Antibody Response to SARS-CoV-2 Vaccination in Patients Following Allogeneic Hematopoietic Cell Transplantation

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Supplementary materials

- Supplementary Methods
- Supplementary Figure Legends
- Supplementary Figures
- Supplementary Table 1-2

SUPPLEMENTARY METHODS

Study design

This study is a prospective single center observational study with a control group recruited from hospital and university staff. SARS-CoV-2 specific antibody response was measured longitudinally in each enrolled patient and in healthy individuals at (i) T0 = baseline (prior to the first vaccination); (ii) T1 = prior to the 2nd dose of vaccination; (iii) T2 = 2-6 weeks after the 2nd vaccination; (iv) T3 = 3 months after the 2nd vaccination and (v) T4 = 6 months after the 2nd vaccination. The primary endpoint of the study was to quantify and characterize the serological response in patients following allo-HCT as compared to a control group comprising healthy individuals. Secondary endpoints were (i) the safety of the vaccines by collecting data of the side effects after administration. For this purpose, patients recorded side effects according to a questionnaire. (ii) To further examine the seroprofile after SARS-CoV-2 vaccination we grouped patients by disease entity (myeloid vs. lymphoid; non-malignant); intensity of the conditioning regimen; stem cell source; patient age and gender; time since allo-HCT (3-6m, 6-12m, >12m post allo-HCT); remission or relapse of the underlying disease; immunosuppressive therapy (IST); and presence or absence of GVHD. Acute GVHD was graded according to the Mount Sinai Acute GVHD International Consortium Criteria (MAGIC) and chronic GVHD according to National Institutes of Health Consensus 2014. Moreover, leukocyte subpopulations in the peripheral blood (lymphocytes, $CD4^+$, recent thymic emigrant $CD4^+$ ($CD31^+$), naive $CD4^+$ ($CD45RA^+$), memory $CD4^+$ ($CD45R0^+$), $CD8^+$, naive $CD8^+$ ($CD45RA^+CD62L^+$), memory $CD8^+$ ($CD45R0^+$), $CD19^+$, $CD20^+$, naive B cells ($CD19^+CD26^-IgD^+$) and plasmablasts) were assessed within 6 months before the first vaccination or during the observation period after the vaccination. The study was conducted according to the Declaration of Helsinki and was approved by the Cantonal Ethics Committee (BASEC No 2021-00261). All patients and healthy individuals participating in this study provided informed consent.[8]

Patient population

We enrolled consecutive patients seen in our transplant outpatient clinic at our single center (University Hospital Zurich, Switzerland) fulfilling the following criteria: (i) history of allo-HCT >3m ago (vaccination is not recommend earlier than 3m post-HCT); (ii) willing to participate in the study; (iii) age >18 years. In addition, healthy volunteers who received the SARS-CoV-2 vaccine were enrolled in the study as a control group. Patients and healthy volunteers were vaccinated through the Swiss national vaccination program, which started in January 2021. Patients with a previous SARS-CoV-2 infection were admitted

to the study. Healthy volunteers who knew they were pre-infected and therefore were given only one dose of the vaccine were excluded.

Serological assessment by multiplex bead assay ABCORA

Longitudinal humoral response to SARS-CoV-2 was measured in EDTA plasma using the multiplex bead assay ABCORA as described.[9] The assay measures IgG, IgA and IgM reactivity to four SARS-CoV-2 antigens RBD, S1, S2 and N (12 SARS-CoV-2 parameters) in addition to IgG, IgA and IgM reactivity to S1 of HCoV-HKU1. In brief, 1:100 diluted plasma was incubated with antigen loaded MagPlex beads (Luminex Corporation, Austin, TX). Secondary PE phycoerythrin (PE)-labeled detector antibodies for IgG, IgA or IgM were used to detect bound immunoglobulins. Results from single dilution measurements are presented as median fluorescence intensity (MFI) corrected for background binding (fold over empty beads). To distinguish SARS-CoV-2-specific from cross reactive antibodies, *signal over cut-off* (SOC) values were defined for each of the 12 SARS-CoV-2 antigen and Ig class combinations, as previously described in.[9] SARS-CoV-2 positive plasma reactivity is defined using the ABCORA 2.3 computational approach achieving 98.20% specificity and 99.91% sensitivity.[9]

