Supplementary Appendix

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This appendix has been provided by the authors to give readers additional information about the work.

Supplementary Appendix

CONTENTS

Neutralization Escape by the SARS-CoV-2 Omicron Variants BA.2.12.1 and BA.4/BA.5

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Supplementary Methods

Study population

A specimen biorepository at Beth Israel Deaconess Medical Center (BIDMC) obtained convenience samples from individuals who received a SARS-CoV-2 vaccine and/or had a history of SARS-CoV-2 infection. The BIDMC institutional review board approved this study (2020P000361). All participants provided informed consent. For the group of individuals receiving three doses of BNT162b2, participants were excluded if they had a history of SARS-CoV-2 infection or a positive nucleocapsid (N) serology by electrochemiluminescence assays (ECLA), or if they received other COVID-19 vaccines or immunosuppressive medications. For the infection cohort, the infecting strain was presumed based on the predominant circulating variant at the time of infection.

Pseudovirus neutralizing antibody assay

The SARS-CoV-2 pseudoviruses expressing a luciferase reporter gene were used to measure pseudovirus neutralizing antibodies. In brief, the packaging construct psPAX2 (AIDS Resource and Reagent Program), luciferase reporter plasmid pLenti-CMV Puro-Luc (Addgene) and spike protein expressing pcDNA3.1-SARS-CoV-2 SΔCT were co-transfected into HEK293T cells (ATCC CRL_3216) with lipofectamine 2000 (ThermoFisher Scientific). Pseudoviruses of SARS-CoV-2 variants were generated by using WA1/2020 strain (Wuhan/WIV04/2019, GISAID accession ID: EPI_ISL_402124), Omicron B.1.1.529 BA.1 (GISAID ID: EPI_ISL_7358094.2), or BA.2 (GISAID ID: EPI_ISL_6795834.2), BA.2.12.1 (GISAID ID: EPI_ISL_12003853.1), and BA.4/BA.5 (GSAID ID: EPI_ISL_12268495.2). The supernatants containing the pseudotype

viruses were collected 48h after transfection; pseudotype viruses were purified by filtration with 0.45-μm filter. To determine the neutralization activity of human serum, HEK293T-hACE2 cells were seeded in 96-well tissue culture plates at a density of 2×10^4 cells per well overnight. Three-fold serial dilutions of heat-inactivated serum samples were prepared and mixed with 50 μl of pseudovirus. The mixture was incubated at 37 °C for 1 h before adding to HEK293T-hACE2 cells. After 48 h, cells were lysed in Steady-Glo Luciferase Assay (Promega) according to the manufacturer's instructions. SARS-CoV-2 neutralization titers were defined as the sample dilution at which a 50% reduction (NT50) in relative light units was observed relative to the average of the virus control wells.

Table S1. Study Population

	BNT162b2 N=27	**Omicron infection** N=27
Age (years), median (range)	35 (23-76)	34 (27-41)
Sex at birth, female	24 (89)	21 (78)
Race		
White	23 (85)	21 (78)
Asian	2 (7)	1 (4)
Black	1 (4)	2 (7)
More than one race	1 (4)	0
Unknown/Other*	0 (0)	3 (11)
Ethnicity		
Hispanic or Latino	2 (7)	1 (4)
Non-Hispanic	24 (89)	24 (89)
Other	1 (4)	2 (7)
Medical condition		
Obese (BMI ≥30 kg/m2)	2 (7)	2 (7)
Hypertension	3 (11)	4 (15)
Diabetes	2 (7)	3 (11)
Pregnant	2 (7)	7 (26)
Lactating	4 (15)	3 (11)
Asthma	0	3 (11)
COVID-19 vaccination history		
BNT162b2 (3 doses)	27 (100)	8 (30)
BNT162b2 (2 doses)/mRNA-1273 (1 dose)	0	2 (7)
mRNA-1273 (3 doses)	0	7 (26)
mRNA-1273 (2 doses)/BNT-1273 (1 dose)	0	2 (7)
mRNA-1273 (2 doses)/Ad26.COV2.S (1 dose)	0	1 (4)
BNT162b2 (2 doses)	0	4 (15)
Ad26.COV2.S (1 dose)/mRNA-1273 (1 dose)	0	1 (4)
Ad5/Ad26 (2 doses)/mRNA-1273 (1 dose)	0	1 (4)
Unvaccinated	0	1 (4)
COVID-19 disease severity (NIH scale)		
Asymptomatic	N/A	1 (4)
Mild	N/A	24 (89)
Moderate	N/A	0
Severe	N/A	2 (7)
Critical	N/A	0
Days from last vaccine dose to positive PCR test, median (IQR)	N/A	83 (37-153)
Days from positive PCR test to sampling, median (IQR)	N/A	29 (14-74)

