CORRESPONDENCE

Adoptive transfer of functional SARS-COV-2-specific immunity from donor graft to hematopoietic stem cell transplant recipients

To the Editor:

Immunocompromised recipients of allogeneic hematopoietic stem cell transplant (HCT) are at increased risk of severe COVID-19.[1] During the first year of a successful HCT, circulating T-cells arise from donor CD34$^+$ cells and can react to antigens exposed to the donor through natural infection or vaccination before transplantation. Therefore, donor pathogen exposure or vaccination pre-graft can be beneficial to the recipient when mounting cellular and humoral response to augment immune reconstitution and control post-HCT natural infection or increase vaccination responses.[2]

Here, we present evidence of transfer and expansion of SARS-CoV-2-specific adaptive immunity from three matched unrelated donors (MUDs), vaccinated with licensed COVID-19 vaccines to unvaccinated and vaccinated recipients. The 10/10 matched (with permissive HLA-DPB1 locus mismatch) MUDs and their recipients did not have COVID-19 history nor developed active infection through study completion (d + 180). All three recipients engrafted and achieved full donor chimerism (>95%)[3] by d + 30.

The patient from MUD/R1 pair (Table 1S) was a 29-year-old Hispanic male, with body mass index of 40.07 kg/m^2, hypertension, diabetes, diagnosed with Philadelphia like B-cell acute lymphoblastic leukemia, with cytokine receptor-like factor 2 rearrangement. The mRNA-1273 vaccinated MUD donor was a 33-year-old male. The recipient underwent a myeloablative HCT soon after CD-19 CAR T-cell therapy, while in second complete remission (CR2) with negative measurable residual disease (MRD), using fractionated total body irradiation with etoposide. He received GVHD prophylaxis of tacrolimus and sirolimus (tacro/siro). He developed grade 1 skin GVHD around d + 24, which resolved with topical therapy. He did not receive a COVID-19 vaccine because prior to HCT, the patient was unstable and not ambulatory.

Patient from MUD/R2 pair was a 74-year-old Caucasian male with history of hypertension, diagnosed with acute myeloid leukemia with deletion Y and SRSF2 mutation, who was in CR1 with negative MRD after receiving hypomethylating agent and venetoclax. The BNT162b2 mRNA vaccinated MUD donor was a 30-year-old female. The recipient underwent reduced intensity HCT using fludarabine and melphalan conditioning (FM), with tacro/siro GVHD prophylaxis in combination with itacitinib JAK-1 inhibitor (NCT04339101). He developed mild chronic GVHD of skin and liver around d + 180. He received a single JNJ-78436735 vaccine dose pre-HCT (d-145).

Patient from MUD/R3 pair was a 65-year-old Caucasian female with hypertension and myelodysplastic syndrome/myeloproliferative neoplasm associated with JAK2, ASXL1, and SRSF2 mutations. The BNT162b2 mRNA vaccinated MUD donor was a 33-year-old male. The recipient underwent reduced intensity HCT using FM, followed by tacro/siro with itacitinib for GVHD prophylaxis. She received the BNT162b2 mRNA COVID-19 vaccine pre- (d-74) and post-HCT (d + 112 and d + 133). On d + 165, the patient received tixagevimab co-packaged with cilgavimab for COVID-19 prophylaxis.

All three MUD donors (Figure 1) developed SARS-CoV-2-specific neutralizing antibodies (NAbs), following vaccination with either the BNT162B2 vaccine or the JNJ-78436735 (Table 1S). Serum levels of receptor-binding domain (RBD)- and Spike (S)-specific antibodies were also similar in the three donors. Low levels of Nucleocapsid (N)-specific IgG were detected in donors from MUD/R1 and MUD/R2 pairs. IgM levels were minimal since all donors received the first vaccine injection >2 months before graft collection. Functional SARS-CoV-2-specific T-cells were mainly CD137$^+$CD3$^+$CD4$^+$, and higher levels were measured in pair 2 donor compared to the other two donors. N-specific T-cells were detectable in pair 1 and 2 donors, analogously to their respective humoral response pattern, which may indicate undocumented exposure to SARS-CoV-2, in these subjects. S-specific IFN-γ had comparable levels in all three donors.

SARS-CoV-2-specific CD137$^+$ T-cells were detected early post-HCT in all three recipients and expanded during immune reconstitution (Figure 1A,B).

