Safety and Immunogenicity of 3 Doses of BNT162b2 and CoronaVac in Children and Adults with Inborn Errors of Immunity

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Abstract

Background Safety and immunogenicity of 3 doses of BNT162b2 and CoronaVac in adult and pediatric patients with inborn errors of immunity (IEIs) remain unknown. Intradermal vaccination may improve immunogenicity in immunocompromised patients. Our study (NCT04800133) aimed to determine the safety and immunogenicity in patients with IEIs receiving a 3-dose primary series of mRNA vaccine BNT162b2 (age 12+) or inactivated whole-virion vaccine CoronaVac (age 3+) in Hong Kong, including Omicron BA.1 neutralization, in a nonrandomized manner. Intradermal vaccination was also studied. Methods Thirty-nine patients were vaccinated, including 16 with homologous intramuscular 0.3ml BNT162b2 and 17 with homologous intramuscular 0.5ml CoronaVac. Two patients received 3 doses of intradermal 0.5ml CoronaVac, and 4 patients received 2 doses of intramuscular BNT162b2 and the third dose with intradermal BNT162b2. Adverse reactions and adverse events were tracked for 7 and 28 days after each dose. Antibody responses assessed included binding IgG antibody to wild-type (WT) spike receptor-binding domain (S-RBD IgG) and surrogate neutralization activity to WT and BA.1 viruses. T cell responses were examined by intracellular cytokine staining following stimulation with SARS-CoV-2 peptide pool(s). Results No safety concerns were identified. Inadequate antibody responses were found after 2 doses in patients with humoral immunodeficiencies and especially so against BA.1. Dose 3 of either vaccine increased S-RBD IgG response. T cell responses against SARS-CoV-2 antigens were detected in vaccinated IEI patients. Intradermal third dose vaccine led to high antibody response in 4 patients. Conclusions The primary vaccination series of BNT162b2 and CoronaVac in adults and children with IEIs should include 3 doses for optimal immunogenicity.

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Running Title: BNT162b2 and CoronaVac in IEI patients

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SUMMARY

Background

Safety and immunogenicity of 3 doses of BNT162b2 and CoronaVac in adult and pediatric patients with inborn errors of immunity (IEIs) remain unknown. Intradermal vaccination may improve immunogenicity in immunocompromised patients. Our study (NCT04800133) aimed to determine the safety and immunogenicity in patients with IEIs receiving a 3-dose primary series of mRNA vaccine BNT162b2 (age 12+) or inactivated whole-virion vaccine CoronaVac (age 3+) in Hong Kong, including Omicron BA.1 neutralization, in a nonrandomized manner. Intradermal vaccination was also studied.

Methods

Thirty-nine patients were vaccinated, including 16 with homologous intramuscular 0.3ml BNT162b2 and 17 with homologous intramuscular 0.5ml CoronaVac. Two patients received 3 doses of intradermal 0.5ml CoronaVac, and 4 patients received 2 doses of intramuscular BNT162b2 and the third dose with intradermal BNT162b2. Adverse reactions and adverse events were tracked for 7 and 28 days after each dose. Antibody responses assessed included binding IgG antibody to wild-type (WT) spike receptor-binding domain (S-RBD IgG) and surrogate neutralization activity to WT and BA.1 viruses. T cell responses were examined by intracellular cytokine staining following stimulation with SARS-CoV-2 peptide pool(s).

Results

No safety concerns were identified. Inadequate antibody responses were found after 2 doses in patients with humoral immunodeficiencies and especially so against BA.1. Dose 3 of either vaccine increased S-RBD IgG response. T cell responses against SARS-CoV-2 antigens were detected in vaccinated IEI patients. Intradermal third dose vaccine led to high antibody response in 4 patients.

Conclusions

The primary vaccination series of BNT162b2 and CoronaVac in adults and children with IEIs should include 3 doses for optimal immunogenicity.

TRIAL REGISTRATION

Clinicaltrials.gov NCT04800133

INTRODUCTION

The COVID-19 pandemic has disproportionately affected patients with inborn errors of immunity (IEIs) and other forms of immune compromise and dysregulation, with higher mortality,[1,2] rare presentations such as multisystem inflammatory syndrome in children (MIS-C),[3] and delayed viral clearance reported.[4] Inborn errors of type I interferon pathway and autoantibody phenocopies have also been found to cause a substantial proportion of previously healthy patients with life-threatening COVID-19 pneumonia.[5,6]

mRNA and inactivated COVID-19 vaccines protect strongly against severe outcomes of COVID-19 at the population level.[7,8]Patients with IEIs or immune dysregulation likely have variable levels of vaccine efficacy, especially for those with defects in adaptive immunity. The initial licensing COVID-19 vaccine trials excluded patients with immunocompromise, driving the need for post-licensure studies to study the immunogenicity of COVID-19 vaccines in IEI patients.

Several studies have reported the immunogenicity of COVID-19 vaccines in IEI patients, but most only focused on 2 doses of COVID-19 mRNA vaccines in adult patients with IEI, mainly common variable immunodeficiency (CVID).[9-14] Inactivated vaccines against COVID-19 such as CoronaVac are extensively used worldwide with more than 4 billion doses distributed, and differ in their ability to induce antibody and T cell responses compared to mRNA vaccines.[15] Moreover, children and adolescents respond to SARS-CoV-2 infection and vaccination differently than adults,[16,17] and we previously reported that adolescents elicit higher antibody response to BNT162b2 and CoronaVac compared with adults.[15] Currently in Hong Kong, immunocompromised patients above the age of 5 are recommended to receive a 3-dose mRNA or inactivated vaccine primary series; or, for those aged 3-4, a 3-dose inactivated vaccine primary series, based on poorer immunogenicity and clinical vulnerability.[18]However, the immunogenicity of three doses of vaccine in patients with heterogeneous IEI remain unclear. In addition, intradermal vaccination of some vaccines such as seasonal flu vaccine has been trialed in immunocompromised patients or older adults, to enhance immunogenicity.[19] This represents a potential option for enhanced immunogenicity of COVID-19 vaccination in patients of IEI as well.

The Omicron BA lineage poses a public health threat with enhanced transmissibility and escape from virus neutralization,[20,21] reducing the effectiveness of COVID-19 vaccines against symptomatic disease but less so for severe outcomes.[7] While T cell epitopes are believed to be preserved,[22] how the neutralizing antibody response in IEI patients is affected by Omicron remains unknown.

To address these questions, we initiated a 3-year nonrandomized study (NCT04800133) to study the safety and immunogenicity of COVID-19 vaccines in children and adults receiving mRNA COVID-19 vaccine BNT162b2 or inactivated whole-virion vaccine CoronaVac in Hong Kong. In the present interim analysis, we focus on patients with IEI who received 3-doses of BNT162b2 (aged 12 and above) or CoronaVac (aged 3 and above). Adverse reactions (ARs) and adverse events (AEs) were monitored after each dose, and humoral and cellular immunogenicity against the wild-type (WT) SARS-CoV-2, as well as neutralization capacity against Omicron BA.1 in IEI patients were studied. Cases of Omicron BA.2 breakthrough infections were also described.

METHODS

Study Design. COVID-19 Vaccination in Adolescents and Children (COVAC; NCT04800133) is a non-randomized study aimed at investigating the immunogenicity of BNT162b2 and CoronaVac, in healthy children and immunocompromised patients as previously described.[15,23,24] The study was approved by the University of Hong Kong (HKU)/Hong Kong West Cluster Hospital Authority Institutional Review Board (UW21-157).

Participants. This prespecified interim analysis included patients aged 3 years and above who were diagnosed with IEIs and received at least one dose of COVID-19 vaccine. Participants with no known history of IEIs were excluded from this analysis.

Procedures. Potential participants were IEI patients diagnosed by the Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, The University of Hong Kong. YLL and JSRD obtained informed consent from participants aged 18 years or above. Underage participants provided informed assent, and consent was obtained from their parents or legally acceptable representatives. Patients were free to elect a homologous intramuscular primary series, a homologous intradermal primary series, or a heterologous primary series with 2 doses of intramuscular vaccine followed by intradermal dose 3. Three doses of 0.3ml BNT162b2 or 0.5 ml CoronaVac were administered via the intramuscular route to the deltoid or anterolateral thigh, or by an intradermal inoculator (MicronJet600, NanoPass Technologies, Nes Ziona, Israel) to the deltoid. Doses 2 and 3 were given at least 14 and 28 days after the preceding dose.

