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Impaired T-cell response to mRNA vaccination heralds risk of COVID-19 in long-term allogeneic hematopoietic stem cell transplantation survivors

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Authorship Contributions: M.L., A.M and K.H were responsible for designing and writing the protocol, conducting the study, extracting data, and analyzing data, interpreting results, writing the manuscript, and updating the reference list. S.E conducted the study, included all patients, participated in the extracting and analyzing of data, updated the reference list, wrote the first draft of the manuscript. J.W extracted and analyzed data, performed statistical analyses, created the figure and table, participated in writing the manuscript. A.M, A.T, J.R, J.S. and S.M. were responsible for the T-cell analyses and participated in the extraction and analysis of data and in writing of the manuscript. All authors have read and approved the last version of the manuscript.

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Initial reports suggested a COVID-19-associated mortality rate approaching 30% among allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients (1). However, patient outcomes have improved with the introduction of effective vaccines and advancements in patient management, such as rapid diagnostic testing and anti-viral therapies. Although new Omicron virus variants show increased transmissibility, they have also been associated with reduced risk of severe disease among vaccinated individuals (2). Recent data suggests that mortality in fully vaccinated allo-HSCT recipients has decreased to as low as 1% (3). Our previous findings show that a weak T-cell response following the first two doses of vaccination leads to reduced humoral immunity (4) and that allo-HSCT recipients who experience vaccine-related adverse events demonstrate augmented T-cell responsiveness (5). While various studies have evaluated COVID-19 vaccination response in this patient cohort (6, 7), to our knowledge, this is the first report of allo-HSCT recipients with poor T-cell response following two doses of mRNA vaccine being at increased risk of acquiring subsequent COVID-19.

This study was conducted between March 2021 and November 2022 at the Sahlgrenska University Hospital in Gothenburg, Sweden (CONSORT diagram in Figure 1). Allo-HSCT recipients without prior history of COVID-19 (n=50), identified in local transplant registries in the Region Västra Götaland (population of approximately 1.7 million), were recruited to participate in this sub-study within the DurIRVac study. All participants gave written informed consent before enrollment. Enrolled allo-HSCT recipients fulfilled the following pre-determined criteria: (i) to be at least three months post allo-HSCT, (ii) to not have received rituximab during the last six months, and (iii) to be without uncontrolled acute GvHD (grade 3-4), all according to EBMT-guidelines for COVID-19 vaccination (Version 7,
Patients were sampled before and after the first two vaccine doses and then every six months. The cohort mainly consisted of long-term survivors with a median time following transplant of 90 months (10-330), with no participant receiving any COVID vaccine dose prior to transplantation. The DurIRVac study was approved by the Swedish Ethical Review Authority (Etikprövningsmyndigheten; permit nos. 2020-03276, 2021-00374 and 2021-00539) and by the Swedish Medical Products Agency (Dnr: 5.1-2021-11118 and EudraCT no. 2021-000349-42) and has been registered at the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT no. 2021-000349-42). The researchers in this study had no influence regarding which mRNA COVID-19 vaccine (mRNA-1273 Moderna Spikevax® or BNT162b2 Pfizer-BioNTech Comirnaty®) participants received nor the number or time-point of vaccinations, as doses were administered in accordance with regional prioritization.

Chemiluminescent microparticle immunoassays (CMIA) were performed on serum using the automated Alinity system for quantitative measurement of IgG antibodies against the receptor-binding-domain (RBD) of the spike protein of SARS-CoV-2 (SARS-CoV-2 IgG II Quant, Abbott, Abbott Park, Illinois, USA) with levels reported in the WHO international standard Binding Antibody Units (BAU) /mL (quantitative detection range: 14 to 5680 BAU/mL (1.14 to 3.75 log10 BAU/ml); samples reaching 5680 BAU/mL were diluted with seronegative serum and reanalyzed). Anti-nucleocapsid antibodies were analyzed at baseline and during follow-up sampling (November 2022).

