



## The 65th ASH Annual Meeting Abstracts

## POSTER ABSTRACTS

## 722.ALLOGENEIC TRANSPLANTATION: ACUTE AND CHRONIC GVHD, IMMUNE RECONSTITUTION

**Lymphocyte Reconstitution after Naïve T Cell-Depleted Hematopoietic Cell Transplantation**

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**Background:** Allogeneic hematopoietic cell transplantation (HCT) is curative for many high-risk hematological malignancies. However, graft-versus-host disease (GVHD) causes morbidity, mortality, and reduced quality of life after HCT. Removal of all T cells from hematopoietic cell grafts (pan-T cell depletion; pan-TCD) significantly reduces GVHD but is associated with impaired immune reconstitution and increased non-relapse mortality due partly to opportunistic infections. We developed a novel strategy to remove CD45RA<sup>+</sup> naïve T cells (T<sub>N</sub>) from peripheral blood stem cell grafts (PBSC) that is associated with a low incidence of serious acute GVHD (aGVHD) and an exceptionally low incidence of chronic GVHD (cGVHD) (Bleakley, JCO 2022). Although CD45RA-depletion removes T<sub>N</sub> and other CD45RA-expressing effector T cell populations from PBSC along with most NK and B cells, T<sub>N</sub>-depletion (T<sub>ND</sub>) does not appear to increase infections or have a major negative impact on immune reconstitution. Understanding subtle differences in lymphocyte population dynamics after T<sub>ND</sub> compared to T cell-replete (TR) HCT will enable further graft engineering advances and may provide broader insights into human HCT immunobiology.

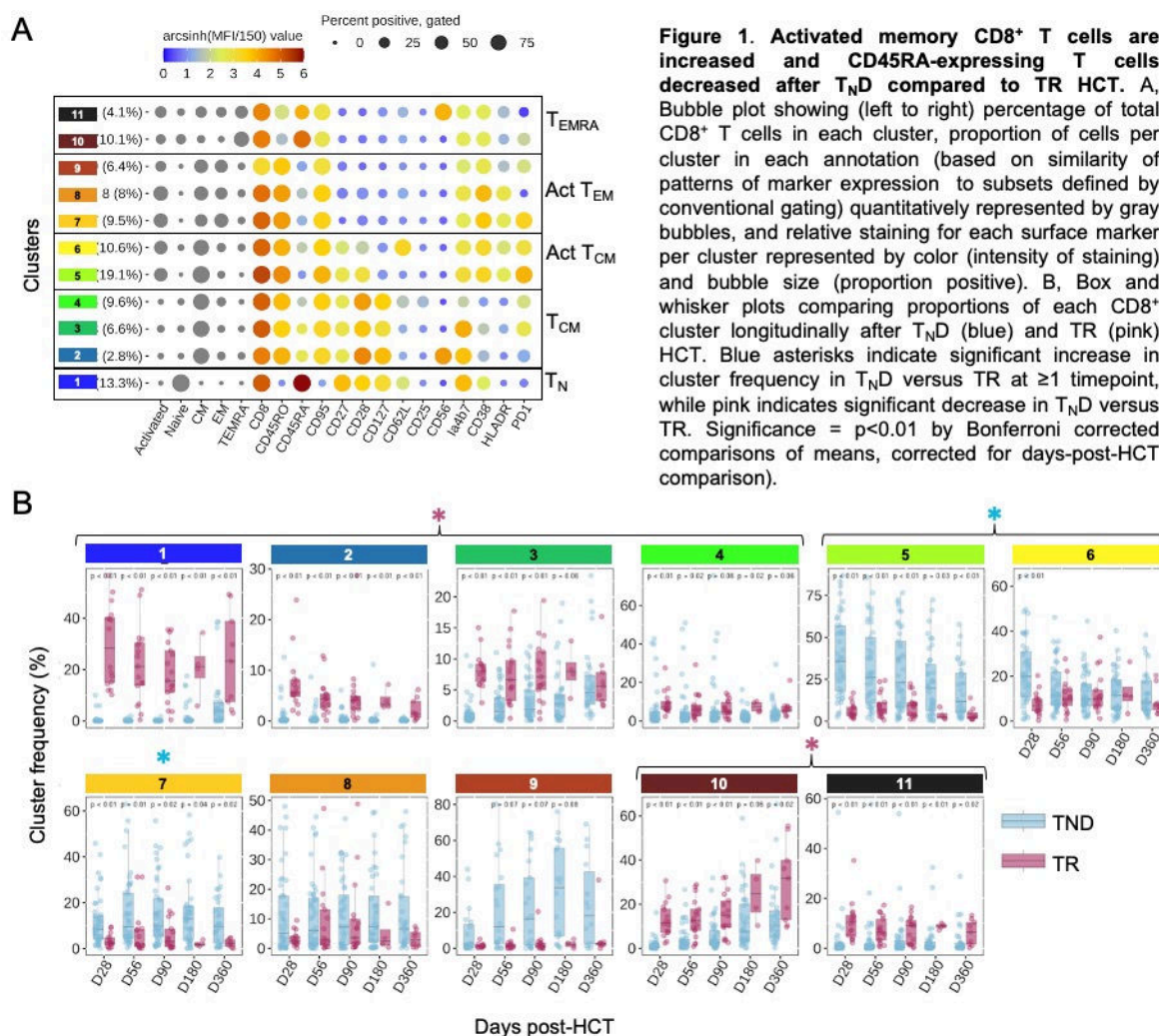
**Objective:** To evaluate lymphocyte reconstitution after T<sub>ND</sub> HCT.

