# Mucosal-associated invariant T cells are functionally impaired in pediatric and young adult patients following allogeneic hematopoietic stem cell transplantation and their recovery correlates with clinical outcomes

Federica Galaverna,[1*] Sara Flamini,[1*] Carmen Dolores De Luca,[2] Ilaria Pili,[1] Emilia Boccieri,[1] Francesca Benini,[2] Francesco Quagliarella,[1] Chiara Rosignoli,[1] Marco Rosichini,[1,3] Shirley Genah,[1] Marialuigia Catanoso,[1] Antonella Cardinale,[1] Gabriele Volpe,[1] Marianna Coccetti,[1] Angela Pitisci,[1] Giuseppina Li Pira,[1] Roberto Carta,[1] Barbarella Lucarelli,[1] Francesca Del Bufalo,[1] Valentina Bertaina,[1] Marco Becilli,[1] Daria Pagliara,[1] Mattia Algeri,[1,4] Pietro Merli,[1] Franco Locatelli[1,5#] and Enrico Velardi[1#]

[1]Research Area of Hematology and Oncology, Cell and Gene Therapy, Bambino Gesù Children's Hospital, IRCCS, Rome; [2]Department of Maternal and Child Health, Sapienza University of Rome, Rome; [3]Department of Molecular Medicine, Sapienza University of Rome, Rome; [4]Department of Health Sciences, Magna Graecia University, Catanzaro and [5]Department of Maternal and Child Health, Catholic University of the Sacred Heart, Rome, Italy

**FG and SF contributed equally as first authors.*

*#FL and EV contributed equally as senior authors.*

**Correspondence:** E. Velardi
enrico.velardi@opbg.net

F. Locatelli
franco.locatelli@opbg.net

**Received:** November 16, 2023.
**Accepted:** May 17, 2024.
**Early view:** May 30, 2024.

**https://doi.org/10.3324/haematol.2023.284649**



## Abstract

Mucosal-associated invariant T (MAIT) cells are innate-like T cells implicated in the response to fungal and bacterial infections. Their contribution to restoring T-cell immunity and influencing hematopoietic stem cell transplant (HSCT) outcomes remains poorly understood. We retrospectively studied MAIT-cell recovery in 145 consecutive children and young adults with hematologic malignancies undergoing allogeneic (allo)-HSCT between April 2019 and May 2022, from unrelated matched donor (MUD, N=52), with standard graft-*versus*-host-disease (GvHD) prophylaxis, or HLA-haploidentical (Haplo, N=93) donor after *in vitro* αβT/CD19-cell depletion, without post-HSCT pharmacological prophylaxis. With a median follow-up of 33 months (range, 12-49 months), overall survival (OS), disease-free survival (DFS), and non-relapse mortality (NRM) were 79.5%, 72%, and 7%, respectively; GvHD-free relapse-free survival (GRFS) was 63%, while cumulative incidence of relapse was 23%. While αβT cells were reconstituted 1-2 years post HSCT, MAIT cells showed delayed recovery and prolonged functional impairment, characterized by expression of activation (CD25, CD38), exhaustion (PD1, TIM3) and senescence (CD57) markers, and suboptimal *ex vivo* response. OS, DFS, and NRM were not affected by MAIT cells. Interestingly, higher MAIT cells at day +30 correlated with higher incidence of grade II-IV acute GvHD (19% *vs*. 7%, *P*=0.06). Furthermore, a greater MAIT-cell count tended to be associated with a higher incidence of chronic GvHD (cGvHD) (17% *vs*. 6%, *P*=0.07) resulting in lower GRFS (55% *vs*. 73%, *P*=0.05). Higher MAIT cells also correlated with greater cytomegalovirus (CMV) reactivation and lower late blood stream infections (BSI) (44% *vs*. 24%, *P*=0.02 and 9% *vs*. 18%, *P*=0.08, respectively). Future studies are needed to confirm the impact of early MAIT-cell recovery on cGvHD, CMV reactivation, and late BSI.

## Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) represents a potentially curative treatment for a wide range of malignant and non-malignant hematologic diseases;[1,2] however, post-transplant immune deficiency represents a major clinical hurdle that both limits a broader use of this treatment and impairs its efficacy.[3] In fact, defective quantitative and qualitative immune recovery, in particular of the T-cell compartment, is associated with an

increased risk of infections, leukemia relapse, and adverse clinical outcomes in patients receiving allo-HSCT.[4-7] This is particularly relevant for patients receiving T-cell-depleted grafts, who experience profound and prolonged immunodeficiency lasting until the T-cell pool is newly generated from donor-derived hematopoietic precursor cells.[8-10] In the setting of unmanipulated HSCT, while mature donor T cells infused with the graft contribute to the early phases of T-cell reconstitution, they are also responsible for the development of graft-*versus*-host disease (GvHD). In addition, immunosuppressive therapies associated with GvHD prophylaxis further delay the process of T-cell reconstitution.

Unconventional T cells have been the subject of major interest in the allo-HSCT field because of their capacity to mediate anti-tumor and anti-infectious effects, without increasing GvHD occurrence. Mucosal-associated invariant T (MAIT) cells are innate-like T cells expressing a semi-invariant T-cell receptor (TCR) (V$\alpha$7.2) and recognizing bacterial- and fungal-derived riboflavin metabolites presented by non-canonical class I-related MHC molecule (MR1).[11,12] MAIT cells are generated in the thymus, and their development requires the presentation of bacteria-derived metabolites by double-positive thymocytes, as well as other cells within the thymic microenvironment.[13] Upon maturation, MAIT cells primarily localize in mucosal tissue, such as the gut, lung, and liver, and are the most abundant innate-like T-cell population in human peripheral blood, accounting for up to 10% of total T cells in healthy adults.[14] MAIT cells have the capacity to rapidly produce several proinflammatory cytokines (including IFN$\gamma$, IL-17, and TNF$\alpha$) and release cytolytic molecules (such as granzyme B and perforin) in a TCR-dependent and -independent manner.[12,15] Previous studies demonstrated that MAIT cells are involved in several immunological diseases, including autoimmune diseases (e.g., multiple sclerosis and inflammatory bowel disease), microbial infections (including *E. coli*, *H. pylori*, *M. tuberculosis* infections), and viral infections (including HIV, hepatitis C and influenza viruses).[14,16]

An increasing number of studies have been investigating the role of MAIT cells during immune reconstitution following allo-HSCT. It has been reported that MAIT-cell recovery is primarily graft-derived after transplantation and that a higher proportion of MAIT cells in the graft is associated with a greater incidence of acute GvHD (aGvHD).[17,18] Previous studies also suggested that reduced post-HSCT MAIT-cell count is associated with the development of aGvHD.[18-20] However, these observations have not been made consistently across studies.[21-23] Importantly, despite their importance in the immune response to pathogens, the potential impact of MAIT cells in the control of bacterial and viral infections post HSCT remains poorly explored.

Here, we examined the reconstitution kinetics and function of MAIT cells in pediatric patients and young adults receiving allo-HSCT from 10 out of 10 HLA-allelic matched unrelated donor (MUD) or HLA-haploidentical (Haplo) family donor and studied their correlation with patient clinical outcomes.

# Methods

## Patient and donor characteristics

We analyzed 145 consecutive patients affected by hematologic malignancies undergoing allo-HSCT between April 2019 and May 2022, fully engrafted at day +30 after receiving allogeneic stem cells from MUD (N=52), with standard GvHD prophylaxis, or from a Haplo-donor (N=93) after T-cell receptor (TCR) alpha-beta ($\alpha\beta$) / CD19-cell depletion. Inclusion criteria were morphological complete remission (CR) at the time of HSCT, with negative or less than $10^{-4}$ minimal residual disease (MRD). Every patient received myeloablative conditioning based on total body irradiation (TBI), busulfan or treosulfan combined with other agents; the preparative regimen was chosen according to the type of disease and patient's comorbidities. GvHD prophylaxis of MUD-HSCT recipients included rabbit anti-T-lymphocyte globulin (ATG, Grafalon, Neovii, 5 mg/kg/day from day -5 to day -3), short-course methotrexate (15-10-10-10 mg/m$^2$ at days +1, +3, +6, +11) and cyclosporin. In the Haplo-HSCT setting, patients received pre-HSCT rituximab and ATG (Grafalon, Neovii, 4 mg/kg/day from day -4 to day -2), as previously described,[24,25] and no post-HSCT pharmacological prophylaxis was given. All patients received antifungal prophylaxis with either fluconazole 6 mg/kg daily or liposomal amphotericin B 3 mg/kg twice weekly (MUD- and Haplo-HSCT, respectively), as well as antiviral prophylaxis with acyclovir 30 mg/kg daily; none of the patients received letermovir as cytomegalovirus (CMV) prophylaxis. Peripheral blood mononuclear cell (PBMC) samples were collected at different time points (+30, +60, +90, +180, +360, +720 days) post HSCT (Figure 1A). To compare the presence and phenotypic characteristics of MAIT cells in the patient's peripheral blood with the different graft sources, we analyzed healthy donor (HD)-derived bone marrow (BM) aspirates (N=11), as well as peripheral blood stem cells (PBSC) obtained from the positive fraction of the TCR$\alpha\beta$/ CD19-depletion procedure (which is enriched in TCR$\alpha\beta$ cells) (N=5). We also evaluated MAIT cells in PBMC of 16 age-matched healthy pediatric donors and 11 adult HD. This observational study was approved by the Ethical Committee of Bambino Gesù Children's Hospital. Written informed consent was obtained from the patient or, if under 18 years of age, from the patient's legal guardian in accordance with the principles of the Declaration of Helsinki.

## Flow cytometry data analysis

All analyses were performed using PBMC previously isolated from blood and stored in liquid nitrogen until use. Monoclonal antibodies used for multi-parametric flow-cy-

tometry analyses and *in vitro* assays are listed in *Online Supplementary Table S1*. In our study, we defined MAIT cells as $CD45^+CD3^+TCR\alpha\beta^+$MR1-5-OP-RU-tetramer positive, as shown in *Online Supplementary Figure S1*. The MR1 tetramer was produced by the NIH Tetramer Core Facility. Data were collected using a BD FACSymphony™ A5 Cell Analyzer (BD Life Sciences). Manual gating and unsupervised clustering analysis were performed using FlowJo v.10.8 Software (LCC). To study post-HSCT MAIT-cell recovery, we included patients in whom at least 2 time points were available for flow cytometry analysis. Data to longitudinally study the reconstitution kinetics of total αβT cells and MAIT cells were available for 126 out of 145 patients (N=76, Haplo; N=50, MUD) at day +30, for 111 at day +60 (N=67, Haplo; N=44, MUD), for 114 at day +90 (N=69, Haplo; N=45, MUD), for 122 at day +180 (N=76, Haplo; N=46, MUD), for 93 at day +360 (N=56, Haplo; N=37, MUD), and for 47 patients at day +720 (N=29, Haplo; N=18, MUD) after HSCT, respectively.

