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Letter

Repeated vaccination against SARS-CoV-2 elicits robust polyfunctional T cell response in allogeneic stem cell transplantation recipients

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SARS-CoV-2 has led to unprecedented global healthcare challenges, with poor outcomes observed in groups with immune deficiency, including allogeneic stem cell transplantation (allo-SCT) recipients (Bakouny et al., 2020). T cell and B cell responses following vaccination against SARS-CoV-2 are important in reducing the risk of severe COVID-19, but the T cell response has not been extensively investigated in this population. We designed a prospective study to evaluate response to vaccination in patients with hematologic malignancies. Herein we report analysis of T cell and humoral response to sequential dosing of vaccination against SARS-CoV-2 in allo-SCT recipients.

Anti-SARS-CoV-2 Spike protein (S) IgG ELISA and neutralizing antibody testing were performed as described previously. The induction of virus-specific T cell responses by vaccination was assessed by flow-cytometric enumeration of antigen-specific $CD8^+$ and $CD4^+$ T lymphocytes using an intracellular cytokine assay for IFNγ and TNFα.

A total of 23 patients were analyzed at one or more time point around the two-dose vaccination schedule (Table S1). Median age was 55 years (range 25–74), and 69.6% (16) were male. Median time from allo-SCT was 55 months (19–172), and BNT162b2 vaccine was given to 81% (21) of patients, while others received ChAdOx1-S.

Following a first dose of vaccine, an anti-S IgG response was assessed in 18 patients at a median of 4.2 weeks after vaccination. Anti-S IgG was detectable in only 38.9% (7), with 4 of these having weak positive results (Figure S1A). A mean anti-S IgG EC_{50} of 76 (range 0–526) was observed at this time point (Figure S1B). Neutralizing antibody analysis was performed in all 7 patients with detectable anti-S IgG at this time point, with a mean ID50 of 292 observed (32–968) (Figure S1C).

Antibody testing was performed in 16 patients following two doses of vaccine, at a median of 12 weeks after the second dose. A detectable anti-S IgG was observed in 81% (13) of patients ($p \leq 0.017$) (Figure S1A), with a mean anti-S IgG of 1043 (0–5594) ($p = 0.025$) (Figure S1B). Neutralizing antibody testing performed in 13 patients with detectable IgG showed a mean ID50 of 747 (107–4707) (Figure S1C). After two doses of vaccine, antibody testing was performed in 10 patients with chronic graft-versus-host disease (GvHD) receiving extracorporeal photopheresis (ECP) and 6 patients not receiving ECP, with a mean EC_{50} of 574 in ECP group, compared with 1826 non-ECP ($p = 0.17$). Similarly, mean neutralizing antibody ID50 was 312 in those requiring ECP compared with 719 in non-ECP. There was a significant correlation between anti-S IgG level and neutralizing ability from paired samples, with r value of 0.83 ($p < 0.0001$) (Figure S1D).

T cell analysis was performed in 17 patients after a single dose of vaccine and in 17 patients after two doses. A T cell response was observed in 35.3% (6) of patients after one dose and in 82.3% (14) of patients after two doses ($p = 0.013$) (Figure S1E). A $CD4^+$ T cell response was observed in 29.4% (5) of patients after one dose and 70.6% (12) of patients after two doses ($p = 0.0.38$), while a $CD8^+$ T cell response was only seen in 17.6% (3) after one dose but 52.3% (9) after two doses ($p = 0.07$). Mean $CD4^+/CD8^+$ TNFα expression after a single dose was 0.12%/0.04%, which increased to 0.42%/0.13% after second dose ($p = 0.17/0.3$). Similarly, mean $CD4^+/CD8^+$ IFNγ expression after a single dose was 0.06%/0.03%, which again increased to 0.07%/0.17% ($p = 0.8/0.1$).

A polyfunctional T cell response, with dual expression of more than one proinflammatory cytokine within the same cell, was observed in 29.4% (5) of patients after one dose and 70.6% (12) after two doses ($p = 0.038$) (Figures S1F and S1G). After a single dose, mean $CD4^+$ polyfunctional T cell response was 0.009%, with an increase to 0.026% after 2 doses ($p = 0.068$) (Figure S1H). Consistently, more than 90% of reactive T cells expressing pro-inflammatory cytokines showed co-expression of CD45RO, a surface protein marker for memory T cells. After a second dose, patients with chronic GvHD requiring ECP had a mean $CD4^+$ TNFα expression of 0.18% compared with 0.86% in those not requiring ECP ($p = 0.09$) (Figure S1I).

