

Reduced Neutralization of SARS-CoV-2 Omicron Variant by Vaccine Sera and monoclonal antibodies

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Abstract:

Due to numerous mutations in the spike protein, the SARS-CoV-2 variant of concern Omicron (B.1.1.529) raises serious concerns since it may significantly limit the antibody-mediated neutralization and increase the risk of reinfections. While a rapid increase in the number of cases is being reported worldwide, until now there has been uncertainty about the efficacy of vaccinations and monoclonal antibodies. Our *in vitro* findings using authentic SARS-CoV-2 variants indicate that in contrast to the currently circulating Delta variant, the neutralization efficacy of vaccine-elicited sera against Omicron was severely reduced highlighting T-cell mediated immunity as essential barrier to prevent severe COVID-19. Since SARS-CoV-2 Omicron was resistant to casirivimab and imdevimab, genotyping of SARS-CoV-2 may be needed before initiating mAb treatment. Variant-specific vaccines and mAb agents may be required to treat COVID-19 due to Omicron and other emerging variants of concern.

The SARS-CoV-2 variant Omicron was first identified in South Africa on November 9, 2021. Due to numerous mutations in the spike protein (S), which is the antigenic target of vaccine-elicited antibodies, Omicron raises serious concerns of a significant reduction in vaccine efficacy and an increased risk of reinfection¹. Compared to the parental variant (B.1), Omicron S has 30 non-synonymous substitutions, three small deletions and an insertion (**Supplementary Figure 1, Supplementary Tables 1-3**). Fifteen of these mutations are in the receptor-binding domain (RBD), a major target of neutralizing antibodies (NAbs)². Several of the S mutations observed in Omicron were reported in preceding variants of concern (VoCs) like Alpha, Beta, Gamma and Delta as well as variants of interest such as Kappa, Zeta, Lambda and Mu (**Supplementary Table 3**) that were associated with higher transmissibility and immune escape. So far, Beta and Mu had the most severe immune evading capacities^{3,4}. Due to the high accumulation of these mutations in Omicron S, synergistic effects are expected and it is unclear whether prior immunity protects against re-infections.

To evaluate the protective capacity, antibody-mediated neutralization efficacy against authentic SARS-CoV-2 Omicron was determined *in vitro* using an isolate obtained from a double 1273-mRNA-vaccinated travel returnee from Zimbabwe and compared to Delta. Neutralization performed with sera from double (no-booster) or triple BNT162b2-vaccinated (sampled 0.5 or 6 months after boosting) revealed an 11.4-, 37.0- and 24.5-fold reduction, respectively (**Figure 1A**). Sera from double mRNA1273-vaccinated (no-booster) and additionally BNT162b2-booster (sampled 0.5 or 6 months after booster vaccination) showed a 20- and 22.7-fold reduction in the neutralization capacity (**Figure 1B**). Poor neutralization against Delta and no efficacy against Omicron were observed using sera from heterologous ChAdOx1 and BNT162b2 vaccinated individuals (**Figure 1C**). Additionally, the BNT162b2-booster group showed a significant increase of NAb titers but a 27.1-fold reduction in neutralization against Omicron. (**Figure 1C**). Convalescent sera poorly neutralize VoCs, however in combination with vaccination provides superior protection. Neutralization of Omicron was 32.8-fold reduced using sera from double BNT162b2-vaccinated and infected individuals (**Figure 1A**).

The currently used monoclonal antibodies imdevimab and casirivimab efficiently prevented Delta infection, however, possibly as a consequence of amino acid substitutions⁵ failed to neutralize Omicron (**Figure 1D**).

In contrast to the currently circulating Delta variant, neutralization efficacy of vaccine-elicited sera against Omicron was severely reduced highlighting T-cell mediated immunity as essential barrier to prevent severe COVID-19. Since Omicron was resistant to casirivimab and imdevimab SARS-CoV-2 genotyping may be needed before initiating mAb treatment. Variant-specific vaccines and mAb agents may be required to treat COVID-19 with emerging variants of concern.

