Supplementary Materials for

Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine–elicited human sera

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MDAR Reproducibility Checklist (.pdf)

Materials and Methods

VSV-SARS-CoV-2 S variant pseudovirus generation

A recombinant replication-deficient vesicular stomatitis virus (VSV) vector that encodes green fluorescent protein (GFP) and luciferase instead of the VSV-glycoprotein (VSV-G) was pseudotyped with SARS-CoV-2 spike (S) derived from either the Wuhan reference strain (NCBI Ref: 43740568) or the variant of concern (VOC)-202012/01 (also known as SARS-CoV-2 lineage B.1.1.7) according to published pseudotyping protocols (Fig. S1) (*12*). In brief, HEK293T/17 monolayers (ATCC® CRL-11268™) cultured in Dulbecco's modified Eagle's medium (DMEM) with GlutaMAX™ (Gibco) supplemented with 10% heat inactivated fetal bovine serum (FBS [Sigma-Aldrich]) (referred to as culture medium) were transfected with SARS-CoV-2 S expression plasmid with Lipofectamine LTX (Life Technologies) following the manufacturer's protocol. At 24 hours VSV-G complemented VSVΔG vector. After incubation for 1 h at 37 °C with 5% CO_2, the inoculum was removed. Cells were washed twice with phosphate buffered saline (PBS) before culture medium supplemented with anti-VSV-G antibody (clone 8G5F11, Kerafast Inc.) was added to neutralize residual VSV-G complemented input virus. VSV-SARS-CoV-2-S pseudotype containing culture medium was harvested 20 h after inoculation, passed through a 0.2 µm filter (Nalgene) and stored at -80 °C. Prior to use in the neutralization test, the pseudovirus batches were titrated on Vero 76 cells (ATCC® CRL-1587™) cultured in culture medium, and the percent infected cells determined on a BD FACSCelesta (Becton Dickinson) flow cytometer (Fig. S3). Individual titers were calculated in transducing units (TU) per mL. Production of the VSV-SARS-CoV-2-S pseudoviruses bearing the Wuhan reference strain or lineage B.1.1.7 strain S protein yielded similar titers (Table S2). The observed small difference in virus output is within the normal variation and not considered to be biologically relevant.

Serum specimens

An unbiased sample set collected in the German phase 1/2 trial (NCT04380701) was investigated representing all younger adults and all older adults vaccinated in the 30 µg BNT162b2 dose escalation cohorts (each n=12), and all samples (n=14 younger adults, n=2 older adults) from a biomarker expansion cohort vaccinated with 30 µg BNT162b2 that were available at the time the pseudovirus neutralization assay was conducted. The immunization was performed on days 1 and 22, and test serum was collected either on day 29 or 43 (7 days and 21 days after dose 2). For subjects with serum collected at day 29, day 43 serum was not yet available. The clinical trial was carried out in Germany in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and with approval by an independent ethics committee (Ethik-Kommission of the Landesärztekammer Baden-Württemberg, Stuttgart, Germany) and the competent regulatory authority (Paul-Ehrlich Institute, Langen, Germany). All participants provided written informed consent.

Pseudovirus neutralization assay

Vero 76 cells were seeded in 96-well white, flat-bottom plates (Thermo Scientific) at 40,000 cells/well in culture medium 4 hours prior to the assay and cultured at 37 °C with 5% CO_2. Each serum was 2-fold serially diluted in culture medium with the first dilution of 1:20 (dilution range of 1:20 to 1:2,560). VSV-SARS-CoV-2-S particles were diluted in culture medium to obtain 1,000 TU in the assay. Serum dilutions were mixed 1:1 with pseudovirus (n=2 technical replicates per serum per pseudovirus) for 30 minutes at room temperature prior to

addition to Vero 76 cell monolayers and incubation at 37 °C with 5% CO_2 for 24 hours. Supernatants were removed, and the cells were lysed with luciferase reagent (Promega). Luminescence was recorded on a Tecan Infinite F200 PRO instrument (Tecan), and neutralization titers were calculated in GraphPad Prism version 9 (GraphPad Software) by generating a 4-parameter logistical (4PL) fit of the percent neutralization at each serial serum dilution. The 50% pseudovirus neutralisation titre (pVNT50) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in luminescence. The full set of n=40 sera was tested for neutralization of SARS-CoV-2 Wuhan and lineage B.1.1.7 spike-pseudotyped VSV in two independent assays covering n=16 and n=24 sera, respectively. A table of the neutralization titers is provided (Table S1).