### *In vitro* functional analysis

Peripheral blood mononuclear cells were cultured in RPMI-1640 (Gibco™) supplemented with 10% Fetal Bovine Serum (Gibco™) and 1% of L-Glutamine (Gibco™). For the activation assay, PBMC were incubated with pre-fixed *E. coli*[26] or αCD3 (Invitrogen) and αCD28 (BD) for 24 hours. Supernatants were collected at the end of the culture and frozen at -80°C. Cells were harvested, washed, stained, and fixed before flow cytometry analysis.

### Enzyme-linked immunosorbent assay

The concentrations of IFNγ, TNFα, Granzyme B and Perforin in cell supernatants were measured by enzyme-linked immunosorbent assay kit HCD8MAG15K17PMX (MILLIPLEX, Merck), used according to the manufacturer's instructions.

### Statistical analysis

Patient and transplant characteristics were expressed as the number and percentage of the total for categorical variables and median with ranges for continuous variables. We considered the whole cohort of patients to perform outcome analyses and to explore the correlation of MAIT cells with post-HSCT clinical course. Clinical variables analyzed included young patient age (≤2 years old), donor type, source of HSC, conditioning regimen, post-HSCT viral infections (any viremia, HHV6, Epstein-Barr virus [EBV], adenovirus [ADV] reactivation, occurrence of viral respiratory infections), CMV, BSI, probable fungal infections, the occurrence of aGvHD of any grade and grade II-IV aGvHD, or cGvHD of any grade and cGvHD grade moderate to severe. A pediatric disease risk score was used as clinical variable when possible (acute leukemia patients) to assess the possible influence on patient outcome.[27] Pre-engraftment BSI was defined as bacteremia with clinical signs of infection occurring between the onset of the conditioning regimen and neutrophil engraftment. One positive blood culture, either from peripheral or from central venous access, was considered sufficient in case of Gram-negative bacteria; 2 or more consecutive positive blood cultures were necessary in case of Gram-positive bacteria. BSI occurring after the engraftment and up to 12 months post HSCT was defined as late BSI. CMV and viral reactivation were defined as any increase of CMV- or viral-DNAemia, determined by polymerase chain reaction (PCR), above the standard reference threshold (>1,000 copies/mL or >500 IU/mL); viral respiratory infections were characterized by positivity to respiratory virus in nasal swab by PCR, with or without clinical upper respiratory symptoms. Fungal infections were defined as probable according to previously published criteria.[28] MAIT-cell-associated variables analyzed included αβT-cell counts at day +30, MAIT-cell count at days +30, +60 and +90, MAIT-cell frequency among αβT-cells at days +30, +60 and +90, $CD161^+$ MAIT-cell count at day +30, +60 and +90, $CD161^+$ cell frequency among MAIT cells at day +30, +60 and +90. All data, including outliers, were included except for flow cytometry cell subset percentages for which the parent subset contained less than 100 events. To determine the impact of the categorical independent variables on categorical and continuous dependent variables in univariate analyses, $\chi^2$ and Mann-Whitney non-parametric tests, respectively, were used. Multivariate analyses were performed by using multiple linear and logistic regression for continuous and binary dependent variables, respectively. The choice of co-variates used in multivariate analysis was based on preliminary univariate analysis results and clinical relevance. Overall survival (OS) was defined as the probability of being alive and was calculated starting from the time of HSCT to patient death or last follow-up; disease-free survival (DFS) was defined as the probability of survival without evidence of disease from the time of HSCT to death, malignancy recurrence or last follow-up; GvHD / relapse-free survival (GRFS) was defined as the probability of survival without occurrence of grade III-IV aGvHD or cGVHD requiring treatment or relapse, whichever occurred first, from the time of HSCT to death or last follow-up. Survival probabilities were estimated by the Kaplan-Meier method and differences between groups were calculated using the log-rank test. The Cox proportional hazard regression model was used to assess the association between patient-, disease- and transplantation-related factors with survival outcomes. Cumulative incidence of relapse (CIR), non-relapse mortality (NRM), aGvHD and cGvHD were calculated using the method of Fine and Gray taking into account the respective competitive risks, namely death in remission for relapse, disease recurrence for NRM, disease recurrence and death for GvHD; comparison between groups was performed with the Gray's test. For flow cytometry data all *P*-values were two-sided and $P<0.05$ was considered statistically significant. Statistical analysis was performed using EZR version 1.32 (Saitama Medical Centre, Jichi Medical Uni-

versity), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria), and Prism software V.9 (GraphPad).

# Results

## Patients and outcome

Fifty-two patients in the MUD-HSCT group and 93 patients in Haplo-HSCT group were included in the analysis. Median age at the time of HSCT was 8 years (range, 0.6-25 years). The clinical characteristics of patients are reported in Table 1. The source of stem cells was PBSC in 103 cases (all Haplo-HSCT and 10 MUD-HSCT) and BM in 42 cases, all MUD-HSCT, while the conditioning regimen was TBI-based or chemo-based in 78% and 22% of the cases, respectively. Thirty-six patients experienced aGvHD with a median time from HSCT of 53 days (range, 15-148 days), with 26 cases of grade II-IV aGvHD, while 15 patients developed cGvHD with a median time of onset of 194 days from HSCT (range, 119-333 days); 11 of them had moderate / severe cGvHD. The cumulative incidence (CI) of all grades and grade II-IV aGvHD was 25% (95%CI: 19-33) and 19% (95%CI: 13-27), respectively (*Online Supplementary Figure S2A*). The CI of cGvHD was 11% (95%CI: 6-17) with a CI of moderate-severe cGvHD of 8% (95% CI: 5-14) (*Online Supplementary Figure S2B*). CI of aGvHD and grade II-IV aGvHD did not differ significantly among different types of HSCT: 31% (95%CI: 20-45) *versus* 22% (95%CI: 15-32) and 21% (95%CI: 12-35) *vs*. 18% (95%CI: 11-28) in MUD and Haplo-HSCT, respectively ($P$=0.27 and $P$=0.78, respectively). Similar results among the two groups were also observed analyzing cGvHD occurrence: 5 MUD-HSCT and 10 Haplo-HSCT experienced cGvHD (CI 10%, 95%CI: 4-22, *vs*. 12%, 95%CI: 6-21, respectively, $P$=0.66), with a CI of moderate / severe cGvHD of 8%, 95%CI: 3-20, *versus* 8%, 95%CI: 4-17, respectively ($P$=0.85).

Post-HSCT respiratory viral infections occurred in 66 cases (45.5% of the whole cohort). Seventy-nine patients (54.5%) experienced at least one viral reactivation, CMV being the most frequent (49 cases, with 11 and 38 cases in MUD and Haplo-HSCT, respectively). The CI of post-HSCT CMV reactivation of the entire cohort resulted in 34% (95%CI: 27-42) (*Online Supplementary Figure S2C*), with a median interval from HSCT of 31 days (range, 1-201 days). Thirteen and 20 patients experienced pre-engraftment or late BSI, with a CI of 8% (95%CI: 5-13) and 14% (95%CI: 9-21), respectively (*Online Supplementary Figure S2C*, and *data not shown*). The median interval between HSCT and the onset of pre-engraftment and late BSI was 7.5 days (range, 0-14 days) and 87 days (range, 34-304 days), respectively. Haplo-HSCT recipients experienced higher CI of CMV reactivation compared to MUD-patients (CI: 41%, 95%CI: 32-52, *vs*. 21%, 95%CI: 12-35, respectively, $P$=0.01) and higher CI of late BSI (19%, 95%CI: 12-29, *vs*. 6%, 95%CI: 2-17, respectively, $P$=0.03). Increased CMV reactivation was also recorded

**Table 1.** Patients' characteristics.

| Patients | N (%) Total, N=145 |
|---|---|
| Gender | |
| Female | 66 (45.5) |
| Male | 79 (54.5) |
| Age in years, median (range) | 8 (0.6-25) |
| Age ≤2 years | 15 |
| Disease | |
| ALL | 91 (62.7) |
| AML | 33 (22.7) |
| NHL | 10 (6.9) |
| MDS | 5 (3.4) |
| JMML | 3 (2) |
| HL | 2 (1.4) |
| CML | 1 (0.7) |
| Disease risk score[27] | 125 (86.2) |
| Low | 20 |
| Intermediate | 92 |
| High | 13 |
| Donor | |
| Haploidentical | 93 (64) |
| MUD | 52 (36) |
| Stem cell source | |
| Peripheral blood | 103 (71) |
| Bone marrow | 42 (29) |
| Conditioning | |
| TBI-based | 113 (78) |
| Chemo-based | 32 (22) |
| Post-transplant GvHD prophylaxis | |
| No post-transplant GvHD prophylaxis | 93 (64) |
| CSA-MTX | 52 (36) |
| GvHD | |
| Acute GvHD/aGvHD grade II/IV | 36 (24.8)/26 (17.9) |
| Chronic GvHD/cGvHD grade moderate/severe | 15 (10.3)/11 (7) |
| Viral reactivation | 79 (54.5) |
| CMV | 49 (33.8) |
| HHV6 | 13 (8.9) |
| ADV | 29 (20) |
| EBV | 3 (2) |
| Viral respiratory infection | 66 (45.5) |
| BSI | 33 (22.7) |
| Pre-engraftment BSI | 13 (8.9) |
| Late BSI | 20 (13.7) |
| Probable fungal infection | 7 (4.8) |
| Relapse | 31 (21.4) |

ADV: adenovirus; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; BSI: blood-stream infection; CML: chronic myeloid leukemia; CMV: cytomegalovirus; CSA: cyclosporin; EBV: Epstein-Barr virus; GvHD: graft-*versus*-host disease; aGvHD: acute GvHD; cGvHD: chronic GvHD; HSCT: hematopoietic stem cell transplantation; HHV6: human herpesvirus 6; HL: Hodgkin lymphoma; JMML: juvenile myelomonocytic leukemia; MDS: myelodysplastic synsdromes; MTX: methotrexate; MUD: matched unrelated donors; NHL: non-Hodgkin lymphoma; TBI: total body irradiation.