Safety data collection

Participants were observed by a study nurse or pediatrician for at least 15 minutes after vaccination. Participants reported prespecified adverse reactions (ARs) in an online or paper-based diary for 7 days after vaccination. Unsolicited adverse events (AEs) were captured for up to 28 days after vaccination. Severe AEs, involving hospitalizations, life-threatening complications, disabilities, deaths and birth defects of their offspring, or breakthrough COVID-19, would be monitored for 3 years after vaccination. Adverse events reported were reviewed by investigators, who determined any possibility of causal relationship with the study vaccine.

Immunogenicity

Immunogenicity outcomes were evaluated at baseline (pre-dose 1), post-dose 1 (pre-dose 2, 21-28 days after dose 1), post-dose 2 (28 days after dose 2), pre-dose 3 (at least 28 days after dose 2), and post-dose 3 (28 days after dose 3). Primary humoral immunogenicity outcomes include wild-type (WT) Spike receptor-binding domain (S-RBD) IgG ELISA and WT surrogate virus neutralization test (sVNT), and secondary outcomes include BA.1 sVNT. WT S-RBD IgG enzyme-linked immunosorbent assay (ELISA) were carried out as previously described and validated.[15,25] sVNT was conducted according to the manufacturer's instructions (GenScript Inc, Piscataway, USA) and as described in our previous publications.[25]IFN-γ^+ or IL-2^+CD4^+ or CD8^+ T cell responses were examined by intracellular cytokine staining after stimulation with SARS-CoV-2 S peptide pool (and N and M peptide pools for CoronaVac recipients) (Miltenyi Biotec, Bergisch Gladbach, Germany) as a primary cellular outcome as described previously.[15,24]Frequencies of T cell responses against S, N and M peptide pools are added together for CoronaVac recipients. Negative values, i.e., below the limit of detection (LOD), limit of quantification (LOQ) or cut-off, are imputed as half the limit/cut-off. All available results from IEI patients who received at least one dose, prior to the analysis, were presented. Additional details are available in the Supplemental Methods.

RESULTS

Participant composition

A total of 39 patients, aged between 5-51 years, received at least one dose of COVID-19 vaccine, including 16 with homologous intramuscular BNT162b2 vaccine (age 13-50 years); 17 with homologous intramuscular CoronaVac vaccine (age 5-51 years). Two elected to receive 3 doses of intradermal CoronaVac, and 4 received

2 doses of intramuscular BNT162b2 and a third dose being intradermal BNT162b2 injection. Patients had a range of IEIs, and their demographics and clinical characteristics are summarized in Table 1. None reported SARS-CoV-2 infection prior to immunization and all available baseline sera tested negative for S-RBD IgG.

TABLE 1. Participant profile.

Patient No.	Vaccine brand	Route	Disease category	Age	Sex	Diagnosis	Mutation	COVID-19 breakthrough	Current medications	ALC 10^9/L
1	BB	MM	Dysregulation	19	M	HIDS	AR *MVK*:c.928G>A,p.V310M	1 mo after dose 3	Anakinra, MMX	3.14
2	BB	MM	Humoral	29	M	XLA	XL *BTK*:c.1567-2A>T (splicing)	3 mo before dose 3	SCIG	1.55
3	BB	MM	Humoral	28	M	XLA	XL *BTK*:c.942A>G (splicing)	/	SCIG	1.25
4	BB	MM	Innate	28	M	STAT1 GOF	AD *STAT1*:c.800C>T,p.A267V	/	/	NA
5	BB	MM	Phagocytic	16	M	X-CGD, post-HSCT	XL *CYBB*:c.1437C>A ,p.Y479*	/	/	NA
6	BBB	MMD	Combined	16	F	STAT3 LOF	AD *STAT3*:c.2134T>C,p.C712R	/	IVIG	1.99
7	BBB	MMD	Dysregulation	26	M	XMEN	XL *MAGT1*:c.916delC,p.L306fs	/	IVIG	1.43
8	BBB	MMD	Humoral	13	M	XLA	XL *BTK*:c.1696C>T,p.P566S	/	IVIG	5.55
9	BBB	MMD	Innate	15	M	STAT1 GOF	AD *STAT1*:c.1170G>A,p.M390I	/	/	NA
10	BBB	MMM	Combined	21	M	AT	/	/	IVIG	0.92
11	BBB	MMM	Combined	49	M	XLT	XL *WAS*:c.134C>T,p.T45M	/	/	1.2
12	BBB	MMM	Dysregulation	18	F	HLH	/	/	IVIG, MMF, pred	0.52
13	BBB	MMM	Humoral	34	M	XLA	XL *BTK*:c.887delA,p.K296Sfs*35	1 wk after dose 3	IVIG	1.01
14	BBB	MMM	Humoral	13	M	XLA	XL *BTK*:c.1111T>C,p.S371P	/	IVIG	1.8
15	BBB	MMM	Humoral	30	M	XLA	XL *BTK*:c.41C>A,p.S14Y	/	IVIG	1.7
16	BBB	MMM	Innate	30	M	STAT1 GOF	AD *STAT1*:c.1151G>A,p.G384D	/	/	0.99
17	BBB	MMM	Innate	19	F	STAT1 GOF	AD *STAT1*:c.1074G>T,p.L358F	/	/	0.45

Patient No.	Vaccine brand	Route	Disease category	Age	Sex	Diagnosis	Mutation	COVID-19 breakthrough	Current medications	ALC 10^9/L
18	BBB	MMM	Phagocytic	50	F	X-CGD carrier	XL *CYBB*:c.469C>T,p.R157*	/	/	1.59
19	BBB	MMM	Phagocytic	23	M	X-CGD, post-HSCT	XL *CYBB*:c.1498G>C ,p.D500H	/	/	2.66
20	BBB	MMM	Phagocytic	14	M	X-CGD	XL *CYBB*:c.469C>T,p.R157*	/	/	2.17
21	CC	MM	Combined	13	M	XLT	XL *WAS*:c.116T>G,p.L39R	/	/	NA
22	CC	MM	Humoral	5	F	Agamma	/	2 wk after dose 1	IVIG	1.32
23	CC	MM	Humoral	11	M	XLA	XL *BTK*:c.3G>T,p.M1I	/	IVIG	2.58
24	CC	MM	Humoral	30	M	XLA	XL *BTK*:c.3G>T,p.M1I	/	IVIG	2.14
25	CC	MM	Phagocytic	10	F	AR-CGD, post-HSCT	AR *CYBA*:c.371C>T,p.A124V	/	/	2.05
26	CCC	DDD	Combined	15	M	X-SCID, post-HSCT	XL *IL2RG*:c.562C>T,p.Q188*	1 mo before dose 3	/	2.23
27	CCC	DDD	Innate	8	M	CARD9	AR het *CARD9*:c.586A>G,p.K196E and c.1526G>A,p.R509K	/	/	1.91
28	CCC	MMM	Dysregulation	8	M	CINCA	AD *NLRP3*:c.1711G>C,p.G571R	/	Canakinumab	NA
29	CCC	MMM	Dysregulation	15	M	CINCA	AD somatic mosaicism *NLRP3*:c.998G>T,p.S333I	1 mo after dose 3	MTX, canakinumab	1.6
30	CCC	MMM	Humoral	5	M	XLA	XL *BTK*:c.1559G>C,p.R520P	/	IVIG	2.7
31	CCC	MMM	Humoral	7	M	XLA	XL *BTK*:c.1559G>C,p.R520P	/	IVIG	2

