COVID-19 vaccine-induced adverse events predict immunogenicity among recipients of allogeneic hematopoietic stem cell transplantation

Recipients of allogeneic hematopoietic stem cell transplantation (allo-HCT) are at elevated risk for severe disease and death from COVID-19[1,2] and mount suboptimal immune responses following COVID-19 vaccination, in particular during the first years after transplantation.[3-7] Previous studies in healthy individuals support an association between adverse reactions after COVID-19 vaccination and induced humoral immunity[8,9] but whether adverse vaccine reactions also are associated with T-cell reactivity remains to be elucidated. Here we report that adverse reactions to COVID-19 vaccination predict the evolvement of virus-specific T cells among recipients of allo-HCT.

This study is part of the DurIRVac study (EudraCT no. 2021 000349 42) that was approved by the Swedish Ethical Review Authority (permit no. 2021 00539) and by the Swedish Medical Products Agency (permit no. 5.1 2021 11118), and followed the European Society for Blood and Marrow transplantation (EBMT) guidelines for COVID-19 vaccination (www.ebmt.org; Version 6.0, May 31, 2021). The study was performed in accordance with the Declaration of Helsinki and all participants gave written informed consent before enrollment. The first cohort constituted 50 patients having undergone allo-HCT 92 months (median; range, 7-340 months) prior to the first COVID-19 vaccine dose. Patients were immunized with two doses of the mRNA-based COVID-19 vaccines BNT162b2 (Pfizer-BioBTech Comirnaty; n=32) or mRNA-1273 (Moderna Spikevax; n=18) with 41 days (median; range, 40-50 days) between doses. Patients with previous polymerase chain reaction (PCR)-confirmed COVID-19 infection or antibodies against SARS-CoV-2 in baseline samples were excluded.

A second cohort comprised 37 COVID-19-naïve allo-HCT recipients who fulfilled the criteria from the Public Health Agency of Sweden for receiving an early third dose of COVID-19 vaccine, i.e., to have undergone transplantation within 3 years, or to currently receive immunosuppressive treatment for graft-*versus*-host disease (GVHD). Patients in this cohort received a third mRNA vaccination (BNT162b2, n=24 or mRNA-1273, n=13) at 127 days (median; range, 56-174 days) after the second dose. Eight patients were included in both cohorts. Further baseline characteristics of these cohorts are provided in [6,7].

Two weeks after each vaccination, patients completed a questionnaire regarding possible adverse events categorized per the Common Terminology Criteria for Adverse Events standards. Adverse events, including occurrence of GVHD, were also retrieved from review of medical records. Thirty-six of 48 (75%) of allo-HCT recipients experienced adverse reactions following the first vaccination and 26 of 49 (53%) following the second vaccine dose. No serious adverse events were recorded. Adverse events were mostly mild and only three patients reported moderate adverse reactions. In the prioritized third-dose cohort 15 of 33 (45%) experienced adverse reactions (2 moderate, 13 mild). The lower frequency of adverse events likely reflects the more pronounced degree of immunodeficiency within cohort 2, with shorter time elapsed since transplantation and higher frequency of patients receiving immunosuppressive treatment. In both cohorts, local reaction at the injection site was the most common adverse event followed by fatigue, malaise, myalgia, and headache (Table 1). Three patients reported worsening of GVHD and two experienced *de novo* onset GVHD. These reactions resolved following topical skin therapy, modest augmentation of the prednisone dose, or spontaneously. The frequency of adverse reactions was similar or slightly higher than that reported in a previous trial in mRNA COVID-19-vaccinated allo-HCT recipients,[10] but lower than reported in the BNT162b2 and mRNA-1273 registration trials enrolling participants from the general population.[11,12] However, larger cohorts of vaccinated allo-HCT recipients and a similar reporting system for adverse reactions as for the registration trials would be needed to elucidate if adverse reactions are less common among allo-HCT.

