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Supplemental information

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

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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

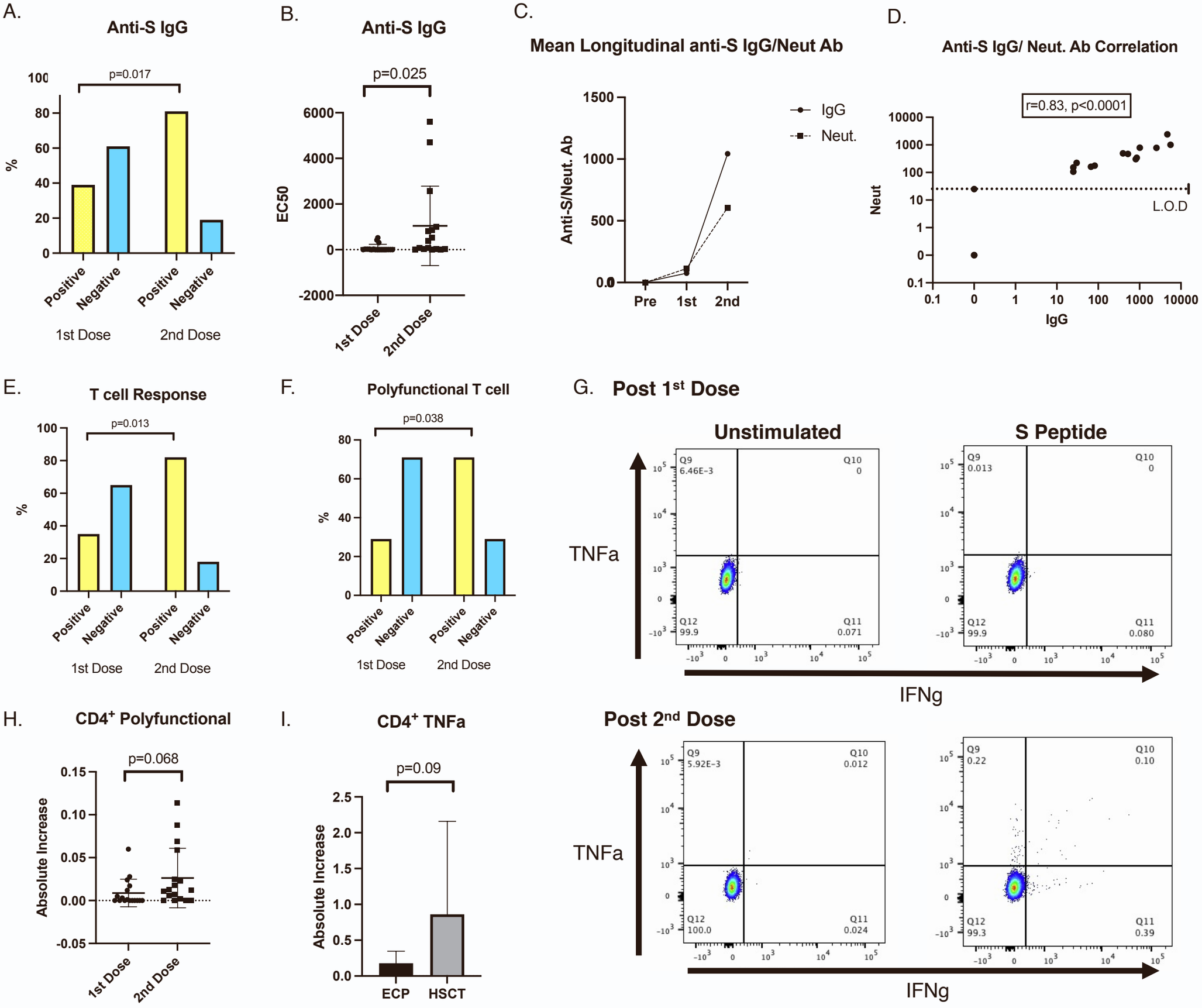
A.
Anti-S IgG
p=0.017
%
Positive
Negative
Positive
Negative
1st Dose
2nd Dose
B.
Anti-S IgG
p=0.025
EC50
1st Dose
2nd Dose
C.
Mean Longitudinal anti-S IgG/Neut Ab
Anti-S/Neut. Ab
IgG
Neut.
Pre
1st
2nd
D.
Anti-S IgG/ Neut. Ab Correlation
r=0.83, p<0.0001
Neut
L.O.D
IgG
E.
T cell Response
p=0.013
%
Positive
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.