Patients with prior allo-SCT who contract COVID-19 infection have poor outcomes, with overall survival reported at 68% at 30 days post diagnosis (Sharma

et al., 2021). Therefore, the development of immunity is particularly important in this patient group. We have previously reported that a single dose of BNT162b2 is sufficient to generate both a humoral and a T cell response in most patients with chronic myeloid malignancies (Harrington et al., 2021). This is in contrast to the response observed in many cancer-patient groups, particularly those with lymphoid malignancies who have received anti-CD20 targeted therapy (Addeo et al., 2021, Greenberger et al., 2021, Thakkar et al., 2021). We demonstrate here how a second dose is required for a significant increase in seroconversion rates and detectable memory T cells in allo-SCT recipients. Through analysis of samples at consecutive time points, including sequential samples from the same patients, we were able to observe the longitudinal response to vaccination and show that a second dose is required for adequate immunogenicity in this population. Our findings are in keeping with that from two studies on isolated antibody responses in allo-SCT patients which reported an anti-S IgG response after a second injection in 83% and 78% of participants, respectively (Redjoul et al., 2021, Le Bourgeois et al., 2021).

Our data report the T cell response to SARS-CoV-2 vaccination in patients with previous allo-SCT. Despite a poor T cell response after a first vaccine injection, a second dose elicited anti-S reactive T cells in most patients. Moreover, a polyfunctional T cell response was also elicited by a second dose, which may have particular functional relevance with regards to anti-viral immunity, with these cells recognized as providing a more effective anti-viral response in the context of COVID-19 infection (Peng et al., 2020). A memory T cell response may play a particularly important role in providing immunity to COVID-19, as studies have shown significant decline in antibody levels in the general population at 3 months post natural infection (Seow et al., 2020).

We have also focused our analysis on patients considered to be particularly immune suppressed with regards to chronic GvHD and ongoing systemic immune suppression. While these patients did show a reduced T cell and antibody response when compared with patients off immune suppression, this was not significant, and most showed an adequate neutralizing antibody response after a second injection. Our study is, however, limited by small sample size, and further longitudinal data are required to evaluate whether the response generated is adequate to provide anti-viral protection.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2021.10.002.

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AUTHOR CONTRIBUTIONS

P.H. and H.d.L. designed the research, performed the research, analyzed the data, and wrote the manuscript. K.J.D., T.L., H.K., and M.M. performed the research and reviewed the manuscript. F.C., R.D., S.S., K.R., D.M., D.A., , DA, S.K., C.H., and V.M. assisted with patient recruitment and reviewed the manuscript. C.S., J.S., A.O.R., and A.E. assisted with patient recruitment and patient interviews and reviewed the manuscript. V.M. and H.d.L. share senior authorship.

DECLARATION OF INTERESTS

P.H. received research funding from Bristol Myers Squibb and speaker fees from Incyte. D.M. received speaker fees and advisory boards Novartis, Celgene, and Jazz pharmaceuticals. S.K. received Celgene and Novartis research grant and Alexion speaker honorarium. C.H. received speaker fees from Novartis, Jannsen, CTI, Celgene, and Medscape and has served on the advisory board for Incyte, CTI, Sierra Oncology, Novartis, Celgene, Roche, AOP Pharma, Geron, and Astra Zenica. H.d.L. has received grants and speakers fees from Bristol Myers Squibb and Incyte and speaker fees from Novartis.

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Supplemental information

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Table S1. Patient Characteristics and Antibody Response Pre-Vaccine and Post 1st and 2nd Vaccine Doses.