SARS-CoV-2 pseudo-neutralization assay

SARS-CoV-2 plasma neutralization activity was recorded using a HIV-based pseudovirus system as previously described.[10] Particles of the env-inactivated HIV-1 reporter construct pHIV-1NL4-3 ΔEnv-NanoLuc (pHIV-1Nanoluc; provided by P. Bieniasz, Rockefeller University, NY, USA) were pseudotyped with codon optimized, truncated SARS-CoV-2 spike expression plasmid (P_CoV2_Wuhan) by co-expression in 293-T cells. Infection of Human ACE2 Stable HeLa (Biogene, Shirley, NY) with SARS-CoV-2 pseudoparticles was detected by measuring the NanoLuc luciferase reporter activity in cell lysates 48h post infection using the Nano-Glo Luciferase Assay System (Promega, Fitchburg, WI) and readout on a Perkin Elmer EnVision reader. Neutralization tests of 1/100 diluted plasma were conducted in 384-wells as described and plasma neutralization titers causing 50% reduction in viral infectivity (NT50) compared to controls without plasma were calculated by fitting a sigmoid dose–response curve (variable slope) to the RLU data, using GraphPad Prism with constraints (bottom=0, top=100).[9] If 50% inhibition was not achieved at the lowest plasma dilution of 1/100, a 'less than' value was recorded. All measurements were conducted in single measurements.

Neutralization prediction

The sum of S1 SOC values (sum S1), defined as the sum of IgG, IgA and IgM S1 SOC values, can be used to predict the neutralization status of a patient using a logistic regression previously developed on a cohort of 467 infected individuals.[9] Indeed, a sum S1 value > 17 is predictive of a neutralization titer NT50>250, with a specificity of 94% and a sensitivity of 67%. The predictive ability of this model was confirmed in the allo-HCT cohort by comparing the model prediction based on the sum S1 value to the measured NT50 value and computing the area under the ROC curve (AUC).

Statistical analysis

Statistical analyses were performed in R (Version 4.0.5). Figures were made using the ggplot2 package. Differences in means between two groups with independent measures were tested using Mann Whitney tests on the log10 transformed sum S1 value. Differences in means between the 4 studied groups (3 transplanted groups + healthy individuals) were tested using a one-way ANOVA with 3 degrees of freedom. Univariable and multivariable linear regression were used to assess the risk factors associated with the immune response to the SARS-CoV-2 vaccine. The outcome was defined as the log10 of the sum S1 at T2. In our models, we accounted for infection by SARS-CoV-2 prior to vaccination: preinfection was either reported by the patient, or determined by a seropositive baseline sample.[9] In each linear regression, a Student t-test with two-sided hypothesis was used to assess if the association between the risk factor and the outcome was significantly different from 0. Significance of Spearman rank correlations were assessed through asymptotic t approximation. When testing several correlation coefficients (Fig 6B), Bonferroni correction for multiple comparisons was used.

We explored clustering approaches to identify different groups of vaccine recipients (allo-HCT patients and healthy individuals), based on their sum S1 SOCs response at T2. A first clustering was made and represented with a heatmap using the heatmap function from the ComplexHeatmap (version 2.6.2) R package.[11] A principal component analysis (PCA) was also realized with the PCA function from the FactoMineR (version 2.4) R package: individuals were represented using the two first axes of the PCA and the three clusters identified with the heatmap.[12]

We analyzed waning of antibody from T2 using a mixed effect single exponential model of the form: log10(sum S1) = $\alpha+\beta$*(day since 2nd dose - T2), an exponential model with change of slope between 30 and 90 days after T2, and a power law model of the form log10(sum S1) = $\alpha+\beta$*ln(day since 2nd dose / T2).[13] We used the Akaike information criterion to compare the models and found that the exponential

model (AIC=558) better fitted the data than the change of slope (AIC between 569 and 574) and power law models (AIC=579), and therefore used the single exponential decline model. A random effect was included on the intercept. Parameters were estimated using the lmer function (lme4 R package).[14] The half-life was computed as: $t_{1/2} = -\log10(2)/\beta$ (in days).

SUPPLEMENTARY FIGURE LEGENDS

Supp Figure S1: Dynamics of binding IgG S2, IgG N and IgA (RBD, S1, S2 and N) responses, represented as SOC values, in allo-HCT patients stratified by time between transplant and vaccination (dark blue: 3-6m, yellow: 6-12m, light blue: >12m) and healthy individuals (grey). Preinfected individuals are represented with triangles and dashed lines.