Patient No.	Vaccine brand	Route	Disease category	Age	Sex	Diagnosis	Mutation	COVID-19 breakthrough	Current medications	ALC 10^9/L
32	CCC	MMM	Humoral	19	F	CVID	/	/	IVIG, HCQ, MMF, pred	2.14
33	CCC	MMM	Humoral	18	M	XLA	XL *BTK*:c.332T>C,p.L111P	/	IVIG	1.7
34	CCC	MMM	Humoral	32	M	XLA	XL *BTK*:c.41C>A,p.S14Y	/	IVIG	1.8
35	CCC	MMM	Humoral	14	M	XLA	XL *BTK*:c.947_948del,p.T316Sfs*6	/	IVIG	3.04
36	CCC	MMM	Humoral	15	M	XLA	XL *BTK*:c.1535T>C,p.L512P	3 mo after dose 3	IVIG	2.12
37	CCC	MMM	Humoral	34	M	XLA	XL *BTK*:EX2-EX3del	/	IVIG	2.7
38	CCC	MMM	Phagocytic	11	M	SCN	AD *ELA2*:c.362T>C,p.L121P	/	G-CSF	3.12
39	CCC	MMM	Phagocytic	51	M	SCN	AD *ELA2*:c.362T>C,p.L121P	/	/	NA

Current immunomodulatory medications, absolute lymphocyte count (ALC) and IgG level around the time of dose 1 are provided. B – BNT162b2, C – CoronaVac, M (route) – intramuscular, D – intradermal, M (sex) – male, F – female, HIDS – HyperIgD syndrome, XLA – X-linked agammaglobulinemia, X-CGD – X-linked chronic granulomatous disease, HSCT – hemopoietic stem cell transplant, X-MEN – X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia, AT – ataxia telangiectasia, XLT – X-linked thrombocytopenia, HLH – hemophagocytic lymphohistiocytosis, AR-CGD – autosomal recessive chronic granulomatous disease, CINCA - chronic infantile, neurologic, cutaneous, and articular syndrome, CVID – common variable immunodeficiency, SCN – severe congenital neutropenia, MTX – methotrexate, SCIG – subcutaneous immunoglobulin, IVIG, intravenous immunoglobulin, MMF – mycophenolate mofetil, pred – prednisolone, HCQ – hydroxychloroquine, G-CSF – granulocyte-colony stimulating factor, NA – not available.

Safety outcomes

As a primary safety outcome, we tracked ARs for 7 days and AEs for 28 days after each dose. IEI patients receiving both vaccines mainly reported mild and moderate ARs (Fig. 1). Severe ARs, e.g. pain at injection site and fatigue, were reported in some patients receiving BNT162b2 but not CoronaVac, in line with previous findings in healthy adolescents of milder reactogenicity with CoronaVac.[15] Eight AEs were reported within 28 days after BNT162b2 (n=4) or CoronaVac (n=4), including rash (n=3), chest discomfort (n=3), nodule in tongue (n=1) and lymphadenopathy (n=1), which were all of mild severity. One patient reported two non-fatal resolved severe AE (asthma exacerbation and poisoning respectively) 107 days after dose 2 and 73 days after dose 3 of BNT162b2. Both severe AEs were deemed not relevant to study vaccination.

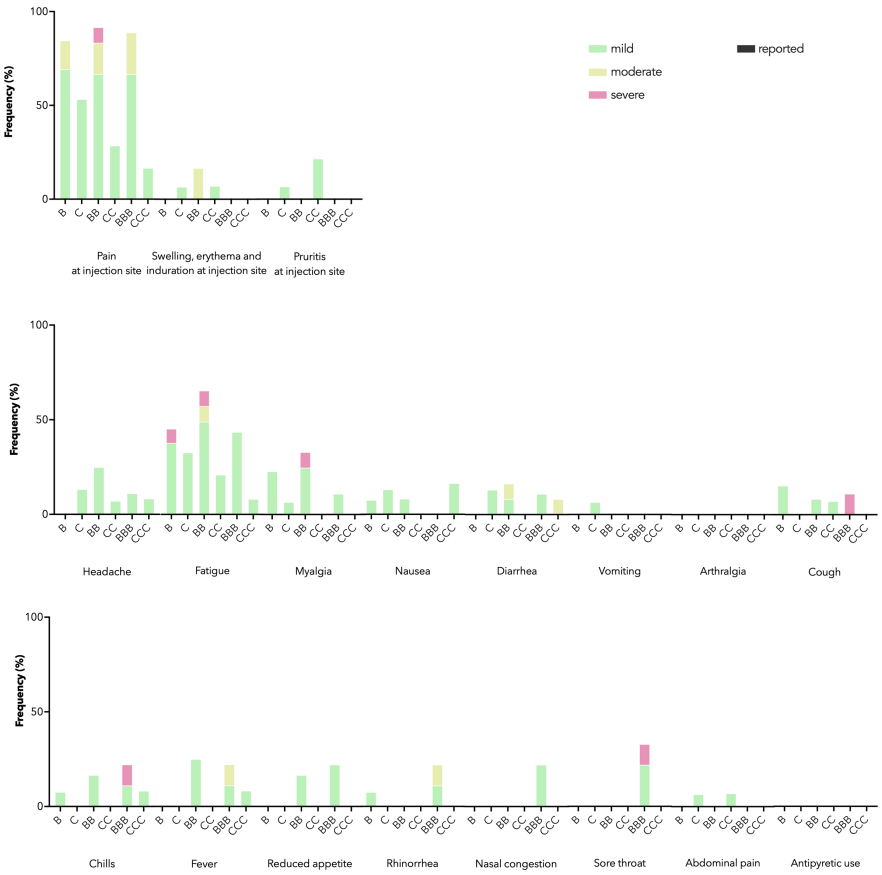


FIG. 1. Adverse reactions (ARs) and antipyretic use reported 7 days after each dose by vaccine brand (B or C). Stacked bar chart shows ARs by maximal severity in different colors.

S-RBD IgG and surrogate virus neutralization by disease category

Humoral immunogenicity outcomes including WT S-RBD IgG and WT sVNT were analyzed at post-dose 2 and post-dose 3 timepoints by disease category in Fig. 2A-D and listed in Table 2. Overall, 55% (18 out of 33 with available results) and 74% (17 out of 23) patients were seropositive (i.e., detectable S-RBD IgG) at post-dose 2 and post-dose 3 respectively. As expected, patients with humoral deficiency had the lowest geometric mean (GM) S-RBD IgG level (Fig. 2A) and sVNT inhibition (Fig. 2B) with 15% (2 of 13) seropositive at the post-dose 2 timepoint. A total of 15 patients, who had X-linked severe combined immunodeficiency (X-SCID) post-hematopoietic stem cell transplant (HSCT) (n=1), *STAT1* gain-of-function (n=1), severe congenital neutropenia (SCN, n=1), ataxia telangiectasia (n=1), and humoral immunodeficiencies (n=11), did not respond and were seronegative after 2 doses, suggesting these IEIs were associated with seroconversion failure (Fig. 2A). Notably, among those with a humoral deficiency, two brothers with X-linked agammaglobulinemia (XLA) seroconverted after dose 2. Six more patients with CVID (n=1) and XLA (n=5) also seroconverted after dose 3 (Fig. 2C), despite the modest level of neutralization attained (Fig. 2D). The highest neutralization responses were observed in patients with immune dysregulation with GM WT sVNT inhibition of 93.6% post-dose 3 (Fig. 2D).