Patient number	Age / Sex	Diagnosis	Time from SCT	GvHD	ECP	Systemic IST	Anti-N IgG OD	Anti-S IgG – Pre	Anti-S IgG – 1st	Neut. Ab – 1st	Anti-S IgG - 2nd	Neut. Ab – 2nd
01	58 / M	MDS	38	Chronic	Yes	CsA	1.5	<25	<25	ND	<25	ND
02	65 / M	MDS	19	Chronic	Yes	CsA	0.6	<25	<25	ND	<25	ND
03	29/ F	B-ALL	19	Resolved	Yes	Nil	0.8	<25	<25	ND	30	222
04	59 /M	AML	68	Chronic	Yes	Nil	0.7	-	-	-	2566	782
05	55 / F	MPAL	59	Chronic	Yes	Pred, MMF, Rux	-	<25	<25	ND	-	-
06	34 / F	AML	92	Chronic	Yes	Nil	0.8	-	-	-	811	303
07	58 / M	AML	70	Chronic	Yes	Hydrocort.	1.0	<25	424	968	856	339
08	71 / M	MDS	32	Chronic	Yes	Nil	-	<25	<25	ND	-	-
09	68 / F	AML	24	Chronic	Yes	Nil	1.4	<25	<25	ND	<25	ND
10	51 / F	T-ALL	74	Chronic	Yes	Nil	1.4	25	25	155	-	-
11	41 / M	AML	59	Chronic	Yes	MMF	1.4	<25	25	53	1012	791
12	58 / M	AML	26	Chronic	Yes	MMF	0.8	-	<25	ND	67	162
13	54 / M	MDS	33	Chronic	Yes	CsA	1.0	-	25	32	396	494
14	53 / F	AML	68	Chronic	Yes	Pred, MMF	-	-	<25	ND	-	-
15	58 / M	AML	57	Chronic	No	MMF, SYK inh.	1.1	-	312	212	5594	1007
16	25 / M	AA	23	Nil	No	Tacro, MMF	2.6	<25	526	533	-	-
17	60 / M	FL	55	Resolved	No	Nil	-	-	<25	ND	-	-
18	74 / M	AML	54	Nil	No	Nil	1.1	-	<25	ND	82	176
19	49 / M	MF	22	Resolved	No	Nil	-	-	-	-	-	-
20	48 / M	CML, CP	77	Nil	No	Nil	1.6	-	-	-	4707	2404
21	58 / M	CML, BP	64	Nil	No	Nil	0.9	-	<25	ND	521	469
22	43 / M	PRCA	172	Nil	No	Nil	1.4	-	-	-	25	153
23	43 / F	AML	20	Nil	No	Nil	1.1	-	-	-	25	107

ECP: extracorporeal photopheresis; IST: immunosuppressive therapy; OD – optical density, MDS – myelodyplastic syndrome, B-ALL – B/T ALL – B/T cell acute lymphoblastic leukaemia, MPAL – mixed phenotype acute leukaemia, AML – acute myeloid leukaemia, AA – aplastic anaemia, FL – follicular lymphoma, MF – myelofibrosis, CML – chronic myeloid leukaemia,
PRCA – pure red cell aplasia, CsA – ciclosporin A, Pred – prednisolone, MMF – mycophenolate mofetil, Rux – ruxolitinib, Tacro – tacrolimus, Imat – imatinib