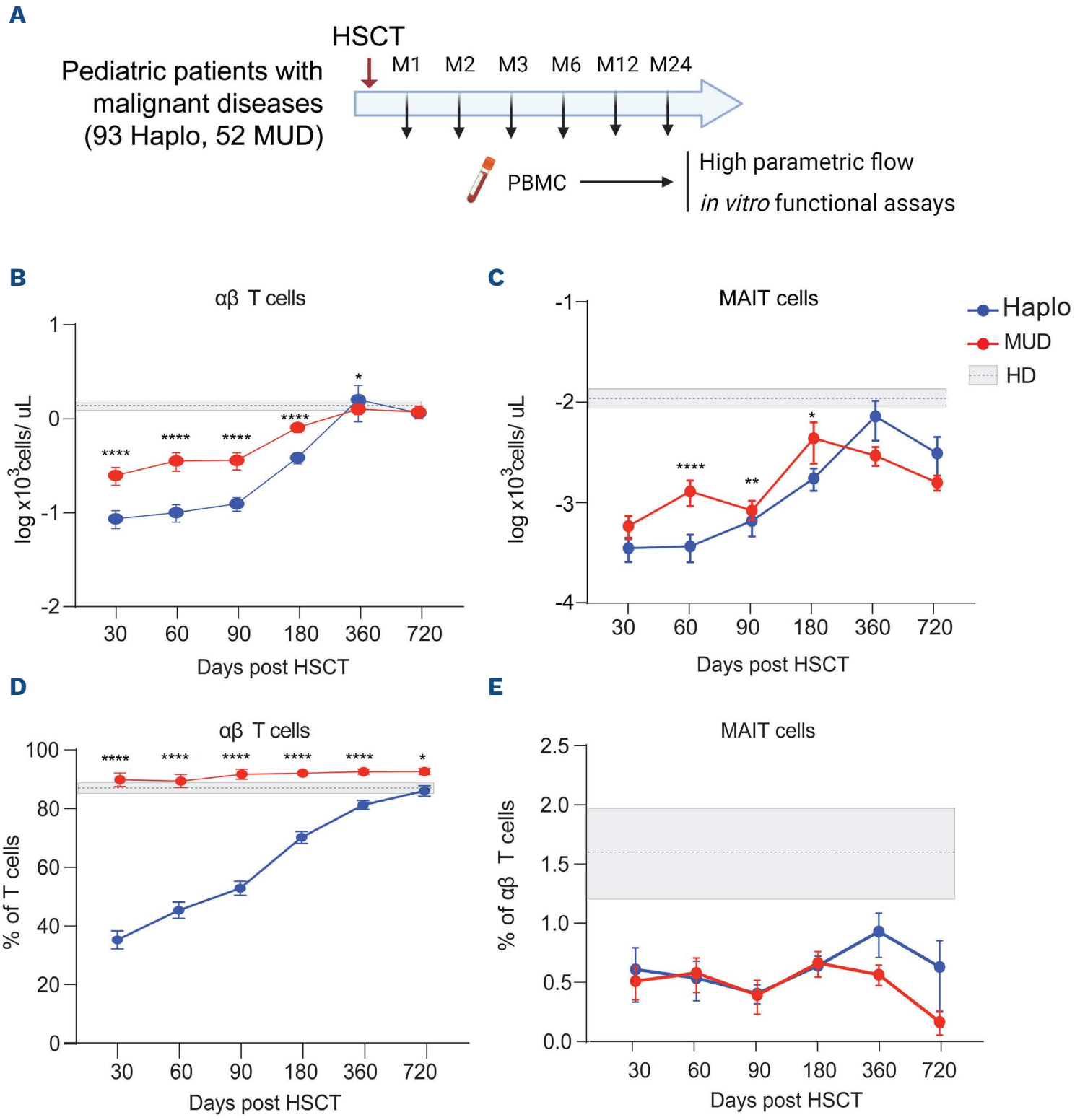


**Figure 1. Mucosal-associated invariant T-cell recovery takes several years to be completed in pediatric and young adult patients after allogeneic-hematopoietic stem cell transplantation.** (A) Representation of the experimental design. Absolute count (B) and frequency (D) of αβ T cells in peripheral blood mononuclear cells (PBMC) of patients after either matched unrelated donor (MUD) (red line) or HLA-haploidentical (Haplo) (blue line) hematopoietic stem cell transplantation (HSCT) at various time points. Absolute count (C) and frequency (E) of mucosal-associated invariant T (MAIT) cells in PBMC of patients after MUD or Haplo-HSCT at various time points. MAIT-cell gating strategies are detailed in *Online Supplementary Figure S1*. Results are shown as mean ± Standard Error of Mean. Full statistical analysis between groups and time points is reported in *Online Supplementary Table S7*. Light gray area shows the physiological interval of cells in 16 age-matched healthy donors (HD). Data were analyzed by Mann-Whitney test. **P*<0.05; **P<0.01; *****P*<0.001.

in patients who experienced pre-engraftment BSI (61%, 95%CI: 37-86, *vs*. 31%, 95%CI: 24-40, respectively, *P*=0.02) and in PBSC recipients (*P*=0.01). Furthermore, 7 patients, all within the Haplo-HSCT group, suffered from probable invasive fungal infection with a median time from HSCT to onset of 90 days (range, 7-660 days).

Thirty-one patients eventually relapsed. With a median follow-up of 33 months (range, 12-49 months) for surviving patients, OS, DFS, and NRM of the entire cohort were 79.5% (95%CI: 71-85), 72% (95%CI: 64-79) and 7% (95%CI: 3-12), respectively (*Online Supplementary Figure S2D, E*). CI of relapse was 23% (95%CI: 17-31), while the GRFS was 63% (95%CI: 52-67) (*Online Supplementary Figure S2D, F*). Pediatric disease risk score was available for 125 out of 145 patients; non-statistically significant trends were observed towards a higher OS, DFS and GRFS for patients

with low-risk score ($P$=0.17, $P$=0.12, $P$=0.16, respectively) (*data not shown*) and higher rates of aGvHD for patients with high-risk score ($P$=0.1) (*data not shown*). Pediatric disease risk score did not influence cGvHD occurrence, nor CMV reactivation, nor late BSI ($P$=0.66, $P$=0.87, $P$=0.83, respectively).

### Mucosal-associated invariant T cells showed prolonged delayed reconstitution and altered phenotypical status following hematopoietic stem cell transplantation

Analyzing the kinetics of immune recovery of the T-cell pool in our cohort of patients, we found that while the αβ T-cell pool gradually recovered in MUD- and Haplo-HSCT patients between one and two years after transplantation (Figure 1B), MAIT cells remained significantly depleted in terms of absolute numbers and frequency in both patient groups, and did not reach normal levels for up to two years following HSCT (Figure 1C, E). Previous studies suggested that early after HSCT, circulating MAIT cells are primarily graft-derived.[18] In line with this observation, we found that MAIT-cell recovery after HSCT in Haplo-recipients was delayed by six months in comparison with MUD-recipients (Figure 1C). These results are consistent with the specific depletion of αβT cells from the Haplo-graft and with their long-term reconstitution which is largely driven by the reactivation of thymic function, as further suggested by the low frequency of αβT cells over the first three months post HSCT (Figure 1D). Together, these data demonstrate that MAIT cells display prolonged delayed reconstitution in pediatric and young adult patients receiving allo-HSCT and their reconstitution kinetics do not reflect that of the conventional αβT-cell pool.

We further characterized the MAIT-cell pool in HSCT recipients by performing UMAP analysis to assess the overall distribution of MAIT-cell subsets according to their expression of a panel of T-cell differentiation and activation markers (*Online Supplementary Table S1*). We found large changes in MAIT-cell cluster composition in patients one year post HSCT (Figure 2A, B). To annotate the MAIT-cell clusters generated in our UMAP, we first investigated the distribution of CD161, as well as of CD4 and CD8 markers, which define phenotypically and functionally distinct MAIT-cell subsets.[29] Interestingly, we observed that a large proportion of MAIT cells detected in patients' PBMC following HSCT were negative for CD161 expression at all time points analyzed (Figure 2C). We also observed large differences in the frequency of MAIT cells expressing CD4 and CD8 after HSCT (Figure 2B, D), since $CD8^+$ MAIT cells were significantly reduced in Haplo-HSCT patients, particularly in the first months following transplantation, when compared to MUD-HSCT patients and healthy donor (HD) controls (Figure 2D). In addition, in MUD-HSCT patients, the $CD8^+$ MAIT-cell subset remained comparable to HD until day 180, when it began to decline until one year following HSCT (Figure 2D). Furthermore, the frequency of $CD4^+$ MAIT cells in Haplo-HSCT had significantly increased by 90 days post-HSCT compared to HD, while in MUD-recipients the frequency of $CD4^+$ MAIT cells started to increase one year following transplantation (Figure 2D). In contrast, the frequency of $CD8^-CD4^-$ double-negative (DN) MAIT cells in Haplo- and MUD-HSCT patients was comparable to that found in the HD samples at all time points analyzed until 180 days after transplantation, when it began to decline (Figure 2D). Given these differences in MAIT-cell phenotype based on CD4 and CD8 expression, and considering that MAIT cells infused with the graft can drive MAIT-cell reconstitution early after HSCT,[18] we considered whether the composition of the BM and PBSC grafts would explain the differences in MAIT-cell subsets expressing $CD161^+$, $CD4^+$, or $CD8^+$. When compared to BM, the frequency of MAIT cells expressing CD161 was significantly reduced in PBSC samples, although no differences were found when comparing CD161 expression in PBSC and PBMC of pediatric and adult HD (Figure 2E). These data suggest that graft sources do not explain the low frequency of $CD161^+$ MAIT cells in MUD- and Haplo-HSCT recipients early after transplantation (Figure 2E). Similarly, no differences were found in the proportion of $CD4^+$ or $CD8^+$ MAIT cells in BM or PBSC graft sources, suggesting that the altered proportion of these 2 markers may reflect the different therapeutic approaches or immune response associated with the 2 transplant procedures.