Supp Figure S2: Dynamics of binding IgM response against 4 SARS-CoV-2 antigens (RBD,S1,S2,N), represented as SOC values, in allo-HCT patients stratified by time between transplant and vaccination (dark blue: 3-6m, yellow: 6-12m, light blue: >12m) and healthy individuals (grey). Preinfected individuals are represented with triangles and dashed lines.

Supp Figure S3: (A) Boxplots showing neutralization titers against WT Wuhan-Hu 1 in allo-HCT patients stratified by time between transplant and vaccination (dark blue: 3-6m, yellow: 6-12m, light blue: >12m) compared to healthy individuals (grey) at T2. Preinfected individuals are represented with triangles. Dashed line corresponds to NT50=250. (B) ROC curve of the model predicting neutralization status (high neutralizer: NT50>250, low neutralizer: NT50<250) as a function of the sum S1 in transplanted patients (dark blue: 3-6m, yellow: 6-12m, light blue: >12m) and healthy controls (grey). (C) Scatterplot of NT50 versus sum S1 values. Each dot correspond to a patient, colored by its group (dark blue: 3-6m, yellow: 6-12m, light blue: >12m, grey: healthy). Horizontal dashed line corresponds to NT50=250 and vertical line to sum S1=17.

Supp Figure 4: (A) Correlation of the Elecsys S titers and sum S1 SOCs values at T1,T2, and T3 (n= 163 at T1, n= 151 at T2, and n=62 at T3). Each dot correspond to a patient, colored by its group (dark blue: 3-6m, yellow: 6-12m, light blue: >12m, grey: healthy). (B-D) Boxplots showing Elecsys S titers in allo-HCT patients stratified by time between transplant and vaccination (dark blue: 3-6m, yellow: 6-12m, light blue: >12m) compared to healthy individuals (grey), at different timepoints: (C) T1: 1m after 1st dose, (D) T2: 1m after 2nd dose, (E) T3: 3m after 2nd dose. Preinfected individuals are represented with triangles.