Statistical analysis

Pseudovirus neutralization titers against VSV-SARS-CoV-2-S bearing the Wuhan or lineage B.1.1.7 spike protein were tested for statistical significance between these two groups by a Wilcoxon matched-pairs signed rank test in GraphPad Prism version 9. Two-tailed p-values are reported. Geometric mean titer (GMT) and 95% confidence intervals are indicated. The difference in distribution of titer ratios (B.1.1.7/Wuhan ref.) between younger and older cohort was tested for statistical significance with a two-tailed Mann-Whitney-U test in GraphPad Prism version 9.

Fig. S1.

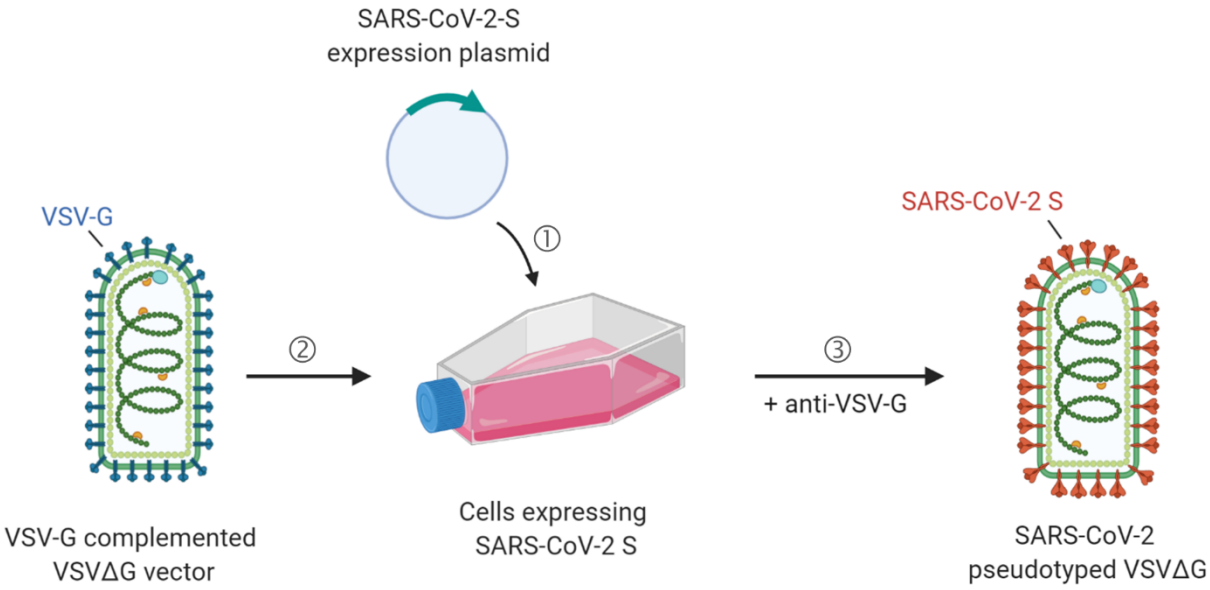

Fig. S1. Schematic illustration of the production of VSV pseudoviruses bearing SARS-CoV-2 S protein. (1) Transfection of SARS-CoV-2-S expression plasmid into HEK293/T17 cells. (2) Infection of SARS-CoV-2 S expressing cells with VSV-G complemented input virus lacking the VSV-G in its genome (VSVΔG) and encoding for reporter genes. (3) Neutralization of residual VSV-G complemented input virus by addition of anti-VSV-G antibody yields SARS-CoV-2 S pseudotyped VSVΔG as a surrogate for live SARS-CoV-2. Schematic was created with BioRender.com

Fig. S2.

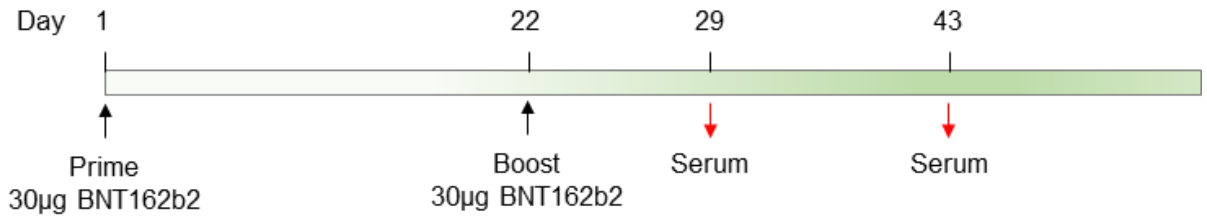

Fig. S2. Schedule of BNT162b2 vaccination and serum sampling.