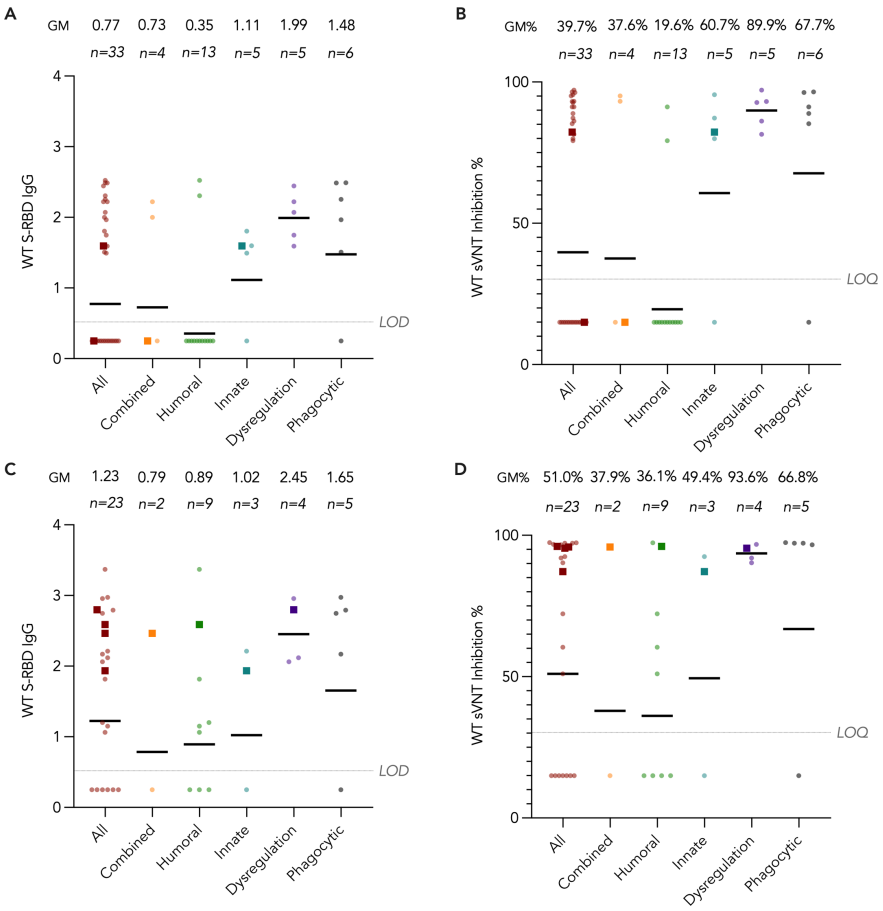


FIG. 2. Wild-type (WT) Spike-receptor binding domain (S-RBD) IgG and surrogate virus neutralization test (sVNT) results after COVID-19 vaccination by disease category. A and B show S-RBD IgG and sVNT results respectively in five disease categories (combined, humoral, innate, dysregulation and phagocytic) after 2 doses, while C and D show S-RBD and sVNT results after 3 doses. Geometric means (GM) are shown with centre lines and stated above each column. Limits of detection and quantification (LOD and LOQ) were drawn as grey lines. Number of analyzed available samples (n) are also stated above each column. A and B included 2 patients (one each in combined and innate) who received first 2 doses intradermally and their datapoints were shown as darkened squares. C and D also included 4 patients (1 each in combined, humoral, innate and dysregulation) who received their third dose intradermally and their datapoints were also shown as darkened squares.

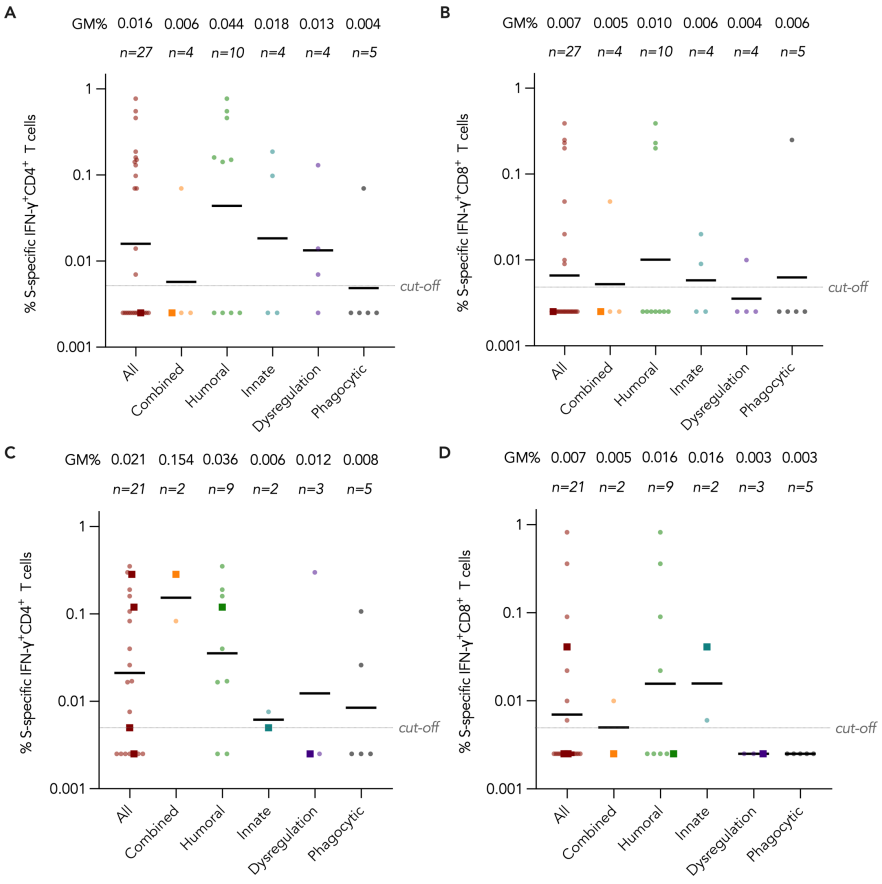
Table 2. Antibody responses in IEI patients. * denotes results samples taken after breakthrough infection. Negative S-RBD IgG, WT sVNT and BA.1 sVNT results were imputed as 0.25, 15 and 10 respectively. B – BNT162b2, C – CoronaVac, M (route) – intramuscular, D – intradermal.

Patient No.	Vaccine brand	Route	Disease category	S-RBD IgG post-dose 2	WT sVNT post-dose 2
1	BB	MM	Dysregulation	2.07	97.14
2	BB	MM	Humoral	0.25	15.00
3	BB	MM	Humoral	0.25	15.00
4	BB	MM	Innate	1.81	95.50
6	BBB	MMD	Combined	2.00	93.15
7	BBB	MMD	Dysregulation	2.22	92.72

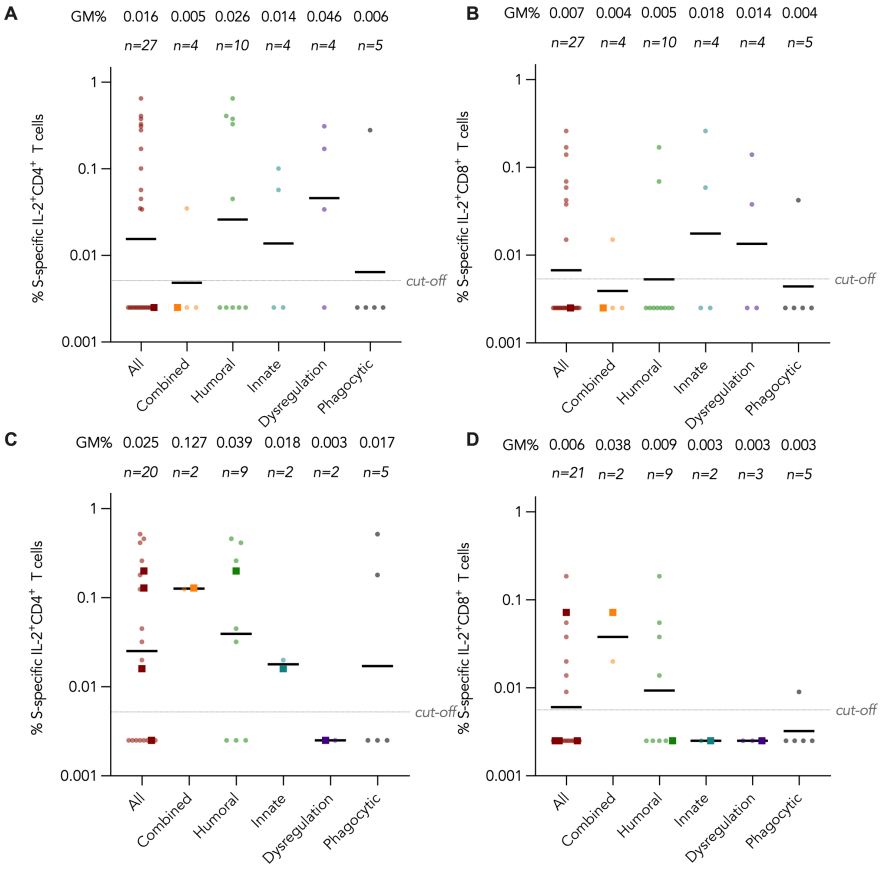
Patient No.	Vaccine brand	Route	Disease category	S-RBD IgG post-dose 2	WT sVNT post-dose 2
8	BBB	MMD	Humoral	/	/
9	BBB	MMD	Innate	1.60	87.19
10	BBB	MMM	Combined	0.25	15.00
11	BBB	MMM	Combined	2.22	95.06
12	BBB	MMM	Dysregulation	1.59	81.51
13	BBB	MMM	Humoral	0.25	15.00
14	BBB	MMM	Humoral	0.25	15.00
15	BBB	MMM	Humoral	0.25	15.00
16	BBB	MMM	Innate	1.49	79.97
17	BBB	MMM	Innate	0.25	15.00
18	BBB	MMM	Phagocytic	2.49	91.22
19	BBB	MMM	Phagocytic	1.97	96.51
20	BBB	MMM	Phagocytic	2.49	96.25
22	CC	MM	Humoral	0.25*	15.00*
23	CC	MM	Humoral	0.25	15.00
25	CC	MM	Phagocytic	2.26	85.24
26	CCC	DDD	Combined	0.25	15.00
27	CCC	DDD	Innate	1.59	82.25
28	CCC	MMM	Dysregulation	2.44	86.13
29	CCC	MMM	Dysregulation	1.75	93.07
30	CCC	MMM	Humoral	2.30	79.20
31	CCC	MMM	Humoral	2.52	91.19
32	CCC	MMM	Humoral	/	/
33	CCC	MMM	Humoral	0.25	15.00
34	CCC	MMM	Humoral	0.25	15.00
35	CCC	MMM	Humoral	0.25	15.00
36	CCC	MMM	Humoral	0.25	15.00
37	CCC	MMM	Humoral	0.25	15.00
38	CCC	MMM	Phagocytic	1.51	88.88
39	CCC	MMM	Phagocytic	0.25	15.00