SUPPLEMENTARY FIGURES

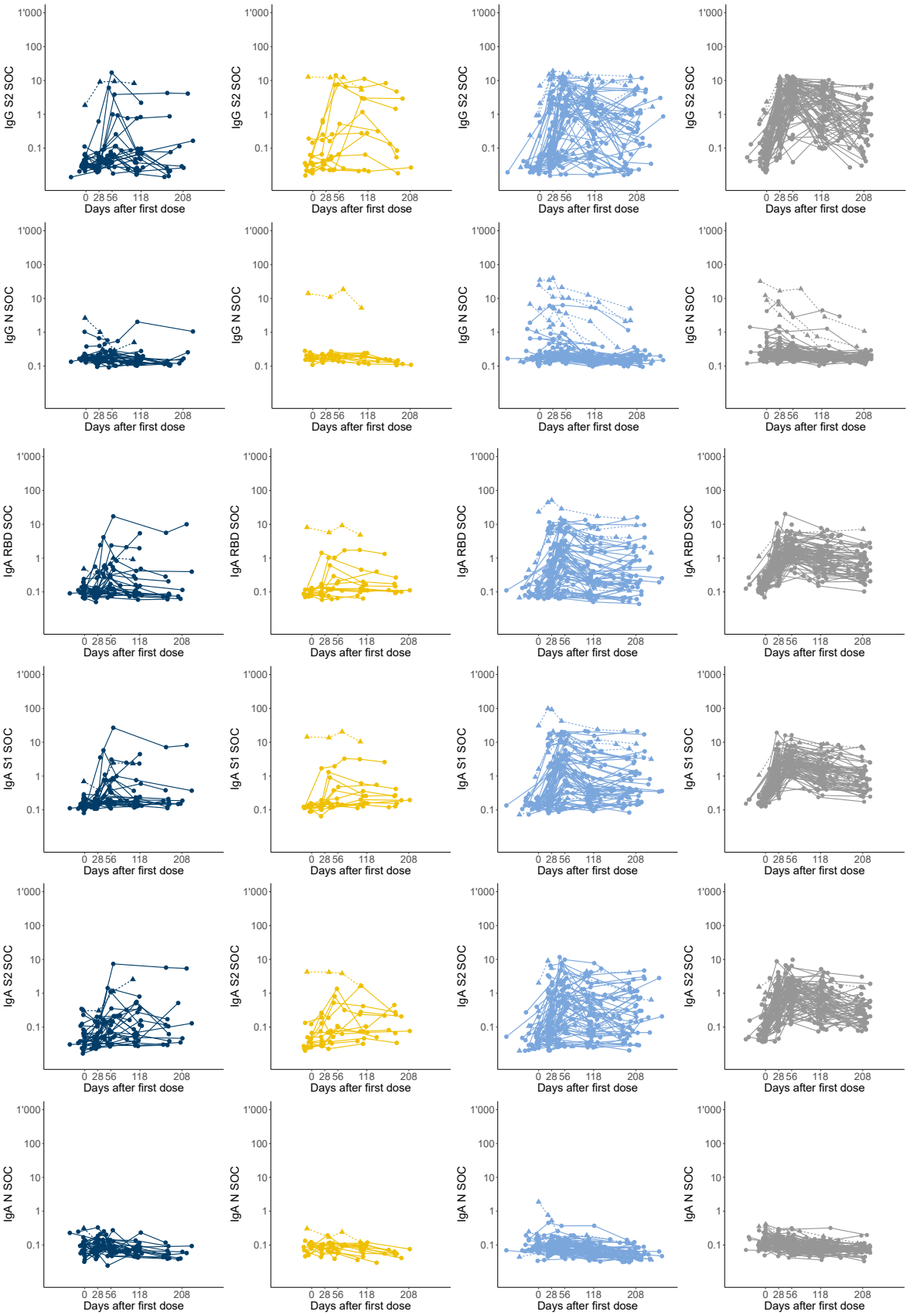

Figure S1

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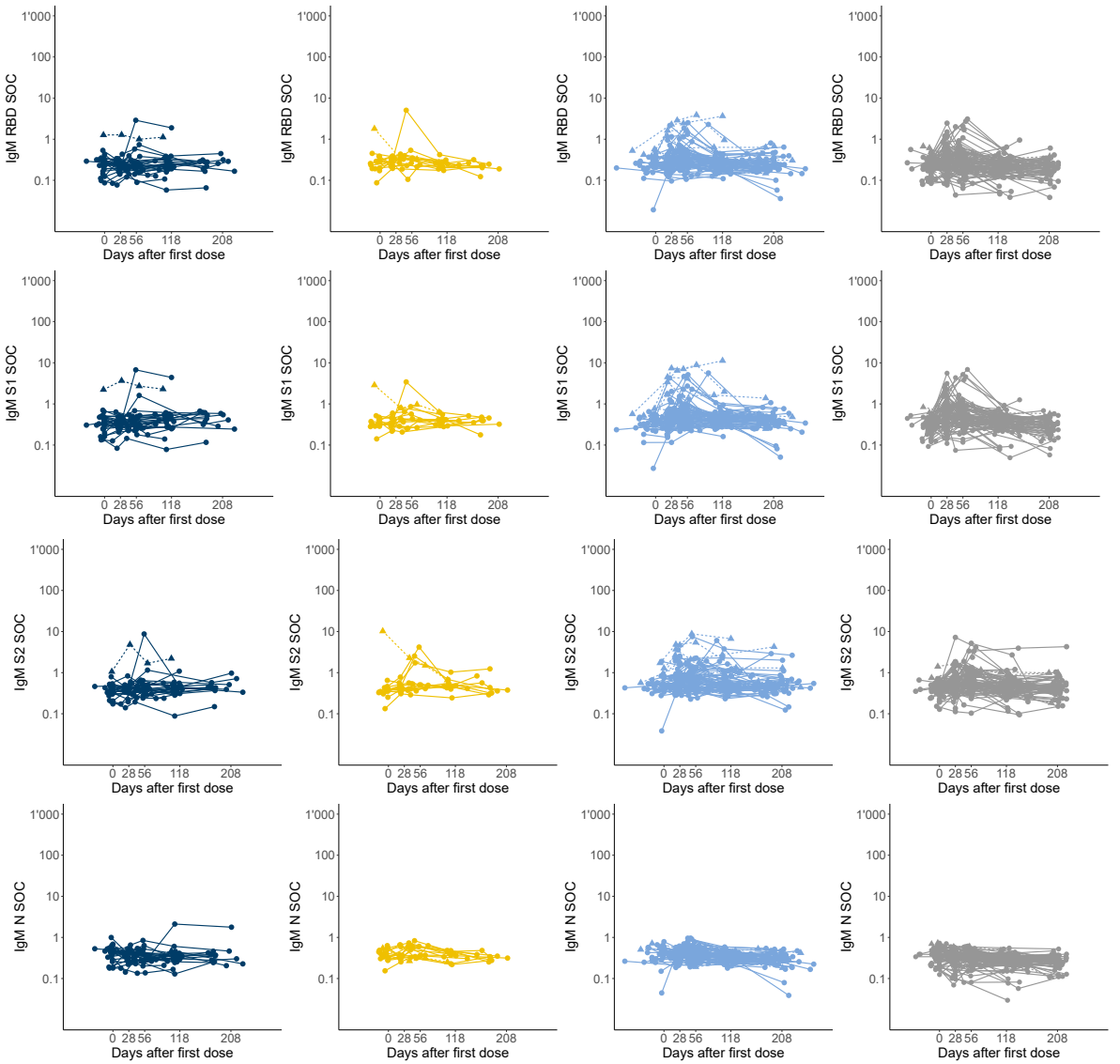
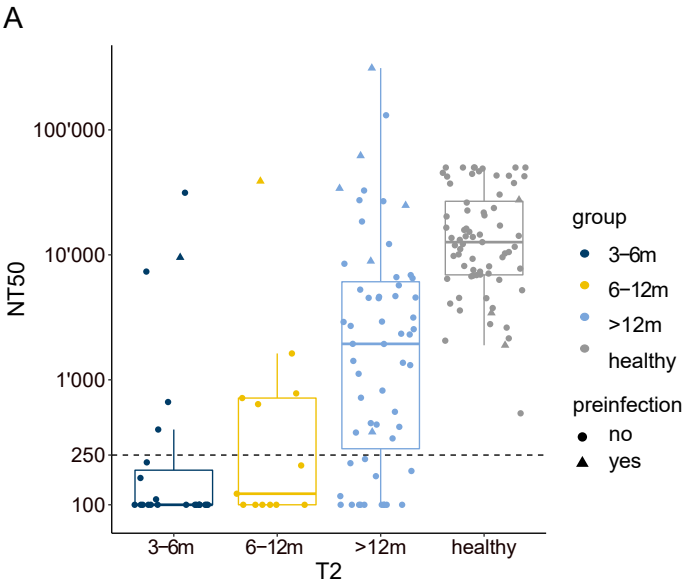