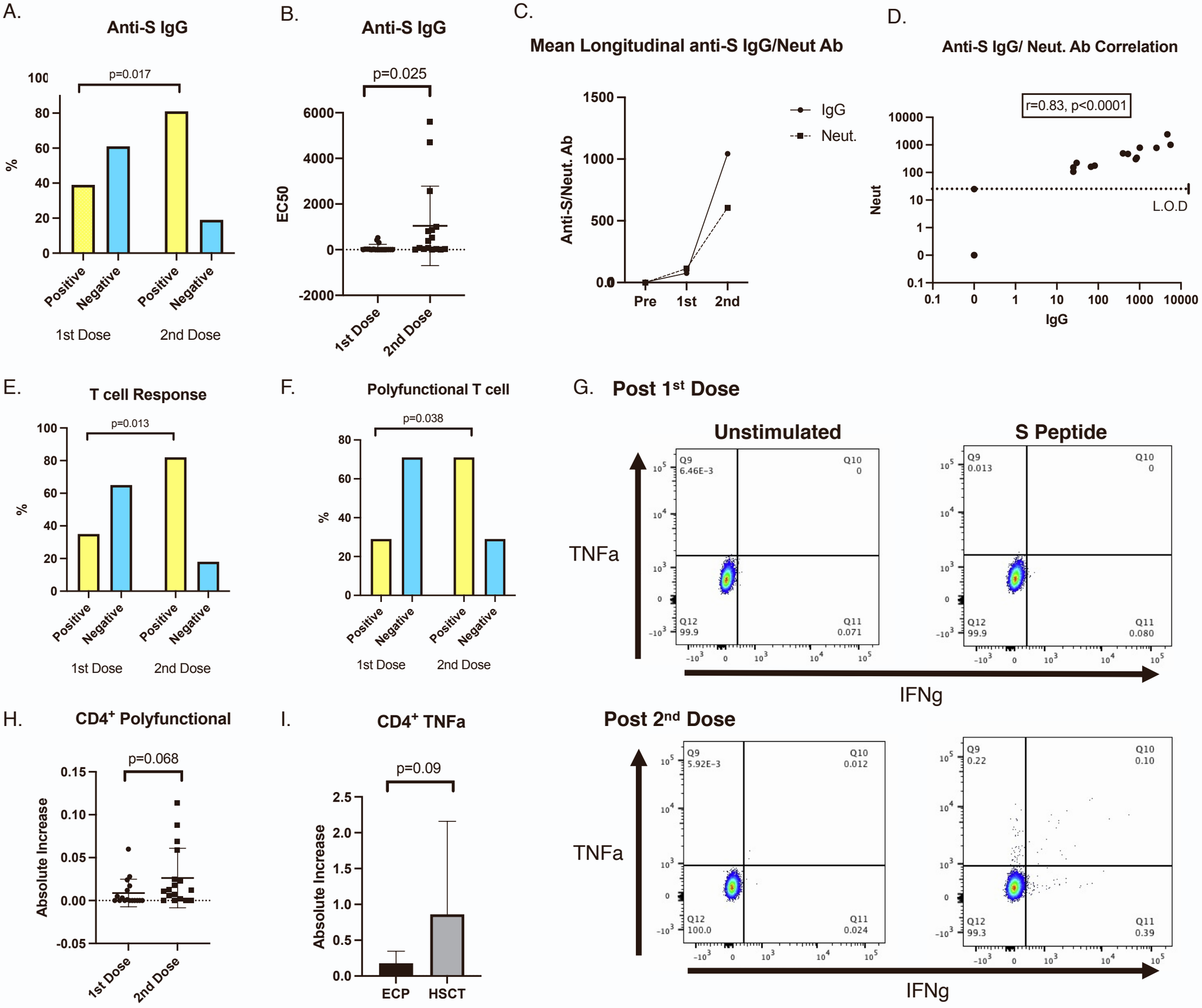
A.
Anti-S IgG
p=0.017
%
Positive
Negative
Positive
Negative
1st Dose
2nd Dose
B.
Anti-S IgG
p=0.025
EC50
1st Dose
2nd Dose
C.
Mean Longitudinal anti-S IgG/Neut Ab
Anti-S/Neut. Ab
IgG
Neut.
Pre
1st
2nd
D.
Anti-S IgG/ Neut. Ab Correlation
r=0.83, p<0.0001
Neut
L.O.D
IgG
E.
T cell Response
p=0.013
%
Positive
Negative
Positive
Negative
1st Dose
2nd Dose
F.
Polyfunctional T cell
p=0.038
%
Positive
Negative
Positive
Negative
1st Dose
2nd Dose
G.
Post 1st Dose
Unstimulated
S Peptide
TNFa
IFNg
Q9
6.46E-3
Q10
0
Q12
99.9
Q11
0.071
Q9
0.013
Q10
0
Q12
99.9
Q11
0.080
H.
CD4+ Polyfunctional
p=0.068
Absolute Increase
1st Dose
2nd Dose
I.
CD4+ TNFa
p=0.09
Absolute Increase
ECP
HSCT
Post 2nd Dose
TNFa
IFNg
Q9
5.92E-3
Q10
0.012
Q12
100.0
Q11
0.024
Q9
0.22
Q10
0.10
Q12
99.3
Q11
0.39

Figure S1. Humoral and T cell response to sequential doses of vaccine against SARS-CoV-2 in allo-SCT Recipients

A) Anti-S IgG response after first and second vaccine dose showing increase in seroconversion rate following second dose of vaccine. Yellow bar represents positive response and blue represents negative response. (Fisher's Exact Test).
B) Anti-S IgG Effective Concentration 50 (EC50) after first and second vaccine doses showing increase in EC50 with second dose of vaccine (Independent samples t-test).
C) Mean anti-S IgG EC50 and Neutralizing Antibody Infectious Dose 50 (ID50) Level pre-vaccine and after 1st and 2nd vaccine doses of vaccine. Solid line represents mean anti-S IgG EC50 and dotted line represents mean neutralising antibody ID50.
D) Correlation of Anti-S IgG and Neutralizing Antibody ID50 from paired samples (Spearman's rank correlation coefficient). Limit of detection (L.O.D) at a dilution of 1:25 represented by dotted line.
E) Total ($CD4^+/CD8^+$) T cell response after first and second vaccine dose showing increase in memory T cell response following second dose of vaccine. Yellow bar represents positive response and blue represents negative response. (Fisher's Exact Test).
F) Polyfunctional T cell response after first and second vaccine dose showing increase in memory T cell response following second dose of vaccine. Yellow bar represents positive response and blue represents negative response. (Fisher's Exact Test).
G) $CD4^+$ T cell polyfunctional ($IFN\gamma/TNF\alpha$) response after 1st vaccine dose (top) and 2nd vaccine dose (bottom). Left plots show unstimulated cell and right plots show cells incubated with S peptides, with polyfunctional response observed in sample stimulated with S peptides post second vaccine dose.
H) Absolute increase in $CD4^+$ T cell polyfunctional cell ($IFN\gamma/TNF\alpha$) showing increase following second dose of vaccine.
I) $CD4^+$ T cell TNFa absolute increase in ECP (extracorporeal photopheresis) cohort vs non ECP SCT cohort showing increase in TNFa expression in SCT patients not undergoing ECP (Independent samples t-test).

