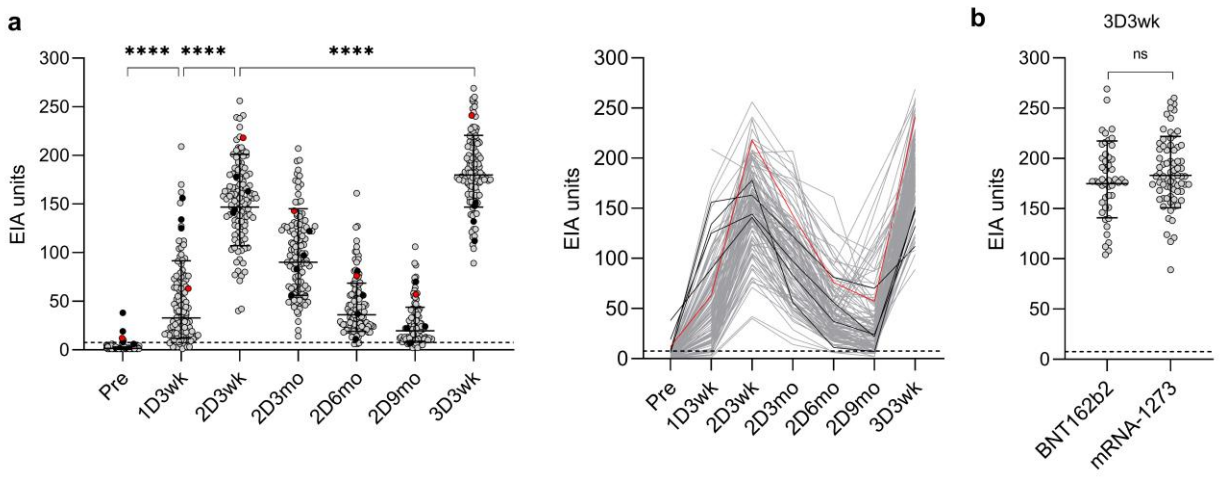


Figure 3. Antibody responses of HCWs after receiving two doses of COVID-19 mRNA vaccines with a short vaccine dose interval and the third dose of mRNA vaccines. a SARS-CoV-2 S1-specific IgG antibody levels were measured with EIA from serum samples collected before the vaccination (Pre; n=108), three weeks after the first vaccine dose (1D3wk; n=119), three weeks (2D3wk; n=120), three months (2D3mo; n=120), six months (2D6mo; n=119), and nine months (2D9mo; n=109) after the second vaccine dose, and three weeks after the third vaccine dose (3D3wk, n=120). All vaccinees received two doses of the BNT162b2 vaccine and the third dose of BNT162b2 or mRNA-1273. Sequential serum samples are connected with a line. HCWs with prior PCR confirmed SARS-CoV-2 infection are represented with black lines and PCR-confirmed breakthrough infection after the second dose is represented with a red line. The dotted line indicates the cut-off value for S1-based EIA. **b** Vaccinated HCWs are separated into two groups based on the third vaccine dose (BNT162b2 vs. mRNA-1273) and anti-S1 IgG antibody responses three weeks after the third vaccine dose are compared. Statistical differences between samples collected before vaccination, and three weeks after each vaccine dose were analyzed with Wilcoxon signed-rank test. Differences between different vaccine groups were compared with unpaired t-test. Two-tailed P-values <0.05 were considered statistically significant. ****<0.0001, ns=not significant