Peripheral blood, collected in vacutainer lithium-heparin tubes (BD, Plymouth, UK), was stimulated with 15-mer peptides with 11-amino acid overlap spanning the S1 domain of the SARS-CoV-2 surface glycoprotein as described previously (8, 9). Plasma from whole blood cultures was recovered and stored at -80°C until analysis of IL-2. Levels of IL-2 in whole
blood supernatants were determined by the FirePlex®-96 Key Cytokines Immunoassay (ab243549 and ab 285173, Abcam) according to the manufacturer’s instructions. Samples were acquired on a BD LSR Fortessa (BD) and analyzed with FirePlex Analysis Workbench (Abcam). S1-specific responses were calculated by subtracting levels of IL-2 in unstimulated control samples from those in S1 stimulated samples. The S1-peptide induced IL-2 has been shown to be contributed foremost by T-cells, in particular CD4+ T-cells (10).

All 41 patients were COVID-19-naïve five months after the 2nd vaccine dose as per polymerase chain reaction (PCR), COVID-19 antigen test, analysis of antibodies against the nucleocapsid protein of SARS-CoV-2 (SARS-CoV-2 IgG, Abbott, Abbott Park, Illinois, USA), review of medical records, or evaluation of participant self-reporting questionnaire.

During the follow-up period after receiving the initial two vaccine doses, the patients were divided into two groups based on whether they had subsequent breakthrough COVID-19 infection or not (baseline characteristics detailed in Table 1). All 12 documented COVID-19 infections had detectable SARS-CoV-2 RNA (n=8), reactive COVID antigen test (n=3), and/or presence of antibodies against the nucleocapsid (n=2) in a sample obtained after the 2nd vaccine dose. No severe breakthrough infections, i.e., requiring hospitalization or leading to death, were observed during the study. The Omicron variants (B.1.1.529) BA.1 and BA.2 dominated the local circulation during this study.

At five months after the second vaccine dose, the group of patients who experienced subsequent breakthrough COVID-19 infection (n=12) had a significantly lower median level of S1-induced IL-2 (52 [7-141] pg/mL) compared to the group without later breakthrough
infection (n=27) (174 [1-2344] pg/mL) (p=0.016, Mann-Whitney) (Figure 2). As baseline characteristics stratified according to COVID-19 breakthrough infection demonstrated a significant difference in the mRNA vaccine used (i.e., Moderna vs. Pfizer-BioNTech) for primary vaccination, this association between T-cell response five months following the second vaccination and risk of infection was confirmed in a subgroup of the cohort consisting of those <65 years of age, where all patients received BNT162b2 (Pfizer-BioNTech Comirnaty®) and were 11/12 infections occurred (Supplementary Figure S1). A receiver operating characteristics (ROC) analysis determined 145 pg/mL of S1-induced IL-2 as a cut-off level that best discriminated patients at risk for subsequent breakthrough infections. No patients with S1-induced IL-2 levels above this value after two doses of vaccination experienced breakthrough infection, compared to 52% of those with levels below (p<0.001, Fischer's exact test).

The antibody levels against RBD within the spike protein after two vaccine doses did not differ significantly between patients who experienced breakthrough infection and those who did not. The median antibody levels were 207 [0-1778] BAU/mL (2.32 [<1-3.25] log10 BAU/ml) in the group with subsequent COVID-19 versus 479 [0-1413] BAU/mL (2.68 [<1-3.15] log10 BAU/ml) in the group without subsequent infection (p=0.89, Mann-Whitney, Figure 2). However, there was a significant correlation between antibody levels and IL-2 response following vaccination (r=0.51, p=0.001, Spearman's rho).

Of the 41 patients, 15 were above or equal to the age of 65 years. This elderly portion of the cohort differed in two ways from younger allo-HSCT recipients; only 1/15 patients had a
breakthrough infection, and all were vaccinated with mRNA-1273 (Moderna Spikevax®). In contrast, all study participants below 65 years of age were vaccinated with BNT162b2 (Pfizer-BioNTech Comirnaty®), and 11/26 experienced breakthrough infection. A subgroup analysis showed that, also in the latter younger cohort, high levels of SARS-CoV-2 specific T-cells, but not antibodies, following dual vaccination were protective against subsequent infection (Supplemental Figure S1). Notably, we did not observe significant differences in the immune responses elicited by two different vaccines (495 vs. 434 BAU/mL (2.69 vs. 2.64 log10 BAU/ml), p=0.8 and 167 vs. 88 IL-2 pg/mL, p=0.3, following two doses of mRNA-1273 and BNT162b2 respectively). This finding suggests that the lower incidence of COVID-19 among individuals over the age of 65 might be attributed to their higher level of adherence to social distancing and other preventive measures, rather than differences regarding vaccine-induced immune responses.

