Homeostasis and expansion of human MAIT cells in the allogeneic setting

Marie Tourret[1]*, Nana Talvard-Balland[1]*, Marion Lambert[1]*, Ghada Ben Youssef[1], Thomas Yvorra[2], Florence Morin[3], Saba Azarnoush[4], Olivier Lantz[5,6,7], Jean-Hugues Dalle[1,4] and Sophie Caillat-Zucman[1,3].

1- Institut national de recherche médicale (INSERM) UMR976, Université de Paris, Paris, France
2- INSERM UMR3666/U1143, Université PSL, Institut Curie, Paris, France
3- Hôpital Saint-Louis, Assistance Publique-Hôpitaux de Paris (AP-HP), Université de Paris, Laboratoire d'Immunologie, Paris, France
4- Hôpital Robert Debré, AP-HP, Université de Paris, Département d'Immuno-Hématologie, Paris, France
5- INSERM U932, Université PSL, Institut Curie, Paris, France.
6- Institut Curie, Laboratoire d'immunologie clinique, Paris, France.
7- Institut Curie, Centre d'investigation Clinique en Biothérapie (CIC-BT1428), Paris, France

* M.T, N.T-B and M.L contributed equally to this work.

Correspondence to:
Sophie Caillat-Zucman, Laboratoire d'Immunologie, Hôpital Saint-Louis,
1 Avenue Claude Vellefaux, 75010, Paris, France Phone: 33-1-42 49 90 81;
E-mail: sophie.caillat-zucman@aphp.fr, sophie.caillat@inserm.fr,

Funding
This work was supported by grants from Agence Nationale de la Recherche (ANR) and Direction Générale de l'Offre de Soins (DGOS) (PRTS 14-CE15-0005, acronym NEOMAIT), Agence de la Biomédecine, and Cent pour Sang la vie. N.T-B was supported by Ligue contre le Cancer and Fondation pour la Recherche Médicale (FRM). Access to samples from the CRYOSTEM collection was made possible thanks to the financial support of Association Laurette Fugain.

The authors declared no conflict of interest

Abstract (221 words)
Mucosal associated invariant T (MAIT) cells are non conventional T cells that recognize microbial riboflavin precursor derivatives presented by the monomorphic MHC class 1-related MR1 molecule. MAIT cells are effectors of anti-microbial defense and exhibit newly-described regulatory and tissue repair functions. MAIT cells are very few at birth, and accumulate gradually over a period of about 6 years during infancy or after cord blood transplantation. The drivers of this low peripheral expansion are unclear. We show that the dynamics of MAIT cell recovery after allogeneic hematopoietic stem cell transplantation recapitulated their physiological expansion in the infancy, regardless of host- or donor-related factors associated with recovery of conventional T cells. Moreover, there was no relationship between MAIT cell numbers and occurrence of acute graft-versus-host disease (GVHD). Using in vitro models, we show that neither homeostatic cytokines nor allogeneic stimulation alone were sufficient to induce sustained MAIT cell expansion, which required additional TCR triggering with MR1 ligand. Furthermore, using a model of T cell-mediated xenogeneic GVHD in immunodeficient mice, we show that human MAIT cells did not expand nor accumulate in tissues, in contrast to conventional T cells. Altogether, these results indicate that even in the allogeneic host, MAIT cell expansion is only driven by microbial-derived MR1 ligands together with inflammatory signals. These data pave the way for harnessing MAIT cell effector and/or regulatory functions for adoptive immune therapy, as they are unlikely to cause GVHD after transfer in allogeneic recipients.

Clinical implications (max 30 words)

As MAIT cells do not participate in allogeneic responses, they can be considered as a novel source of immune cells for adoptive therapy in unrelated recipients.

Capsule summary (max 35 words)

MAIT cells do not expand nor accumulate in tissues in response to allogeneic stimulation. These data open the way for leveraging their effector functions in an allogeneic context because they are unlikely to cause GVHD.

Key words

Mucosal associated invariant T (MAIT) cells; hematopoietic stem cell transplantation (HSCT); allogeneic response; graft-versus-host disease (GVHD); lymphocyte homeostasis; cytokines; adoptive therapy.

Abbreviations

CFSE: Carboxyfluorescein succinimidyl ester
GVHD: graft-versus-host disease
GVL: graft-versus-leukemia
HSCT: hematopoietic stem cell transplantation
MAIT: Mucosal associated invariant T
MA: myeloablative; NMA: non myeloablative
MSD: matched sibling donor; MUD: matched unrelated donor
TCR: T cell receptor
Tconv: conventional T cells

Introduction

MAIT cells are innate-like T cells that express a semi-invariant TCR (Vα7.2-Jα33/20/12 in humans, combined to a limited set of Vβ chains) restricted by the monomorphic, highly conserved during evolution, MHC class Ib molecule, MR1 (1)(*Franciskiewicz et al, I. Review*). MAIT cells respond to riboflavin (vitamin B2) precursor derivatives such as 5-OP-RU presented by MR1 (2-4). Because these derivatives are produced by most bacteria and yeast, but not by mammal cells, the MAIT-MR1 recognition pathway is a sophisticated discriminatory mechanism to target microbes while sparing the host. Upon TCR engagement, MAIT cells proliferate, release proinflammatory cytokines (IFNγ, TNFα, IL-17) and mediate perforin-dependent killing of target cells, supporting their role in antimicrobial defense (5-7). MAIT cells can also be activated in a TCR-independent fashion in response to IL-12 and IL-18 (8-10), extending their potential participation to inflammatory conditions in which these cytokines are produced. Recently, a novel TCR-dependent tissue repair potential of MAIT cells was evidenced (11-15), supporting that MAIT may exhibit broader functions. Whether the nature of MAIT cell functions is modulated by the timing and mode of stimulation remains to be determined.

MAIT cells are predominantly localized in the liver and barrier tissues, including lung, intestine and skin, in agreement with their expression of various chemokine receptors. MAIT cells are also abundant in the adult peripheral blood where they represent up to 10% of CD3 T cells, while they are very few in cord blood. We recently showed that the postnatal expansion of MAIT cells was a very slow process, taking up to 6 years to reach levels observed in adults (16). This progressive expansion likely occurred through repeated exposure of a few MAIT cell clones to microbial antigens.

The curative effect of allogeneic hematopoietic stem cell transplantation (allo-HSCT) in hematological malignancies is based on the capacity of donor T cells to mediate graft-versus-leukemia (GVL) reaction and eliminate residual tumor cells in the host. A serious drawback is that donor T cells may also recognize normal nonhematopoietic cells, leading to potentially life-threatening graft-versus-host disease (GVHD) (17-21). GVHD is characterized by activation, expansion and migration to target tissues of donor alloreactive T cells. In the first weeks after HSCT, the T cell compartment recovers through peripheral expansion of graft -derived T cells in response to increased levels of homeostatic cytokines and to host's allogeneic antigens. However, reconstitution of a fully diversified T-cell repertoire occurs only later by resumed thymic output of newly generated naïve T cells derived from donor stem cells (22). Even under favorable conditions, it takes at least 2 months to produce naive T cells, and a plateau of thymic output is reached only after 1 to 2 years. Rapid and efficient T cell recovery after allogeneic HSCT is a critical determinant for decreasing the risk of infections and relapse.

The role, if any, of human MAIT cells after HSCT remains unclear. Given their abundance in the liver, lungs, intestine and skin, which are the classical GVHD tissue targets, and their ability to secrete both proinflammatory cytokines and tissue repair factors, MAIT cells might exacerbate or protect from GVHD. In major MHC-mismatched mouse HSCT models, recipient's residual MAIT cells protected from acute intestinal GVHD through microbial-induced IL-17 secretion and subsequent inhibition of alloreactive donor-derived T cell proliferation (23). In humans, MAIT cell numbers fail to normalize in the first year after HSCT, but the impact on GVHD occurrence is controversial and long-term recovery was not analyzed (24-26). Notably, we found that MAIT cell recovery was strongly delayed after cord blood transplantation, mimicking the postnatal expansion period of 5-6 years. Moreover, MAIT cells were undetectable in intestinal biopsies taken at time of acute GVHD, suggesting that their defect in the circulation was not related to their recruitment to target tissues and that

MAIT cells did not participate to GVHD tissue damage (16).

To better decipher human MAIT cell homeostatic expansion and potential contribution to the alloimmune response, we analyzed long-term MAIT cell recovery in different HSCT settings and used relevant *in vitro* and *in vivo* models of alloreactivity.

Patients and Methods

Human samples

The prospective children cohort consisted of 40 consecutive children who received unmanipulated bone marrow transplant from genoidentical sibling (n= 19), or HLA-matched (n= 15) or single antigen-mismatched (n= 6) unrelated donor for a hematological malignancy at Robert Debré Hospital between January 2013 and December 2015. Mean age at time of transplantation was 10.5 years (range 0.8-16 yrs). Myeloablative conditioning regimen was provided either with VP16 and total body irradiation (TBI), or with cyclophosphamide and busulfan. In vivo T-cell depletion by ATG day -3 to day -1 was given in the majority of MUD recipients. Primary prophylaxis of GVHD consisted of a calcineurin inhibitor alone (MSD recipients) or with methotrexate (MUD recipients). Patient characteristics are listed in Table 1. Blood samples were collected prior to conditioning and at approximately 1, 3, 6, 12 and 24 months after HSCT as the standard of care for assessment of immunologic recovery. Patients who died before day 180 were not included in the analysis.
To evaluate long-term recovery of MAIT cells, we analyzed blood samples taken 2 to 16 years after HSCT in 60 additional HSCT recipients (32 sibling and 32 unrelated donors) at time of a routine hospital visit.
The adult recipient group consisted of 49 HSCT recipients from the French CRYOSTEM biobank (http://www.cryostem.org/en/resources-cryostem/biological-resources/) and SFGM-TC (Société Française de Greffe de Moelle et Therapie Cellulaire). Among those, 24 patients were given myeloablative conditioning and 25 received non-myeloablative conditioning prior to infusion of T-cell replete bone marrow (n= 19) or peripheral blood stem cells (n= 30) from an HLA-matched sibling donor. Samples were collected prior to conditioning, and at 3 and 12 months in the absence of aGVHD (n= 27 patients). For the 22 patients with aGVHD, samples were collected at the time of aGVHD diagnosis before any treatment (mean 32 days after transplant, range 10-100), one month later, and at 12 months. Frozen PBMCs were obtained retrospectively for all recipients and their donors. Patient characteristics are listed in Table 2.