MUD/R1 pair patient, who did not receive COVID-19 vaccine, had measurable functionally activated S-specific CD137$^+$CD3$^+$CD4$^+$ T-cells early post-HCT (d + 30). They subsequently declined and then gradually expanded to levels comparable to those of the donor by d + 150 when patient's lymphopenia resolved. S-specific IFN-γ was detectable starting d + 60 and markedly increased through d + 150.

Frequencies of both S- and N-specific donor derived CD137$^+$CD3$^+$CD4$^+$ T-cells were 3–5 times lower in the MUD/R2 recipient than in the donor, by d + 30. However, during immune reconstitution, they steadily increased, and by six months post-transplant, they surpassed levels detected in the donor blood draw. Moreover, low but measurable levels of S-specific CD137$^+$CD8$^+$ T-cells were detected in the MUD/R2 pair, which peaked by study end in the recipient. T-cells were actively producing high levels of S-specific IFN-γ, though
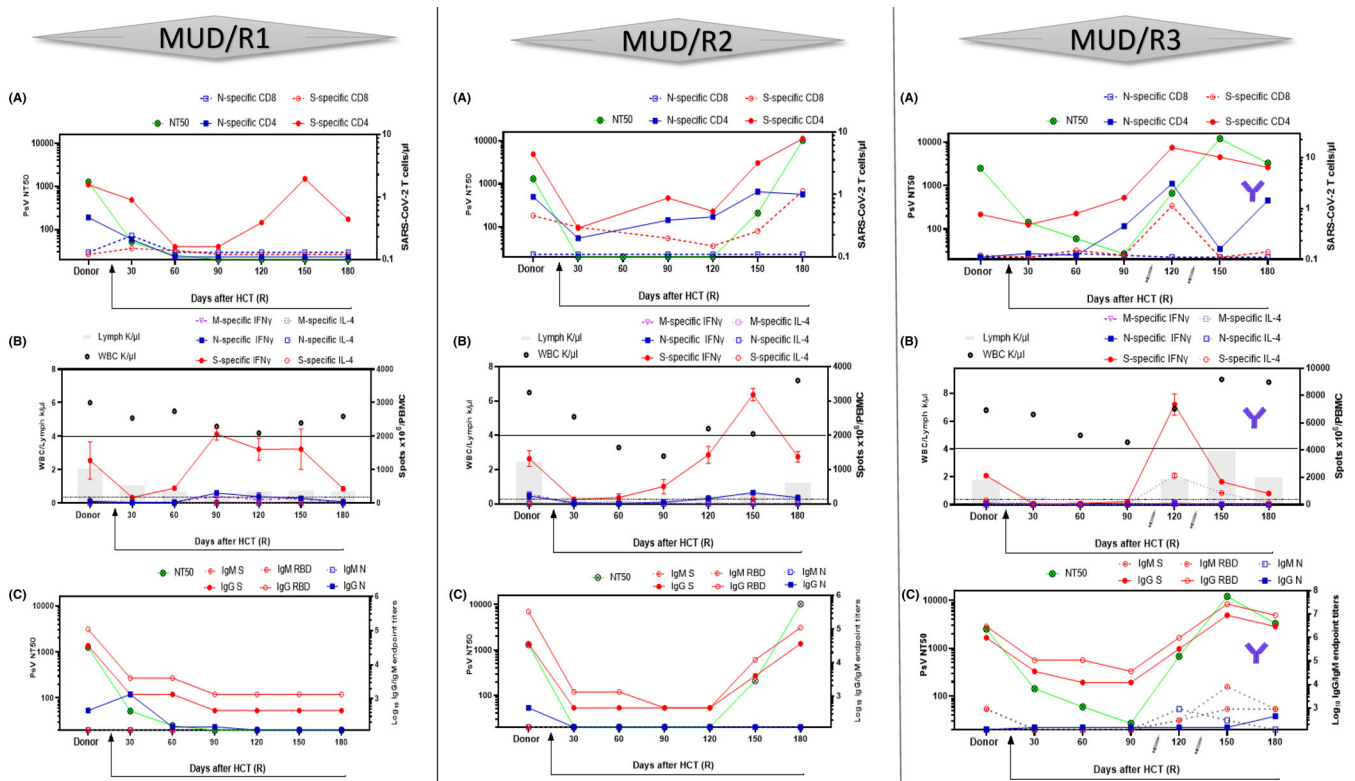
FIGURE 1 Longitudinal SARS-CoV-2-specific adaptive humoral and cellular profiles in each donor/recipient (MUD/R) HCT pair. Description of the immunological assays used is provided in the Supporting Information. Panels A show the levels of Spike (S)- and Nucleocapsid (N)-specific CD137$^+$ T cells/μl (right y-axes) measured by multiparameter cytofluorimetry in peripheral blood mononuclear cells (PBMC); and SARS-CoV-2-neutralizing antibodies, as serum dilution that neutralized 50% of the SARS-CoV-2 pseudo virus (pv NT50, left y-axes). Panels B show S-, N-, and membrane (M)-specific interferon (IFN)γ and interleukin (IL)4 spots/10^6 PBMC (right y-axes), measured by ELISPOT; left y-axes, white blood cells and lymphocyte, k/μl. Panels C show serum concentrations of S-, receptor-binding domain (RBD)-, N-specific IgG and IgM measured using indirect ELISA, and expressed as endpoint titers; left y-axes, as specified for panels A. For A, B, and C panels, x-axes show post-HCT blood draw day for R; pre-HCT blood draw day for D is reported in Table 1S. Arrows indicates, HCT (day 0); dotted lines, lower limit of the normal range (0.5/4.1 k/μl) for lymphocyte (Lymph) counts; solid lines, lower limit of the normal range (4–11 k/μl) for white blood cells (WBC) counts; syringe symbol, approximate post-HCT day of BNT162b2 vaccination (exact days are reported in Table 1S); purple antibody symbol, administration of tixagevimab co-packaged with cilgavimab long-acting antibody (LAAB) combination at day +165 post-HCT