The immunogenic mRNA COVID-19 vaccines elicit robust innate and adaptive immune responses (11). Although T and B-cells crosstalk, they have partially independent roles (10). Higher neutralizing antibody levels against SARS-CoV-2 in healthy adults have been associated with protection against infection (12). However, in the Omicron era, studies have reported a lack of association between antibody concentrations and the incidence of infection (13), which was supported by our findings. The magnitude of the T-cell response has been shown to negatively correlate with disease severity (14). However, an absolute protective effect against infection due to high T-cell activity in the absence of antibodies has not been established. A recent study of 572 vaccinated allo-HSCT recipients found a one-year cumulative incidence of breakthrough infection of 15%, with antibody titers associated with both breakthrough infections and disease severity, though data on T-cell response were not
reported (15). As an increasing number of T cell assays for SARS-CoV-2 (e.g., Qiagen QuantiFERON® SARS-CoV-2, Hyris’ T-cell Test, T-SPOT®.COVID, etc.) are becoming commercially available as complements to already accessible COVID-19 antibody tests, our findings might be easier to generalize to other healthcare settings.

Despite the small sample size, which may have resulted in some numerically very different results being statically similar, and the inevitable risk of not detecting asymptomatic COVID-19 despite analyzing anti-nucleocapsid antibodies at baseline and follow-up, we conclude that a strong T-cell response might protect against acquiring breakthrough COVID-19. Additionally, antibody and T-cell responses following mRNA COVID vaccination were correlated in allo-HSCT recipients. This study suggests that antigen-specific T-cells play a pivotal role in protection against COVID-19 and warrants future studies of their protective role against COVID-19 and infection with other respiratory viruses.
References


**Table 1.** Baseline characteristics of allo-HSCT recipients stratified by Covid-19 breakthrough infection status after receiving two mRNA vaccine doses and analyzing the complete sample data.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All patients (n=41)</th>
<th>Covid-19&lt;sup&gt;†&lt;/sup&gt; following 2&lt;sup&gt;nd&lt;/sup&gt; vaccination (n=12)</th>
<th>No Covid-19&lt;sup&gt;†&lt;/sup&gt; following 2&lt;sup&gt;nd&lt;/sup&gt; vaccination (n=29)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median, years (range)</td>
<td>54 (29-78)</td>
<td>46.5 (29-78)</td>
<td>62 (40-75)</td>
<td>0.16&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>21 (51)</td>
<td>5 (42)</td>
<td>16 (55)</td>
<td>0.51**</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>20 (49)</td>
<td>7 (58)</td>
<td>13 (45)</td>
<td></td>
</tr>
<tr>
<td>Vaccine, Moderna / Pfizer-BioNTech, n (%)</td>
<td>15 (37) / 26 (63)</td>
<td>1 (8) / 11 (92)</td>
<td>14 (48) / 15 (52)</td>
<td>0.03**</td>
</tr>
<tr>
<td>Time from transplant to first vaccine dose (months, min-max)</td>
<td>90 (10-330)</td>
<td>108 (13-330)</td>
<td>89 (10-246)</td>
<td>0.58*</td>
</tr>
<tr>
<td>Conditioning intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIC / MAC, n (%)</td>
<td>20 / 21 (49)</td>
<td>5 / 7 (42)</td>
<td>15 / 14 (52)</td>
<td>0.73**</td>
</tr>
<tr>
<td>Donor source,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD / URD (%)</td>
<td>10 / 31 (24)</td>
<td>4 / 8 (33)</td>
<td>6 / 23 (21)</td>
<td>0.44**</td>
</tr>
</tbody>
</table>

