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Supplementary appendix

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Supplementary Appendix

Heterologous prime-boost vaccination with ChAdOx1 nCoV-19 and BNT162b2 mRNA

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1. Table S1. Characteristics of cohorts analyzed in laboratory 1 (Munich, Germany)

	ChAdOx1 nCoV-19 prime, BNT162b2 mRNA boost **CGN N = 183; MUC N = 49; total N = 232**	**BNT162b2 mRNA prime, BNT162b2 mRNA boost** **MUC N = 410**
Volunteer source	General population at vaccination center	Healthcare workers
Age in years, median (IQR) [range]	47 (33-55) [18-65]	38 (31-48) [20-78]
Sex, n (%) Female	190 (81.9%)	248 (60.5%)
Sex, n (%) Male	42 (18.1%)	162 (39.5%)
Time from prime to boost dose in days, median (IQR) [range]	63 (63-77) [54-85]	21 (21-22) [18-25]
Time from boost dose to blood collection in days, median (IQR) [range]	14 (13-15) [13-15]	14 (13-15) [9-62]

CGN, Cologne; MUC, Munich; IQR: interquartile range.

2. Characteristics of cohorts analyzed in laboratory 1 (Munich, Germany)

To evaluate standard mRNA vaccination, employees of the University Hospital rechts der Isar of the Technical University of Munich were offered to have their antibody responses to SARS-CoV-2 analyzed after vaccination with two doses of BNT162b2 mRNA. Persons with known or accidentally detected prior SARS-CoV-2 infection were excluded. Serum samples obtained from 410 persons at the time of booster vaccination and 14±1 day after the second vaccination were analyzed.

Given the uncertain immunogenicity of heterologous prime boost vaccinations, 232 vaccinees, who received a first vaccination with ChAdOx1 nCoV-19 and - according to current recommendation in Germany - a booster vaccination with BNT162b2 mRNA at the vaccination center in Cologne or in Munich, Germany, were offered to register for blood sampling appointments at the day of booster vaccination with BNT162b2 mRNA and 13 to 15 days after the second vaccination. Information on age, sex, and dates of first and second vaccination were obtained. A summary of the characteristics of the different groups of vaccinees is shown in Table S1.

3. Table S2. Characteristics of cohorts analyzed by laboratory 2.

	BNT162b2 mRNA prime, BNT162b2 mRNA boost ERL N = 127	ChAdOx1 nCoV-19 prime, ChAdOx1 nCoV-19 boost ERL N = 66	ChAdOx1 nCoV-19 prime, BNT162b2 mRNA boost ERL N = 250
Volunteer source	Healthcare workers	General population at vaccination center	General population at vaccination center
Age in years, median (IQR) [range]	41 (27-52) [20-65]	57 (45-62) [31-64]	52 (31-59) [19-59]
Sex, n (%) Female	90 (70.87%)	43 (65.2%)	160 (64.0%)
Sex, n (%) Male	37 (29.13%)	23 (34.8%)	90 (36.0%)
Time from prime to boost dose in days, median (IQR) [range]	23 (21-25) [14-29] *	63 (63-63) [63-63]	63 (63-63) [57-71]
Time from boost dose to blood collection in days, median (IQR) [range]	14 (14) [10-21]	15 (13-15) [13-16]	14 (13-15) [6-21]

ERL, Erlangen; IQR: interquartile range.; n* data available for 126 out of 127 volunteers

4. Characteristics of cohorts analyzed in laboratory 2 (Erlangen, Germany)

Healthcare workers were offered analysis of their neutralizing antibody responses to SARS-CoV-2 two to three weeks after the second BNT162b2 mRNA vaccination. Given the uncertain immunogenicity of heterologous prime boost vaccinations, recipients of a priming vaccination with ChAdOx1 nCoV-19, who had received a second vaccination with ChAdOx1 nCoV-19 or BNT162b2 mRNA on April 29th, 2021, at the public vaccination center in Erlangen were offered to register by phone for blood sampling appointments 14 to 16 days after the second vaccination. Blood samples and information on age, sex, and dates of first and second vaccination were obtained from 316 volunteers (for Summary see Table S2). Volunteers reporting prior SARS-CoV-2 infection were excluded.