Fig. S3.

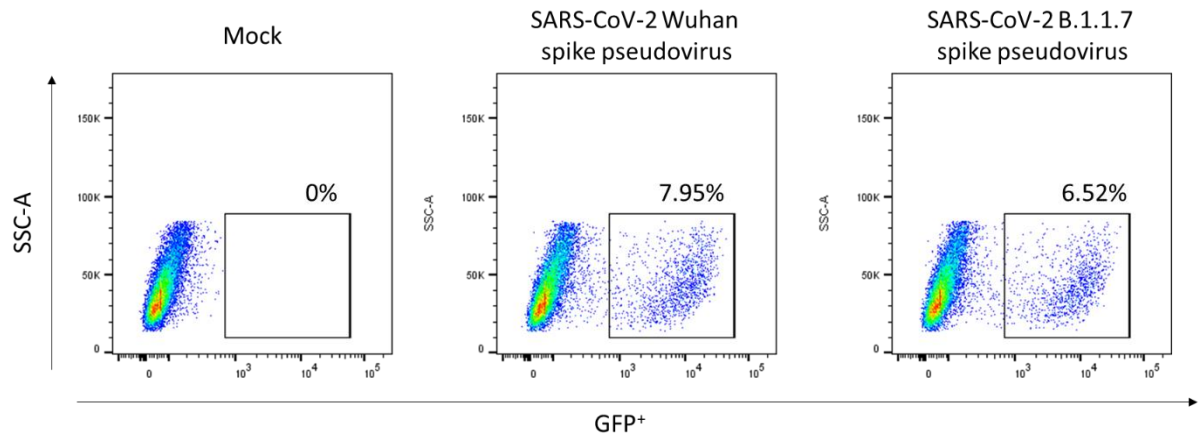

Fig. S3. Titration of SARS-CoV-2 Wuhan reference strain and lineage B.1.1.7 spike-pseudotyped VSV on Vero 76 cells using GFP-infected cells as read-out.

Table S1. $pVNT_{50}$ values of 40 BNT162b2 post-immunization sera against SARS-CoV-2 Wuhan reference strain spike-pseudotyped and lineage B.1.1.7 spike-pseudotyped VSV.
*, older adult; #, serum collected at day 29 (7 days after dose 2)

Serum ID	$pVNT_{50}$		$pVNT_{50}$ ratio (B.1.1.7/Wuhan ref.)
	Wuhan ref.	B.1.1.7	
1	160	161.2	1.01
2	114.1	85.75	0.75
3	223.2	128.6	0.58
4	193	268.4	1.39
5	111.9	64.26	0.57
6	128	99.08	0.77
7	278.1	226.8	0.82
8	203.6	185	0.91
9*	94.91	58.4	0.62
10*	209.7	126.8	0.60
11*	50.82	41.66	0.82
12*	241.3	486.1	2.01
13*	174	84.84	0.49
14*	292.5	136.7	0.47
15*	186.7	121.6	0.65
16*	86.28	116.2	1.35
17*	229.8	220.8	0.96
18*	951.5	1215	1.28
19*	253.1	162	0.64
20*	142.9	152	1.06
21	308.3	158.9	0.52
22	245.7	181.6	0.74
23	241.7	276.9	1.15
24	473.1	262.2	0.55
25	546.8	280.8	0.51
26	174.2	208.3	1.20
27*	151.9	136.1	0.90
28	242.8	132.7	0.55
29	442.6	631.1	1.43
30	417.8	243.1	0.58
31	188.2	94.29	0.50
32#	317.9	249.1	0.78
33*#	155	119.3	0.77
34#	352.1	262.4	0.75
35#	257.4	157.9	0.61
36#	410.5	323.4	0.79
37#	1066	961.1	0.90
38	655	1009	1.54
39	103	79.05	0.77
40#	196	160	0.82

Table S2. Titers of SARS-CoV-2 Wuhan reference strain and lineage B.1.1.7 spike-pseudotyped VSV in transducing units (TU) per mL.

VSV pseudovirus bearing	Titer [TU/mL]
Wuhan strain SARS-CoV-2 S	1.59×10^5
Lineage B.1.1.7 SARS-CoV-2 S	1.30×10^5

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