Cells and reagents
Peripheral blood mononuclear cells were isolated by Pancoll density gradient centrifugation (PanBiotech) and used immediately, or frozen. 5-OP-RU was synthesized as described in (27-29). Human MAIT cells were expanded *in vitro* for 6 days in human T-cell culture medium (RPMI-1640 (Invitrogen, Life Technologies) containing 10% human AB serum (EuroBio) and 1% penicillin/streptomycin (100U/mL, Invitrogen) and supplemented on day 0 with human IL-2 (100 U/mL, Miltenyi) and 300 nM 5-OP-RU.

Mice
NOD-Scid-IL-2Rγ^{null} (NSG) mice (Jackson laboratory, Bar Harbor, MI) were housed under specific pathogen-free conditions in the animal facility of the St-Louis Research Institute (Paris). Female mice aged 8-10-weeks were used in experiments, after approval of all procedures and protocols by the Institutional animal Care and Use Ethics Committee (CE121#16624).

Flow cytometry
MAIT cells were analyzed on 100 µl residual whole blood within 24 hours after collection, or on isolated PBMCs where indicated. Multiparametric 14-color flow cytometry analyses were performed as described in Supplementary data. MAIT cells were defined as $CD3^{+}CD4^{-}$ $CD161^{high}V\alpha7.2^{+}$ T cells in the first part of the study (HSCT patients). Thereafter, we used

the specific MR1:5-OP-RU tetramer (29) when it became available (NIH tetramer core facility). Data were acquired on a BD LSR Fortessa or FACSCelesta flow cytometer. A total of at least 100,000 events in a live gate were collected. Gates were defined through isotype and fluorescence minus one (FMO) stains. MAIT frequencies were expressed as a percentage of $CD3^+$ lymphocytes. Absolute numbers (per microliter) were calculated from the absolute lymphocyte count determined on the same sample with a hematology automated analyzer. Data were analyzed using FlowJo software.

In vitro stimulations

Human carboxyfluorescein succinimidyl ester (CFSE)-labeled (1μM) PBMCs were cultured *in vitro* for 6 days in human T-cell culture medium supplemented on day 0 with human IL-2 (20 or 100 U/mL, Miltenyi), human IL-15 (50 ng/mL, Miltenyi), human IL-7 (10 ng/mL, Miltenyi) human IL-12/IL-18 (50 ng/mL each, R&D Systems) and/or with 300 nM 5-OP-RU. Cells were harvested on day 6 and stained as above. MAIT and Tconv cell proliferation was analyzed by flow cytometry.

For mixed lymphocyte reactions, CFSE-labeled (1 μM) bulk PBMCs used as responders ($1x10^6$/ml) were incubated with γ-irradiated allogeneic stimulator PBMCs (1:1 ratio) in 96-well round-bottom plates for 6 days. Cells were harvested, stained as above and analyzed by flow cytometry.

Adoptive transfer of xenogeneic cells

NSG mice (Jackson laboratory, Bar Harbor, MI) were housed in the pathogen-free animal facility of Institut de Recherche Saint-Louis (Paris). Eight- to 10-week-old female NSG were irradiated (1.3 Gy) 24 hours prior to injection of $3x10^6$ human PBMCs in the caudal vein. Mice clinically monitored and weighed 3 times per week. Development of GVHD was monitored based on weight loss, posture (hunching), reduced mobility and hair loss. Human chimerism in peripheral blood (percent of human $CD45^+$ cells) was assessed weekly by flow cytometry. Where indicated, mice were given synthetic 5-OP-RU (1 nmol i.p. every 3 days from the day of PBMCs infusion) and/or human IL-15 (0.5 μg i.p. every 3 days or every week) until euthanized. Mice were sacrificed at the indicated time after adoptive transfer, or when weight loss was >15%. Peripheral blood, spleen, liver, lungs and intestine were harvested. Cells were isolated as described in Supplementary data and were analyzed by flow cytometry.

Statistics

Differences between groups were analyzed using non-parametric tests for paired (Wilcoxon) and unpaired (Mann-Whitney or Kruskall-Wallis) groups, or two-way ANOVA. Correlations were assessed using the Spearman's rank correlation. Two-sided P values < 0.05 were considered significant. No statistical method was used to predetermine sample size. Analyses were performed using Prism software v.6 (GraphPad).

All data including outliers were included with one pre-determined exception: flow cytometry cell-subset percentages were considered non-evaluable if the parent subset contained <100 events (i.e. the denominator was <100).

Study approval

The study was carried out with the approval of the Robert Debré Hospital Ethics Committee (HREC 2013/49) and the CPP Ile de France IV (2015/03NICB), in agreement with the principles of the Declaration of Helsinki and French legislation. All subjects (or their parents for the children cohort) provided written informed consent. The study was registered in a public trial registry: ClinicalTrials.gov number NCT0240.

Results

1/ MAIT cell reconstitution is defective for several years in HSCT recipients

We previously showed that it takes up to 6 years to recover normal MAIT cell values after cord blood transplantation in children (16). Because MAIT cells numbers are 1-2 logs less in cord blood than in adult blood, we set out to determine the profile of MAIT cell recovery in other HSCT settings.

We conducted a prospective analysis in 40 children with hematological malignancy who received unmanipulated bone marrow transplant from HLA-matched sibling donor (MSD, n= 19) or unrelated donor (MUD, n= 21) after myeloablative conditioning. Patients and transplant characteristics are summarized in Table 1. Absolute peripheral blood counts and percentages of lymphocytes were determined before initiation of the conditioning regimen (± day -15 before HSCT) and up to 24 months after HSCT. MAIT cells were identified as $CD3^+$ $CD4^-$ $Va7.2^+$ $CD161^{high}$ cells. This population fully overlapped with the population labeled by MR1:5-OP-RU tetramers (29), which were not available at the beginning of this study (Figure 1A).

Absolute counts of conventional T cells (Tconv) and MAIT cells were significantly lower in pre-HSCT patients compared to age-matched healthy children, as expected in such heavily pretreated patients. However, while the number of Tconv gradually increased from 1 month after transplantation and returned almost to normal after one year, there was virtually no increase in the number of MAIT cells during the study period. Two years after HSCT, MAIT cell values remained 5 times lower than in age-matched donors (7.2 and 36.6 cells/μL, respectively) (Figure 1B).

To extend our findings beyond 2 years after transplant, we performed a cross-sectional analysis at time of a routine hospital visit in 60 additional children maintaining stable remission 2 to 16 years after HSCT (n= 83 samples). In line with our previous study on post-natal MAIT cell development (16), we found that the number of MAIT cells very slowly increased to reach a plateau corresponding to normal values only about 6 years after HSCT (Figure 1C)

Because of the large interindividual variability in the number of MAIT cells, we sought to determine whether characteristics of the recipients influenced the rate and kinetics of MAIT cell recovery. Slow recovery was found regardless of the underlying malignancy (acute lymphoblastic leukemia versus others), gender or age of the recipient, pre-HSCT conditioning (with/without TBI or cyclophosphamide), or duration of immunosuppressive treatment (data not shown). The number of Tconv and MAIT cells before and up to 3 months after HSCT was lower in MUD compared to MSD recipients, likely due to a longer time to transplant and more frequent use of in vivo T-cell depletion with anti-thymocyte globulin (ATG) in the MUD group. However, while Tconv reached comparable values after 3 months, MAIT cell numbers remained 2-fold lower in MUD compared to MSD recipients at all time points after HSCT (2-way ANOVA, p= 0.0005) (Figure 1D). There was no significant correlation between the number of MAIT cells in the recipient before conditioning and 1 or 3 months after HSCT (Figure 1E).

T cell reconstitution is impaired in patients with aGVHD, at least in part because of defective thymic production of HSC-derived naïve T cells (22). We previously showed that a rebound in naïve MAIT cells occurred between 6 and 12 months after transplantation, indicating the emergence of new thymus-derived cells, and this was not influenced by the occurrence of aGVHD (16). In the present study, grade 2-4 aGVHD developed in 26 patients at a mean of

31 days after HSCT (range 14-64). MAIT cell recovery was not different between patients with or without aGVHD (Figure 1G).
Microbial infections have been associated with modifications of MAIT cell frequencies in the peripheral blood of patients (6, 30-32). Six children presented severe microbial infection in the first 3 months after HSCT. There was no impact of this potential source of MR1 ligands on MAIT cell recovery (Figure 1H).
Altogether, these results show that MAIT cell reconstitution is delayed for several years after HSCT in children regardless of factors generally associated with impaired recovery of conventional T cells.