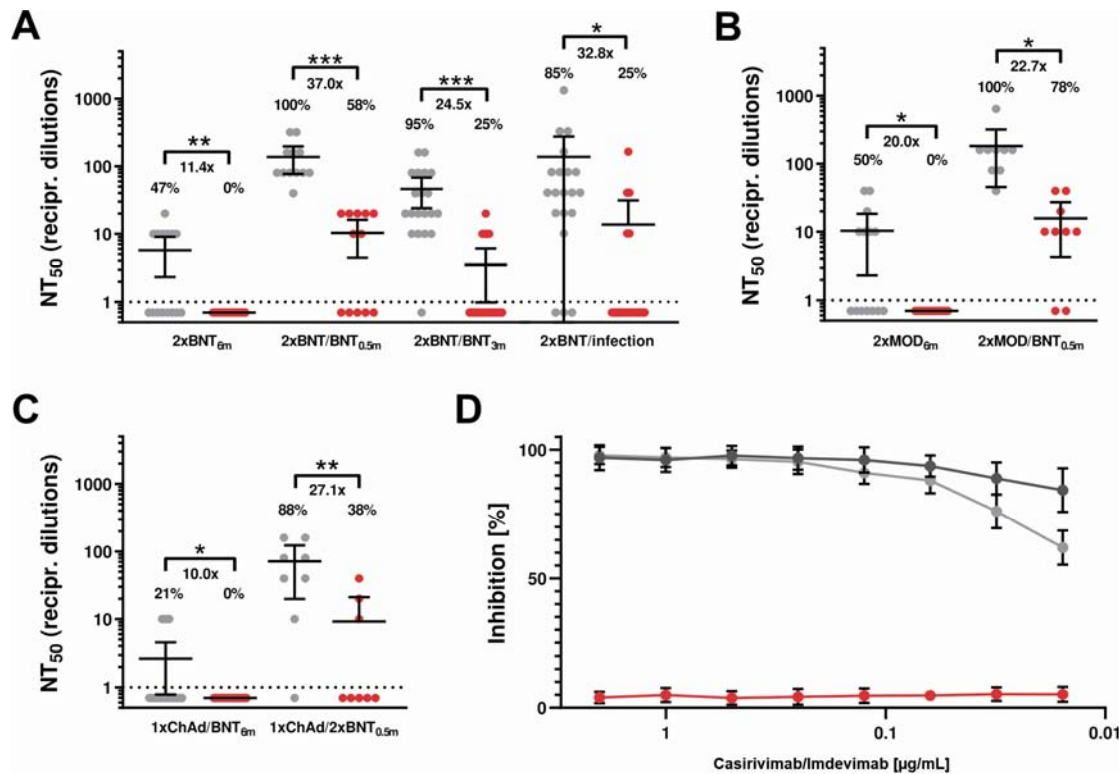


Figure 1 - Antibody-mediated neutralization efficacy against authentic SARS-CoV-2 variants Delta and Omicron. Values represent reciprocal dilutions of SARS-CoV-2 variants Delta (grey) and Omicron (red) micro-neutralization titers resulting in 50% virus neutralization (NT₅₀). **A**) Neutralization assays were performed using serum samples obtained from individuals double BNT162b2 vaccinated (2xBNT). Sera from additionally BNT162b2 boosted individuals were sampled 0.5 month (2xBNT/BNT_{0.5m}) or 3 month (2xBNT/BNT_{3m}) as well as sera from double BNT162b2 vaccinated and SARS-CoV-2 infected individuals (2xBNT/infection). **B**) Neutralization assays with sera from double mRNA-1273 vaccinated (2xMOD) and additionally BNT162b2 boosted (2xMOD/BNT_{0.5m}). **C**) Neutralization titers for sera from heterologous ChAdOx1 and BNT162b2 vaccinated (1xChAd/1xBNT_{6m}) and BNT162b2 boosted (1xChAd/2xBNT_{0.5m}) individuals. The x-fold reduction was determined using the difference between NT₅₀ values for Delta and Omicron. Only Delta neutralizing samples were considered for the calculation. Negative titers were handled as 1. The percentages indicate the relative number of sera that achieved a measurable titer. Information regarding the sera donors (sex, age, antibody titers test and sampling dates) are summarized in the Supplementary Appendix. **D**) Neutralization efficacy of monoclonal antibodies imdevimab and casirivimab against SARS-CoV-2 Omicron (red), B (dark grey), and Delta (grey). The indicated concentrations of mAbs casirivimab and imdevimab were applied in a 1:1 ratio. Mean values of two technical replicates per sample are depicted with 95% confidence intervals and SD. All experiments were verified using a second SARS-CoV-2 strain (Supplementary Table 4). Statistical significance compared to Delta was calculated by two-tailed, paired student's t-tests. Asterisks indicate p-values as * (p < 0.05), ** (p < 0.01), and *** (p < 0.001).