the patient remained lymphopenic, and at times also leukopenic until d + 150.

S-specific CD137$^+$CD3$^+$CD4$^+$ T-cells were detectable early post-HCT in the recipient of the MUD/R3 pair. T-cells consistently expanded from the donor graft during immune reconstitution, and further increased when post-transplant COVID-19 vaccine was administered. S-specific CD137$^+$CD3$^+$CD8$^+$ and N-specific CD137$^+$CD3$^+$CD4$^+$ T-cells proliferation peaked at around three months post-HCT vaccination. Very high levels of S-specific IFN-γ and modest levels of S-specific IL-4 were detected after the first post-HCT COVID-19 vaccine dose.

Functionally activated SARS-CoV-2-specific T-cells were characterized for their memory phenotypes (Figure 1S). Most of the S- and N-specific CD137$^+$CD3$^+$CD4$^+$ were central memory T-cells (TCM) expressing high levels of CD28 and minimal levels of CD45RA. S- and N-specific CD137$^+$CD3$^+$CD4$^+$ T-cells exhibited stable phenotypes, with modest increasing levels in TCM for S-specific CD137$^+$CD3^{+-}CD4$^+$ T-cells in all three MUD/R pairs. For MUD/R pair 2 and

3 patients, frequency of S-specific CD137$^+$ CD3$^+$ CD8$^+$ T-cells was at some time points ≥0.2%, consequently memory phenotype could be measured[9]. Persistent levels of less differentiated effectors T-cell subsets (TEMRA) with expansion plasticity phenotypic signature (CD45RA$^+$ CD28$^-$) were detected in the CD8 arm of functionally activated S-specific T-cells.

Antibody-mediated SARS-CoV-2 specific immunity was detected in all three MUD/R pairs (Figure 1A,C). Transfer of donor-derived SARS-CoV-2 specific humoral immunity can be surmised for MUD/R1 pair, since no COVID-19 infection nor vaccination was reported for the patient. Declining levels of S- and RBD- IgG binding antibodies were measurable through 6 months post-HCT. Transfer of N-IgG and low levels of SARS-CoV-2 specific NAbs was detectable only on d + 30.

In MUD/R2 and MUD/R3 pairs in whom recipients received pre-HCT SARS-CoV-2 vaccines, early post-HCT detection of adaptive humoral immunity was probably of both donor and recipient origin. In recipient of MUD/R2 pair, lymphopenia resolution (Figure 1B) may

have led to the expansion of donor-derived SARS-CoV-2-specific B-cells with consequent increase in S- and RBD-IgG binding titers and NAbs levels by d + 150. In MUD/R3 pair, post-HCT COVID-19 vaccination of the recipient greatly boosted both S- and RBD-IgG binding titers and NAbs which peaked by d + 150. Low titer levels of SARS-CoV-2-specific IgM antibodies were also detectable following COVID-19 vaccination. Subsequent administration of tixagevimab/cilgavimab at d + 165 did not further increase levels of SARS-CoV-2 adaptive humoral immunity in the recipient.