2/ MAIT cell recovery is not influenced by the type of conditioning regimen and is not correlated with the occurrence of GVHD

Despite their expression of the multidrug efflux protein (5), MAIT cells did not survive the pre-transplant myeloablative (MA) conditioning, as indicated by their absence in the peripheral blood and tissues early after HSCT (16). Non-myeloablative (NMA) conditioning causes lower bone marrow suppression and mucosal injury than MA conditioning, and the MAIT cell compartment might, at least in part, recover through proliferation of conditioning-resistant residual MAIT cells from the host's bone marrow and tissues. We evaluated the impact of conditioning on MAIT cell recovery by retrospectively analyzing 49 adult HSCT recipients (25 given NMA and 24 MA) from the CRYOSTEM biobank, a national collection of biological samples from donors and HSCT recipients before and after transplant. Characteristics of the patients are shown in Table 2.
There was no difference in MAIT cell frequencies among patients who received MA or NMA regimen at any time point after HSCT (Figure 2A). To confirm that the conditioning regimen had no impact on post-HSCT expansion, we used Ki67 staining to quantify proliferating MAIT cells. The frequency of $Ki67^+$ proliferating MAIT cells was low at all time points after HSCT, regardless of the intensity of the conditioning regimen (Figure 2B).
As the profile of MAIT cell recovery was similar in patients given MA or NMA conditioning, the two groups were combined thereafter. One year after HSCT, MAIT cell values remained 4 times lower in the recipients than in their respective donor (mean 7.6 vs 29.3 cells/µL, Wilcoxon non parametric paired test $P < 0.0001$) (Figure 2C), while the number of Tconv strongly increased (Figure 2D).
We next evaluated the potential relationship between MAIT cells and aGVHD. In this series, 26 patients presented grade 2-4 aGVHD diagnosed at a mean of 29 days after transplantation. MAIT cells were quantified at the time of aGVHD diagnosis before any treatment. As observed above in children HSCT recipients, the kinetics of MAIT cell recovery was not different between patients who showed no or mild (grade 1) aGVHD and those with grade 2-4 aGVHD (Figure 3A).
The majority of HSCT recipients (30 out of 49) received peripheral blood stem cells, but others received bone marrow. We did not always have access to the bone marrow (BM) sample to quantify MAIT cells in the bone marrow graft. In 6 available paired BM and PB samples, MAIT cell frequencies were comparable in these two compartments (Figure 3B). Therefore, we used the number of MAIT cells in the donor PB as a surrogate for that in the BM. There was no difference in the number of donor MAIT cells between patients with or without aGVHD (Figure 3C).
Notably, the proportion of Ki67+ cells at time of aGVHD, which might represent alloreactive proliferating cells, was significantly higher in CD4 than in MAIT cells (6.5%, 5.5% and 2.2%, respectively). One year after HSCT however, no more difference was observed (Figure 3D).

Taken together, our results show that the recovery of MAIT cells after HSCT is influenced neither by the nature of conditioning nor by aGVHD. In contrast with Tconv, MAIT do not proliferate at time of aGVHD. Moreover, the number of MAIT cells in the donor, or in the recipient before conditioning, shows no relationship with occurrence of aGVHD.

3/ MAIT cell proliferation in response to cytokines or alloantigen stimulation

Cytokines produced in the lymphopenic environment early after HSCT, in particular IL-7 and IL-15, induce extensive homeostatic expansion of lymphocytes. The lack of expansion of MAIT cells after HSCT, even at time of aGVHD, might be related to their unresponsiveness to these cytokines, or to cytokine consumption by other lymphocytes.
First, we treated human peripheral blood mononuclear cells (PBMCs) with IL-7 or IL-15 alone, or in combination with the archetypal MR1 ligand 5-OP-RU (300 nM) for 6 days, and monitored the proliferation of MR1 tetramer^{+} MAIT cells in comparison to that of Tconv cells. In the absence of cytokine, MAIT cells hardly responded to 5-OP-RU alone. Although the vast majority of MAIT cells proliferated in response to IL-15 or IL-7 alone, proliferation strength (as assessed by the number of divisions) was significantly increased when 5-OP-RU was added to IL-15, and to a lesser extent to IL-7. By comparison, cytokine-induced proliferation was low in Tconv cells, and not influenced by the presence of MR1 ligand, as expected (Figure 4A).
Chemotherapy and radiation induce the secretion of IL-12 and IL-18, which may trigger early TCR-independent activation of MAIT cells transferred with the graft. A small proportion of MAIT cells proliferated following stimulation with IL-12+IL-18. This proportion increased in the presence 5-OP-RU, although the division index remained lower than that observed with IL-15 (Figure 4A).
To directly explore the capacity of MAIT cells to recognize allogeneic cells, we used a CFSE-based method to quantify T cell proliferation in mixed lymphocyte reaction (MLR). As shown in Figure 4B, MAIT cells barely proliferated in response to irradiated allogeneic PBMCs, while a mean of 40% of conventional T cells proliferated during the 6-day MLR (P= 0.005).
Activated alloreactive T cells may produce IL-2 during the 6-day MLR. MAIT cells did not proliferate at all in response to IL-2 alone (20 or 100 U/mL). However, as observed with other cytokines, a strong proliferation was observed when IL-2 was combined to 5-OP-RU (Figure 4C). Notably, adding 5-OP-RU in the MLR induced a strong MAIT cell proliferation, suggesting that IL-2 produced by the neighboring alloreactive T cells during the MLR was sufficient to make MAIT cells proliferate in combination with MR1 ligand (Figure 4D).
Taken together, these results suggest that neither lymphopenic condition nor allogeneic stimulation alone is sufficient to induce sustained MAIT cell expansion in the post-HSCT period. In any case, the presence of MR1 ligand together with homeostatic or inflammatory cytokine is required for MAIT cells to proliferate.

4/ Human MAIT cells do not expand in immunodeficient mice and do not cause xenogeneic GVHD

To further document the capacity of MAIT cells to expand and mediate tissue damage after HSCT, we used a model of xenogeneic GVHD (xeno-GVHD) in which low doses of human PBMCs are injected into irradiated immunodeficient NOD/LtSz-Prkdc^{scid}IL2Rg^{tm1Wjl} (NSG) mice. In this model, human CD3 T cells consistently expand after 2-3 weeks and mediate an acute GVHD-like syndrome with extensive CD3 T-cell tissue infiltration and damage of mouse skin, liver, intestine and lungs, resulting in death by 30-50 days.

We analyzed the presence of human transferred cells in the different recipient lymphoid and nonlymphoid organs, including those where MAIT cells are known to preferentially reside. At day 28 after injection, flow cytometry analysis of human CD45+ leucocytes demonstrated the presence of a variable proportion of CD45+ cells among nucleated cells in the peripheral blood and tissues, among which almost all represented Tconv cells, but less than 0.05% were MAIT cells (Figure 5A).

Mice were monitored to evaluate aGVHD progression (weight loss, reduced mobility, hunched posture, hair loss), and euthanized when weight loss was >15%, at a mean of 45 days after injection. Consistent with previous reports, we found a massive accumulation of conventional T cells which represented >98% of human $CD45^+$ cells in all mice compartments. Remarkably, MAITs were barely detectable in all compartments analyzed (Figure 5B).

Since MAIT cells did not proliferate significantly *in vitro* in the absence of 5-OP-RU, it is possible that their expansion or survival after the *in vivo* transfer of huPBMCs may require the presence of more MR1 ligands. Mice were given 5-OP-RU (1 nmol) intraperitoneally every 3 days from the day of huPBMC injection, a dose previously shown to activate endogenous MAIT cells (33). This did not lead to significant increase in MAIT cell numbers in the peripheral blood or tissues (Figure 5C).

To determine if absence of MAIT cell detection in NSG mice was not secondary to their low proportion within adoptively transferred human PBMCs (mean 3% of CD3 T cells, i.e around $5x10^4$ infused MAIT cells), we first cultured PBMCs with 5-OP-RU and IL-2 for 6 days. As shown in Figure 5D, this procedure made it possible to expand up to 32% of MAIT cells among CD3 T cells. Mice were given $3x10^6$ of these MAIT-enriched PBMCs, together with 5-OP-RU i.p every 3 days. At day 52 after infusion, mice were sacrificed, although they were still healthy. Human $CD45^+$ cells were not detectable in the peripheral blood and tissues, indicating absence of engraftment of MAIT-enriched PBMCs (Figure 5D).

It is not clear whether mouse cytokines can sustain proliferation and survival of human MAIT cells in NSG mice due to a species barrier between human lymphoid cells and recipient microenvironment (34-36). This is a key question for IL-15, as it is mostly mouse-derived in the xeno-GVHD model given the low human myeloid chimerism (main source of IL-15).

Human MAIT cells cultured *in vitro* with mouse IL-15 did not proliferate at all, at contrast with human IL-15. Proliferation was partially restored when 5-OP-RU was added to mouse IL-15, although it remained lower than with human IL-15. By comparison, murine and human IL-7 had similar effects, regardless of the presence of 5-OP-RU (Figure 6A). These results suggested that IL-15 availability might be suboptimal in NSG mice. We thus treated NSG mice with human IL-15 (0.5 μg i.p every 3 days from the day of huPBMC injection). In such condition, mice developed signs of severe aGVHD earlier than in the absence of IL-15, and were therefore euthanized at day 25 after huPBMC injection. Although a massive infiltration of CD3 T cell was observed in all compartments, MAIT cell proportions were not increased (Figure 6B). We next treated NSG mice with human IL-15 once a week (to limit the toxicity) together with 5-OP-RU, and monitored the presence of MAIT and CD3 T cells in the peripheral blood up to 50 days after huPBMC transfer. While the proportions of conventional T cells increased, MAIT cells remained undetectable in the recipient mice (Figure 6C).