T cell responses by disease category

IFN-γ^+ and IL-$2^+$$CD4^+$ and $CD8^+$ T cell responses were examined by intracellular cytokine staining after stimulation with SARS-CoV-2 S peptide pool (and N and M peptide pools for CoronaVac recipients) as primary cellular outcomes, as tabulated in Table 3. We analyzed S-specific IFN-γ^+ $CD4^+$ and $CD8^+$ T cell responses at post-dose 2 and post-dose 3 timepoints by disease category in Fig. 3A-D; results for S-specific IL-2^+ $CD4^+$ and $CD8^+$ T cell responses are shown in the Supp. Fig 1A-D. When all IEI patients were considered, 48% and 30% patients respectively had a positive S-specific IFN-$\gamma^+$$CD4^+$ and $CD8^+$ T cell after 2 doses, with low geometric mean frequencies of 0.016% and 0.007% (Fig. 3A-B). As T cell responses after vaccine were also variable in the healthy population, we were not powered to interpret T cell findings in disease categories with very small sample sizes. Patients with humoral deficiencies mounted a robust T cell response with a geometric mean frequency of 0.044% and 0.01% S-specific IFN-$\gamma^+$$CD4^+$ and $CD8^+$ T cell responses at the post-dose 2 timepoint. At the post-dose 3 timepoint, 62% and 33% of all patients showed a positive S-specific IFN-$\gamma^+$$CD4^+$ and $CD8^+$ T cell responses respectively. Patients with humoral deficiencies had S-specific IFN-γ^+ $CD4^+$ and $CD8^+$ T cell responses of 0.036% and 0.016%, respectively, at the post-dose 3 timepoint.



ΦΙΓ. 3. Ωιλδ-τψπε (ΩΤ) Σ πεπτιδε ποολ-σπεςιφις ιντερφερον-γ (ΙΦΝ-γ)$^+$ ˜Δ4$^+$ ανδ ˜Δ8$^+$ Τ ςελλς αφτερ ˜Ο˝ΙΔ-19 αςςινατιον βψ δισεασε ςατεγορψ. A and B show S-specific IFN-γ^+CD4$^+$ and CD8$^+$ results respectively in five disease categories (combined, humoral, innate, dysregulation and phagocytic) after 2 doses, while C and D show S-specific IFN-γ^+ CD4$^+$ and CD8$^+$ results after 3 doses. Geometric means (GM) are shown with center lines and stated above each column. Cut-offs were drawn as grey lines. Number of analyzed available samples (n) are also stated above each column. A and B included 1 patient (in combined) who received first 2 doses intradermally and their datapoints were shown as darkened squares. C and D also included 4 patients (1 each in combined, humoral, innate and dysregulation) who received their third dose intradermally and their datapoints were also shown as darkened squares.



SUPP FIG. 1. Wild-type (WT) S peptide pool-specific interleukin-2 (IL-2)$^+$ CD4$^+$ and CD8$^+$ T cells after COVID-19 vaccination by disease category. A and B show S-specific IL-2$^+$CD4$^+$ and CD8$^+$ results respectively in five disease categories (combined, humoral, innate, dysregulation and phagocytic) after 2 doses, while C and D show S-specific IL-2$^+$ CD4$^+$ and CD8$^+$ results after 3 doses. Geometric means (GM) are shown with centre lines and stated above each column. Cut-offs were drawn as grey lines. Number of analyzed available samples (n) are also stated above each column. A and B included 1 patient (in combined) who received first 2 doses intradermally and their datapoints were shown as darkened squares. C and D also included 4 patients (1 each in combined, humoral, innate and dysregulation) who received their third dose intradermally and their datapoints were also shown as darkened squares.

Table 3. S-specific and SNM-specific IFN-γ^+CD4 and CD8 T cell responses in IEI patients. * denotes results from samples taken after breakthrough infection. Negative S-specific and SNM-specific T cell results were imputed as 0.0025% and 0.0075% respectively. B – BNT162b2, C – CoronaVac, M (route) – intramuscular, D – intradermal.

Patient No.	Vaccine brand	Route	Disease category	Σ IΦN-γ$^+$ ˚Δ4 ποστ-δοσε 2 (%)	Σ IΦN-γ$^+$ *
1	BB	MM	Dysregulation	0.014	0.0025
2	BB	MM	Humoral	0.46	0.20
3	BB	MM	Humoral	0.14	0.0025
4	BB	MM	Innate	0.19	0.0025
6	BBB	MMD	Combined	0.070	0.0025
7	BBB	MMD	Dysregulation	0.0070	0.010
8	BBB	MMD	Humoral	/	/

Patient No.	Vaccine brand	Route	Disease category	Σ IΦN-γ$^+$ ˚Δ4 ποστ-δοσε 2 (%)	Σ IΦN-γ$^+$ ˚
9	BBB	MMD	Innate	0.0025	0.0090
10	BBB	MMM	Combined	0.0025	0.0025
11	BBB	MMM	Combined	0.0025	0.048
12	BBB	MMM	Dysregulation	0.13	0.0025
13	BBB	MMM	Humoral	0.55	0.39
14	BBB	MMM	Humoral	0.16	0.0025
15	BBB	MMM	Humoral	0.0025	0.0025
16	BBB	MMM	Innate	0.0025	0.020
17	BBB	MMM	Innate	0.098	0.0025
18	BBB	MMM	Phagocytic	0.0025	0.0025
19	BBB	MMM	Phagocytic	0.0025	0.0025
20	BBB	MMM	Phagocytic	0.0025	0.0025
26	CCC	DDD	Combined	0.0025	0.0025
29	CCC	MMM	Dysregulation	0.0025	0.0025
32	CCC	MMM	Humoral	/	/
33	CCC	MMM	Humoral	0.15	0.0025
34	CCC	MMM	Humoral	0.0025	0.0025
35	CCC	MMM	Humoral	0.0025	0.0025
36	CCC	MMM	Humoral	0.77	0.23
37	CCC	MMM	Humoral	0.0025	0.0025
38	CCC	MMM	Phagocytic	0.0025	0.0025
39	CCC	MMM	Phagocytic	0.070	0.25

Longitudinal analysis of antibody responses by vaccine brand and route, including Omicron BA.1 neutralization

We tracked these antibody and T cell responses longitudinally from pre-vaccine baseline to post-dose 3 by vaccine brand in Fig. 4 and 5. Very few (6 out of 30, 20%) participants seroconverted after a single dose of vaccine. Two doses of both vaccines significantly induced WT S-RBD IgG (both $P<0.01$) and WT sVNT (both $P<0.01$) (Fig. 4A-B). Bloods were additionally drawn at pre-dose 3 for patients who received dose 3 at least 70 days (mean 150 days) after dose 2, and significant decline of sVNT levels was observed after BNT162b2 only ($P=0.022$). WT S-RBD IgG levels were also significantly increased by a third dose of BNT162b2 ($P=0.037$) and increasing trends of WT S-RBD for CoronaVac and WT sVNT for both vaccines were also observed post-dose 3 compared to post-dose 2. Neutralization responses in those who received an intradermal third dose appeared high (all four >85% inhibition), and all were higher than the respective GM by vaccine brand and dose.