Supplementary Methods

Intracellular cytokine flow cytometry assay

T cell functionality was assessed using intracellular cytokine staining after incubation with SARS-CoV-2 specific peptides covering the immunogenic domains of the Spike (S) protein (Miltenyi Biotech). Cells were thawed, then rested for 18 hours at 37°C, 5% CO2. Specific peptides (0.25 µg/ml) and anti-CD28 (BD bioscience) were added for 3 hours, followed by Brefeldin-A (BFA) for an additional 3 hours. Unstimulated cells were utilised as negative controls and PMA and Ionomycin (Miltenyi Biotech) was added separately as a positive control. Cells were stained with a viability dye, stained with antibodies directed against surface markers, and fixed and permeabilised (BD CytoFix/Cytoperm) prior to staining with antibodies directed against intracellular cytokines. Directly conjugated monoclonal antibodies with the following specificities were used; CD3 BUV395 (clone SK37), CD4 PE (clone M-T477), CD45RO BV711, TNFa (clone MAB11) and IFNg APC (clone B27). Live dead staining was performed using Zombie NIR amine reactive fluorescent dye (Biolegend). Gating on the lymphocyte population, single cells, live cells, CD3+ cells, CD4+ cells and CD4- (CD8+) was performed. T cell analysis was performed on a BD Fortessa cytometer and results processed using Flowjo version 10.5. Statistical analysis was performed using Prism, version 8.

ELISA protocol

ELISAs were conducted as previously described1. All plasma samples were heat-inactivated at 56 °C for 30 min before use. High-binding ELISA plates (Corning, 3690) were coated with antigen (Nuclear (N) protein or the S glycoprotein at 3µg ml−1 (25 µl per well) in PBS, either overnight at 4 °C or for 2 h at 37 °C. Wells were washed with PBS-T (PBS with 0.05% Tween-20) and then blocked with 100 µl of 5% milk in PBS-T for 1 h at room temperature. The wells were emptied and serial dilutions of plasma (starting at 1:25, 6-fold dilution) were added and incubated for 2 h at room temperature. Control reagents included CR3009 (2 µg ml−1)(N-specific monoclonal antibody), CR3022 (0.2 µg ml−1)(S-specific monoclonal antibody), negative control plasma (1:25 dilution), positive control plasma (1:50), and blank wells. Wells were washed with PBS-T. Secondary antibody was added and incubated for

1h at room temperature. IgG was detected using goat-anti-human-Fc-AP (alkaline phosphatase) (1:1,000) (Jackson, catalogue no. 109-055-098) and wells were washed with PBS-T and AP substrate (Sigma) was added and plates read at 405 nm. EC50 values were calculated in GraphPad Prism. Where an EC50 was not reached at 1:25, a plasma was considered seropositive if the OD at 405nm was 4-fold above background and a value of 25 was assigned.

Neutralization assay with SARS-CoV-2

HIV-1 (human immunodeficiency virus type-1) based virus particles, pseudotyped with SARS-CoV-2 Wuhan Spike were prepared in HEK-293T/17 cells and neutralization assays were conducted as previously described3. Serial dilutions of plasma samples (heat inactivated at 56 °C for 30 min) were prepared in DMEM complete media (10% foetal bovine serum -FBS, 1% Pen/Strep (100 IU/mL penicillin and 100 mg/mL streptomycin) and incubated with pseudotyped virus for 1 h at 37 °C in 96-well plates. Next, HeLa cells stably expressing the ACE2 receptor (provided by Dr James Voss, Scripps Research, La Jolla, CA) were added (12,500 cells/50µL per well) and the plates were left for 72 hours. Infection level was assessed in lysed cells with the Bright-Glo luciferase kit (Promega), using a Victor™ X3 multilabel reader (Perkin Elmer). Measurements were performed in duplicate and the duplicates used to calculate the serum dilution that inhibits 50% infection (ID50) using GraphPad Prism.