Taken together, these results indicate that in contrast to conventional CD3 T cells, human MAIT cells do not accumulate in the periphery and do no not participate to T cell-mediated xeno-GVHD in immunodeficient mice, regardless of the presence of MR1 ligand and homeostatic cytokines.

Discussion

Human MAIT cells are very few at birth and accumulate gradually during infancy, with an expansion of about 30 times to reach a plateau around 6 years of age (16). The drivers of this peripheral expansion remain elusive, although it is proposed that successive encounters with microbes lead to accumulation of MAIT cell clonotypes that will constitute the future MAIT cell pool (37). A wide range of bacteria and fungi are able to synthesize riboflavin and hence provide MR1 ligand (38). However, certain bacteria do not activate MAIT cells, likely due to the lack of an intact riboflavin biosynthetic pathway in these strains (30). MAIT are absent in germ-free mice and are very few in laboratory mice, but dramatically expand following challenge with various riboflavin-producing microbes (1, 30, 33). In humans with controlled intranasal infection with live Salmonella enteritica serovar Paratyphi A, MAIT cells show evidence of expansion of select MAIT cell clonotypes, that could be related to higher functional avidity of their TCR to the bacterial metabolite antigen (39).
Here, we show that the reconstitution of the MAIT cell population in different HSCT settings occurs over a period of at least 6 years, thus recapitulating their physiological expansion in the infancy. These results extend our previous observation after cord blood transplantation, a procedure in which the number of MAIT cells contained in the donor graft is extremely low. We show that MAIT cell reconstitution is delayed regardless of recipient- or donor-related factors such as age, underlying disease, stem cell source, conditioning regimen, or occurrence of aGVHD. In all circumstances, MAIT cell numbers very slowly increase during several years and this is not due to impaired thymopoiesis, which may be secondary to conditioning or aGVHD itself (22). Previous studies reported early defective MAIT cell recovery following HSCT (24-26), but none of them investigated the long-term reconstitution (i.e. more than 2 years). Moreover, there were conflicting results on the potential relationship between MAIT cell numbers and aGVHD. In agreement with Solders et al. (25), we do not find the association between low MAIT cell numbers and occurrence of aGVHD observed by others (24, 26). It must be stressed that the numbers of MAIT cells during the first 100 days after HSCT, i.e. the time to develop aGVHD, were in all cases extremely low, raising questions on the relevance of such small differences.
Loss of diversity and increased bacterial domination early after HSCT (in particular by Enterococcus, a strain unable to synthesize riboflavin) has been associated with increased risk of aGVHD and decreased survival, an effect dependent on the presence of T cells (40-42). One might speculate that blooming of Enterococcus could prevent from early expansion of donor-derived MAITs due to lack of MR1 ligands. In order to definitely determine if *in vivo* availability of riboflavin metabolites influences MAIT cells recovery, the gut metagenome or metatranscriptome of HSCT recipients should be investigated for the presence of Rib biosynthesis genes.
In a recent study conducted in mouse models of allogeneic HSCT, conditioning-resistant host residual MAIT cells protected from intestinal aGVHD through microbial-induced IL-17 secretion and subsequent inhibition of alloreactive donor-derived T cell proliferation (23). Whether these findings are directly translatable to human HSCT is however unlikely due to specificities of HSCT models in mice, including total body irradiation, use of splenocytes as donor cells and major MHC-mismatched setting. Moreover, mouse MAIT cells differ from human ones by their low frequency, their phenotype (mostly $CD4^-CD8^-$), their tissue localization (absent from bone marrow), and their persistence in the colon after conditioning (while residual human MAIT cells are undetectable in the recipient's intestine)(23, 43, 44).

The lack of long-term MAIT cell recovery after HSCT raised the question of their responsiveness to homeostatic cytokines and allogeneic antigens. Early after HSCT, IL-7 and

IL-15 are produced but little consumed because of the lymphopenic state induced by the conditioning regimen. This results in high serum levels of these cytokines, allowing the little number of T cells present after transplantation to undergo expansion. It has been demonstrated that cytokine signals alone are sufficient to induce expansion of memory T cells transferred with the graft (45). To determine if donor T cells might outcompete MAIT cells by limiting the availability of cytokines, we stimulated PBMCs with IL-7 or IL-15 and compared the proliferative response of MAIT and Tconv cells. MAIT cells proliferated at least as much as Tconv cells. This suggests that their low peripheral expansion after HSCT is not related to cytokine consumption by other lymphocytes. Moreover, it may explain the small early expansion of donor-derived MAIT cells observed by others in the first month after HSCT, before they plateau at very low values (24). However, this cytokine-induced proliferation is not sufficient to maintain their prolonged expansion.

Although TCR triggering by MR1 ligand alone was insufficient to drive MAIT cell proliferation, we found that combining cytokine and TCR signals had a clear synergistic effect, as previously observed for cytokine production (11-13, 46).

Thus, cytokines produced in the first weeks after transplant in the lymphopenic recipient may provide early but limited proliferation of graft-derived MAIT cells. However, sustained expansion of mature and newly thymus-derived MAIT cells will only occur if MR1 ligands are present together with inflammatory signals. Such restricted conditions are likely to be crucial in controlling the balance between healthy and pathological processes. In steady state conditions, such as the postnatal period, MAIT cells are continuously exposed to MR1 ligands derived from the commensal bacteria present in the microbiome. In the absence of inflammatory signals provided during microbial infections, this will only drive minimal proliferation associated with normal homeostasis and survival of MAIT cells. It is likely that the size of the adult MAIT cell pool is determined by sequential accumulation of MAIT cell clones that expand upon successive infections by riboflavin-producing microbes, which provide both MAIT-specific TCR triggering and inflammatory signals.

MAIT cells do not appear to play a role in allogeneic responses, as anticipated due to the very limited diversity of their MR1-restricted TCR. Using a classical *in vitro* model of alloreactivity, we show that MAIT cells do not proliferate except in the simultaneous presence of MR1 ligand and soluble factors (such as IL-2) produced by activated alloreactive conventional T cells. Moreover, MAIT cells do not participate in the development of xeno-GVHD in irradiated NSG mice infused with human PBMCs. In this model, expansion of human T cells relies both on lymphopenia-driven proliferation in the immunedeficient host, and on recognition of murine MHC by xenoreactive T cells. It is likely that the xeno-GVHD is caused by a fraction of T cells having a low frequency in donor PBMCs, which subsequently expand in mouse organs (47). MAIT cells were never detected at significant values in the xeno-GVHD model, even when additional MR1 ligand was provided from the day of human PBMCs infusion. They were absent from the peripheral blood, but also from GVHD target tissues, which are precisely those where MAIT cells are preferentially localized in the physiological setting (lungs, liver, intestine). One cannot exclude that the irradiated NSG host with an ablated immune compartment may be less likely to provide conditions for presentation of MR1 ligands to MAIT cells or the delivery of costimulatory signals. However, MR1 is highly conserved between species, and murine MR1 can present 5-OP-RU to human MAIT cells as efficiently as human MR1 (30). That donor T cells may outcompete MAIT cells by limiting the availability of IL-15 seems unlikely, as demonstrated by the failure of exogenous human IL-15 to increase MAIT cell numbers in NSG recipients, even in the presence of MR1 ligand. Whether MAIT cells fail to traffic or find their niche in the host due to species barriers between chemokine receptors and their ligands is also unlikely given the variety of tissue homing molecules expressed on MAIT cells (5, 48).

In conclusion, the mechanisms that regulate homeostasis and expansion of MAIT cells in allogeneic conditions are profoundly different from those of conventional T cells. While survival of MAIT cells may be accounted for by cytokines alone, their expansion is likely controlled by the conjunction of inflammatory signals and sufficient availability of MR1 ligands. However, MAIT cells do not expand nor accumulate in tissues in response to allogeneic stimulation. These data pave the way for harnessing novel MAIT cell immunoregulatory functions in the allogeneic setting. MAIT cells are also excellent candidates for producing universal chimeric antigen receptor-engineered effector cells (CAR-MAIT), as they are abundant in peripheral blood, can migrate to tissues, have potent effector functions and are unlikely to cause GVHD after transfer in allogeneic recipients.

Acknowledgments

The authors thank all the patients and their physicians and the nurse and technician staff from Hôpital Robert Debré who helped with this study, as well as all members of the CRYOSTEM Consortium for providing patients samples used in this study : University Hospital of Angers, University Hospital of Dijon Bourgogne, University Hospital of Besançon, University Hospital of Grenoble, University Hospital of Lille, University Hospital of Lyon, University Hospital of Bordeaux, Paoli-Calmettes Institute, AP-HM, University Hospital of Nantes, AP-HP (Saint Louis Hospital, La Pitié-Salpêtrière Hospital, Saint-Antoine Hospital, Robert Debré Hospital, Necker Hospital, Groupe Hospitalier Henri Mondor), INSERM, University Hospital of Toulouse, University Hospital of Tours, University Hospital of Rennes, University Hospital of Clermont-Ferrand, University Hospital of Saint-Etienne, Lucien Neuwirth Cancer Institute, University Hospital of Poitiers, University Hospital of Nice, University Hospital of Brest, Military Hospital of Percy, University Hospital of Montpellier, Gustave Roussy Institute, University Hospital of Limoges, University Hospital of Caen, Etablissement Français du Sang.
The authors are grateful to Véronique Parietti (Plateforme d'expérimentation animale, IRSL, Hôpital Saint-Louis).

Author contributions

M. Tourret, N Talvard-Balland, M. Lambert, G. Ben Youssef, F Morin and S Azarnoush designed research studies, conducted experiments, analyzed data, and/or provided patient samples. T. Yvorra produced synthetic 5-OP-RU. O Lantz and J-H. Dalle analyzed data, and wrote the manuscript. S. Caillat-Zucman designed research studies, supervised the study, analyzed data, and wrote the manuscript with the help of other coauthors.