Peripheral blood was collected immediately before the first and third vaccine doses, 4 weeks (median 28; range, 16-38 days) after the first, second and third doses and at 5.5 months (median; range, 3.8-5.6 months) after the second dose. In order to quantify vaccine-specific cellular responses, the whole blood samples were stimulated *ex vivo* with multimer peptides spanning the S1 portion of the spike protein to induce the release of T-cell-derived IFN-γ from SARS-CoV-2 specific T cells. This assay captures virus-specific T cells ($CD4^+$ and $CD8^+$) with high sensitivity and specificity.[13] In brief, 1 mL of peripheral blood, collected in lithium-heparin tubes, was stimulated with 1 μg/mL/peptide of 170 15-mer peptides with 11-amino acid overlap spanning the N-terminal SARS-CoV-2 spike 1 (S1) domain (product number: 130-

Table 1. Adverse reactions after COVID-19 vaccination.

	Cohort 1 (N=50)		Cohort 2 (N=37)
	Dose 1	Dose 2	Dose 3
Evaluable patients,[1] N (%)	48 (96)	48 (96)	33 (90)
No adverse events, N (%)	12 (25)	22 (46)	18 (55)
Local reaction at injection site, N (%)	30 (62)	17 (35)	14 (42)
Systemic reaction,[2] N (%)	23 (48)	17 (35)	7 (21)
Fatigue	10 (21)	13 (27)	3 (9)
Myalgia	9 (19)	7 (14)	3 (9)
Headache	7 (15)	7 (14)	3 (9)
Malaise	7 (15)	13 (27)	3 (9)
Fever	3 (6)	2 (4)	3 (9)
Nausea	1 (2)	0 (0)	1 (3)
GVHD[3]-related	5 (10)	0 (0)	0 (0)

[1]Patients completing the questionnaire regarding adverse events. [2]Any adverse event other than local reactions. [3]Graft-*versus*-host disease (GVHD).