### Peripheral mucosal-associated invariant T cells were functionally impaired up to one year post hematopoietic stem cell transplantation

To gain further insight into MAIT-cell functional status following HSCT, we measured the expression of several markers associated with T-cell activation, proliferation and exhaustion at one year after transplantation. Our analysis revealed differential regulation of several T-cell markers (Figure 3A). Manual gating demonstrated a significant decrease in $CD28^+$ MAIT-cell frequency and a significant increase in the frequency of MAIT cells expressing the activation markers CD25 and CD38 (Figure 3B). In addition, we identified a substantial increase in MAIT cells expressing the exhaustion markers PD-1 and TIM-3, and the senescence marker CD57 (Figure 3B). As additional evidence of cell exhaustion, the proportion of MAIT cells expressing PD-1 in combination with TIM-3 or CD57 was also significantly increased in transplanted recipients (Figure 3B). To characterize MAIT-cell functional competence, we performed *in vitro* stimulation with PBMC isolated from patients 1-year post HSCT. We assessed IFNγ, TNFα, Granzyme B and Perforin production in response to *E. coli* challenge, and we observed that, when compared to HD PBMC, PBMC from transplanted patients showed a suboptimal response to microbial stimulation (Figure 4A). As cytokines measured in culture supernatants could not be solely attributed to MAIT cells (Figure 4A), we further investigated the activation

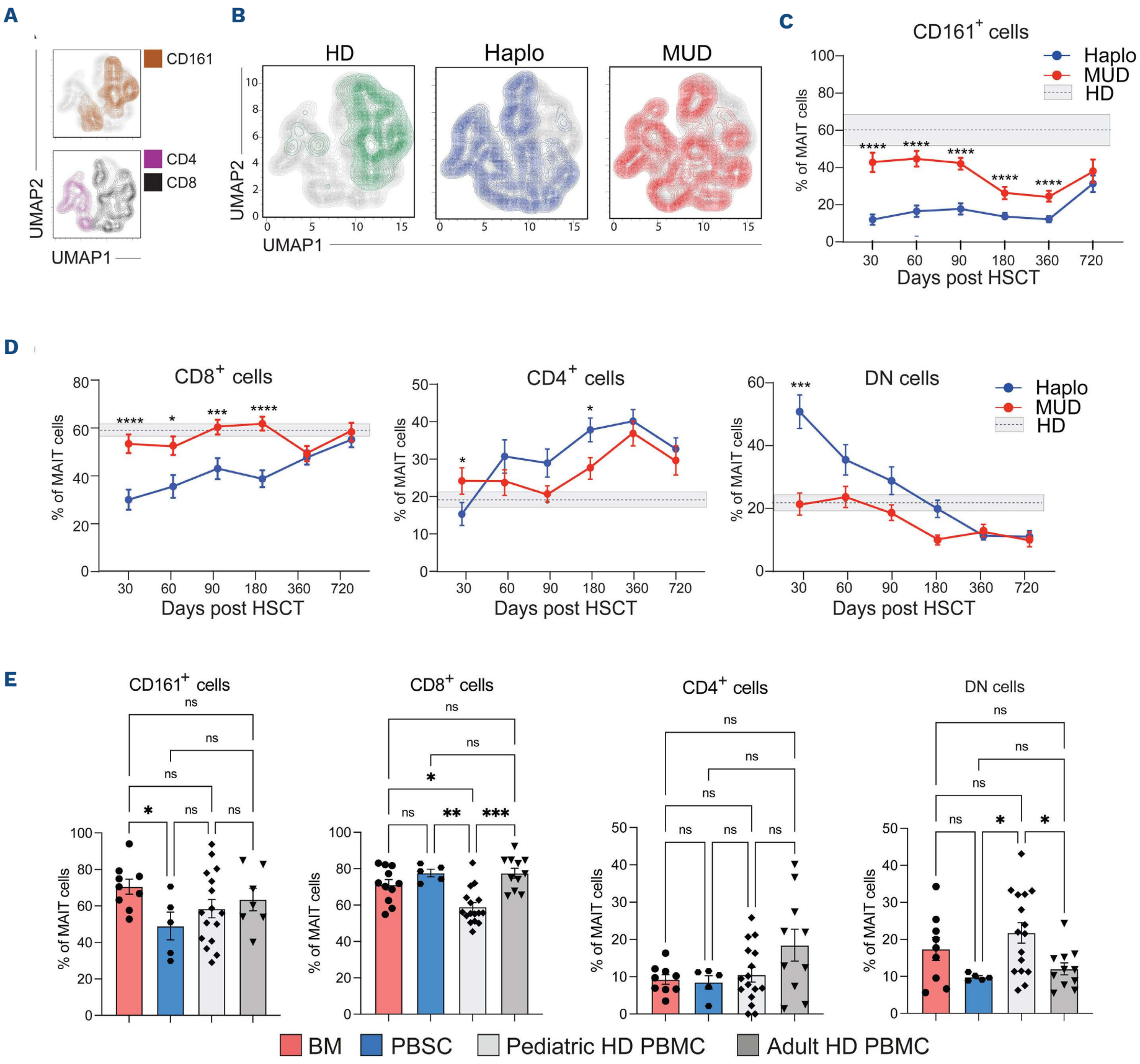


**Figure 2. Mucosal-associated invariant T-cell subset recovery in allogeneic-hematopoietic stem cell transplantation patients.** (A) Uniform Manifold Approximation and Projection (UMAP) embedding of merged mucosal-associated invariant T (MAIT) cells derived from allogeneic-hematopoietic stem cell transplantation (allo-HSCT) samples of healthy donor (HD), and HLA-haploidentical (HAPLO) and matched unrelated donor (MUD) patients one year following hematopoietic stem cell transplantation (HSCT). UMAP were generated on MAIT cells by gating on $CD45^+CD3^+TCR\alpha\beta^+MR1\text{-}5OP\text{-}RU\text{-}tetramer^+$ cells, and using phenotypic and functional T-cell markers, defined in *Online Supplementary Table S1*. Distribution of $CD161^+$, $CD4^+$ or $CD8^+$ MAIT-cell subsets is represented. (B) UMAP distribution of MAIT cells derived from peripheral blood mononuclear cells (PBMC) samples of HD (green), and Haplo- (Blu) and MUD- (red) HSCT patients one year following HSCT. Each plot is representative of 5-6 concatenated samples. (C) Frequency of $CD161^+$ cells among MAIT cells at different time points in PBMC of Haplo- (blue line) or MUD- (red line) HSCT recipients. (D) Frequency of $CD8^+$, $CD4^+$, and $CD8^-CD4^-$ double negative (DN) cells among MAIT cells in PBMC of Haplo- (blue line) or MUD- (red line) HSCT recipients. Results are shown as mean ± Standard Error of Mean. Light gray area shows the physiological interval of cells in 16 age-matched HD. (E) Percentage of MAIT cells expressing CD161, CD8, CD4, and $CD8^-$ $CD4^-$ DN, in bone marrow (BM) from HD (N=11), peripheral blood stem cells (PBSC) derived from the positive fraction of the TCRαβ/ CD19-depletion procedure (N=5), PBMC from aged-matched HD (N=16), and adult HD (N=11). Results show individual values and median (horizontal bar). Data were analyzed by Mann-Whitney test. **P*<0.05, ***P*<0.01, ****P*<0.005, *****P*<0.001; ns: not significant.

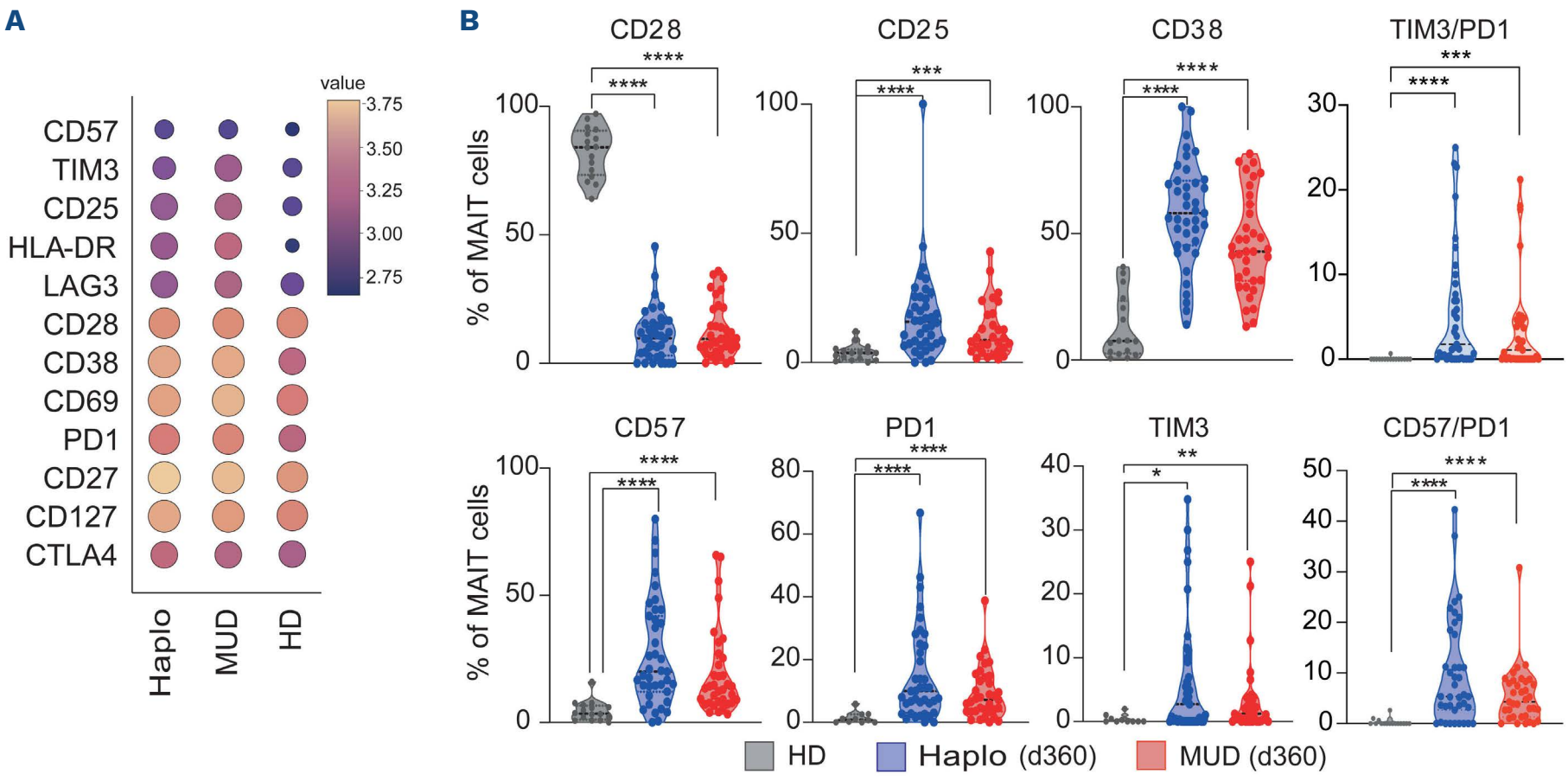


**Figure 3. Peripheral mucosal-associated invariant T cells express activation and exhaustion markers one year post-hematopoietic stem cell transplantation patients.** (A) Bubble diagram showing the expression of different activation markers among mucosal-associated invariant T (MAIT) cell subsets in peripheral blood mononuclear cells (PBMC) of patients one year post hematopoietic stem cell transplantation (HSCT). A gradient of violet to light yellow indicates an increase in expression. The size of the bubble indicates the number of cells. (B) Percentage of MAIT cells expressing CD28, CD25, CD38, CD57, PD-1, and TIM-3 among healthy donors (HD) (gray dots, N=16), and HLA-haploidentical (Haplo)-HSCT (blue dots, N=39) or matched unrelated donor (MUD)-HSCT (red dots, N=35) patients one year after transplantation. Results show individual values and median (horizontal bar). Gating strategies are shown in *Online Supplementary Figure S3*. Data were analyzed by Mann-Whitney test. **P*<0.05, ***P*<0.01, ****P*<0.005, *****P*<0.001. d: day.