Data displayed as median (range or interquartile range, IQR) and n (%). BMI, body mass index; NIH, National Institutes of Health; PCR, polymerase chain reaction; pregnant designation reflects time of sampling. *Self-identified race as Jamaican, Brazilian, and Colombian.

Table S2. SARS-CoV-2 Infected Participants

ID	Dominant variant*	Age	Sex at birth	Medical history	COVID-19 vaccine history (primary/boost)	Days from boost or last dose to PCR	Days from PCR to sampling	COVID-19 NIH disease severity
1	BA.1	32	F		BNT162b2	69	106	mild
2	BA.1	42	M		BNT162b2/ mRNA-1273	26	81	mild
3	BA.1	42	M	Asthma	BNT162b2/ mRNA-1273	40	74	mild
4	BA.1	30	F	Pregnant	mRNA-1273/ mRNA-1273	59	52, 102	mild
5	BA.1	31	F		mRNA-1273/ mRNA-1273	67	13, 113	mild
6	BA.1	35	F	Pregnant	mRNA-1273/ mRNA-1273	21	12	mild
7	BA.1	59	F	HTN	BNT162b2/ BNT162b2	88	25, 109	mild
8	BA.1	35	F	Pregnant, Obesity	BNT162b2	287	17	mild
9	BA.1	39	M	Obesity	Ad26.COV2.S/ mRNA-1273	11	15, 102	mild
10	BA.1	24	F		BNT162b2/ BNT162b2	99	22	mild
11	BA.1	23	F		mRNA-1273/ Ad26.COV2.S	67	51, 94	mild
12	BA.1	50	F	DM, HTN	mRNA-1273/ mRNA-1273	6	8	asymptomatic
13	BA.1	59	M	DM	BNT162b2	210	9	severe
14	BA.1	22	F	Pregnant	Unvaccinated	N/A	4	severe
15	BA.1	35	F	Pregnant	mRNA-1273/ mRNA-1273	77	35	mild
16	BA.1	33	F	Pregnant	BNT162b2	228	2	mild
17	BA.1	27	M	Asthma	BNT162b2/ BNT162b2	101	13, 71	mild
18	BA.1	29	F	Asthma	mRNA-1273/ BNT162b2	15	14	mild
19	BA.1	50	F		mRNA-1273/ mRNA-1273	11	33	mild
20	BA.1	34	F	Lactating	BNT162b2/ BNT162b2	116	10, 54	mild
21	BA.1	34	F		mRNA-1273/ BNT162b2	42	16, 29, 91	mild
22	BA.1	32	F	Lactating, HTN	BNT162b2/ BNT162b2	102	18, 74	mild
23	BA.1	25	F	Pregnant	Sputnik V (Ad5/Ad26)/ mRNA-1273	239	32	mild
24	BA.1	36	F	Lactating	BNT162b2/ BNT162b2	113	41	mild
25	BA.1	25	F	DM	BNT162b2/ BNT162b2	164	12	mild
26	BA.2	41	M	HTN	BNT162b2/ BNT162b2	169	16	mild
27	BA.2	24	F		mRNA-1273/ mRNA-1273	149	16	mild

* >50% of sequences in New England (Region 1) according to CDC; PCR, polymerase chain reaction for SARS-CoV-2; NIH, National Institutes of Health; HTN, hypertension; DM, diabetes mellitus.