To our knowledge, this is the first reported evidence of donor-derived SARS-CoV-2 immune transfer, expansion, and vaccine boosting in HCT recipients. Substantial SARS-CoV-2-specific IFN-γ was measurable in all three HCT recipients. In contrast, IL-4 levels remained minimal which was indicative of a polarized Th1 response, associated with protection from severe COVID-19.[4] Memory phenotype for both S- and N-specific CD137+ T-cells showed elevated frequencies of TCM, which can home to lymph nodes, where they help B cells undergo affinity maturation.[4] Increasing percentages of S-specific CD137+ CD8+ TEMRA effectors characterized by proliferative and self-renewal capacity were also detected, which are typically found in convalescing COVID-19 patients and vaccinated individuals.[4] Our data confirm recent studies suggesting that T-cell responses in immunosuppressed patients can be preserved and may provide an essential role in vaccine-mediated protection.[5] Hence, the prompt surge in levels of donor-derived functional SARS-CoV-2-specific T-cells and IFN-γ in MUD/R3 patient indicate that in T-cell replete HCT recipients, a graft from a vaccinated donor can favor successful booster-like cellular response even early post-HCT, when humoral responses are blunted by ongoing immunosuppressive regimens.

In the three recipients, NAb titers remained low or undetectable early post-HCT, confirming delayed B-cell functional reconstitution and adaptive humoral immune recovery post-HCT. Increases in SARS-CoV-2-specific IgG and NAb followed SARS-CoV-2-specific CD4 T-cell reconstitution. The critical role of CD4 T-cells[6] in promoting robust, long lived SARS-CoV-2-specific antibody levels, and in response to mRNA vaccines has been shown including in HCT and cellular therapy recipients, in whom COVID-19 vaccines are not precluded even when B-cell aplasia occurs. In summary, pre-HCT vaccination of MUD/R2 and MUD/R3 pairs potentiated immune reconstitution and stimulated proliferation of functional donor-derived T-cells, which were likely the primary cause of the robust SARS-CoV-2-specific humoral responses observed in the recipients.

Detection of low levels of S-specific and RBD-specific IgM after COVID-19 re-vaccination could be explained as the inability of the immunosuppressed patient to mount an efficient antibody response. No effect of tixagevimab/cilgavimab prophylaxis was observed in MUD/R3 patient, likely because the treatment was administered immediately after the post-HCT vaccination rise in humoral response.

Our data suggest that choosing a donor with SARS-CoV-2-specific immunity could be decisive as an alternative prophylaxis strategy to mitigate COVID-19 severity in HCT recipients and can promote a functional vaccine response early post-HCT. Finally, none of the three recipients described in this report developed COVID-19 post-HCT, which is a limitation of this study. Therefore, further investigation in different transplant settings is needed to verify that SARS-CoV-2-specific adoptive immunity from a COVID-19-seropositive donor is protective for the recipient after transplantation. Such clinical studies can constitute a critical, essential step toward improvement of the remarkably poor recovery from COVID-19 observed in the HCT setting.

CONFLICT OF INTEREST

Corinna La Rosa received consulting fees and research funding from Helocyte Inc.; Don J. Diamond consulting fees, patent royalties, research funding, and fees for serving on the advisory board of Helocyte Inc. In addition, Don J. Diamond has two patents 8 580 276 and 9 675 689 that are licensed to Helocyte. Don J. Diamond and Flavia Chiuppesi are co-inventors of the Patent Cooperation Treaty (PCT) application that covers the development of a COVID-19 vaccine (PCT/US2021/032821) licensed to GeoVax Labs Inc. Don J. Diamond receives consulting fees and research support from GeoVax Labs Inc. Ryotaro Nakamura is a consultant for Omeros, Bluebird, Viracor Eurofins, Magenta Therapeutics, Kadmon, Napajen Pharma; received research funding from Helocyte, Miyarisan Pharmaceutical; and travel, accommodations, expenses from Kyowa Hakko Kirin, Alexion Pharmaceuticals. [Correction added on September 9, 2022, after first online publication: The preceding statement was removed in this version.] The remaining authors declare no relevant competing financial interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CLINICAL TRIAL REGISTRATION

This study is registered on clinicaltrials.gov, as NCT04666025.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.