of MAIT cells by flow cytometry after *in vitro* stimulation. We observed that while MAIT cells derived from patients at one year post transplantation efficiently responded to αCD3/αCD28 and, with decreased efficiency, to *E. Coli* stimulation by up-regulating the activation markers CD25 and CD69, their activation capacity was suboptimal, as the proportion of MAIT cells co-expressing CD25 and CD69 was significantly reduced when compared to HD controls (Figure 4B, C). Overall, our data demonstrate that along with a considerable delay in recovery following HSCT, MAIT cells displayed a significant increase in the activation / exhaustion profile up to one year after HSCT, indicating a dysfunctional or hyperactivated state, and suboptimal response to APC-dependent (*E. coli*) and APC-independent (αCD3/αCD28) TCR stimulation.

### Mucosal-associated invariant T cells are associated with an increased risk of chronic graft-*versus*-host disease and cytomegalovirus reactivation in hematopoietic stem cell transplantation patients

Next, we sought to examine whether MAIT-cell assessment in patient peripheral blood post HSCT could provide an insight into clinical outcomes. We observed no impact of the absolute number or frequency of MAIT at early (day +30) or later (days +60, +90) time points on OS, DFS, NRM, and CI of relapse (*data not shown*). As CD161 has been used as an additional marker to evaluate MAIT cells and their association with clinical outcomes, we further analyzed the absolute number of $CD161^+$ MAIT cells and their frequency within the $MR1^+$ population at days +30, +60 and +90 post HSCT. However, even in this case, no correlation with OS, DFS, NRM, and CI of relapse was observed (*data not shown*). We next evaluated a possible association between post-HSCT MAIT cells and the occurrence of GvHD. As the median time of aGvHD onset in our cohort was 53 days, we focused on day +30 to evaluate if MAIT cells could represent an early marker associated with aGvHD. Therefore, we did not consider the 7 patients who developed aGvHD before day +30 to perform aGvHD analysis. While there was no significant difference in CI of any grade aGvHD in patients with higher MAIT count (25%, 95%CI: 16-38, *vs*. 16%, 95%CI: 9-27, *P*=0.27) (Figure 5A), we found that a greater absolute number of MAIT cells on day +30 was associated with higher CI of grade II-IV aGvHD (19%, 95%CI: 11-30, *vs*. 7%, 95%CI: 3-16, *P*=0.06) (Figure 5B). Multivariate analysis incorporating the absolute number of αβT cells at day +30 post HSCT (significantly associated with the occurrence of aGvHD in univariate analysis) revealed that none of the variables

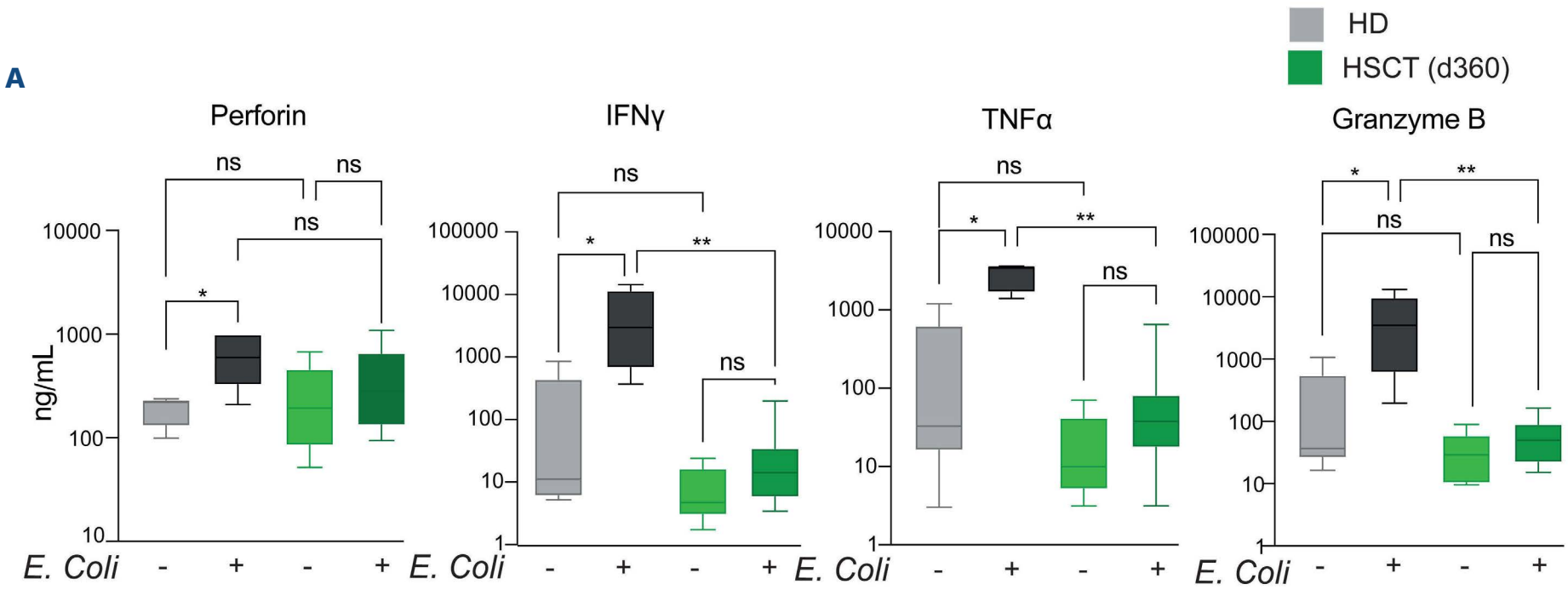


**Figure 4. Altered functional status of mucosal-associated invariant T cells in patients one year after hematopoietic stem cell transplantation patients.** (A) Box plots summarizing the concentration (ng/mL) of cytokines (IFNγ, TNFα, Granzyme B, and Perforin) in the culture supernatants after *E. coli* stimulation for 24 hours of peripheral blood mononuclear cells (PBMC) derived from healthy donors (HD) (N=5) or HSCT patients (matched unrelated donor [MUD], N=5; HLA-haploidentical [Haplo], N=5) one year after transplantation. Total cytokine levels in the cell culture supernatants were assessed using ELISA assay. Results are shown as mean ± Standard Error of Mean. (B, C) Frequency of $CD25^{+}CD69^{+}$ cells among mucosal-associated invariant T (MAIT) cells following aCD3 / aCD28 (B) and prefixed *E. coli* (C) *in vitro* activation assay. Results show individual values; dots represent MAIT cells derived from PBMC of HD (N=10) or HSCT (MUD, N=5; Haplo, N=10) patients one year after transplantation. Data were analyzed by Wilcoxon matched pairs signed rank test per row, with individual ranks computed for each comparison. **P*<0.05, ***P*<0.01, ****P*<0.005, ns: not significant. d: day; MFI: median fluorescence intensity.

was an independent risk factor (*Online Supplementary Table S2*). Thus, the higher number of MAIT cells in aGvHD patients could be the result of a greater number of total T cells. Interestingly, in univariate analysis, we observed that patients with higher absolute number of MAIT cells at day +30 post HSCT showed a higher risk of developing all grade cGvHD (17%, 95%CI: 9-28, *vs*. 6%, 95%CI: 2-15, *P*=0.06) (Figure 5C), with a non-significant similar trend on moderate-severe cGvHD (13%, 95%CI: 6-23, *vs*. 5%, 95%CI 1-13, *P*=0.11) (Figure 5D). Multivariate analysis incorporating prior occurrence of grade II-IV aGvHD and conditioning regimen (chemo-based *vs*. TBI-based) did not confirm

