provide the niche space for B and T cell diversification, these findings suggest that GVHD, even in sub-clinical stages, may impair diversification of the immune repertoire. We evaluated post-HCT B cell immunoglobulin heavy chain (IGH) gene diversity in 42 patients with chronic lymphocytic leukemia who received unmanipulated mobilized peripheral blood grafts following total lymphoid irradiation and anti-thymocyte globulin (TLI/ATG). Twenty-one patients also received 4 weekly doses of Rituximab beginning 2 months post-HCT. Post-HCT peripheral blood samples were prospectively archived at pre-scheduled intervals. The IGH repertoire was quantified in over 400 samples using the LymphoSIGHT(TM) method, which employs consensus IGH V and J segment primers to universally amplify rearranged IGH genes, followed by massively parallel high-throughput sequencing (IGH-HTS) and bioinformatic analysis. Donor graft material was analyzed in the same manner. A median 1.8x10e6 (2.6x10e4 – 2.7x10e6) leukocyte genomes per sample with a median 6.4x10e5 (12 – 7.6x10e5) IGH molecules were analyzed. Compared to healthy donors, patients receiving only TLI/ATG exhibited significantly decreased diversity at days +30 and +90 (p<0.001) (Figure 1A), whereas IGH diversity remained significantly decreased at days +30, +90, +180, +270 (each p <.001) and +365 (p<0.05) in those who received post-HCT Rituximab (Figure 1B). Clonotype analysis confirmed significant (p<0.009) but incomplete depletion of adoptively transferred IGH clones in those receiving post-HCT Rituximab (Figure 1C). The incidences of relapse (38 vs. 52%) and new-onset GVHD (28 vs. 33%) were similar in the two groups. Amongst patients at risk for new onset GVHD (after censoring for relapse and Rituximab treatment), IGH diversity was significantly decreased at day +30 (54 +/- 13 vs. 18818 +/- 11576 unique IGH clones; P = .03) and day +90 (2693 +/- 2657 vs. 40316 +/- 16480 unique IGH clones; P = .02) in those who subsequently developed GVHD in comparison to those who did not (Figure 1D). IGH-HTS analysis of the adoptively transferred and reconstituting B cell repertoires after allo-HCT may provide a method for predicting new onset GVHD and for identifying pathogenically involved adoptively transferred B cell clones.

Figure 1. Box and whisker plots demonstrating post-HCT IGH clonotypic diversity in the peripheral blood of patients allografted after conditioning with TLI/ATG (A) or TLI/ATG + post-HCT Rituximab (B). Mean (+s.e.m.) number of adoptively transferred IGH clones in patients conditioned with TLI/ATG (solid line) or TLI/ATG Rituximab (dotted line) (C). IGH clonotype diversity at days +30 and +90 post-HCT in patients who did (GVHD+; red) or did not (No GVHD; black) subsequently develop GVHD after censoring for relapse, Rituximab treatment, and prior GVHD (D). Numbers on graph indicate evaluable patients at each time point.

**Marketers of Angiogenesis and Neovascularization Correlate with Functional Recovery of Thymic Epithelial Cells and T Cell Immune Reconstitution After Cord Blood Transplantation in Adults**

Ioannis Politikos 1, Haesook Kim 2, Julia Brown 1, Sean M. McDonough 3, Legion Li 1, Corey Cutler 4, Robert J. Soiffer 5, Joseph H. Antin 1, Karen Ballen 6, Jerome Ritz 1, Vassiliki A. Boussiotis 1, 1 Division of Hematology-Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA; 2 Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA; 3 Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA; 4 Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA; 5 Harvard Medical School, Dana-Farber Cancer Institute, Boston, MA; 6 Hematology/Oncology, Massachusetts General Hospital, Boston, MA

Umbilical cord blood transplantation (UCBT) is increasingly used as an alternative source for allogeneic hematopoietic stem cell transplantation. A significant component of immune reconstitution after UCBT involves thymic regeneration, which can be measured by T-cell receptor rearrangement excision circles (TRECs). TREC levels are a strong predictive factor for overall survival in adult recipients of UCBT. Injury of the thymic microenvironment, particularly of thymic epithelial cells (TEC), during H SCT compromises thymopoiesis. Human UCB is enriched in endothelial precursors that can sustain thymopoiesis in immunodeficient mice transplanted with human thymic grafts, where they engraft and promote neovascularization and wound healing. We examined markers of angiogenesis and neovascularization ANG1 and VEGF associated with TEC function, thymic regeneration and T cell reconstitution in patients after double reduced intensity UCBT. 27 evaluable patients with a median age of 50 years with hematopoietic malignancies were treated with (Flu/Mel/rATG) conditioning followed by double UCBT; GVHD prophylaxis was tacrolimus and sirolimus. At various time points Which ones after UCBT, serum levels of IL-7 and SCF that are produced by TEC displayed a strong correlation (p<0.005) with ANG1 and VEGF indicating that functional recovery of TEC is associated with angiogenesis and neovascularization. In contrast, serum levels of IL-7 and SCF displayed a strong inverse correlation (p<0.001) with TREC, CD4+, CD8+ and Treg numbers suggesting that uptake of these cytokines by cognate IL-7R and Kit receptor on immature T cell progenitors resulted in their differentiation and expansion. IL-7 and SCF also displayed a strong inverse correlation (p<0.001) with the ability of T cells to differentiate into pathogen-specific effectors as determined by CMV-specific IFN-γ ELISpot. Conversely, VEGF and ANG1 levels positively correlated (p<0.001) with CD4+, CD4+CD25+ and CD4+CD45RA+ numbers. Our results are consistent with a model in which ANG1 and VEGF support TEC recovery leading to production of SCF and IL-7 by TEC and uptake of these cytokines by T cell progenitors resulting in their differentiation and expansion. These sequential steps in the process of thymic regeneration might represent novel therapeutic targets for improvement of immune reconstitution after UCBT.