Neutralization capacity of patient sera against Omicron BA.1 was evaluated by sVNT which found most patients who received 2 doses of vaccine (89% for BNT162b2 and 100% for CoronaVac) could not neutralize BA.1 (Fig. 4C), indicating that BA.1 markedly evades neutralization in IEI patients. BA.1 sVNT levels were significantly increased by dose 3 of BNT162b2 ($P=0.043$) but not CoronaVac, although dose 3 CoronaVac did elicit BA.1 neutralization response in 1 out of 6 patients tested.

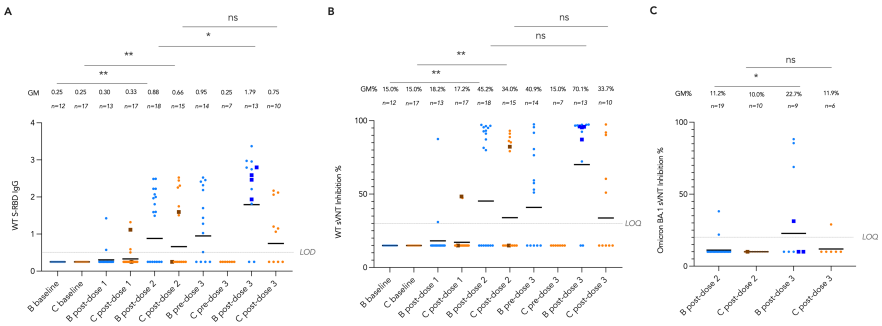
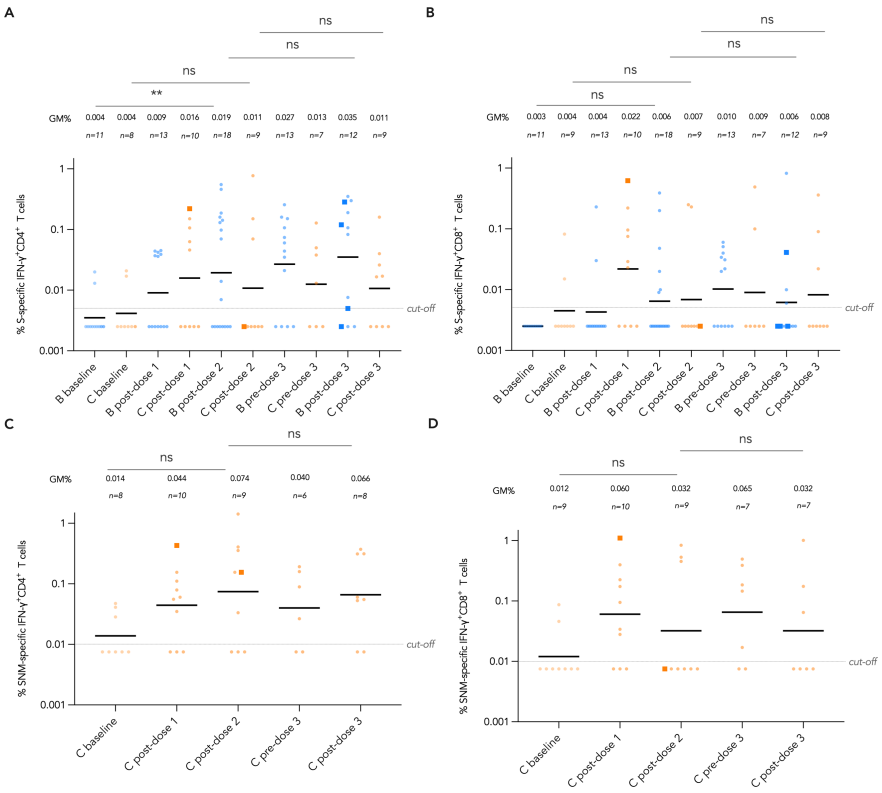


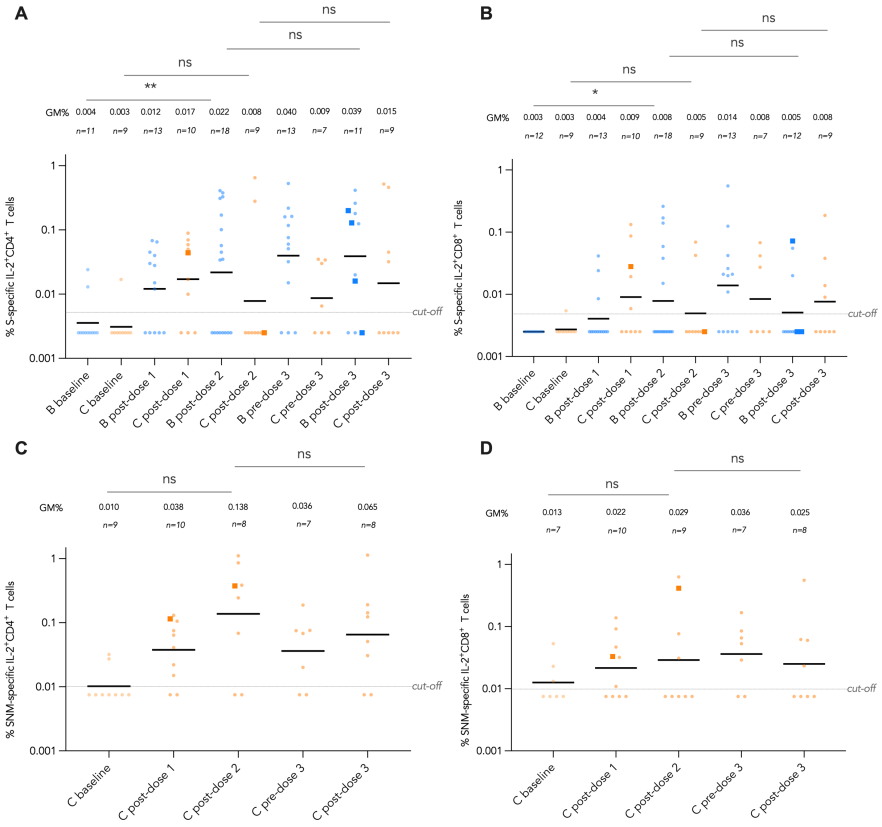
FIG. 4. Longitudinal analysis of A, wild-type (WT) Spike-receptor binding domain (S-RBD) IgG, B, WT surrogate virus neutralization test (sVNT) results, and C, Omicron BA.1 sVNT by vaccine type. Geometric means (GM) are shown with center lines and stated above each column. Limits of detection and quantification (LOD and LOQ) and cut-offs were drawn as grey lines. Data from participants receiving intradermal vaccination were shown as darkened squares beginning at their initial intradermal dose. Data from the same participant were analyzed longitudinally by paired t test after natural logarithmic transformation, and the P values are denoted by asterisks (*, P<0.05; **, P<0.01; ns, not significant).

Longitudinal analysis of T cell responses by vaccine brand and route

Longitudinal analysis of IFN-γ^+ CD4$^+$and CD8$^+$T cell responses against SARS-CoV-2 S peptide pool in BNT162b2 recipients and S, N and M peptide pools in CoronaVac recipients from baseline to post-dose 3 are shown in Fig. 5A-D. Responses were compared longitudinally between baseline and post-dose 2 by vaccine brand, as well as post-dose 2 with post-dose 3 by paired t test after natural logarithmic transformation. We only found weak increases that were statistically insignificant, except between baseline and post-dose 2 in BNT162b2 recipients for S-specific IFN-γ^+ CD4$^+$ T cells (P=0.0064; Fig. 5A). Analysis of IL-2$^+$ CD4$^+$ and CD8$^+$ T cells also yielded similar findings (Supp. Fig. 2A-D). Four patients received an intradermal dose 3 appeared to have a similar distribution with patients who received intramuscular vaccination.