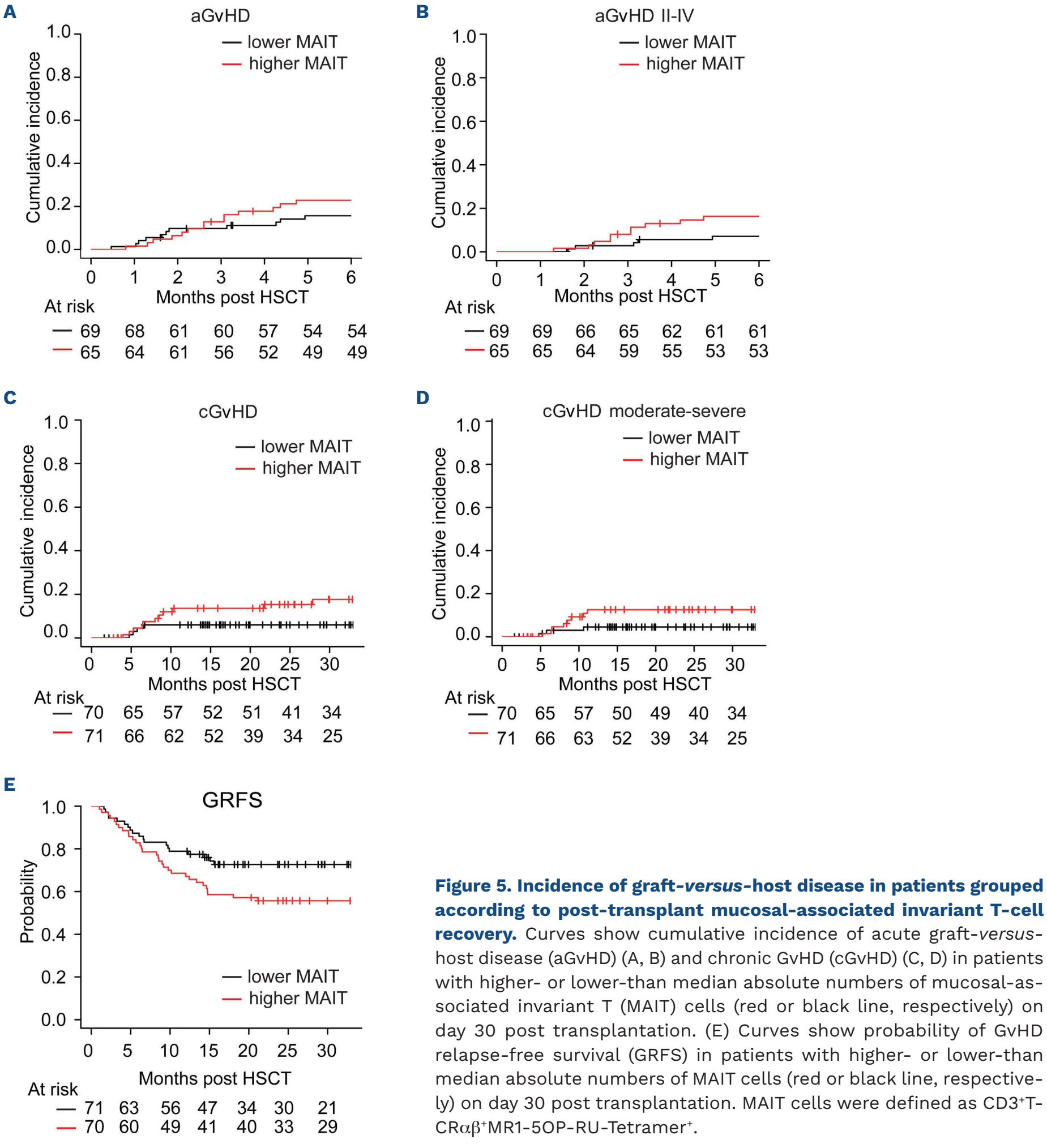


**Figure 5. Incidence of graft-*versus*-host disease in patients grouped according to post-transplant mucosal-associated invariant T-cell recovery.** Curves show cumulative incidence of acute graft-*versus*-host disease (aGvHD) (A, B) and chronic GvHD (cGvHD) (C, D) in patients with higher- or lower-than median absolute numbers of mucosal-associated invariant T (MAIT) cells (red or black line, respectively) on day 30 post transplantation. (E) Curves show probability of GvHD relapse-free survival (GRFS) in patients with higher- or lower-than median absolute numbers of MAIT cells (red or black line, respectively) on day 30 post transplantation. MAIT cells were defined as $CD3^+$T-CR$\alpha\beta^+$MR1-5OP-RU-Tetramer$^+$.

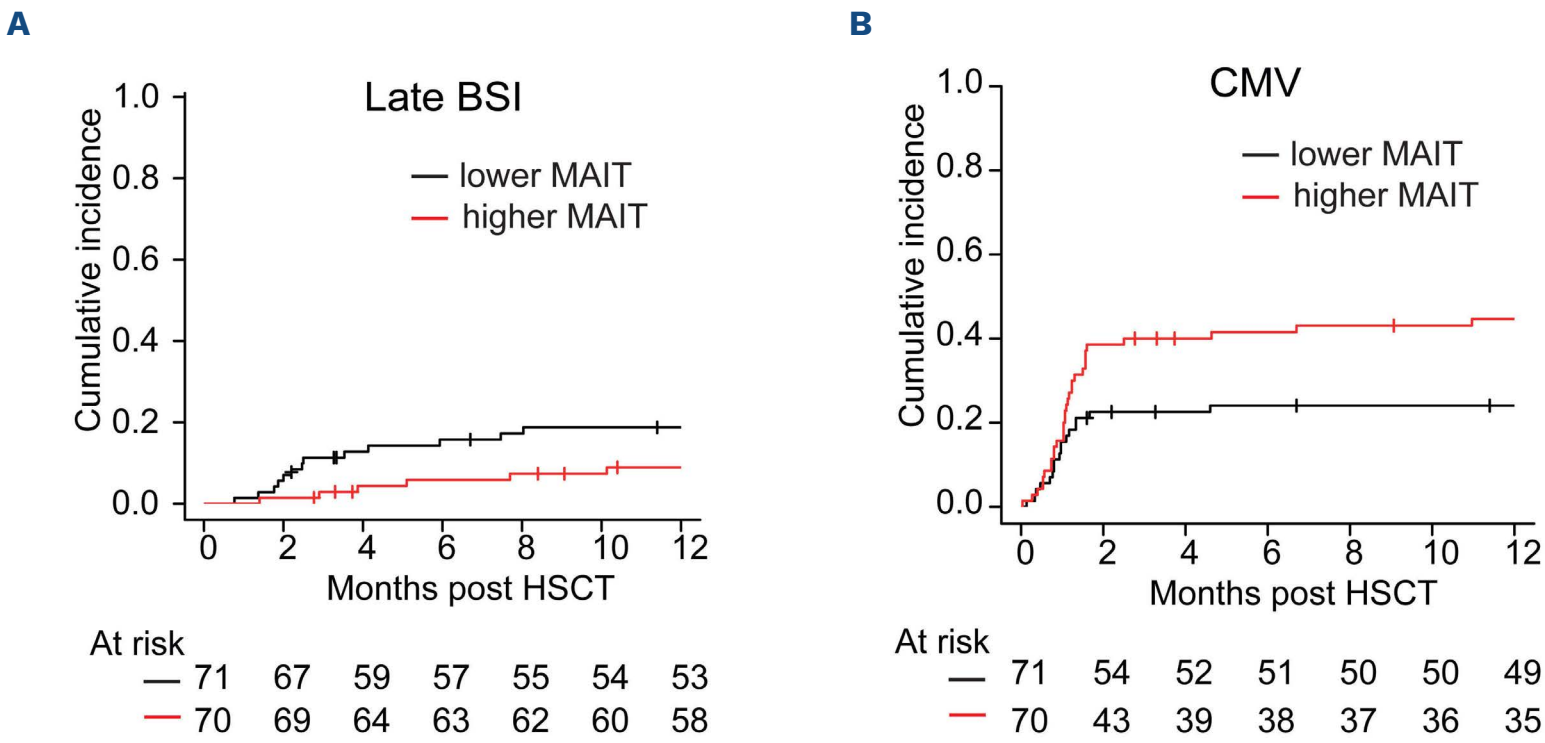


**Figure 6. Incidence of late blood-stream infection and cytomegalovirus reactivation in patients grouped according to post-transplant mucosal-associated invariant T-cell recovery.** Curves show cumulative incidence of late blood-stream infection (BSI) (A) and cytomegalovirus (CMV) (B) in patients with higher- or lower-than median absolute numbers of mucosal-associated invariant T (MAIT) cells (red or black line, respectively) on day 30 post transplantation. MAIT cells were defined as $CD3^{+}TCR\alpha\beta^{+}MR1\text{-}5OP\text{-}RU\text{-}Tetramer^{+}$. HSCT: hematopoietic stem cell transplantation.

these findings, although a non-significant trend was reported towards higher MAIT cells at day +30 and higher incidence of cGvHD (*P*=0.14) (*Online Supplementary Table S3*). In line with this observation, a greater number of MAIT cells at this early time point was correlated with a lower GRFS (*P*=0.06) (Figure 5E, *Online Supplementary Table S4*) in multivariate analysis. Furthermore, a tendency towards higher GRFS was observed for patients with a low disease risk score (*P*=0.09) (*Online Supplementary Table S4*).

Given the reported role of MAIT cells in controlling bacterial infection, we next assessed the correlation between MAIT cells and the incidence of BSI. While no significant association was found analyzing patients experiencing pre-engraftment BSI, we observed trends towards an association between higher absolute MAIT cell count at day +30 and lower CI of late BSI (9%, 95%CI: 4-19, *vs*. 19%, 95%CI: 11-30, *P*=0.08) (Figure 6A). Multivariate analysis did not confirm these findings, although it again showed a tendency towards an association between higher values of MAIT cells and reduced incidence of post-engraftment BSI (*P*=0.14) (*Online Supplementary Table S5*).

We also investigated the impact of MAIT cells in detecting post-HSCT viral infections. In particular, we studied a possible association between early MAIT-cell count and the occurrence of CMV reactivation, as it represents the most frequent viral infection in our cohort, and a major cause of morbidity and mortality in transplanted patients. Interestingly, we observed that higher values of MAIT cells at day +30 correlated with higher CMV reactivation (44%, 95%CI: 34-57, *vs*. 24%, 95%CI: 16-36, *P*=0.02) (Figure 6B). These results were confirmed in multivariate analysis which indicated that the MAIT-cell count at day +30 and the occurrence of prior pre-engraftment BSI were independently associated with CMV reactivation (*P*=0.01 and *P*=0.03, respectively) (*Online Supplementary Table S6*).

# Discussion

Delayed post-HSCT T-cell reconstitution is associated with impaired immune responses to several antigens and an increased risk of severe infections, leukemia relapse, and adverse clinical outcomes. Unconventional T cells, such as γδ T cells and invariant natural killer T (iNKT) cells, have been found to play a critical role in allo-HSCT, with the potential to minimize the risk of unfavorable clinical outcomes.[30-33] Given their innate ability to rapidly release proinflammatory molecules upon activation and potent antimicrobial activity, there is a growing interest in studying MAIT-cell biology and exploring their function in both health and disease. In our study, we longitudinally monitored post-HSCT MAIT T-cell recovery and their association with clinical outcomes in 2 different pediatric settings: haplo-HSCT (in which MAIT cells are depleted from the graft) and MUD-HSCT. We found that MAIT-cell recovery is delayed for up to two years in both transplant settings. We also found that MAIT cells exhibit an altered functional status when profiled one year post HSCT, as demonstrated by the expression of activation / senescence markers, as well as the suboptimal response to TCR stimulation. Together, our study offers novel insights into the biology and clinical impact of MAIT cells in the context of allo-HSCT.