preinfection no ●

yes ▲

Figure S2


A
100'000
10'000
1'000
250
100
NT50
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6–12m
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healthy
T2
group
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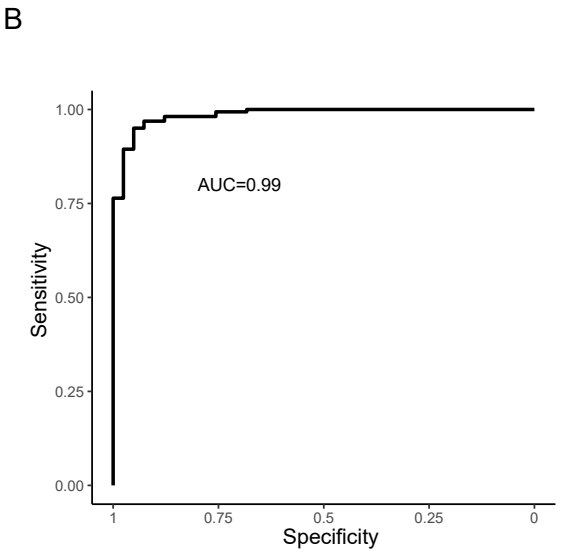
6–12m
>12m
healthy
preinfection
no
yes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0.00
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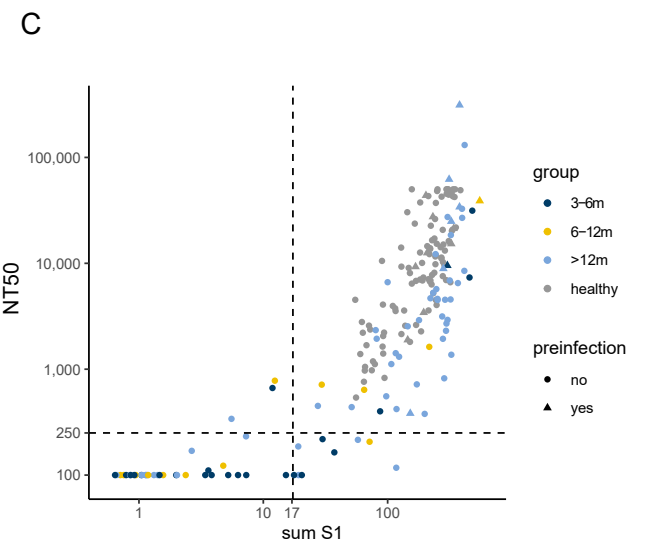
0.25
0
Specificity