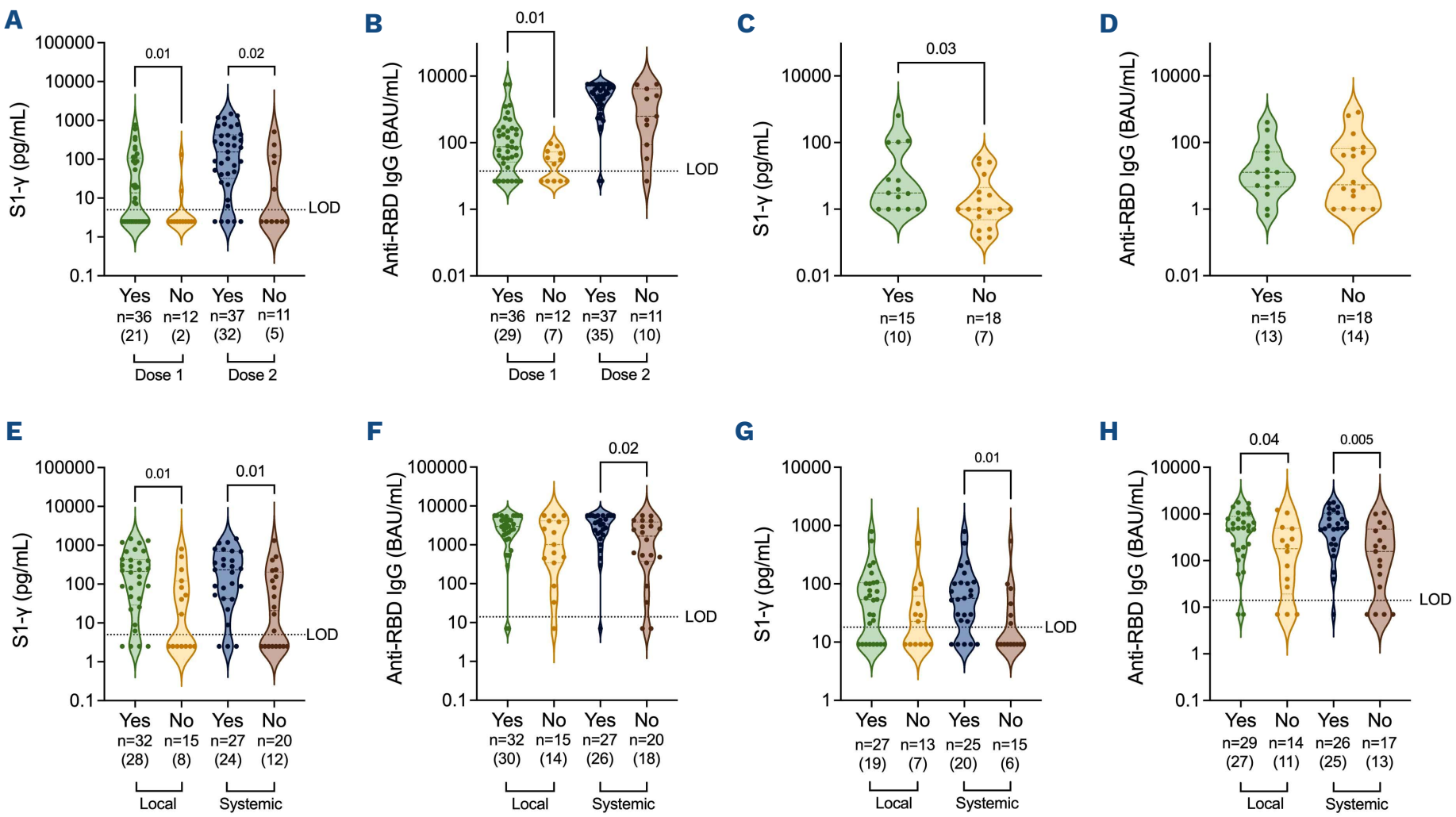

Figure 1. Adverse events following COVID-19 vaccination predict evolving vaccine-specific T-cell and antibody responses. Samples from allogeneic hematopoietic stem cell transplantation (allo-HCT) patients who experienced (Yes) or did not experience (No) adverse events to COVID-19 vaccination were analyzed for vaccine-specific T-cell and antibody responses. (A, C, E, and G) Whole blood was stimulated with S1 peptides for 48 hours and analyzed for T-cell-induced interferon-γ (IFN-γ). (B, D, F and H) IgG serum antibody levels of the receptor-binding domain (RBD) within S1 were measured. (A and B) Immunogenicity in samples from allo-HCT cohort 1, retrieved 1 month after the first and second vaccine dose. (C and D) Immunogenicity in samples from allo-HCT cohort 2, retrieved 1 month after the third vaccine dose. (E to H) Patients in cohort 1 are separated as experiencing local adverse events (local), or at least 1 systemic adverse event (systemic) to any of the 2 first vaccine doses. (E and F) Results 1 month after the second vaccine dose. (G and H) Results 5-6 months after the second vaccine dose. The number of samples above the cut-off for each assay (>5 pg/mL IFN-γ detected by enzyme-linked immunosorbent assay (A, C and E), >18 pg/mL IFN-γ detected by Fireplex (G) and >14 BAU/mL IgG), are shown in brackets below the number of observations (n). Statistics by Mann-Whitney test. Dashed lines show the limit of detection (LOD) for each assay.

Table 2. Patient characteristics versus adverse reactions after COVID-19 vaccination.

	Cohort 1						Cohort 2		
	Dose 1			Dose 2			Dose 3		
Adverse reaction[1]	**No (N=12)**	**Yes[a] (N=36)**	***P*[2]**	**No (N=11)**	**Yes[b] (N=37)**	***P***	**No (N=18)**	**Yes[c] (N=15)**	***P***
Median age in years (range)	65 (40-75)	50 (29-78)	0.03[d]	65 (41-75)	48 (29-78)	<0.01[d]	61 (32-70)	64 (31-78)	0.67[d]
<24 months since allo-HCT[3], N (%)	4 (33)	2 (6)	0.03[e]	4 (36)	2 (5)	0.02[e]	11 (61)	5 (33)	0.17[e]
Female sex, N (%)	2 (17)	21 (58)	0.02[e]	1 (9)	22 (59)	<0.01[e]	6 (33)	9 (60)	0.17[e]
Received Pfizer[4] vaccine, N (%)	4 (33)	26 (72)	0.04[e]	3 (27)	27 (73)	0.01[e]	12 (67)	9 (60)	0.73[e]
cGVHD[5], N (%)	2 (17)	15 (42)	0.17[e]	2 (18)	15 (41)	0.28[e]	8 (44)	12 (80)	0.07[e]
Ongoing IST[6], N (%)	4 (33)	5 (14)	0.20[e]	4 (36)	5 (14)	0.18[e]	10 (56)	12 (80)	0.27[e]

[1]Any adverse reaction to (a) the first vaccine dose, (b) at least 1 of the first 2 doses, (c) the third vaccine dose. [2]Statistics by (d) Mann-Whitney test or (e) Fisher's exact test, [3]Allogenic hematopoietic cell transplantation, [4]BNT162b2 (Pfizer-BioNTech), [5]chronic graft-*versus*-host disease (GVHD), [6]immunosupressive treatment (IST).