In our study, we observed that a large proportion of MR1-restricted T cells were CD161 negative after transplantation. CD161 (in combination with the TCR Vα7.2) has been used by several investigators to evaluate MAIT cells.[17,18,20,23] However, the development of the MR1 tetramer loaded with the 5-OP-RU (a derivative of the microbial vitamin B2 precursor 5-A-RU) would allow more specific identification of MAIT cells based on their restricted specificity.[34-36] Some studies have suggested that the expression of CD161 could change in particular pathological conditions, such as systemic infections or autoimmune diseases.[37-39] Given that CD161 also plays a role in cell trafficking,[40] a possible explanation for the decreased levels of CD161$^+$ MAIT cells following HSCT could lie in their recruitment to lymphoid organs, damaged tissues, and GvHD target organs. In addition, Koay *et al.* demonstrated that the frequency of CD161$^-$ MAIT cells is much higher in the thymus than in peripheral blood and that the acquisition of CD161 is associated with a more mature status of MAIT cells.[41] Consequently, the observed decline in CD161 expression post transplantation could be indicative of a less mature status of MAIT cells. This hypothesis aligns with the observation that MUD-HSCT patients exhibited a gradual decrease in the presence of CD161$^+$ MAIT cells, which may reflect the progressive dilution of graft-derived CD161$^+$ MAIT cells in favor of an increase in CD161$^-$ MAIT cells (Figure 2C). Thus, comparing the reconstitution kinetics of patients receiving MUD or Haplo-HSCT, in which αβT cells are either infused or depleted from the graft, respectively, our study also provides new insights into the endogenous process of MAIT-cell generation in adult life.

As MAIT-cell development is largely dependent on antigens expressed by gut flora,[13] one possible explanation for their delayed reconstitution may be attributed to the long-lasting alterations in the gut mucosa and microbiota composition of transplanted recipients. Notably, conditioning regimens for allo-HSCT disrupt host-microbiota balance leading to mucositis, organ dysfunction, and increased susceptibility to infections. Furthermore, the gut microbial dysbiosis that occurs in patients following HSCT has been extensively documented over the past years in adult[42-44] and pediatric[45-49] patients. Nevertheless, as emphasized by other studies,[21,50] it is still remarkable that MAIT cells do not recover to normal levels even up to two years post HSCT. Given that the reactivation of thymic functionality post transplant requires several months to occur,[3] and that the presentation of bacteria-derived metabolites by double positive thymocytes, thymic epithelial cells, and dendritic cells (all cellular subsets dramatically damaged by antineoplastic therapies and conditioning regimens) is necessary for MAIT-cell development,[13] it is plausible that the combination of microbial dysbiosis and impaired thymic function contribute to the prolonged impairment of MAIT-cell count recovery. Compared to other published studies describing MAIT-cell recovery and their association with clinical outcomes in adult patients receiving HSCT,[18,19,21,34] our findings in a cohort of pediatric / young adult patients further extend those studies and offer new perspectives. In particular, we observed prolonged post-transplant MAIT-cell insufficiency even in young patients in which immune recovery, and in particular thymic-dependent T-cell reconstitution, is thought to proceed much faster than in adult patients. However, MAIT-cell development and homeostatic maintenance, not only in the context of allo-HSCT, but also in adulthood, remains largely unknown.

Previous studies in adult patients receiving allo-HSCT identified a correlation between MAIT cells and aGvHD development.[17-23] It has been reported that a higher frequency of MAIT cells in the graft can be linked to the occurrence of GvHD.[17,18] However, this observation has not been consistent across studies as others reported that a lower number of MAIT cells in the graft correlated with a higher incidence of intestinal GvHD.[23] Data on the correlation between MAIT cells and the occurrence of GvHD in pediatric recipients of allo-HSCT are limited. Tourret *et al.* showed that the number of MAIT cells six months after allo-HSCT was lower in pediatric patients experiencing severe aGVHD.[50] Our results differ from this study, as we focused on the use of early (day +30) MAIT-cell evaluation as a predictive marker of transplant complications. In addition, compared to other studies,[20,21,50] we identify MAIT cells with the MR1-5-OP-RU tetramer, which can make the comparison with results generated with the combination of TCR Vα7.2 and CD161 markers more difficult. Given that the recovery of MAIT cells in the early post-transplant phase is primarily graft-derived,[18] our data collected profiling MAIT cells at day +30 post HSCT are more in agreement with a detrimental role on the risk of GvHD when higher values of MAIT cells are present in the graft. Although we compared the phenotypic differences of MAIT cells in the different graft sources, we did not systematically analyze MAIT cells in the infused products, and this represents a major limitation of this study. Importantly, as demonstrated in the multivariate analysis, the elevated incidence of aGvHD in patients with higher values of MAIT cells was driven by an increased number of total αβT cells in these patients.

Of particular interest are our data on the occurrence of cGvHD. Previous studies showed that the number of MAIT cells is lower in patients with cGvHD compared to patients without cGvHD.[51,52] However, in contrast to these studies in which patient samples were analyzed at the time of cGvHD occurrence / treatment or many months later, we performed the correlative analysis between MAIT cells and the risk of cGvHD development in samples collected one month post HSCT. Thus, our results offer a more predictive value, rather than a suggestion of the effect of the disease or the effect of the pharmacological treatment on MAIT-cell reconstitution.

Our findings further revealed that a higher level of MAIT cells was associated with a lower risk of late BSI under univariate analysis. While further investigations with larger

patient cohorts are needed to consolidate these results, our data align with the anti-bacterial properties of MAIT cells. Moreover, there is emerging evidence to suggest that MAIT cells undergo expansion and activation during acute viral infections through a TCR-independent mechanism, contributing to anti-viral response.[53-55] Notably, we observed a correlation between higher MAIT cells in patients experiencing CMV reactivation, indicating potential activation and expansion of MAIT cells during the early phase of viral replication.

Similar to other studies, in this work we aimed to investigate the associations between HSCT outcomes and the number and frequency of MAIT cells in patients' peripheral blood. However, MAIT cells localize to sites (including gastrointestinal tracts, skin, lung, and oropharynx) where they undergo terminal differentiation and acquire specific transcriptomic programs that dictate distinct functions.[56-58] In agreement with this, peripheral blood MAIT cells respond differently from tissue-derived MAIT cells following TCR stimulation.[59,60] Thus, due to the lack of validated tissue-specific MAIT-cell markers, and the limited information on the balance between tissue-resident and circulating MAIT cells, it remains to be elucidated whether results gathered from PBMC studies reflect the quantitative and quantitative immunological status in tissue.

In conclusion, our study on the reconstitution kinetics of MAIT cells in pediatric patients receiving MUD-HSCT with standard pharmacological prophylaxis or Haplo-HSCT after TCRαβ/CD19-cell depletion revealed that MAIT-cell recovery is significantly impaired in terms of absolute number and function in both transplant settings for up to two years. In addition, we provide novel insights into the clinical value of monitoring MAIT cells, and their association with complications associated with allo-HSCT.

**Disclosures**

*PM reports personal fees from Sobi and Jazz, outside the submitted work. MA served on the Scientific Advisory Board for Vertex Pharmaceuticals and as Steering Committee member for Vertex Pharmaceuticals, outside the submitted work. FL reports personal fees from Amgen, personal fees from Novartis, other from Bellicum Pharmaceutical, other from Neovii, personal fees from Miltenyi, personal fees from Medac, personal fees from Jazz Pharmaceutical, personal fees from Takeda, outside the submitted work.*

**Contributions**

*GF designed, collected data, performed the statistical analysis and wrote the paper. FS designed and performed the experiments, and wrote the paper. RM and GS performed experiments and cryopreserved patients' blood samples. CDL performed the statistical analysis and collected data. PI, BE, CM, RC, DBF, QF, BM, PD, BF and CR were involved in the clinical management of patients and collected data. BV and VG performed the flow cytometric analysis. CM, CA and PA cryopreserved patients' blood samples. AM and MP supervised the project. LF and VE conceived and supervised the project, wrote and edited the manuscript. All authors contributed to the intellectual content of this article, and reviewed and approved the final manuscript for publication.*

**Acknowledgments**

*We thank the NIH tetramer core facility for the provision of the MR1 tetramer complex. We gratefully acknowledge the assistance of the Flow Cytometry Core and, in particular, we thank Dr. Ezio Giorda and his team. We would also like to thank Dr. Tiziana Franchin and the Research Biobank for their support.*

**Funding**

*VE was supported by grants from the Amy Stelzer Manasevit Research Program, the Italian Association for Cancer Research, and the Ministero della Salute. LF was supported by grants from AIRC (Special Program Metastatic disease: the key unmet need in oncology 5 per mille 2018 Project Code 21147 and Accelerator Award 2017 INCAR), the Ministero dell'Istruzione, dell'Università e della Ricerca, PRIN ID 2017 WC8499_004, Ministero della Salute, RF-2016-02364388. FS and SG were supported by an AIRC fellowship for research in Italy. MR was supported by an AIRC fellowship for research abroad.*

**Data-sharing statement**

*Data generated during this study are available from the corresponding author upon reasonable request.*