Table 1. Characteristics of the children HSCT recipients

	Sibling donor	**Unrelated donor**
Donor origin	19 (47.5%)	21 (52.5%)
Female gender	6 (31.6%)	10 (47.7%)
Median age of recipient, yrs (range)	11 (0.67-16)	10.5 (2-15)
Donor age, yrs	14 (1-21)	>18
Hematological disease: - **ALL** - **AML** - **JMML** - **Lymphoma** - **Myelodysplasia** - **CML**	 - 14 (73.7%) - 3 (15.8%) - 1 (5.3%) - 0 - 1 (5.25%) - 0	 - 9 (42.9) - 8 (38%) - 2 (9.5%) - 1 (4.8%) - 0 - 1 (4.8%)
Myeloablative conditioning **In vivo T-cell depletion (ATG)**	19 (100%) 0	21 (100%) 12 (57%)
Acute GVHD - **Stage 0-1** - **Stage 2-3-4**	 -6 (32%) -13 (68%)	 -8 (38%) -13 (62%)

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphoblastic leukemia; JMML: juvenile myelomonocytic leukemia

Table 2: Characteristics of the adult HSCT recipients from the CRYOSTEM biobank

	Myeloablative conditioning	**Non-myeloablative conditioning**
Pre-transplant conditioning	24 (49%)	25 (51%)
Female gender	8 (33.3%)	10 (40%)
Median age of recipient, yrs (range)	45 (17-56)	45 (23-65)
Hematological disease: - **AML** - **ALL** - **Hodgkin lymphoma** - **Non Hodgkinlymphoma** - **MDS or MPN** - **Multiple myeloma** - **Secondary acute leukemia** - **CLL** - **CML**	 - 8 (33.3%) - 6 (25%) - 2 (8.3%) - 4 (16.6%) - 1 (4.2%) - 2 (8.3%) - 1 (4.2%) - 0 - 0	 - 11 (44%) - 0 - 4 (16%) - 3 (12%) - 4 (16%) - 1 (4%) - 0 - 1 (4%) - 1 (4%)
Stem cell source: - **Peripheral blood** - **Bone marrow**	 -8 (33.3%) -16 (66.7%)	 -22 (88%) -3 (12%)
Acute GVHD - **Stage 0-1** - **Stage 2-4**	 - 16 (66.7%) - 8 (33.3%)	 - 17 (68%) - 8 (32%)
Mean time from transplant to GVHD, days (range)	34 (16-102)	30 (10-51)
In vivo T-cell depletion (ATG)	5 (20.8%)	13 (52%)

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CML: chronic myeloid leukemia; CLL: chronic lymphoblastic leukemia; MDS: Myelodysplastic syndrome; MPN: myeloproliferative neoplasm

Legend to Figures

Figure 1: MAIT cell recovery takes a very long time after HSCT
(A) Representative staining of MAIT cells in a donor and a recipient 3 months after HSCT. Numbers indicate the percentage of $V\alpha7.2^+$ $CD161^{high}$ cells in the $CD3^+CD4^-$ T cell gate (left quadrants) and the fraction of $V\alpha7.2^+$ MR1:5-OP-RU $tetramer^+$ cells in the corresponding gate (right quadrants)
(B) Absolute counts of CD3 T cells (Tconv) and MAIT cells detected in the peripheral blood of 40 children before conditioning (pre) and followed from month 1 to month 24 after HSCT. Light boxes show the reference physiological cell count interval obtained from 36 age-matched healthy donors. Data are shown as mean ± SEM.
(C) Relationship between log10-based transformed MAIT cell absolute numbers and time from transplantation up to 16 years after HSCT (n= 83 samples).
(D) Recovery dynamics of MAIT cells (left panel) and CD3 T cells (right panel) in transplant recipients of matched sibling donors (MSD) and unrelated donors (MUD). Results show the mean absolute numbers ± SEM during the first 24 months after HSCT. Dashed lines represent values in age-matched healthy donors. 2-way ANOVA, p= 0.0005.
(E) Correlation of MAIT cell numbers in the recipient before conditioning (pre) and 1 month (M1, Spearman's r= 0.31, p= 0.12) or 3 months (M3, r= 0.19, p= 0.30) after HSCT. Each point corresponds to one patient and lines connect matched samples.
(F) Comparison of MAIT cell recovery in patients with grade 0-1 and grade 2-4 aGVHD. Results show the mean absolute numbers ± SEM.
(G) MAIT cell recovery is not influenced by the presence or absence of severe microbial infection occurring during the first 3 months after HSCT. Mean absolute numbers ± SEM
*** P = 0.0005, n.s, not significant.

Figure 2: MAIT cell recovery is not influenced by the conditioning regimen
(A) MAIT cell recovery kinetics from individual patients given myeloablative (MA, n= 24) or nonmyeloablative (NMA, n=25) conditioning. Each point corresponds to one patient and lines connect matched samples. Bold lines represent mean ± SEM. 2-way ANOVA, p= 0.85.
(B) Individual values and means (horizontal bars) of frequencies of $Ki67^+$ proliferating MAIT cells in patients given MA or NMA conditioning. 2-way ANOVA, p= 0.43
(C) MAIT cell numbers in the graft donors and their recipients 1 year after HSCT. Wilcoxon non parametric paired test P <0.0001.
(D) Absolute counts of CD3 T cells (Tconv) and MAIT cells in the whole CRYOSTEM cohort (n= 49 patients). Dashed lines represent values (mean ± SEM) observed in the donors.

Figure 3 : Absence of relationship between MAIT cell numbers and aGVHD
(A) Kinetics of MAIT cell recovery in patients with grade 0-1 and grade 2-4 aGVHD. Results show the mean absolute numbers ± SEM. 2-way ANOVA, p= 0.63
(B) Comparison of MAIT cell frequency in paired bone marrow and peripheral blood samples from 6 healthy donors (Wilcoxon matched-pairs signed rank test, p= 0.06).
(C) Comparison of MAIT cell numbers in the donors from patients who developed grade 0-1 and grade 2-4 aGVHD. Mann-Whitney test, p= 0.6.
(D) Quantification of $Ki67^+$ proliferating cells among MAIT, CD4 and CD8 T cells at time of aGVHD (left panel) and 12 months after HSCT (right panel). Scatter plots with bars showing mean ± SEM of the percentage of $Ki67^+$ cells. Kruskall Wallis, ** p= 0.002, * p= 0. 04.

Figure 4: MAIT cell proliferative response to homeostatic cytokines and allogeneic cells

Carboxyfluorescein succinimidyl ester (CFSE)-labeled human PBMCs (10^6/mL) were cultured for 6 days in the indicated conditions. Proliferation of MR1 tetramer$^+$ MAIT (red lines) and conventional T (Tconv, black lines) cells was quantified by CFSE dilution (% of CFSE^{low} cells) at the end of the culture.
(A) Proliferative response to stimulation by IL-7 (10ng/mL), IL-15 (50 ng/mL) and IL-12/18 (50 ng/mL each) in the presence or absence of the synthetic MR1 ligand, 5-OP-RU (300 nM). *Left panels*: Representative CFSE staining gated on MAIT and Tconv cells after 6-day culture. Numbers in the quadrants indicate the percentage of proliferating (CFSE^{low}) cells among each population. *Right panel*: Division index (number of divisions in the total population) of MAIT and Tconv cells. Mean ± SEM of 3 independent experiments. P value (paired t test) is indicated
(B) Mixed lymphocyte reaction: CFSE-labeled responder PBMCs were cultured with irradiated allogeneic PBMCs at 1:1 ratio. Responder T cells were identified at the end of the 6-day culture by gating on CD3$^+$ T cells following exclusion of dead cells.
Left panel: Representative CFSE staining gated on MAIT and Tconv cells after 6-day culture in the presence of allogeneic (upper panel) or autologous (lower panel) PBMCs. Numbers in the quadrants indicate the percentage of proliferating (CFSE^{low}) cells among each population. *Right panel*: Individual values and means ± SEM of proliferating Tconv and MAIT cells (n= 6 experiments using different recipient/donor pairs). P value (paired t test) is indicated.
(C) PBMCs were stimulated with IL-2 at the indicated concentration, with or without 5-OP-RU (300 nM). *Left panel*: representative CFSE staining gated on MAIT and Tconv cells. *Right panel*: Division index of MAIT and Tconv cells. Mean± SEM of 4 independent experiments.
D) Mixed lymphocyte reaction was performed in the absence or presence of 300 nM 5-OP-RU. Representative CFSE staining gated on MAIT and Tconv cells after 6-day culture.

Figure 5: Absence of MAIT cell expansion in a xenogeneic GVHD model
Irradiated (1.3 Gy) NSG mice injected with 3.10^6 huPBMCs were monitored to evaluate aGVHD progression, and euthanized at the indicated time. Peripheral blood, spleen, liver, lungs and colon were harvested, and cells were isolated.
(A-C) The proportion of human CD45+ leukocytes among viable cells (i.e. human chimerism, upper panels), and the proportion of Tconv (black dots) and MAIT (red dots) cells among human CD45+ cells (lower panels) were determined by flow cytometry. Results show individual values and median (horizontal bar). (A) Mice were sacrificed at day 28 after injection (n= 4 mice). (B) Mice were euthanized when they exhibited signs of aGVHD (± day 45, n= 8 diseased mice). (C) Mice were given 5-OP-RU every 3 days (1nmol i.p.) from the day of huPBMC injection and euthanized at time of aGVHD (n=7 diseased mice).
(D) PBMCs were cultured with 5-OP-RU and IL-2 for 6 days (left panel). Mice were given $3x10^6$ of these MAIT-enriched PBMCs, together with 5-OP-RU i.p every 3 days. At day 52 after infusion, mice were sacrificed although they were still healthy (n=2). Human CD45$^+$ cells were barely detectable in the peripheral blood and tissues (right panel).