ΦΙΓ. 5. Λονγιτυδιναλ αναλψσις οφ ωιλδ-τψπε (ΩΤ) Σ ανδ Σ, Ν ανδ Μ προτειν πεπτιδε ποολ-σπεσιφις ιντερφερον-γ (ΙΦΝ-γ)$^{+}$˜Δ4^{+} ανδ ˜Δ8^{+} Τ ςελλς βψ αςςινε βρανδ ανδ ρουτε. S-specific IFN-γ^{+}CD4^{+} and CD8^{+} T cell responses are shown in A and B respectively for both BNT162b2 and CoronaVac recipients, while added SNM-specific IFN-γ^{+}CD4^{+} and CD8^{+} T cell responses are shown in C and D for CoronaVac recipients. Samples from the same patient were paired between baseline and post-dose 2 timepoints as well as post-dose 2 and post-dose 3 timepoints, and compared with paired t test after natural logarithmic transformation with p-values denoted (**, P<0.01; ns, not significant).



SUPP FIG. 2. Longitudinal analysis of wild-type (WT) S and S, N and M protein peptide pool-specific IL-2 (IL-2)$^+$CD4$^+$ and CD8$^+$ T cells by vaccine brand and route. S-specific IL-2$^+$CD4$^+$ and CD8$^+$ T cell responses are shown in A and B respectively for both BNT162b2 and CoronaVac recipients, while added SNM-specific IL-2$^+$CD4$^+$ and CD8$^+$ T cell responses are shown in C and D for CoronaVac recipients. Samples from the same patient were paired between baseline and post-dose 2 timepoints as well as post-dose 2 and post-dose 3 timepoints, and compared with paired t test after natural logarithmic transformation with p-values denoted (*, P<0.05; **, P<0.01; ns, not significant).

Breakthrough COVID-19 cases

Breakthrough COVID-19 reported by participants would be monitored for 3 years after vaccination. In early 2022, Hong Kong had its first major wave of COVID-19, dominated by the Omicron BA.2.2 variant. Seven participants, in the humoral (n=5), combined (n=1) and dysregulation (n=1) categories, had reported COVID-19 during that time after a partial or complete primary series (Table 1). All participants reported mild infections without need for hospitalization. Contingency analyses by Fisher exact test showed that proportion of infected participants did not differ by vaccine brand (B 15% vs C 21%, P=0.69), age group (adults 15% vs children 21%, P=0.69) or disease category (humoral 29% vs non-humoral 9%, P=0.21). Immunogenicity assessments were available for 3 of them pre- and post-infection (patients 13, 22, and 26), all of whom were seronegative for S-RBD IgG both immediately before and after infection (Tables 2 and 3).

DISCUSSION

Studying immunogenicity to COVID-19 vaccines enables us to understand protection conferred by vaccination on IEI patients. Three doses of BNT162b2 and CoronaVac were well-tolerated by our pediatric and adult IEI patients. Antibody responses to COVID-19 vaccines were found to be lowest in patients with humoral deficiencies, yet non-responders were also found in other IEIs not affecting adaptive immunity. Antibody

responses were enhanced by a third dose of vaccine, especially cross-neutralization against SARS-CoV-2 variant Omicron. T cell responses were detected in many patients after vaccination, yet there is heterogeneity in responses. Patients who received intradermal vaccination appeared in general to have higher antibody levels but had similar T cell response, though sample sizes were small. Breakthrough infections with BA.2 were mild in vaccinated IEI patients.

Antibody responses were a primary outcome measured in many COVID-19 vaccine studies, including this study, as antibody responses have been shown to correlate with protection against symptomatic COVID-19.[26] Our finding of seroconversion failure in 45% patients after 2 doses of COVID-19 vaccines, which was reduced to 26% after a third dose, strongly support the need for a 3-dose primary series in patients with IEIs. Another study also found seroconversion failure was reduced from 39% to 24% after a third dose in a heterogeneous cohort of IEI patients with mostly humoral immunodeficiencies.[27] Many of these IEI patients, especially those with humoral deficiencies, mounted a detectable T cell response to COVID-19 vaccines. While understudied and controversial in the virology and vaccinology fields,[28] adaptive immunity against severe viral illnesses, except for enteroviruses, depends on T cells rather than B cells, as exemplified by recurrent and life-threatening viral infections in patients with combined immunodeficiencies but not in those with agammaglobulinemia.[29] The presence of both common seroconversion and T cell response in triple-vaccinated IEI patients suggests 3 doses may be adequate for primary series in IEI patients in general. A fourth dose is likely required a few months after the primary series as a booster, dependent on circulation of SARS-CoV-2 in the community, degree of antibody waning, and potency of cellular memory.

Although numbers of participants in each IEI category was small, trends could be observed. For example, those with humoral immunodeficiencies developed the lowest S-RBD IgG and sVNT after 2 vaccine doses, seroconversion failure was also found in a patient with *STAT1* gain-of-function after 2 doses and another with SCN after 3 doses of vaccine as well. Both patients did not undergo HSCT, and neither was on immunosuppressive medications. While functional antibody deficiency is known to associate with *STAT1* gain-of-function,[30] impairment of humoral immune response in phagocytic disorders is not well delineated. Within our study, 5 out of 6 enrolled patients with phagocytic disorders (2 with SCN, 2 with chronic granulomatous disease, CGD, and 1 X-linked CGD carrier) did not seroconvert to a single dose of BNT162b2 (n=3) or a single dose of CoronaVac (n=2), which contrasts with the 100% seroconversion to a single dose of BNT162b2 in healthy adolescents.[15] Our findings suggest a partially impaired B cell response to vaccines in patients with phagocytic disorders, which may be rescued by dose 2 or more additional doses. While conventional knowledge dictates vaccine response may only be impaired in patients with adaptive defects, seroconversion failure may be found in patients with different IEIs, and patients with any specific IEI should be recommended to complete the 3-dose primary series with booster vaccination, irrespective of disease category.

CoronaVac is widely used globally, and this is likely to include patients with IEI patients as well, yet little data have been published on IEI patients. Studies in adult patients with secondary immunodeficiencies or immune dysregulation disorders showed reduced antibody responses to 2 doses of CoronaVac.[31-33] S-RBD IgG and sVNT results in our study showed 3 doses of CoronaVac elicited antibody response in IEI patients. Eleven of 17 patients with humoral deficiencies in our study opted for CoronaVac. Whole-virion inactivated vaccines could elicit T cell response against other structural proteins such as N and M unelicited by S-only mRNA vaccines,[15,34] which correlate with protection against severe disease and infection,[35,36] and are not susceptible to mutations in Omicron.[24] In our patients with humoral immunodeficiencies and no breakthrough COVID-19, 4 out of 5 patients in our study had a detectable SNM-specific IFN-γ^+ $CD4^+$ T cell response after just 2 doses of CoronaVac; the single patient who did not have a detectable IFN-γ^+ $CD4^+$ T cell response had an IL-2^+ $CD4^+$ T cell response. The effectiveness of CoronaVac in IEI patients was further supported by the 4 patients who received CoronaVac and experienced a mild breakthrough COVID-19. Our results support that CoronaVac is safe and effective in IEI patients.