Materials and Methods

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board of the Ethics Committee of the Faculty of Medicine at Goethe University Frankfurt (2021-201, 20-864 and 250719).

Human sera

Peripheral blood was collected from vaccinated individuals before and two weeks or three months after the booster vaccination with BNT162b2 (Pfizer-BioNTech). All sera (Supplementary Table 4) were prepared by centrifugation 2000 x g for 10 min. All sera were inactivated at 56°C for 30 min and stored at -20°C until use.

Virus identification and Sequencing

SARS-CoV-2 isolates were obtained from nasopharyngeal swabs of travel returnees from South Africa as screened by the Public Health Department of the City of Frankfurt am Main, Germany. Swab material was suspended in 1.5 mL phosphate-buffered saline (PBS) and split for RNA-Isolation and viral outgrowth assay. RNA was isolated using the QIAamp Viral RNA Mini Kit (QIAGEN, Hilden, Germany) according to manufacturer's instructions. RNA was subjected to variant specific RT-qPCR genotyping and Oxford Nanopore sequencing.

Library preparation, sequencing and bioinformatics analysis

RNA samples extracted from swabs were used for library preparation according to NEBNext ARTIC Standard Protocol (New England Biolabs Ipswich, Massachusetts, USA) (dx.doi.org/10.17504/protocols.io.budxns7n) using the Artic nCoV-2019 V4 primers (IDT, Coralville, Iowa, USA). Libraries were generated using ligation sequencing kit SQK-LSK109, native barcoding expansion kit EXP-NBD104 and FLO-MIN106D R9.4.1 flow cell according to the standard protocol (Oxford Nanopore Technologies, UK) and sequenced on MinION MK1c (Oxford Nanopore Technologies, UK) for 8 h with basecalling and demultiplexing options enabled. The obtained FASTQ files were filtered and analyzed using ARTIC pipeline (<https://artic.network/ncov-2019/ncov2019-bioinformatics-sop.html>).

See **Supplementary Figure 1** for schematic representation of the SARS-CoV-2 genome indicating spike positions.

Sequences are available on GISAID (www.gisaid.org, access date 12/2021), under the following accession numbers. Omicron strains used in this study are as follows: B.1.1.529a (EPI_ISL_6959868), B.1.1.529b (EPI_ISL_6959871). GenBank accession number for the SARS-CoV-2 B.1.617.1 (Delta) isolate FFM-IND5881/2021 (MZ315140).

Cell culture and Virus Propagation

A549-AT cells⁶ stably expressing ACE2 and TMPRSS2 and Caco2 cells (DSMZ, Braunschweig, Germany, no: ACC 169) were maintained in Minimum Essential Medium (MEM) supplemented with 10% fetal calf serum (FCS), 4 mM L-glutamine, 100 IU/mL of penicillin, and 100 µg/mL of streptomycin at 37°C and 5% CO₂. All culture reagents were purchased from Sigma (St. Louis, MO, USA).

As described previously SARS-CoV-2 isolates were propagated using Caco-2 cells, which were selected for high permissiveness to SARS-CoV-2 infection by serial dilution and passaging⁷. Cell-free cell culture supernatant containing infectious virus was harvested after complete cytopathic effect (CPE) and aliquots were stored at -80°C. Titers were determined by the median tissue culture infective dose (TCID₅₀) method as described by Spearman⁸ and Kaerber⁹ using Caco-2 cells. All cell culture work involving infectious SARS-CoV-2 was performed under biosafety level 3 (BSL-3) conditions. Sample inactivation for further processing was performed with previously evaluated methods¹⁰.