Figure 6: Human IL-15 does not improve MAIT cell accumulation in the NSG model
(A) CFSE-labeled human PBMCs were cultured for 6 days with mouse (open histograms) or human (grey histograms) IL-15 (50 ng/mL) or IL-7 (10 ng/mL) in the absence or presence of 5-OP-RU. Representative experiment showing CFSE^{low} proliferating MAIT cells (gated on CD3 T cells).
(B) Mice were treated with huIL-15 (0.5 μg i.p. every 3 days) from the day of huPBMC injection, and euthanized at time of aGVHD (±day 26, n= 3 diseased mice). Results show the proportion of human CD45+ leukocytes among viable cells, and the proportion of Tconv and

MAIT cells among human CD45+ cells in the indicated compartments. Individual values and median (horizontal bar) are shown.

(C) Mice were treated with huIL-15 (0.5 μg i.p. every week) and 5-OP-RU (1 nmol i.p. every 3 days) from the day of huPBMC injection. Peripheral blood samples were taken at day 30 and 50 (n= 2 mice). Results show mean percentages of the indicated population.

References

1. Godfrey DI, Koay HF, McCluskey J, Gherardin NA. The biology and functional importance of MAIT cells. Nat Immunol. 2019;20(9):1110-28.
2. Treiner E, Duban L, Bahram S, Radosavljevic M, Wanner V, Tilloy F, et al. Selection of evolutionarily conserved mucosal-associated invariant T cells by MR1. Nature. 2003;422(6928):164-9.
3. Kjer-Nielsen L, Patel O, Corbett AJ, Le Nours J, Meehan B, Liu L, et al. MR1 presents microbial vitamin B metabolites to MAIT cells. Nature. 2012;491(7426):717-23.
4. Corbett AJ, Eckle SB, Birkinshaw RW, Liu L, Patel O, Mahony J, et al. T-cell activation by transitory neo-antigens derived from distinct microbial pathways. Nature. 2014;509(7500):361-5.
5. Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, et al. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood. 2011;117(4):1250-9.
6. Gold MC, Cerri S, Smyk-Pearson S, Cansler ME, Vogt TM, Delepine J, et al. Human mucosal associated invariant T cells detect bacterially infected cells. PLoS Biol. 2010;8(6):e1000407.
7. Le Bourhis L, Dusseaux M, Bohineust A, Bessoles S, Martin E, Premel V, et al. MAIT cells detect and efficiently lyse bacterially-infected epithelial cells. PLoS Pathog. 2013;9(10):e1003681.
8. Loh L, Wang Z, Sant S, Koutsakos M, Jegaskanda S, Corbett AJ, et al. Human mucosal-associated invariant T cells contribute to antiviral influenza immunity via IL-18-dependent activation. Proc Natl Acad Sci U S A. 2016;113(36):10133-8.
9. Ussher JE, Bilton M, Attwod E, Shadwell J, Richardson R, de Lara C, et al. CD161++ CD8+ T cells, including the MAIT cell subset, are specifically activated by IL-12+IL-18 in a TCR-independent manner. Eur J Immunol. 2014;44(1):195-203.
10. Wilgenburg BV, Loh L, Chen Z, Pediongco TJ, Wang H, Shi M, et al. MAIT cells contribute to protection against lethal influenza infection in vivo. Nat Commun. 2018;9(1):4706.
11. Leng T, Akther HD, Hackstein CP, Powell K, King T, Friedrich M, et al. TCR and Inflammatory Signals Tune Human MAIT Cells to Exert Specific Tissue Repair and Effector Functions. Cell Rep. 2019;28(12):3077-91 e5.
12. Lamichhane R, Schneider M, de la Harpe SM, Harrop TWR, Hannaway RF, Dearden PK, et al. TCR- or Cytokine-Activated CD8(+) Mucosal-Associated Invariant T Cells Are Rapid Polyfunctional Effectors That Can Coordinate Immune Responses. Cell Rep. 2019;28(12):3061-76 e5.
13. Hinks TSC, Marchi E, Jabeen M, Olshansky M, Kurioka A, Pediongco TJ, et al. Activation and In Vivo Evolution of the MAIT Cell Transcriptome in Mice and Humans Reveals Tissue Repair Functionality. Cell Rep. 2019;28(12):3249-62 e5.
14. Constantinides MG, Link VM, Tamoutounour S, Wong AC, Perez-Chaparro PJ, Han SJ, et al. MAIT cells are imprinted by the microbiota in early life and promote tissue repair. Science. 2019;366(6464).
15. Salou M, Lantz O. A TCR-Dependent Tissue Repair Potential of MAIT Cells. Trends Immunol. 2019;40(11):975-7.
16. Ben Youssef G, Tourret M, Salou M, Ghazarian L, Houdouin V, Mondot S, et al. Ontogeny of human mucosal-associated invariant T cells and related T cell subsets. J Exp Med. 2018;215(2):459-79.
17. Zeiser R, Blazar BR. Acute Graft-versus-Host Disease - Biologic Process, Prevention, and Therapy. N Engl J Med. 2017;377(22):2167-79.

18. Blazar BR, Hill GR, Murphy WJ. Dissecting the biology of allogeneic HSCT to enhance the GvT effect whilst minimizing GvHD. Nat Rev Clin Oncol. 2020.
19. Magenau J, Runaas L, Reddy P. Advances in understanding the pathogenesis of graft-versus-host disease. Br J Haematol. 2016;173(2):190-205.
20. Zeiser R, Socie G, Blazar BR. Pathogenesis of acute graft-versus-host disease: from intestinal microbiota alterations to donor T cell activation. Br J Haematol. 2016;175(2):191-207.
21. Socie G, Ritz J. Current issues in chronic graft-versus-host disease. Blood. 2014;124(3):374-84.
22. Chaudhry MS, Velardi E, Malard F, van den Brink MR. Immune Reconstitution after Allogeneic Hematopoietic Stem Cell Transplantation: Time To T Up the Thymus. J Immunol. 2017;198(1):40-6.
23. Varelias A, Bunting MD, Ormerod KL, Koyama M, Olver SD, Straube J, et al. Recipient mucosal-associated invariant T cells control GVHD within the colon. J Clin Invest. 2018;128(5):1919-36.
24. Bhattacharyya A, Hanafi LA, Sheih A, Golob JL, Srinivasan S, Boeckh MJ, et al. Graft-Derived Reconstitution of Mucosal-Associated Invariant T Cells after Allogeneic Hematopoietic Cell Transplantation. Biol Blood Marrow Transplant. 2018;24(2):242-51.
25. Solders M, Erkers T, Gorchs L, Poiret T, Remberger M, Magalhaes I, 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.
26. Kawaguchi K, Umeda K, Hiejima E, Iwai A, Mikami M, Nodomi S, 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.
27. Soudais C, Samassa F, Sarkis M, Le Bourhis L, Bessoles S, Blanot D, et al. In Vitro and In Vivo Analysis of the Gram-Negative Bacteria-Derived Riboflavin Precursor Derivatives Activating Mouse MAIT Cells. J Immunol. 2015;194(10):4641-9.
28. Mak JY, Xu W, Reid RC, Corbett AJ, Meehan BS, Wang H, et al. Stabilizing short-lived Schiff base derivatives of 5-aminouracils that activate mucosal-associated invariant T cells. Nat Commun. 2017;8:14599.
29. Reantragoon R, Corbett AJ, Sakala IG, Gherardin NA, Furness JB, Chen Z, et al. Antigen-loaded MR1 tetramers define T cell receptor heterogeneity in mucosal-associated invariant T cells. J Exp Med. 2013;210(11):2305-20.
30. Le Bourhis L, Martin E, Peguillet I, Guihot A, Froux N, Core M, et al. Antimicrobial activity of mucosal-associated invariant T cells. Nat Immunol. 2010;11(8):701-8.
31. Grimaldi D, Le Bourhis L, Sauneuf B, Dechartres A, Rousseau C, Ouaaz F, et al. Specific MAIT cell behaviour among innate-like T lymphocytes in critically ill patients with severe infections. Intensive Care Med. 2014;40(2):192-201.
32. Ghazarian L, Caillat-Zucman S, Houdouin V. Mucosal-Associated Invariant T Cell Interactions with Commensal and Pathogenic Bacteria: Potential Role in Antimicrobial Immunity in the Child. Front Immunol. 2017;8:1837.
33. Legoux F, Bellet D, Daviaud C, El Morr Y, Darbois A, Niort K, et al. Microbial metabolites control the thymic development of mucosal-associated invariant T cells. Science. 2019;366(6464):494-9.
34. Roychowdhury S, Blaser BW, Freud AG, Katz K, Bhatt D, Ferketich AK, et al. IL-15 but not IL-2 rapidly induces lethal xenogeneic graft-versus-host disease. Blood. 2005;106(7):2433-5.