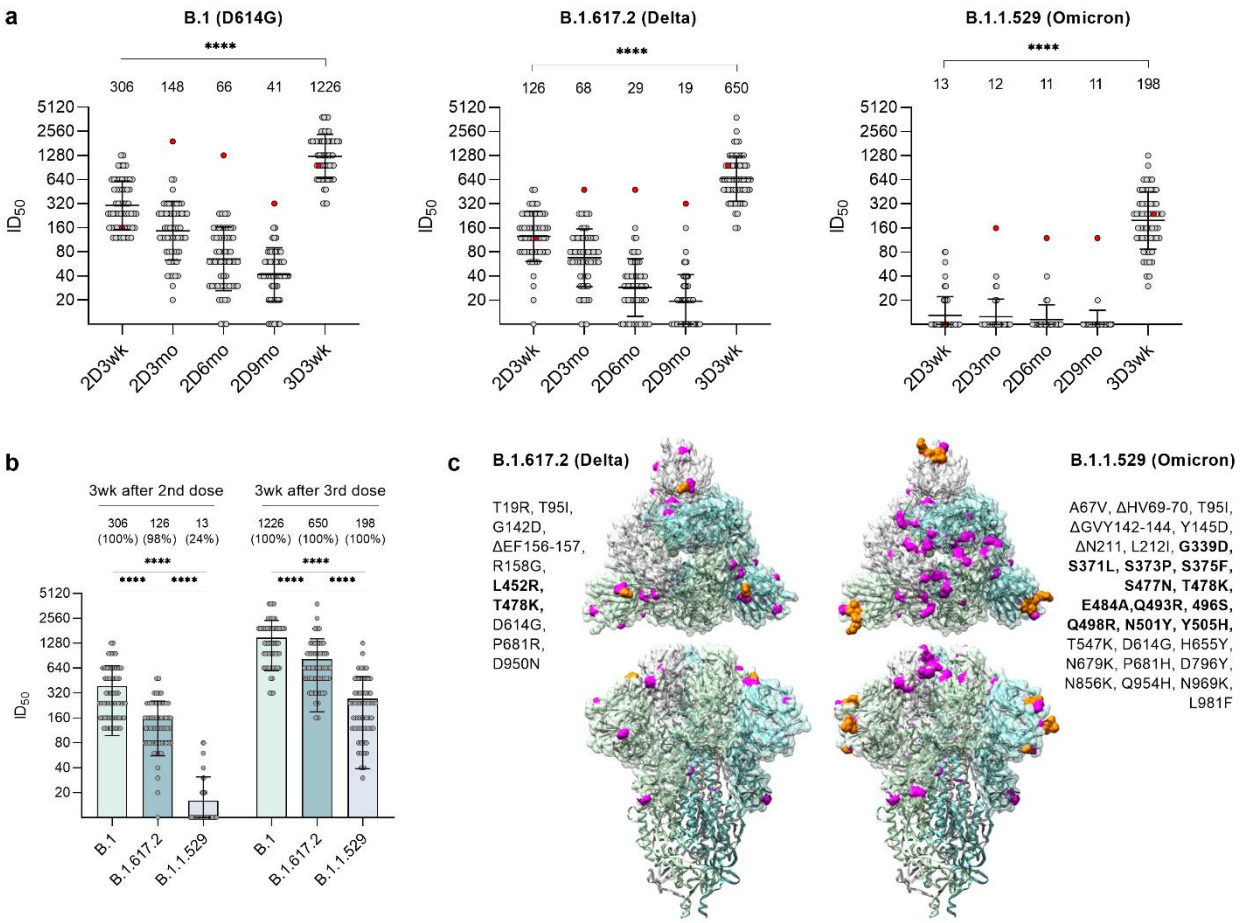
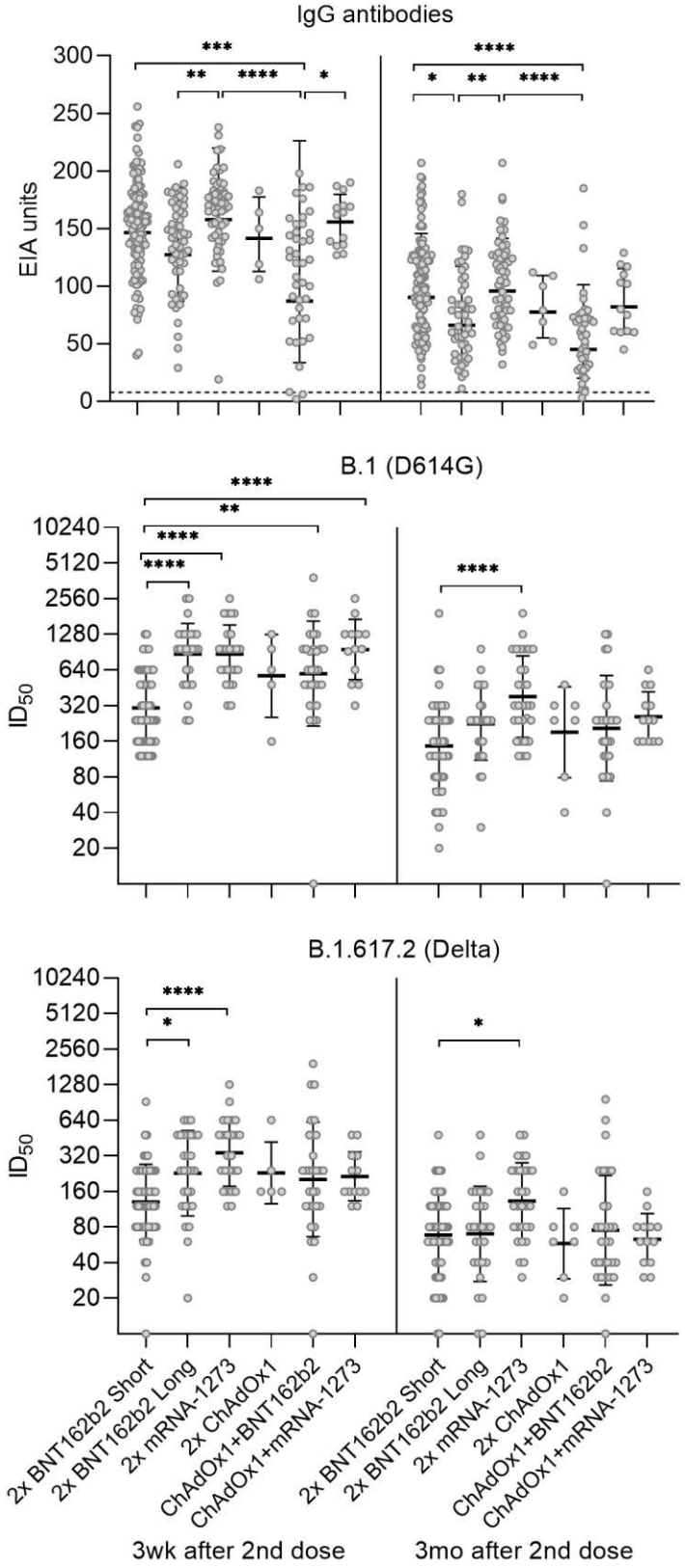