We hypothesized intradermal vaccination may elicit better antibody and T cell responses in IEI patients. While there seemed to be no appreciable difference with T cell response of intradermal vaccination in IEI

patients, we found a trend toward higher antibody responses in intradermal vaccinees. Additional studies involving healthy children or IEI patients may confirm our findings. We also examined the immunogenicity outcomes in patients receiving intradermal vaccination on a case-by-case basis as "n-of-1 trials". Strikingly, one patient, who is a 15-year-old boy with post-HSCT X-SCID, did not seroconvert after receiving 3 doses of intradermal CoronaVac and contracting COVID-19 during the Omicron BA.2-dominant period in Hong Kong. Chart review revealed that the patient had a successful allogenic hematopoietic stem cell transplant with a 10/10 matched unrelated donor and conditioning regimen of fluradabine, melphalan, anti-thymocyte globulin and rituximab by the age of 1 year, resulting in 51% donor chimerism in the peripheral blood and him being generally healthy without immunoglobulin replacement. Last follow-up showed normal T and B cell counts (1829 cells/ul and 406 cells/ul) and normal IgG level (1289 mg/dl). The patient also previously seroconverted to intramuscular vaccines with seropositivity for measles, mumps, rubella, and tetanus. He has been on penicillin prophylaxis after splenectomy at age of 1 year as disseminated BCGosis involved his spleen. Interestingly, 2 years prior to COVID-19 vaccination, the patient developed cryotherapy-resistant verruca vulgaris on his right hand, a known complication after HSCT in X-SCID patients. That led us to hypothesize that seroconversion failure to intradermal vaccine is also due to mutated keratinocytes in the skin, with impaired chemotactic functions, not corrected by HSCT.[37,38] This finding suggests that while intradermal vaccination may enhance seroconversion in most immunocompromised vaccinees, X-SCID patients who underwent HSCT may not benefit from intradermal vaccination.

Our study had several strengths and limitations. In addition to antibodies, we also studied T cell responses, which protect against disease progression. We were able to track both antibody and T cell responses sequentially in vaccinees from pre-vaccine to post-dose 3. The utility of longitudinal antibody testing in predicting infections deserves to be studied in larger multi-cohort studies. Our sample size was limited due to rarity of IEIs, yet studies from other centers could corroborate our findings. We could not assess clinical effectiveness.

In conclusion, our findings support the need for 3 doses of mRNA or inactivated COVID-19 vaccines for IEI patients and this should be regarded as the primary series of vaccination. Future studies should focus on the longevity of immune response and effect of a fourth dose and hybrid immunity in these patients.

SUPPLEMENTAL METHODS

S-RBD IgG and surrogate virus neutralization test (sVNT)

The SARS-CoV-2 S receptor-binding domain (S-RBD) IgG enzyme-linked immunosorbent assay (ELISA) were carried out as previously described and validated.[15,25] sVNT was conducted according to the manufacturer's instructions (GenScript Inc, Piscataway, USA) and as described in our previous publications.[25,39] All sera were heat-inactivated at 56° C for 30 minutes before testing.

In brief, S-RBD IgG ELISA plates were coated overnight with 100 ng/well of purified recombinant S-RBD in PBS buffer, followed by addition of 100 µL Chonblock Blocking/Sample Dilution (CBSD) ELISA buffer (Chondrex Inc, Redmond, USA). This was incubated at room temperature (RT) for 2 hours. Serum was tested at a dilution of 1:100 in CBSD ELISA buffer, then added to the wells for 2 hours at 37°C. After washing with PBS containing 0.1% Tween 20, horseradish peroxidase (HRP)-conjugated goat anti-human IgG (1:5,000) (GE Healthcare, Chicago, USA) was added for 1 hour at 37°C, followed by washing five times with PBS containing 0.1% Tween 20. HRP substrate (Ncm TMB One, New Cell & Molecular Biotech Co. Ltd, China) of 100 µL was added for 15 minutes, and the reaction was stopped by 50 µL of 2 M H_2SO4. The OD was analysed in a Sunrise absorbance microplate reader (Tecan, Männedorf, Switzerland) at 450 nm wavelength. The background OD in PBS-coated control wells with the participant's serum was subtracted from each OD reading. Values at or above an OD450 of 0.5 were considered positive and values below were imputed as 0.25.

The sVNT was performed using 10 µL of each serum, positive and negative controls, which were diluted at 1:10 and mixed with an equal volume HRP conjugated to the WT or BA.1 SARS-CoV-2 S-RBD (6 ng). The mixture was incubated for 30 minutes at 37°C, then 100 µL of each sample was added to microtitre plate wells coated with angiotensin-converting enzyme-2 (ACE-2) receptor. This plate was sealed for 15 minutes at

37°C and then washed with wash-solution, tapped dry, and 100 μL of 3,3',5,5'-tetramethylbenzidine (TMB) was added and incubated in the dark at RT for 15 minutes. This reaction was stopped with 50 μL of Stop Solution and the absorbance at 450 nm was detected by a microplate reader. The % inhibition of each serum was calculated as (1 - sample OD value/negative control OD value) x100%. Inhibition (%) of at least 30%, the limit of quantification (LOQ), was regarded as positive, and values below 30% were imputed as 15%.

T cell responses

Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient separation then frozen in liquid nitrogen until use. Thawed PBMCs were rested for 2 hours in 10% human AB serum supplemented RPMI medium. Next, the cells were stimulated with sterile ddH2O or 1 μg/mL overlapping peptide pools representing the WT SARS-CoV-2 S, N and M proteins (Miltenyi Biotec, Bergisch Gladbach, Germany) for 16 hours in the presence of 1 μg/mL anti-CD28 and anti-CD49d costimulatory antibodies (clones CD28.2 and 9F10, Biolegend, San Diego, USA). After 2 hours of stimulation, 10 μg/mL brefeldin A (Sigma, Kawasaki, Japan) was added.[40] The cells were then washed and subjected to immunostaining using a fixable viability dye (eBioscience, Santa Clara, USA, 1:60) and antibodies against CD3 (HIT3a, 1:60), CD4 (OKT4, 1:60), CD8 (HIT8a, 1:60), IFN-γ (B27, 1:15) and IL-2 (MQ1-17H12, 1:15) antibodies (Biolegend, San Diego, USA). Data acquisition was carried out using flow cytometry (LSR II; BD Biosciences, Franklin Lakes, USA) and analyzed by Flowjo v10 software (BD, Ashland, USA). The antigen-specific IFN-γ^+ or IL-2^+ T cells were calculated by subtracting the background (sterile ddH2O) data, and presented as the percentage of $CD4^+$ or $CD8^+$ T cells.[41] T cell response against a single peptide pool was considered positive when the frequency of cytokine-expressing cells was higher than or equal to 0.005% and the stimulation index was higher than 2; negative values were imputed as 0.0025%. Total T cell responses against S, N and M peptide pools were also added together, with a cut-off of 0.01%.

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STATEMENT OF CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

FUNDING

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STATEMENT OF CONTRIBUTION

Y.L. Lau conceptualized the study. Y.L. Lau, M. Peiris, W. Tu, D. Leung, J.S. Rosa Duque, and X. Wang designed the study. Y.L. Lau led the acquisition of funding. Y.L. Lau, W. Tu, and M. Peiris supervised the project. S.M. Chan, D. Leung, X. Mu, S.M.S. Cheng, I.Y.S. Tam, and J.H.Y. Lam led the study administrative procedures. W.H.S. Wong provided software support. S.M. Chan, and W.H.S. Wong contributed to recruitment of participants. Y.L. Lau and J.S. Rosa Duque provided study-related clinical assessments and follow-up. D. Leung, S.M. Chan, C.H. Cheang, J.H.Y. Lam, J.S. Rosa Duque, and Y.L. Lau collected safety data. S.M.S. Cheng, S. Chaothai, L.C.H. Tsang, and M. Peiris developed and performed S-RBD IgG, and sVNT. X. Mu, Y. Zhang, M. Wang, W. Zhang, Y. Chung, H.H.W. Wong, A.M.T. Lee, W.Y. Li, X. Wang, and W. Tu developed and performed the T cell assays. D. Leung, T.S.S. Lee, and J.H.Y. Lam curated and analyzed the data. D. Leung and J.H.Y. Lam visualized the data. D. Leung, X. Mu, S.M.S. Cheng, J.S. Rosa Duque, J.H.Y. Lam, and S.M. Chan validated the data. J.S.R. Duque, G.T. Chua, K.N.

Cheong, E.Y.L. Au, J.S.Y. Kwok, P.C.Y. Chong, P.P.W. Lee, M.H.K. Ho, T.L. Lee, and Y.L. Lau provided clinical care. D. Leung wrote the first draft supervised by Y.L. Lau, with input from J.S. Rosa Duque, X. Mu, and S.M.S. Cheng. All authors reviewed and approved the final manuscript.

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