35. Huntington ND, Legrand N, Alves NL, Jaron B, Weijer K, Plet A, et al. IL-15 trans-presentation promotes human NK cell development and differentiation in vivo. J Exp Med. 2009;206(1):25-34.
36. Herndler-Brandstetter D, Shan L, Yao Y, Stecher C, Plajer V, Lietzenmayer M, et al. Humanized mouse model supports development, function, and tissue residency of human natural killer cells. Proc Natl Acad Sci U S A. 2017;114(45):E9626-E34.
37. Lantz O, Legoux F. MAIT cells: an historical and evolutionary perspective. Immunol Cell Biol. 2018;96(6):564-72.
38. Mondot S, Boudinot P, Lantz O. MAIT, MR1, microbes and riboflavin: a paradigm for the co-evolution of invariant TCRs and restricting MHCI-like molecules? Immunogenetics. 2016;68(8):537-48.
39. Howson LJ, Napolitani G, Shepherd D, Ghadbane H, Kurupati P, Preciado-Llanes L, et al. MAIT cell clonal expansion and TCR repertoire shaping in human volunteers challenged with Salmonella Paratyphi A. Nat Commun. 2018;9(1):253.
40. Taur Y, Jenq RR, Perales MA, Littmann ER, Morjaria S, Ling L, et al. The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. Blood. 2014;124(7):1174-82.
41. Jenq RR, Taur Y, Devlin SM, Ponce DM, Goldberg JD, Ahr KF, et al. Intestinal Blautia Is Associated with Reduced Death from Graft-versus-Host Disease. Biol Blood Marrow Transplant. 2015;21(8):1373-83.
42. Stein-Thoeringer CK, Nichols KB, Lazrak A, Docampo MD, Slingerland AE, Slingerland JB, et al. Lactose drives Enterococcus expansion to promote graft-versus-host disease. Science. 2019;366(6469):1143-9.
43. Cui Y, Franciszkiewicz K, Mburu YK, Mondot S, Le Bourhis L, Premel V, et al. Mucosal-associated invariant T cell-rich congenic mouse strain allows functional evaluation. J Clin Invest. 2015;125(11):4171-85.
44. Rahimpour A, Koay HF, Enders A, Clanchy R, Eckle SB, Meehan B, et al. Identification of phenotypically and functionally heterogeneous mouse mucosal-associated invariant T cells using MR1 tetramers. J Exp Med. 2015;212(7):1095-108.
45. Geginat J, Lanzavecchia A, Sallusto F. Proliferation and differentiation potential of human CD8+ memory T-cell subsets in response to antigen or homeostatic cytokines. Blood. 2003;101(11):4260-6.
46. Kurioka A, Ussher JE, Cosgrove C, Clough C, Fergusson JR, Smith K, et al. MAIT cells are licensed through granzyme exchange to kill bacterially sensitized targets. Mucosal Immunol. 2015;8(2):429-40.
47. Ehx G, Somja J, Warnatz HJ, Ritacco C, Hannon M, Delens L, et al. Xenogeneic Graft-Versus-Host Disease in Humanized NSG and NSG-HLA-A2/HHD Mice. Front Immunol. 2018;9:1943.
48. Walker LJ, Kang YH, Smith MO, Tharmalingham H, Ramamurthy N, Fleming VM, et al. Human MAIT and CD8alphaalpha cells develop from a pool of type-17 precommitted CD8+ T cells. Blood. 2012;119(2):422-33.

Figure 1

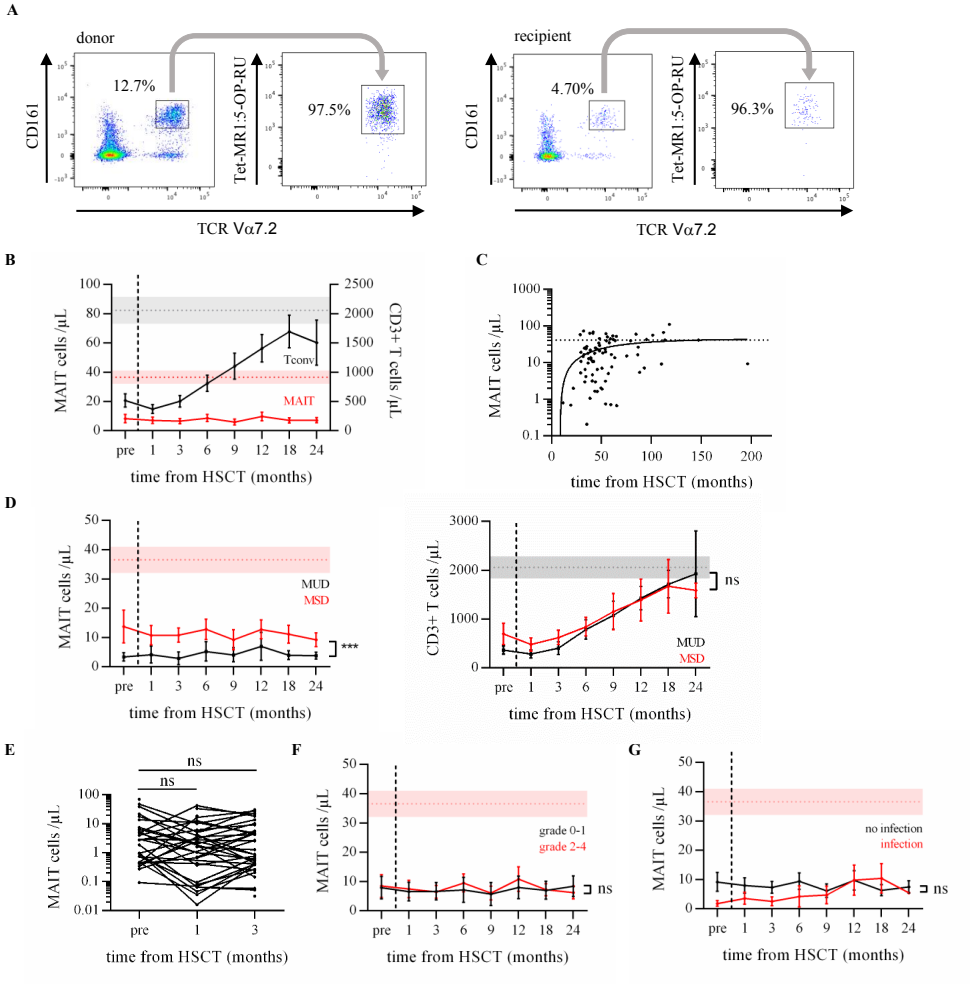

A
donor
recipient
CD161
Tet-MR1:5-OP-RU
12.7%
97.5%
4.70%
96.3%
TCR Vα7.2
B
MAIT cells /µL
CD3+ T cells /µL
Tconv
MAIT
time from HSCT (months)
C
MAIT cells /µL
time from HSCT (months)
D
MAIT cells /µL
MUD
MSD
***
time from HSCT (months)
CD3+ T cells /µL
ns
MUD
MSD
time from HSCT (months)
E
ns
ns
MAIT cells /µL
time from HSCT (months)
F
MAIT cells /µL
grade 0-1
grade 2-4
ns
time from HSCT (months)
G
MAIT cells /µL
no infection
infection
ns
time from HSCT (months)

Figure 2

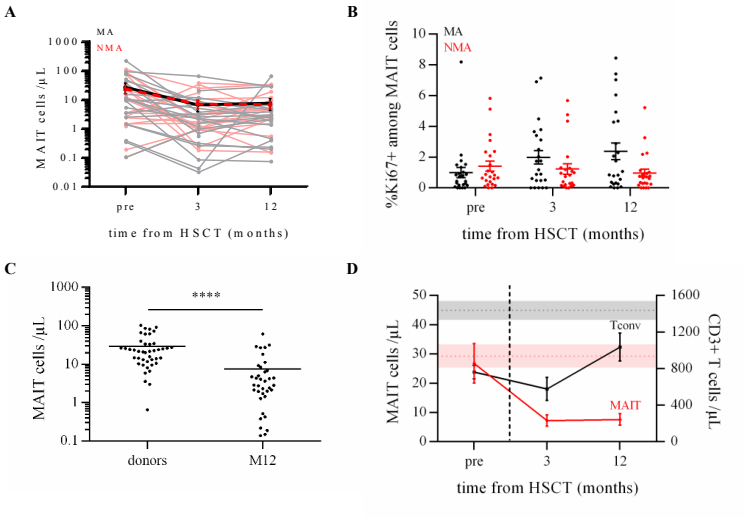

A
MA
NMA
MAIT cells /µL
1000
100
10
1
0.1
0.01
pre
3
12
time from HSCT (months)
B
MA
NMA
%Ki67+ among MAIT cells
10
8
6
4
2
0
pre
3
12
time from HSCT (months)
C
MAIT cells /µL
1000
100
10
1
0.1
****
donors
M12
D
MAIT cells /µL
50
40
30
20
10
0
CD3+ T cells /µL
1600
1200
800
400
0
Tconv
MAIT
pre
3
12
time from HSCT (months)