C
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1,000
250
100
NT50
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100
sum S1
group
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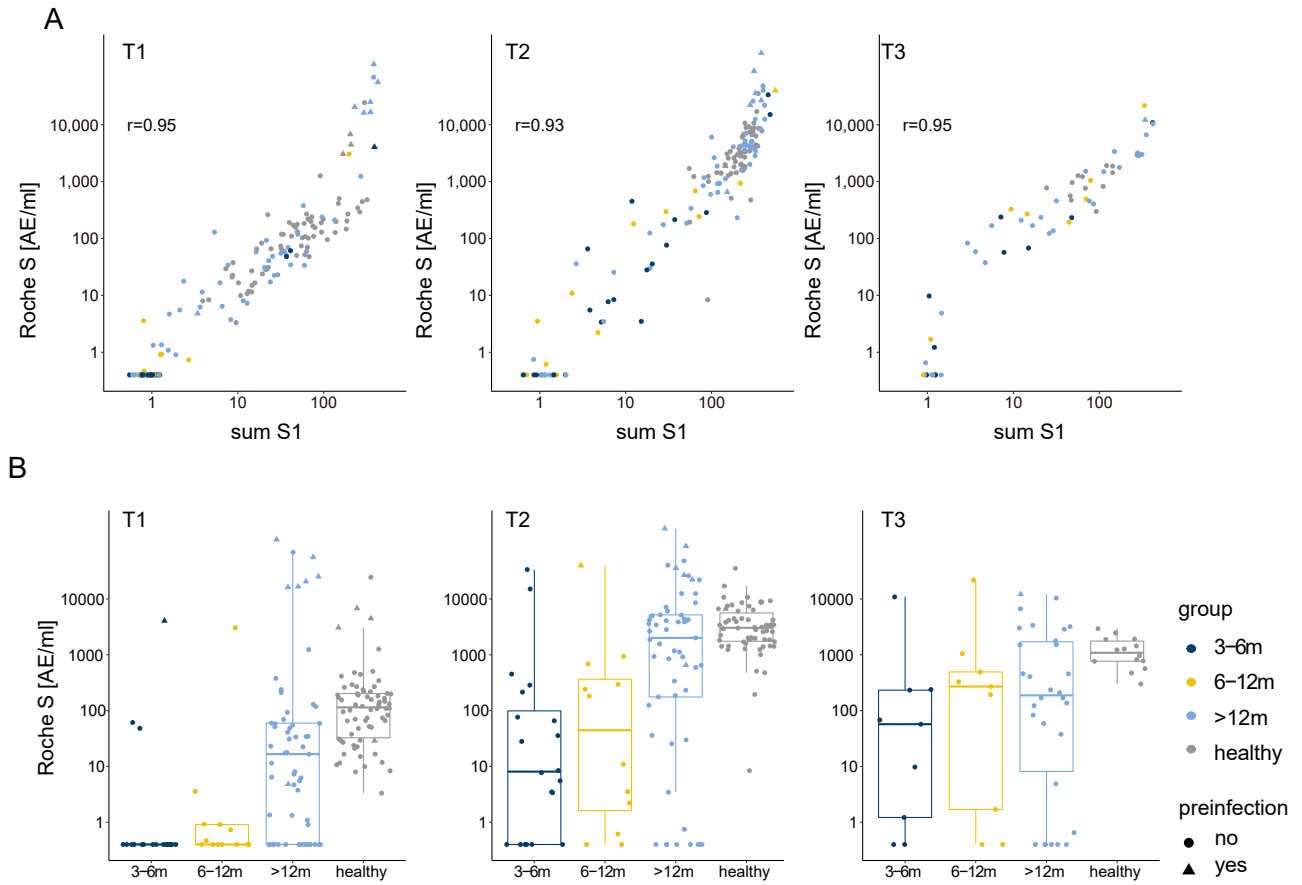
6–12m
>12m
healthy
preinfection
no
yes