Neutralization and antiviral assays

SARS-CoV-2 antibody concentrations were determined using the SARS-CoV-2 IgG II Quant assay and the Alinity I device (Abbott Diagnostics, Wiesbaden, Germany) with an analytical measurement range from 2.98–5680 binding antibody units per mL (BAU/mL). All sera were serially diluted (1:2) and incubated with 4000 TCID₅₀/mL of SARS-CoV-2 Delta or Omicron. Infected cells were monitored for cytopathic effect (CPE) formation 48 h post inoculation. Monoclonal antibody solutions containing imdevimab and casirivimab alone or in combination in equal ratios (1:1) were serially diluted (1:2) and incubated with 4000 TCID₅₀/mL of the indicated SARS-CoV-2 variant. After 48 h CPE formation was evaluated microscopically. Evaluation of monoclonal antibodies was quantified using Spark Cyto 400 multimode imaging plate reader (Tecan) as

described before^{6,11}.

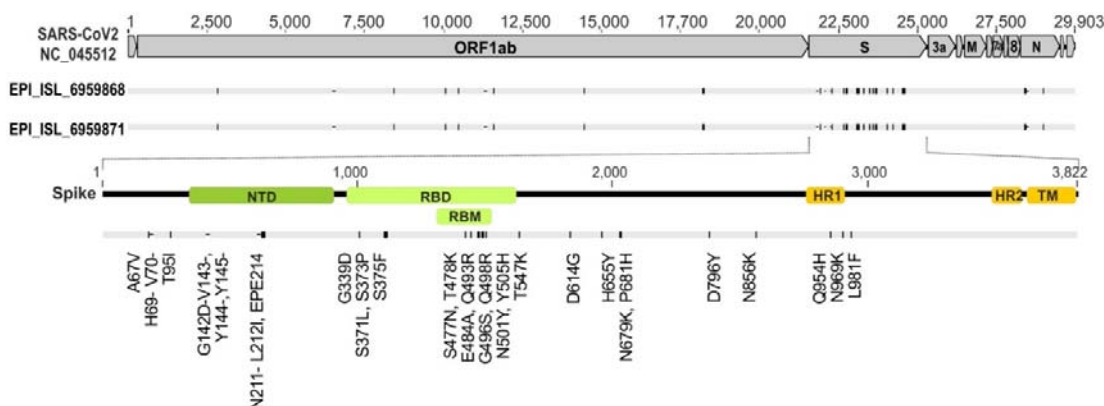
References:

1. Pulliam JRC, van Schalkwyk C, Govender N, et al. Increased risk of SARS-CoV-2 reinfection associated with emergence of the Omicron variant in South Africa. medRxiv 2021:2021.11.11.21266068. DOI: 10.1101/2021.11.11.21266068.
2. Harvey WT, Carabelli AM, Jackson B, et al. SARS-CoV-2 variants, spike mutations and immune escape. Nature reviews Microbiology 2021;19(7):409-424. DOI: 10.1038/s41579-021-00573-0.
3. Uriu K, Kimura I, Shirakawa K, et al. Neutralization of the SARS-CoV-2 Mu Variant by Convalescent and Vaccine Serum. The New England journal of medicine 2021. DOI: 10.1056/NEJMc2114706.
4. Widera M, Wilhelm A, Hoehl S, et al. Limited neutralization of authentic SARS-CoV-2 variants carrying E484K in vitro. The Journal of infectious diseases 2021. DOI: 10.1093/infdis/jiab355.
5. Barnes CO, Jette CA, Abernathy ME, et al. SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies. Nature 2020;588(7839):682-687. DOI: 10.1038/s41586-020-2852-1.
6. Widera M, Wilhelm A, Toptan T, et al. Generation of a Sleeping Beauty Transposon-Based Cellular System for Rapid and Sensitive Screening for Compounds and Cellular Factors Limiting SARS-CoV-2 Replication. Frontiers in microbiology 2021;12(2034):701198. (Original Research) (In English). DOI: 10.3389/fmicb.2021.701198.
7. Widera M, Muhlemann B, Corman VM, et al. Surveillance of SARS-CoV-2 in Frankfurt am Main from October to December 2020 Reveals High Viral Diversity Including Spike Mutation N501Y in B.1.1.70 and B.1.1.7. Microorganisms 2021;9(4). DOI: 10.3390/microorganisms9040748.
8. Spearman C. The method of right and wrong cases (constant stimuli) without Gauss's formulae. British journal of psychology 1908;2(3):227.
9. Kärber G. Beitrag zur kollektiven Behandlung pharmakologischer Reihenversuche. Naunyn-Schmiedeberg's Archiv für experimentelle pathologie und pharmakologie 1931;162(4):480-483.
10. Widera M, Westhaus S, Rabenau HF, et al. Evaluation of stability and inactivation methods of SARS-CoV-2 in context of laboratory settings. Medical microbiology and immunology 2021;210(4):235-244. DOI: 10.1007/s00430-021-00716-3.
11. Wilhelm A, Toptan T, Pallas C, et al. Antibody-Mediated Neutralization of Authentic SARS-CoV-2 B.1.617 Variants Harboring L452R and T478K/E484Q. Viruses 2021;13(9). DOI: 10.3390/v13091693.
12. Elbe S, Buckland-Merrett G. Data, disease and diplomacy: GISAID's innovative contribution to global health. Glob Chall 2017;1(1):33-46. DOI: 10.1002/gch2.1018.

