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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 $^{+}$ naïve T cells (T_N) 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_N and other CD45RA-expressing effector T cell populations from PBSC along with most NK and B cells, T_N-depletion (T_ND) does not appear to increase infections or have a major negative impact on immune reconstitution. Understanding subtle differences in lymphocyte population dynamics after T_ND 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_ND HCT.
Methods and Results: We compared blood lymphocyte populations in T_ND 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 $^{+}$ and CD4 $^{+}$ T cells, NK cells, and B cells) over scores of batches of recipient and control samples. Absolute numbers of total CD8 $^{+}$ T cells were similar in T_ND compared to TR at all timepoints, whereas total CD4 $^{+}$ T cell numbers were consistently lower in T_ND until 1 year post-HCT. Using conventional gating and high-dimensional clustering analyses, we identified several key differences in T cells after T_ND. CD45RA-expressing populations (T_N and effector memory re-expressing CD45RA [T_{EMRA}]) in CD8 $^{+}$ and CD4 $^{+}$ T cells were rare in CD45RA-depleted PBSC and remained decreased in T_ND HCT recipients until around 1 year (CD8 $^{+}$, Fig. 1). Activated CD8 $^{+}$ and CD4 $^{+}$ central and effector memory (T_{CM}, T_{EM}) T cells were increased proportionally and in absolute numbers in T_ND early post-HCT (CD8 $^{+}$, Fig. 1). Absolute numbers of regulatory T cells (Treg) were decreased in T_ND; consequently, ratios of activated CD8 $^{+}$ and CD4 $^{+}$ to Treg were both markedly increased early post-HCT, despite a similar incidence of aGVHD in T_ND 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_ND 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_ND 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_ND HCT. Despite the major effect CD45RA-depletion has on graft composition-removing all T_N and T_{EMRA} 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_ND 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_{CM} and T_{EM} CD4 $^{+}$ and CD8 $^{+}$ T cells early after T_ND 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 $_N$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 $_N$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 Therapuetics:* 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.

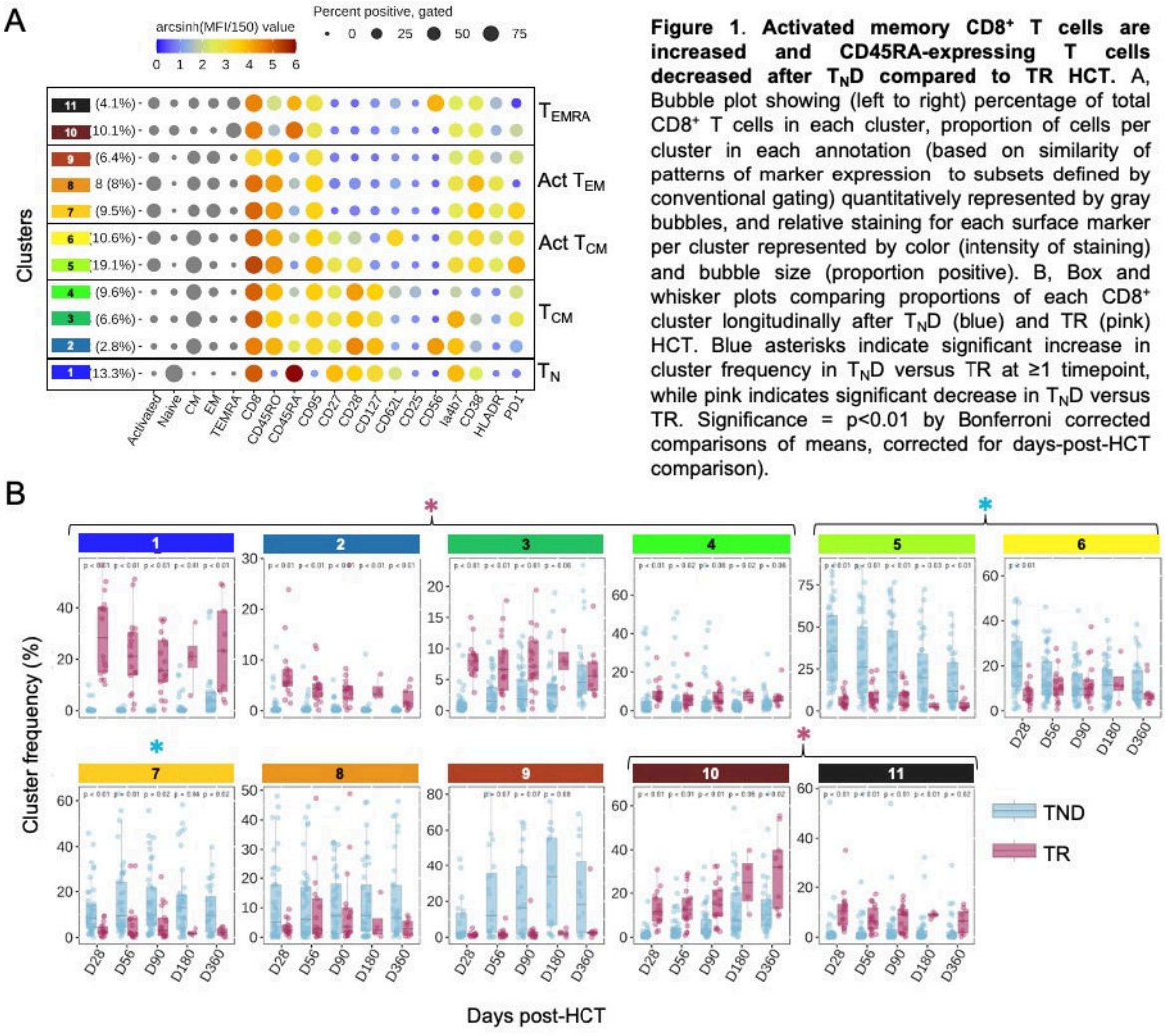

Figure 1. Activated memory CD8⁺ T cells are increased and CD45RA-expressing T cells decreased after T$_N$D compared to TR HCT. A, Bubble plot showing (left to right) percentage of total CD8⁺ T cells in each cluster, proportion of cells per cluster in each annotation (based on similarity of patterns of marker expression to subsets defined by conventional gating) quantitatively represented by gray bubbles, and relative staining for each surface marker per cluster represented by color (intensity of staining) and bubble size (proportion positive). B, Box and whisker plots comparing proportions of each CD8⁺ cluster longitudinally after T$_N$D (blue) and TR (pink) HCT. Blue asterisks indicate significant increase in cluster frequency in T$_N$D versus TR at ≥1 timepoint, while pink indicates significant decrease in T$_N$D versus TR. Significance = p<0.01 by Bonferroni corrected comparisons of means, corrected for days-post-HCT comparison).

Figure 1

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