Figure S3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T2
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T3
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Roche S [AE/ml]
sum S1
B
T1
T2
T3
Roche S [AE/ml]
3–6m
6–12m
>12m
healthy
group
3–6m
6–12m
>12m
healthy
preinfection
no
yes

Figure S4

SUPPLEMENTARY TABLES

Supplementary Table 1: Side effects of the SARS-CoV-2 vaccines

Collected questionnaires	**1st vaccination n = 75/110**	**2nd vaccination n = 74/110**
Local adverse events		
Redness at injection site	3 (4%)	2 (2.7%)
Swelling at injection site	9 (12%)	7 (9.5%)
Pain at injection site	39 (52%)	38 (51.4%)
Calor at injection site	3 (4%)	1 (1.4%)
Systemic adverse events		
Myalgia	10 (13,3%)	5 (6.8%)
Chills	3 (4%)	2 (2.7%)
Headaches	9 (12%)	10 (13.5%)
Fever	1 (1,3%)	2 (2.7%)
No side effects	30 (40%)	32 (43.2%)

Supplementary Table 2: Detailed characteristics of immunosuppression and GVHD in low-responders to the SARS-CoV-2 vaccine

Patient	Age		Timepoint post allo-HCT			IST			GVHD		GVHD organs			Relapse		cGVHD duration	comment
	<45y	> 45 y	3-6m	6-12m	>12m	no	proph_ IST	Th_IST	no/mild	Moderate /severe	skin/enoral /eyes	HE/GI	LU	no	yes		
Th_IST m/sGVHD																	
P1		x	x					x		x	x	x		x			LU_GVHD in FU
P2		x	x					x	x			x		x			relapse in the follow-up
P3		x	x					x		x		x		x		2m	Ruxolitinib
P4	x			x				x	x			x		x			Ruxolitinib
P5		x			x			x		x	x			x		>12m	Ruxolitinib
P6		x			x			x		x			x	x		>12m	Ruxolitinib, ECP
P7	x		x					x		x	x			x		3m	LU_GVHD in FU
P8		x			x			x	x			x		x			Ruxolitinib
P9		x	x					x		x		x		x		2m	Ruxolitinib
P10		x		x				x	x		x	x		x			..
P11		x	x					x	x		x			x			..
P12		x			x			x		x	x			x		8m	Ruxolitinib
P13	x				x			x		x		x	x	x		>12m	Death
P14		x			x			x		x	x	x		x		2m	GVHD after DLI
P15		x			x			x		x	x			x		2m	Ruxolitinib
Relapse																	
P16		x	x			x			x						x		molecular persistence (DLI)
P17		x			x	x			x						x		DAC+venetoclax
P18		x			x	x			x						x		HyperCVAD
P19		x			x	x			x						x		KRd
P20		x		x		x			x						x		Gilteritinib
P21		x			x	x			x						x		Ivosidenib
P22		x	x			x			x			x			x		Ponatinib, Ruxolitinib before vaccination
P23		x			x	x			x						x		Gilteritinib
<12m post allo-HCT																	

P24		x	x				x		x					x			..
P25		x	x				x		x			x		x			..
P26		x	x				x		x		x			x			..
P27	x		x				x		x		x	x		x			..
P28		x	x			x			x					x			..
P29		x	x				x		x					x			..
P30		x	x				x		x			x		x			..
P31		x	x				x		x					x			..
P32	x		x			x			x					x			
P33		x	x				X		X					x			
others																	
P34		x		x		x			x					x			..
P35	x			x			x		x					x			..
P36		x			x	x			x		x	x		x			..
P37		x			x	x			x					x			..
P38		x			x	x			x					x			

Abbreviations: Th IST, therapeutic immunosuppressive treatment; proph_IST, prophylactic immunosuppressive treatment; y, years; m, month; HE, hepatic; GI, gastrointestinal; LU, lung; cGVHD, chronic GVHD; KRd, carfilzomib, lenalidomide, dexamethasone; FU, follow-up; DLI, donor lymphocyte infusion