# References

1. Jenq RR, Van Den Brink MRM. Allogeneic haematopoietic stem cell transplantation: individualized stem cell and immune therapy of cancer. Nat Rev Cancer. 2010;10(3):213-221.
2. Algeri M, Merli P, Locatelli F, Pagliara D. The role of allogeneic hematopoietic stem cell transplantation in pediatric leukemia. J Clin Med. 2021;10(17):3790.
3. Velardi E, Clave E, Arruda LCM, Benini F, Locatelli F, Toubert A. The role of the thymus in allogeneic bone marrow transplantation and the recovery of the peripheral T-cell compartment. Semin Immunopathol. 2021;43(1):101-117.
4. Bartelink IH, Belitser SV, Knibbe CAJ, et al. Immune reconstitution kinetics as an early predictor for mortality using various hematopoietic stem cell sources in children. Biol blood Marrow Transplant. 2013;19(2):305-313.
5. Fedele R, Martino M, Garreffa C, et al. The impact of early CD4+ lymphocyte recovery on the outcome of patients who undergo allogeneic bone marrow or peripheral blood stem cell transplantation. Blood Transufs. 2012;10(2):174-180.
6. de Koning C, Prockop S, van Roessel I, et al. CD4+ T-cell reconstitution predicts survival outcomes after acute graft-

versus-host-disease: a dual-center validation. Blood. 2021;137(6):848-855.
7. Dekker L, de Koning C, Lindemans C, Nierkens S. Reconstitution of t cell subsets following allogeneic hematopoietic cell transplantation. Cancers (Basel). 2020;12(7):1-21.
8. Keever C, Small T, Flomenberg N, et al. Immune reconstitution following bone marrow tansplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts. Blood. 1989;73(5):1340-1350.
9. Eyrich M, Lang P, Lal S, et al. A prospective analysis of the pattern of immune reconstitution in a paediatric cohort following transplantation of positively selected human leucocyte antigen-disparate haematopoietic stem cells from parental donors. Br J Haematol. 2001;114(2):422-432.
10. Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2012;18(12):1835-1844.
11. Treiner E, Duban L, Bahram S, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422(6928):164-169.
12. Kjer-Nielsen L, Patel O, Corbett AJ, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012;491:717-723.
13. Legoux F, Bellet D, Daviaud C, et al. Microbial metabolites control the thymic development of mucosal-associated invariant T cells. Science. 2019;366(6464):494-499.
14. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nat Immunol. 2019;20(9):1110-1128.
15. Dusseaux M, Martin E, Serriari N, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161 hi IL-17-secreting T cells. Blood. 2011;117(4):1250-1259.
16. Salio M. Unconventional MAIT cell responses to bacterial infections. Semin Immunol. 2022;61-64:101663.
17. Wang Z, Zhang S, Zhang X, et al. Mucosal-associated invariant T cells predict increased acute graft-versus-host-disease incidence in patients receiving allogeneic hematopoietic stem cell transplantation. Cancer Cell Int. 2022;22(1):1-16.
18. Bhattacharyya A, Hanafi LA, Sheih A, et al. Graft-derived reconstitution of mucosal-associated Invariant T cells after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transpl. 2018;24(2):242-251.
19. Andrlova H, Miltiadous O, Kousa AI, et al. MAIT and Vdelta2 unconventional T cells are supported by a diverse intestinal microbiome and correlate with favorable patient outcome after allogeneic HCT. Sci Transl Med. 2022;14(646):eabj2829.
20. Kawaguchi K, Umeda K, Hiejima E, et al. Influence of post-transplant mucosal-associated invariant T cell recovery on the development of acute graft-versus-host disease in allogeneic bone marrow transplantation. Int J Hematol. 2018;108(1):66-75.
21. Solders M, Erkers T, Gorchs L, et al. Mucosal-associated invariant T cells display a poor reconstitution and altered phenotype after allogeneic hematopoietic stem cell transplantation. Front Immunol. 2017;8:1861.
22. Ben Youssef G, Tourret M, Salou M, et al. Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. J Exp Med. 2018;215(2):459-479.
23. Gao MG, Hong Y, Zhao XSXY, et al. The potential roles of mucosa-associated invariant T cells in the pathogenesis of gut graft-versus-host disease after hematopoietic stem cell transplantation: Front Immunol. 2021;12:720354.
24. Locatelli F, Merli P, Pagliara D, et al. Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion. Blood. 2017;130(5):677-685.
25. Merli P, Algeri M, Galaverna F, et al. TCRαβ/CD19 cell–depleted HLA-haploidentical transplantation to treat pediatric acute leukemia: updated final analysis. Blood. 2024;143(3):279-289.
26. Hagel JP, Garner LC, Bilton M, et al. Human MAIT cell activation in vitro. In: Kaipe H, Magalhaes I, editors. MAIT cells: methods and protocols. New York, NY: Springer US; 2020. p97-124.
27. Qayed M, Ahn KW, Kitko CL, et al. A validated pediatric disease risk index for allogeneic hematopoietic cell transplantation. Blood. 2021;137(7):983-993.
28. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Clin Infect Dis. 2008;46(12):1813-1821.
29. Martin E, Treiner E, Duban L, et al. Stepwise development of MAIT cells in mouse and human. PLoS Biol. 2009;7(3):e54.
30. Airoldi I, Bertaina A, Prigione I, et al. γδ T-cell reconstitution after HLA-haploidentical hematopoietic transplantation depleted of TCR-αβ+/CD19+ lymphocytes. Blood. 2015;125(15):2349-2358.
31. Ravens S, Schultze-Florey C, Raha S, et al. Human γδ T cells are quickly reconstituted after stem-cell transplantation and show adaptive clonal expansion in response to viral infection. Nat Immunol. 2017;18(4):393-401.
32. Chaidos A, Patterson S, Szydlo R, et al. Graft invariant natural killer T-cell dose predicts risk of acute graft-versus-host disease in allogeneic hematopoietic stem cell transplantation. Blood. 2012;119(21):5030-5036.
33. Moreira-teixeira L, Bachy E, Bouillie M, et al. Early posttransplantation donor-derived invariant natural killer T-cell recovery predicts the occurrence of acute graft-versus-host disease and overall survival. Blood. 2012;120(10):2144-2154.
34. Reantragoon R, Corbett AJ, Sakala IG, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. J Exp Med. 2013;210(11):2305-2320.
35. Varelias A, Bunting MD, Ormerod KL, et al. Recipient mucosal-associated invariant T cells control GVHD within the colon. J Clin Invest. 2018;128(5):1919-1936.
36. Gherardin NA, Souter MNT, Koay HF, et al. Human blood MAIT cell subsets defined using MR1 tetramers. Immunol Cell Biol. 2018;96(5):507-525.
37. Nicol B, Salou M, Vogel I, et al. An intermediate level of CD161 expression defines a novel activated, inflammatory, and pathogenic subset of CD8 þ T cells involved in multiple sclerosis. J Autoimmun. 2018;88:61-74.
38. Cosgrove C, Ussher JE, Rauch A, et al. Early and nonreversible decrease of CD161++ /MAIT cells in HIV infection. Blood. 2013;121(6):951-961.
39. Park Y, Lim J, Kim SY, Kwon GC, Koo SH, Kim J. Changes of frequency and expression level of CD161 in CD8+ T cells and natural killer T cells in peripheral blood of patients with systemic lupus erythematosus. Microbiol Immunol. 2020;64(7):532-539.
40. Fergusson JR, Smith KE, Fleming VM, et al. CD161 defines a transcriptional and functional phenotype across distinct human T cell lineages. Cell Rep. 2014;9(3):1075-1088.
41. Koay HF, Gherardin NA, Enders A, et al. A three-stage intrathymic development pathway for the mucosal-associated

invariant T cell lineage. Nat Immunol. 2016;17(11):1300-1311.
42. Peled JU, Gomes ALC, Devlin SM, et al. Microbiota as predictor of mortality in allogeneic hematopoietic-cell transplantation. N Engl J Med. 2020;382(9):822-834.
43. Holler E, Butzhammer P, Schmid K, et al. Metagenomic analysis of the stool microbiome in patients receiving allogeneic stem cell transplantation: loss of diversity is associated with use of systemic antibiotics and more pronounced in gastrointestinal graft-versus-host disease. Biol Blood Marrow Transplant. 2014;20(5):640-645.
44. Jenq RR, Ubeda C, Taur Y, et al. Regulation of intestinal inflammation by microbiota following allogeneic bone marrow transplantation. J Exp Med. 2012;209(5):903-911.
45. Elgarten CW, Margolis EB, Kelly MS. The microbiome and pediatric transplantation. J Pediatric Infect Dis Soc. 2024;13(Suppl 1):S80-S89.
46. Biagi E, Zama D, Nastasi C, et al. Gut microbiota trajectory in pediatric patients undergoing hematopoietic SCT. Bone Marrow Transplant. 2015;50(7):992-998.
47. Masetti R, Leardini D, Muratore E, et al. Gut microbiota diversity before allogeneic hematopoietic stem cell transplantation as a predictor of mortality in children. Blood. 2023;142(16):1387-1398.
48. Ingham AC, Kielsen K, Cilieborg MS, et al. Specific gut microbiome members are associated with distinct immune markers in pediatric allogeneic hematopoietic stem cell transplantation. Microbiome. 2019;7(1):131.
49. Ingham AC, Kielsen K, Mordhorst H, et al. Microbiota long-term dynamics and prediction of acute graft-versus-host disease in pediatric allogeneic stem cell transplantation. Microbiome. 2021;9(1):148.
50. Tourret M, Talvard-Balland N, Lambert M, et al. Human MAIT cells are devoid of alloreactive potential: prompting their use as universal cells for adoptive immune therapy. J Immunother Cancer. 2021;9(10):e003123.
51. Stikvoort A, Chen Y, Rådestad E, et al. Combining flow and mass cytometry in the search for biomarkers in chronic graft-versus-host disease. Front Immunol. 2017;8:717.
52. Konuma T, Kohara C, Watanabe E, et al. Reconstitution of circulating mucosal-associated invariant T cells after allogeneic hematopoietic cell transplantation: its association with the riboflavin synthetic pathway of gut microbiota in cord blood transplant recipients. J Immunol. 2020;204(6):1462-1473.
53. Van Wilgenburg B, Scherwitzl I, Hutchinson EC, et al. MAIT cells are activated during human viral infections. Nat Commun. 2016;7:11653.
54. Sandberg JK, Leeansyah E, Eller MA, Shacklett BL, Paquin-Proulx D. The emerging role of MAIT cell responses in viral infections. J Immunol. 2023;211(4):511-517.
55. Phetsouphanh C, Phalora P, Hackstein CP, et al. Human MAIT cells respond to and suppress HIV-1. Elife. 2021;10:e50324.
56. Salou M, Legoux F, Gilet J, et al. A common transcriptomic program acquired in the thymus defines tissue residency of MAIT and NKT subsets. J Exp Med. 2019;216(1):133-151.
57. Constantinides MG, Link VM, Tamoutounour S, et al. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. Science. 2019;366(6464):eaax6624.
58. Khuzwayo S, Mthembu M, Meermeier EW, et al. MR1-restricted MAIT cells from the human lung mucosal surface have distinct phenotypic, functional, and transcriptomic features that are preserved in HIV infection. Front Immunol. 2021;12:631410.
59. Garner LC, Amini A, FitzPatrick MEB, et al. Single-cell analysis of human MAIT cell transcriptional, functional and clonal diversity. Nat Immunol. 2023;24(9):1565-1578.
60. Sobkowiak MJ, Davanian H, Heymann R, et al. Tissue-resident MAIT cell populations in human oral mucosa exhibit an activated profile and produce IL-17. Eur J Immunol. 2019;49(1):133-143.