Figure 4. Neutralizing antibodies of HCWs receiving three doses of BNT162b2 vaccine.

Neutralizing antibody responses of HCWs (n=59) were analyzed with a microneutralization test against SARS-CoV-2 isolates representing B.1, B.1.617.2 and B.1.1.529 variants. Serum samples were collected before the first vaccine dose and three weeks after the second and third vaccine dose. Additionally, follow-up serum samples were collected 3, 6, and 9 months after the second vaccine dose. A vaccinee with a PCR-confirmed SARS-CoV-2 infection after vaccination is marked with red dots. Neutralization titers against B.1, B.1.617.2, and B.1.1.529 variants after the second and third vaccine doses were compared. Geometric mean is indicated above the figure with the percentage of positive samples when neutralization titer ≥20 is considered positive. Differences between neutralization titers were analyzed with Wilcoxon signed-rank test and two-tailed p-values <0.05 were considered statistically significant. ****<0.0001 **c** Amino acid changes in B.1.617.2

and B.1.1.529 compared to Wuhan Hu-1 sequence are shown in trimeric SARS-CoV-2 spike protein structure (PDB: 6VXX). Substitutions are highlighted with magenta and deletions with orange. Changes located in the receptor binding domain are bolded.


IgG antibodies
EIA units
300
250
200
150
100
50
0
***
**
****
*
****
*
**
****
B.1 (D614G)
ID50
10240
5120
2560
1280
640
320
160
80
40
20
****
**
****
****
****
B.1.617.2 (Delta)
ID50
10240
5120
2560
1280
640
320
160
80
40
20
****
*
*
2x BNT162b2 Short
2x BNT162b2 Long
2x mRNA-1273
2x ChAdOx1
ChAdOx1+BNT162b2
ChAdOx1+mRNA-1273
2x BNT162b2 Short
2x BNT162b2 Long
2x mRNA-1273
2x ChAdOx1
ChAdOx1+BNT162b2
ChAdOx1+mRNA-1273
3wk after 2nd dose
3mo after 2nd dose

501

Figure 5. SARS-CoV-2 neutralizing antibodies after two doses of different combinations of COVID-19 vaccines. Serum samples collected 3 weeks (3wk) and three months (3mo) after the second vaccine dose from HCWs who were vaccinated with 2x BNT162b2 with short (n=120) or long vaccine dose interval (n=49), 2x mRNA-1273 (n=68), 2x ChAdOx1 (n=7), ChAdOx1+BNT162b2 (n=44), or ChAdOx1+mRNA-1273 (n=14) compared for IgG antibodies against SARS-CoV-2 S1 (EIA units). The dotted line indicates the cut-off value for S1-based EIA. Neutralizing antibody responses against B.1 and B.1.617.2 variants were analysed for vaccinees with two doses of vaccine combinations: 2x BNT162b2 with short vaccine dose interval (n=60), 2x BNT162b2 long vaccine dose interval (n=30), 2x mRNA-1273 (n=30), 2x ChAdOx1 (n=7), ChAdOx1+BNT162b2 (n=30), or ChAdOx1+mRNA-1273 (n=11). Half-maximal inhibitory dilutions (ID50) were calculated and titers <20 marked as 10. Geometric mean titers for each vaccine group are shown as a line with geometric SD. Differences in IgG antibody levels and neutralization titers between different groups were analysed with Kruskal-Wallis rank test and P-values <0.05 were considered statistically significant. ****<0.0001

Supplementary Files

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