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Supplementary Material:



Supplementary Figure 1 - Schematic representation of the SARS-CoV-2 genome indicating spike positions. The numbers denote nucleotide positions based on the reference strain NC_045512. NTD RBD and RBM are highlighted by green boxes. HR1, HR2, and TM are indicated by orange boxes. ORF based on reference sequence NC_045512 are shown as grey boxes. Nucleotide substitutions compared to the reference sequence are indicated in the lower section. For sequencing coverage see **Supplementary Table 2**. Dropouts and low coverage regions: Spike: (22796-22983), (23621-23885); E/M gene: (26348-27186). For affected Nanopore Primers see **Supplementary Table 1**.

Supplementary Table 1 - Dropouts and low coverage Regions: Spike: (22796-22983), (23621-23885) E/M gene: (26348-27186)

Position in SARS-CoV-2 genome	Affected Nanopore Primer
22673	SARS-CoV-2_76_LEFT
22674	SARS-CoV-2_76_LEFT
23040	SARS-CoV-2_76_RIGHT
23048	SARS-CoV-2_76_RIGHT
23055	SARS-CoV-2_76_RIGHT
23948	SARS-CoV-2_79_RIGHT
26270	SARS-CoV-2_88_LEFT
26577	SARS-CoV-2_89_LEFT
27259	SARS-CoV-2_90_RIGHT

Supplementary Table 2 – Sequencing coverage, identity and amino acid (aa) changes of SARS-CoV-2 Omicron samples.

Best reference hit	%id	%coverage	#Δs	List of aa changes
NSP1 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP2 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP3 hCoV-19/Wuhan/WIV04/2019	99.8%	99.9%	4	K38R, S1265del, L1266I, A1892T
NSP4 hCoV-19/Wuhan/WIV04/2019	99.8%	100%	1	T492I
NSP5 hCoV-19/Wuhan/WIV04/2019	99.7%	100%	1	P132H
NSP6 hCoV-19/Wuhan/WIV04/2019	99.7%	99.0%	4	L105del, S106del, G107del, I189V
NSP7 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP8 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP9 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP10 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP11 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP12 hCoV-19/Wuhan/WIV04/2019	99.9%	99.0%	1	P323L
NSP13 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP14 hCoV-19/Wuhan/WIV04/2019	99.8%	100%	1	I42V
NSP15 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NSP16 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NS3 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
E hCoV-19/Wuhan/WIV04/2019	87.1%	41.3%	4	T9I, A22X, F23X, F26X
(no ref hits for M)	0%	0%	0	no coverage
NS6 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NS7a hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NS7b hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
NS8 hCoV-19/Wuhan/WIV04/2019	100%	100%	0	no aa changes
N hCoV-19/Wuhan/WIV04/2019	99.3%	99.3%	6	P13L, E31del, R32del, S33del, R203K, G204R

Supplementary Table 3. Mutations in the spike proteins of SARS-CoV-2 variants of concern. Compared to the parental SARS-CoV-2 isolate B.1, Omicron gains additional substitutions, insertions and deletions. Amino acid substitutions already found in other variants are highlighted in red. Altered positions but with different distinct substitutions are indicated in blue.