127-041, Miltenyi Biotec). After 2 days of incubation at 37°C, samples were centrifuged and IFN-γ content of recovered plasma was determined by enzyme-linked immunosorbent assay (DY285B, R&D systems) or by FirePlex (Abcam, ab285173) according to the manufacturer's instructions. Humoral responses were assessed by quantification of serum anti-RBD IgG (SARS-CoV-2 IgG II Quant, Abbott, Illinois, USA) using a chemiluminescent microparticle immunoassay in an automated Alinity system, as described [13].

Patients in cohort 1 experiencing adverse reactions to the first vaccination showed significantly higher levels of virus-specific T cells as reflected by increased S1-induced IFN-γ in plasma supernatants. The enhanced T-cell response among patients experiencing adverse events to at least one vaccine dose remained significant also after the second vaccination (Figure 1A). Adverse reactions to the first vaccine dose were also associated with significantly higher anti-RBD IgG levels with a similar non-significant trend after two vaccine doses (Figure 1B). Among the third dose-prioritized patients (cohort 2) induction of SARS-CoV-2 specific T cells was superior in patients experiencing adverse reactions to third dose vaccination (Figure 1C) with a similar trend for induced anti-RBD-IgG (Figure 1D).

Systemic adverse events, referring to any event other than local reactions, appeared particularly predictive of vaccine immunogenicity. Hence, patients experiencing at least one systemic adverse reaction to the first or second immunization showed significantly higher levels of virus-specific T cells as well as anti-RBD IgG at 1 month and 5.5 months after the second vaccine dose (Figure 1E to H). Concordantly, vaccine-induced fever has been linked to robust antibody responses towards human papillomavirus (HPV) and COVID-19.[8,14] None of the systemic side effects significantly predicted evolving immune responses when analyzed separately, likely explained by the small sample size. Also, no significant association between systemic adverse events and immunogenicity was seen in the third dose cohort, in which only seven patients experienced systemic reactions.

In accordance with previous studies of mRNA COVID-19-vaccinated healthy subjects,[8,9] female sex and low age was associated with enhanced frequency of adverse reactions also among vaccinated allo-HCT recipients (Table 2). In cohort 1, patients receiving the BNT162b2 vaccine reported more adverse reactions compared with patients receiving the mRNA-1273 vaccine (Table 2). This was unexpected, but likely explained by the higher age of patients receiving the mRNA-1273 vaccine in this cohort (median age 63 years; range, 46-71 *vs*. 35 years; range, 18-58 years for BNT162b2-vaccinated patients). Patients transplanted within the last 2 years showed reduced frequency of adverse reactions, and patients with chronic GVHD tended to report more adverse events (Table 2).

The association between adverse vaccine reactions and T-cell immunogenicity remained significant in multivariate linear regression analysis when taking potential confounders (age, sex, vaccine type, immunosuppressive therapy and chronic GVHD) into account. Thus, the presence of adverse reactions independently predicted T-cell responses in cohort 1 following the first immunization (P=0.019) and following the third vaccine dose (P=0.032) in cohort 2.

To conclude, this study shows that recipients of allo-HCT who experience adverse reaction to mRNA COVID-19 vaccination were more likely to mount durable SARS-CoV-2-specific immune responses. Systemic adverse reactions appeared more predictive of immunoreactivity compared with local adverse reactions, but further studies are needed to learn which side effects are predictive of adaptive immune responses for improved vaccine formulations.

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Contributions
HGW conducted experiments, analyzed data, made figures and wrote the manuscript. SE designed and conducted the clinical trial. AT, MA and NI conducted experiments. JW analyzed data. JR conducted experiments and analyzed data. MLi designed the clinical trial. MLa and KH designed the clinical trial and wrote the manuscript. AM designed the clinical trial, conducted experiments, analyzed data and wrote the manuscript. All authors have edited and approved the manuscript.

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Data-sharing statement
Inquiries regarding sharing of de-identified data shall be addressed to the corresponding author.

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