**Methods and Results:** We compared blood lymphocyte populations in T<sub>ND</sub> HCT recipients (n=47) to those in TR HCT controls (n=19) at days 28, 56, 90, 180, and 360 post-HCT using multiparametric flow cytometry. To complement standard Boolean analysis, we employed a metaclustering approach to simultaneously evaluate ~20 cell surface markers for each class of lymphocytes (CD8<sup>+</sup> and CD4<sup>+</sup> T cells, NK cells, and B cells) over scores of batches of recipient and control samples. Absolute numbers of total CD8<sup>+</sup> T cells were similar in T<sub>ND</sub> compared to TR at all timepoints, whereas total CD4<sup>+</sup> T cell numbers were consistently lower in T<sub>ND</sub> until 1 year post-HCT. Using conventional gating and high-dimensional clustering analyses, we identified several key differences in T cells after T<sub>ND</sub>. CD45RA-expressing populations (T<sub>N</sub> and effector memory re-expressing CD45RA [T<sub>EMRA</sub>]) in CD8<sup>+</sup> and CD4<sup>+</sup> T cells were rare in CD45RA-depleted PBSC and remained decreased in T<sub>ND</sub> HCT recipients until around 1 year (CD8<sup>+</sup>, Fig. 1). Activated CD8<sup>+</sup> and CD4<sup>+</sup> central and effector memory (T<sub>CM</sub>, T<sub>EM</sub>) T cells were increased proportionally and in absolute numbers in T<sub>ND</sub> early post-HCT (CD8<sup>+</sup>, Fig. 1). Absolute numbers of regulatory T cells (Treg) were decreased in T<sub>ND</sub>; consequently, ratios of activated CD8<sup>+</sup> and CD4<sup>+</sup> to Treg were both markedly increased early post-HCT, despite a similar incidence of aGVHD in T<sub>ND</sub> and TR recipients. Although most NK cell populations were removed from CD45RA-depleted PBSC, absolute numbers of NK cells in all compartments, including both activated and inhibited, were increased in T<sub>ND</sub> recipients early post-HCT compared to TR. Like NK cells, most B cells except for plasmablasts and plasma cells were removed by CD45RA-depletion of PBSC. Immature, naïve, and memory B cells were reduced very early post-T<sub>ND</sub> HCT, but substantial B cell recovery occurred within the first 2-3 months.

**Conclusions:** We present the first detailed description of lymphocyte reconstitution after T<sub>ND</sub> HCT. Despite the major effect CD45RA-depletion has on graft composition-removing all T<sub>N</sub> and T<sub>EMRA</sub> from the T cell compartment and most NK and B cells-NK and B cell recovery occurred early, and by 1 year post-HCT there was little difference in lymphocyte populations between T<sub>ND</sub> and TR. This is consistent with hematopoietic progenitors, not mature lymphocytes, as the source of B and NK cell reconstitution. Among T cells, the most striking findings were an increase in activated T<sub>CM</sub> and T<sub>EM</sub> CD4<sup>+</sup> and CD8<sup>+</sup> T cells early after T<sub>ND</sub> HCT, and increased ratios of activated T cells to Treg. Although elevated blood T effector/Treg

ratios have been associated with cGVHD, cGVHD was uncommon and steroid-responsive after T<sub>N</sub>D HCT. Whether increased activated T cells reflect a reduction in Treg-mediated suppression after HCT, bystander T cell activation, activation by antigen (e.g., alloantigen, latent herpes viruses, microbiota), or multiple factors are questions we are now investigating. We are also exploring associations between lymphocyte populations early post-T<sub>N</sub>D HCT and clinical outcomes; these could serve as biomarkers to facilitate early intervention to prevent or treat GVHD, relapse, or infection.

**Disclosures Shlomchik:** BlueSphere Bio: Current Employment, Current holder of stock options in a privately-held company, Membership on an entity's Board of Directors or advisory committees, Patents & Royalties, Research Funding; Orca Bio: Consultancy, Current holder of stock options in a privately-held company. **Newell:** Immunoscape: Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; Neogene Therapeutics: Current equity holder in private company, Membership on an entity's Board of Directors or advisory committees; Nanostring Technologies: Membership on an entity's Board of Directors or advisory committees. **Bleakley:** Orca Bio: Consultancy; Miltenyi Biotec: Research Funding.



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