Figure 3

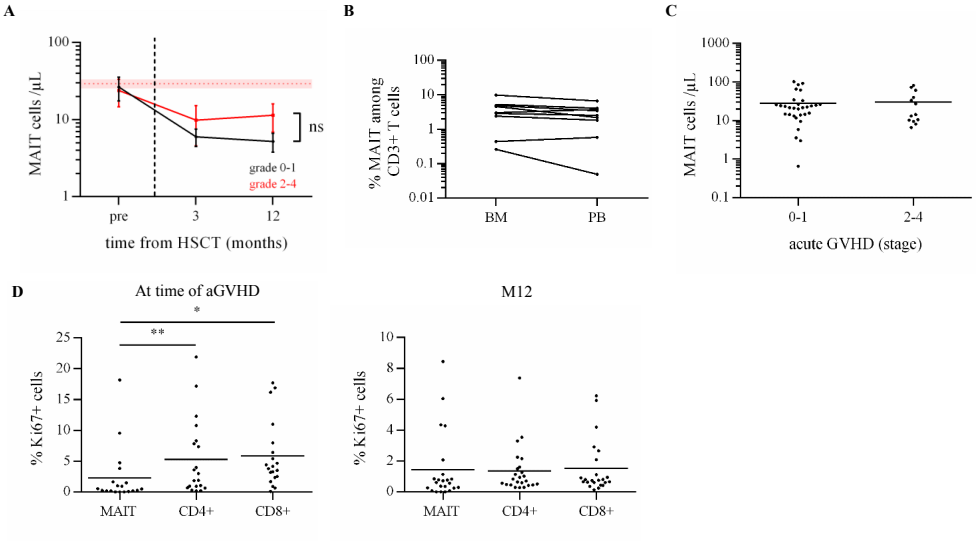

A
MAIT cells /µL
100
10
1
ns
grade 0-1
grade 2-4
pre
3
12
time from HSCT (months)
B
% MAIT among CD3+ T cells
100
10
1
0.1
0.01
BM
PB
C
MAIT cells /µL
1000
100
10
1
0.1
0-1
2-4
acute GVHD (stage)
D
At time of aGVHD
M12
% Ki67+ cells
25
20
15
10
5
0
*
**
MAIT
CD4+
CD8+
% Ki67+ cells
10
8
6
4
2
0
MAIT
CD4+
CD8+

Figure 4

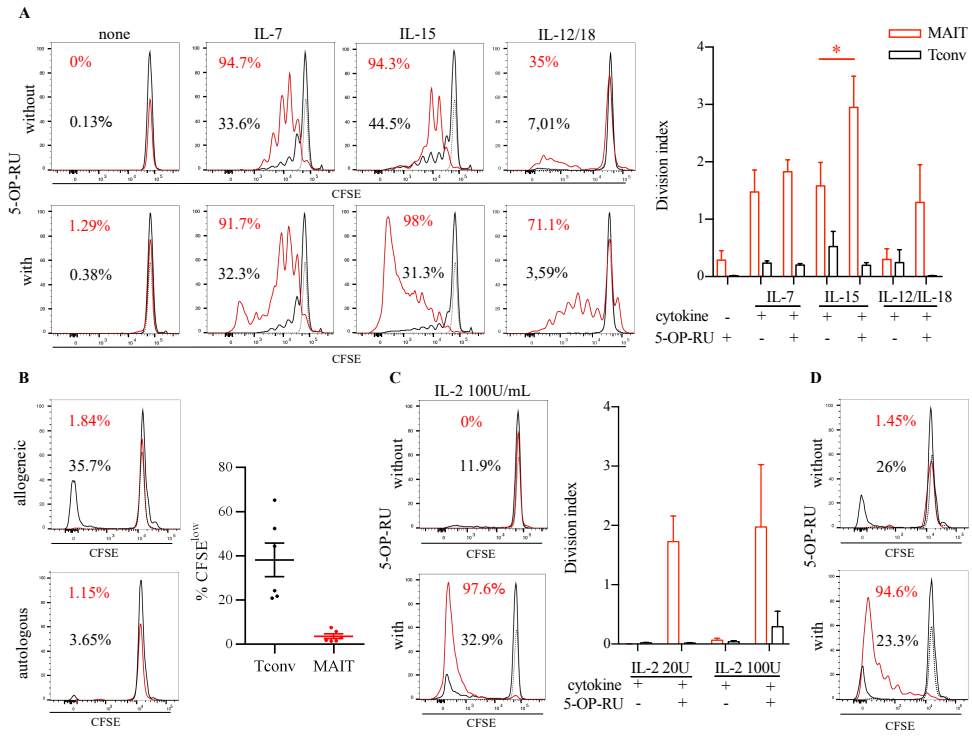

A
none
IL-7
IL-15
IL-12/18
without
with
5-OP-RU
0%
0.13%
94.7%
33.6%
94.3%
44.5%
35%
7,01%
1.29%
0.38%
91.7%
32.3%
98%
31.3%
71.1%
3,59%
CFSE
MAIT
Tconv
Division index
*
IL-7
IL-15
IL-12/IL-18
cytokine
5-OP-RU
B
allogeneic
autologous
1.84%
35.7%
1.15%
3.65%
% CFSE low
Tconv
MAIT
C
IL-2 100U/mL
0%
11.9%
97.6%
32.9%
IL-2 20U
IL-2 100U
D
1.45%
26%
94.6%
23.3%

Figure 5

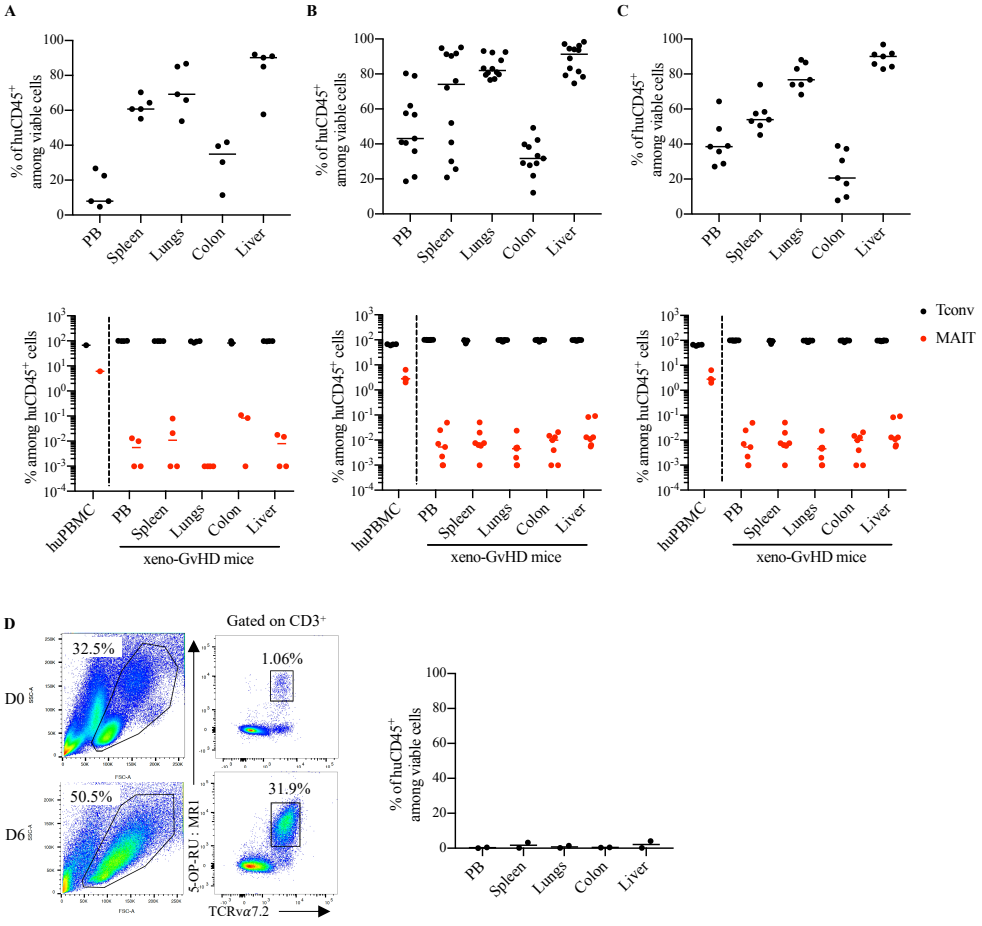
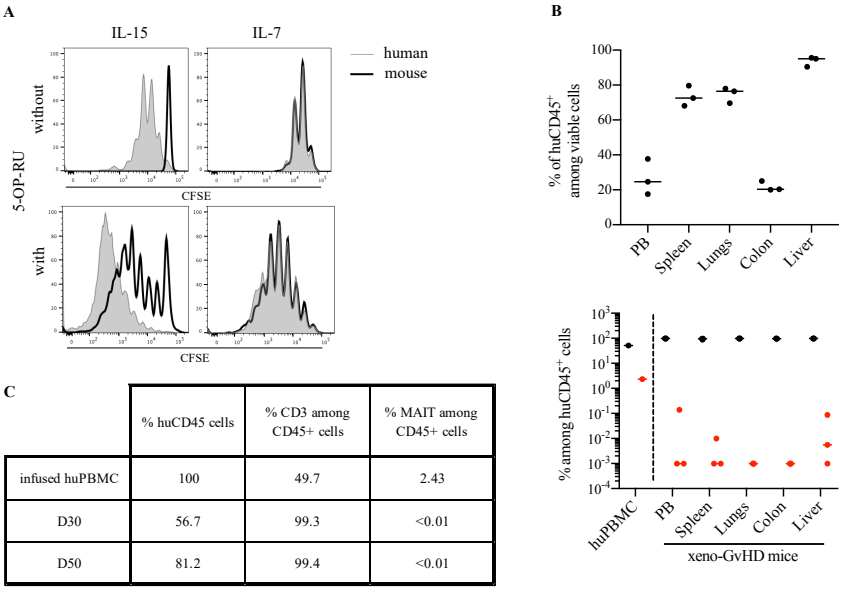

A
B
C
% of huCD45+ among viable cells
PB
Spleen
Lungs
Colon
Liver
% among huCD45+ cells
huPBMC
xeno-GvHD mice
Tconv
MAIT
D
Gated on CD3+
D0
D6
32.5%
50.5%
1.06%
31.9%
5-OP-RU : MR1
TCRvα7.2
FSC-A
SSC-A

Figure 6



C

	% huCD45 cells	% CD3 among CD45+ cells	% MAIT among CD45+ cells
infused huPBMC	100	49.7	2.43
D30	56.7	99.3	<0.01
D50	81.2	99.4	<0.01