<sup>†</sup> indicates Covid-19 breakthrough infection status; <sup>**</sup> indicates statistical significance at the 0.01 level; <sup>*</sup> indicates statistical significance at the 0.05 level.
<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>YES, n (%)</th>
<th>NO, n (%)</th>
<th>YES, n (%)</th>
<th>NO, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML, n (%)</td>
<td>18 (44)</td>
<td>7 (44)</td>
<td>11 (44)</td>
<td>1.0**</td>
</tr>
<tr>
<td>CML, n (%)</td>
<td>7 (17)</td>
<td>2 (13)</td>
<td>5 (20)</td>
<td>0.31**</td>
</tr>
<tr>
<td>MDS, n (%)</td>
<td>4 (10)</td>
<td>1 (6)</td>
<td>3 (12)</td>
<td>1.0**</td>
</tr>
<tr>
<td>Other, n (%)</td>
<td>12 (29)</td>
<td>6 (38)</td>
<td>6 (24)</td>
<td>0.13††</td>
</tr>
<tr>
<td>GvHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES, n (%)</td>
<td>15 (38)</td>
<td>4 (33)</td>
<td>11 (39)</td>
<td>1.0**</td>
</tr>
<tr>
<td>NO, n (%)</td>
<td>25 (63)</td>
<td>8 (67)</td>
<td>17 (61)</td>
<td></td>
</tr>
<tr>
<td>Ongoing</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>immunosuppression</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>YES (%)</td>
<td>7 (18)</td>
<td>2 (17)</td>
<td>5 (18)</td>
<td>1.0**</td>
</tr>
<tr>
<td>NO (%)</td>
<td>33 (83)</td>
<td>10 (83)</td>
<td>23 (82)</td>
<td></td>
</tr>
</tbody>
</table>

†Definition: Positive SARS-CoV-2 PCR, antigen test and or antibodies to nucleocapsid antigen.

RIC: Reduced intensity conditioning; MAC: maximal intensity conditioning; RD: Related donor; URD: Unrelated donor; AML: Acute myeloid leukemia; CML: Chronic myeloid leukemia; MDS: Myelodysplastic syndrome; GvHD: Graft-versus-host disease. The presence of GvHD was assessed at the visit prior to the first vaccine dose. Ongoing immunosuppression was registered if present at first vaccination. Statistics using Mann-Whitney U test (*) or Fischer’s exact test (**). Statistical analyses were performed using SPSS statistical software package (version 28). P-values are designated as follows: *P<0.05 and **P<0.01. All indicated P-values are two-sided.
Figure Legends

Figure 1. CONSORT-diagram for allo-HSCT recipients in the study.

Figure 2. Humoral and T-cell-mediated immune responses 5 months after 2nd mRNA COVID vaccination in allo-HSCT recipients stratified according to subsequent SARS-CoV-2 infection or not. Spike 1 (S1) specific immune responses in allo-HSCT recipients 3 months after the second vaccine dose demonstrated as T-cell production of interleukin-2 (IL-2) in supernatant plasma following stimulation of whole blood with S1 peptides and IgG antibody levels in serum against the receptor-binding domain (RBD) in S1. Statistical comparisons were calculated by Mann-Whitney U-test. Statistical analyses were performed using GraphPad Prism software (version 9). P-value is two-sided and designated as *P<0.05.
Assessed for eligibility:
Allo-HSCT recipients screened at baseline
N=82

Not included, n=24¹

Included allo-HSCT recipients
N=58

Excluded, n=8²

No signs of earlier COVID-19 at baseline
N=50

Excluded, n=9³

Sampled after two doses of vaccine and subsequent complete data available⁴

Subsequent COVID-19 infection
N=12

No subsequent COVID-19 infection
N=29⁵

¹Previously COVID-19 vaccinated (n=13), Recent treatment with rituximab (n=4), Hesitant to COVID-19 vaccine (n=2), Current infection (n=2), Declined participation (n=3).

²Previously confirmed COVID-19 infection by PCR (n=5) or seropositive at baseline (n=3).

³PCR positive between dose 1 and 2 (n=1), Dead due to complications of severe GvHD (n=1), Relapse of underlying disease (n=3), Lost-to follow-up (n=4).

⁴At least two blood samples available after the first two vaccine doses and data available on occurrence of COVID-19.

⁵Complete data available on IL-2 (n=27) and anti-nucleocapsid IgG (n=28).
Supplementary Figure S1. Humoral and T-cell-mediated immune responses 5 months after 2nd mRNA COVID vaccination in allo-HSCT recipients aged below 65 years (all were given BNT162b2 (Pfizer-BioNTech Comirnaty®)) stratified according to subsequent SARS-CoV-2 infection or not. Spike 1 (S1) specific immune responses in allo-HSCT recipients 3 months after the second vaccine dose demonstrated as T-cell production of interleukin-2 (IL-2) in supernatant plasma following stimulation of whole blood with S1 peptides and IgG antibody levels in serum against the receptor-binding domain (RBD) in S1. Statistical comparisons were calculated by Mann-Whitney U-test. Statistical analyses were
performed using GraphPad Prism software (version 9). P-value is two-sided and designated as *P<0.05.