Parental:	B.1	D614G
Alpha:	B.1.1.7	HV69-70del, Y144del, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H
Beta:	B.1.351	L18F, D80A, D215G, R246I, K417N, E484K, N501Y, D614G, A701V
Gamma:	P.1	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T10277I
Delta:	B.1.617.2	T19R, T95I, G142D, E156-, F157-, R158G, L452R, T478K, D614G, P681R, D950N
Omicron:	B.1.1.529	A67V, HV69-70del, T95I, del142-144, Y145D, del211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, 446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F

GISAID¹² Lineage Comparison: Alaa Abdel Latif, Julia L. Mullen, Manar Alkuzweny, Ginger Tsueng, Marco Cano, Emily Haag, Jerry Zhou, Mark Zeller, Emory Hufbauer, Nate Matteson, Chunlei Wu, Kristian G. Andersen, Andrew I. Su, Karthik Gangavarapu, Laura D. Hughes, and the Center for Viral Systems Biology. **outbreak.info** (available at <https://outbreak.info/compare-lineages?pango=B.1.617.2&pango=P.1&pango=B.1.351&pango=B.1.1.7&pango=B.1&pango=Omicron&gene=S&threshold=94&sub=true&dark=false>). Accessed 7 December 2021.

Supplementary Table 4. Patient characteristics and overview of sera used in this study.

Group	Original study	Immunization scheme	Group size n	Age Median (range)	Sex (f/m)	BAU/mL Median (IQR)	BAU/mL Mean (range)	months after last vaccination	months after SARS-CoV-2 infection	NT (Delta Omicron) Median (range)
2xBNT	Impf Care Study	2x BNT162b	15	51 (24 – 64)	11 / 4	178 (116.9-290.2)	289.8 (34.2-1012)	6 - 7	n.a.	0 (0-20) / 0(0-0)
2xBNT / BNT _{0.5m}	Bürgerhospital	2x BNT162b + 1x BNT162b	12	38 (28 – 59)	10 / 2	2143 (1355-4033)	2729 (825.3-6250)	0.5	n.a.	80 (40-320) / 10 (0-20)
2xBNT / BNT _{3m}	Bürgerhospital	2x BNT162b + 1x BNT162b	20	42,5 (26 – 63)	12 / 8	1354 (690.4-2178)	1838 (167.1-7377)	2 (+ / - 0.8)	n.a.	20 (0-260) / 0 (0-20)
2xMOD	Internal study	2x mRNA-1273	14	28 (23 – 50)	7 / 7	355 (174-691.1)	588.8 (90.6-2364)	6	n.a.	5 (0-40) / 0 (0-0)
2xMOD / BNT _{0.5m}	Internal study	2x mRNA-1273 1x BNT162b	9	29 (23 – 50)	4 / 5	2600 (1879-4089)	3155 (1422-6540)	0.5	n.a.	160 (40-640) / 10 (0-40)
1x ChAd / 1xBNT _{5m}	Heterologous Vaccination study	1x ChAdOx1 1x BNT162b	19	43 (20 – 59)	14 / 5	162.1 (95.5-236.5)	161.9 (48.6-276.4)	6	n.a.	0 (0-10) / 0 (0-0)
1x ChAd / 2xBNT _{0.5m}	Heterologous Vaccination study	1x ChAdOx1 2x BNT162b	8	48 (30 – 59)	6 / 2	1544 (523.9-2931)	1728 (186.8-3676)	0.5	n.a.	60 (0-160) / 0 (0-40)
2xBNT / infection	Impf Care Study	2x BNT162b + SARS-CoV-2	20	87.5 (68 – 93)	17 / 3	1065 (548.7-4407)	2616 (58.7-11400)	6-7	0.7 – 7.6	40 (0->1380) / 0 (0-160)