5. Supplementary Figures.

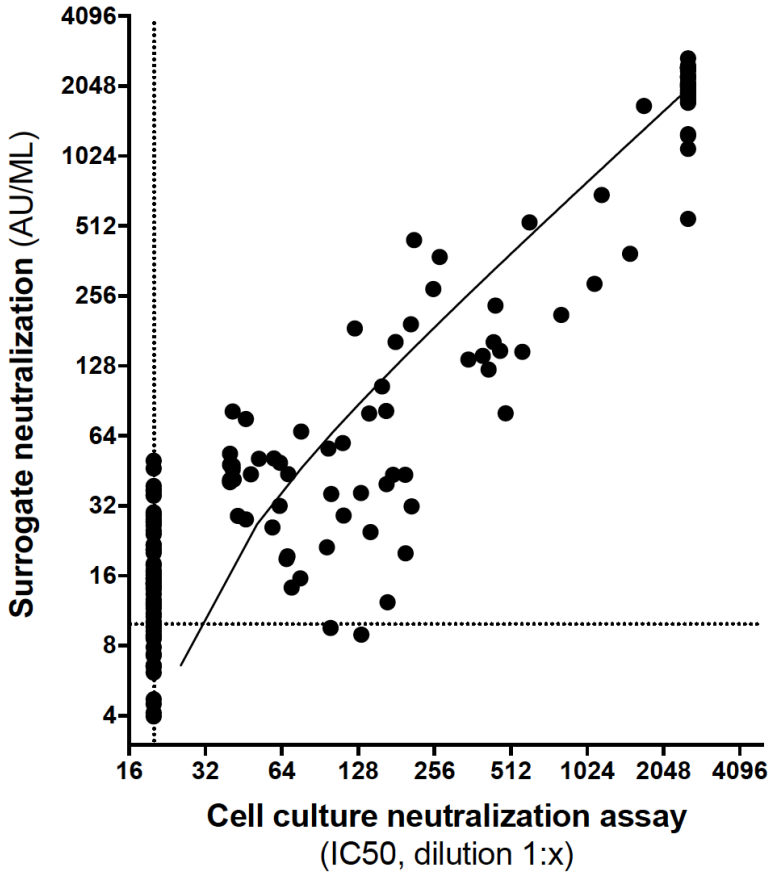

Figure S1: Correlation of the surrogate neutralizing antibody assay and a cell-culture based SARS-CoV-2 infection inhibition assay. 220 sera obtained from COVID-19 convalescent or vaccinated donors were analyzed 1) in a cell-culture based assay using SARS-CoV-2 wild-type and 2) in a competitive chemo-luminescence immunoassay (iFlash-2019-nCoV NAb assay, Yhlo, Shenzen, China). The surrogate neutralization assay detects antibodies that are able to compete with binding of a recombinant extracellular domain of the SARS-CoV-2 receptor ACE2 to a recombinant receptor-binding domain of the SARS-CoV-2 S1 protein coated on microparticles and thus indicates the neutralization activity of sera. Activity is determined in arbitrary units (AU). Cut-off is 10 AU/ml, indicated by the horizontal dotted line. For the cell-culture assay, Vero E6 cells were infected with SARS-CoV-2 (Wuhan strain, D614G variant) at a multiplicity of infection of 0.06 incubated with test sera in a serial 2-fold dilution form 1:20 to 1:2560. Lowest dilution (1:20) is indicated by the vertical dotted line. After 24 hours, cells were fixed for an in-cell ELISA using anti-dsRNA J2 antibody (Jena Bioscience, Jena, Germany) to detect SARS-CoV-2 infection. Using the resulting inhibition curve, the inhibitory 50% concentration (IC50) given as dilution 1:x was determined. All positive values obtained are plotted. Simple linear regression analysis revealed a slope of 0.7863 (0.7542 to 0.8135) and an R squared of 0.9256 being significantly non-zero ($p<0.0001$). To ensure cell survival, an initial 1:20 dilution of serum had to be used precluding calculation of IC50 if serum neutralization activity is low. The surrogate neutralization assay, in contrast, was done with undiluted serum and therefore allowed a quantification also when serum neutralization activity was low.

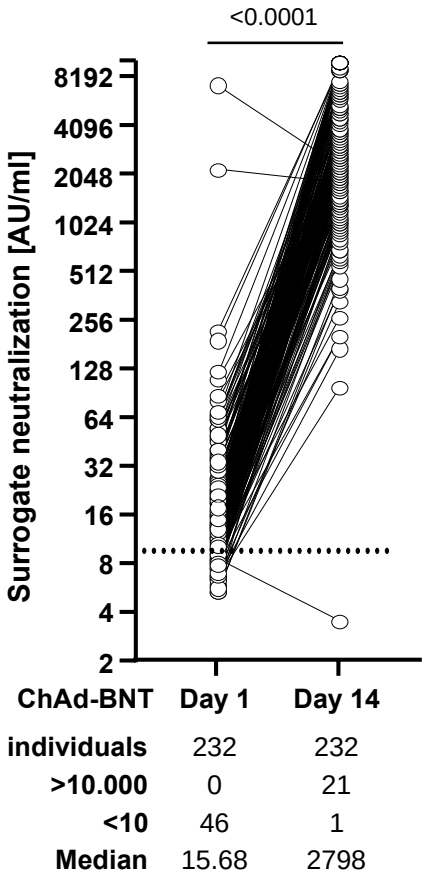

Figure S2. Comparison of surrogate neutralization activity following ChAdOx1 nCoV-19 prime and BNT162b2 mRNA (BNT) boost vaccination. Vaccinees received one dose of the ChAdOx1 nCoV-19 (ChAd) vaccine and, with few exceptions, 9 weeks later a BNT162b2 mRNA (BNT) boost vaccination (for details see Suppl. Table S1). In all vaccinees, serum samples were obtained at the day of boost vaccination and at day 14±1 after the heterologous booster. Surrogate neutralization activity is given in paired samples for each individual. Dots represent single vaccinees. P values from a two-tailed Wilcoxon matched-pairs signed rank test are shown above the graph. Descriptive statistics shown below the graph include numbers of individuals for which results are given, numbers of results below the lower (<10) and above the upper (>10.000) cut-off of the surrogate neutralization assay and median values.

6. Ethics Statement

The retrospective analysis plan on the comparison of the SARS-CoV-2 antibody levels in response to different vaccine regimens was approved by the local ethics committees in Erlangen (Az. 202_21 Bc) and Munich (Az. 26/21 und Az. 330/21 S).

7. Funding Statement

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