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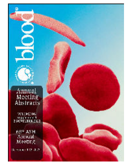

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POSTER ABSTRACTS

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

Deciphering Antigen Specificity of Protective Regulatory T Cell Clones in Patients after Allogeneic Stem Cell Transplantation

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Introduction: Clonal expansion of donor regulatory T cells (Tregs) in recipients of allogeneic stem cell transplantations (allo-SCT) is associated with the absence of acute graft-versus-host disease (aGVHD). Therefore, we hypothesize that antigen-specific donor Tregs prevent aGVHD. In this project, we aim to identify the cognate antigen of expanded Treg clones in patients after allo-SCT.
Methods: We recruited 177 consecutive patients undergoing allo-SCT for acute myeloid leukemia without severe infection and CMV-reactivation for this study. Healthy adults matching patients' sex and age served as the control group. We sorted $CD3^{+}CD4^{+}CD25^{hi}CD127^{low}$ Tregs from peripheral blood mononuclear cell samples collected early after transplantation (21 to 36 days, median 27 days), and we performed single-cell RNA sequencing (scRNAseq) to profile Treg transcriptome including both TCR alpha and beta chain. To determine the antigen specificity of a given TCR, we adapted a cytokine-capturing antigen-screening system (Lee and Meyerson, Sci Immunol, 2021). We built the system by 1) expressing designated TCR$\alpha\beta$ chains in Jurkat cells, and 2) expressing cell-surface interleukin-2 capture mAb, corresponding MHC-II molecules, and CD74-fused epitope libraries in HEK293T cells serving as antigen-presenting cells (APCs). By co-culturing both modified cell lines, we can perform high-throughput screening of pooled candidates. To obtain epitope-encoding minigenes, we either used RNA fragmentation to generate candidates randomly or customized oligonucleotide synthesis to encode the tiled human proteome. To scale down the diversity of the synthetic library, we utilized NetMHCIIpan (DTU Health Tech) for MHC-II binding affinity prediction. By sequencing the CD74-fused minigene after screening out APCs with the highest cytokine intensity on the cell surface, we can decode the peptide sequence recognized by the given TCR. We have validated the cytokine-capturing antigen-screening system using a CMV-specific TCR and its cognate antigen.
Results: In total, we analyzed six patients with and six patients without aGVHD. ScRNAseq showed that the $FOXP3^{+}$ Treg population in patients without aGVHD shared more transcriptional similarities with healthy control as compared to patients with aGVHD. Due to lymphocytopenia, Tregs are extremely low in numbers early after allo-SCT, but clustering analysis revealed highly clonal expanded populations of Tregs with various degrees of activation. Notably, one patient without aGVHD was found to have a distinct Treg-dominant CD4 population, and a markedly abundant Treg clone (>15% of the entire Treg repertoire) was found to be parts of both $FOXP3^{+}$ and $FOXP3^{-}$ clusters. We then transduced the most abundant TCR into JM-T, the TCR-deficient Jurkat cell. We used an RNA-fragmented randomized library for the first screening, in which we identified one shared epitope in three independent screenings of high frequency. We then moved on to perform screenings for synthesized candidate pools predicted by NetMHCIIpan, representing human proteome and common post-allo-SCT infections. Currently, we are analyzing sequencing reads from parallel screenings and attempting direct expansion of $IL\text{-}2^{+}$ sort-outs.
Conclusion: By expanding our established screening system, we aim to elucidate the antigens of multiple clonal expanded TCR candidates from the same patient. Identification of specific antigens leading to clonal Treg expansion and protection from GVHD holds the potential for the development of targeted cell therapies.

Disclosures Schultze-Florey: *Neovii:* Other: Travel support. **Koenecke:** *Miltenyi Biotec:* Consultancy; *Glaxo Smith Kline:* Consultancy; *Amgen:* Consultancy; *Novartis:* Consultancy, Speakers Bureau; *Kite/Gilead:* Consultancy; *Roche:* Consultancy,

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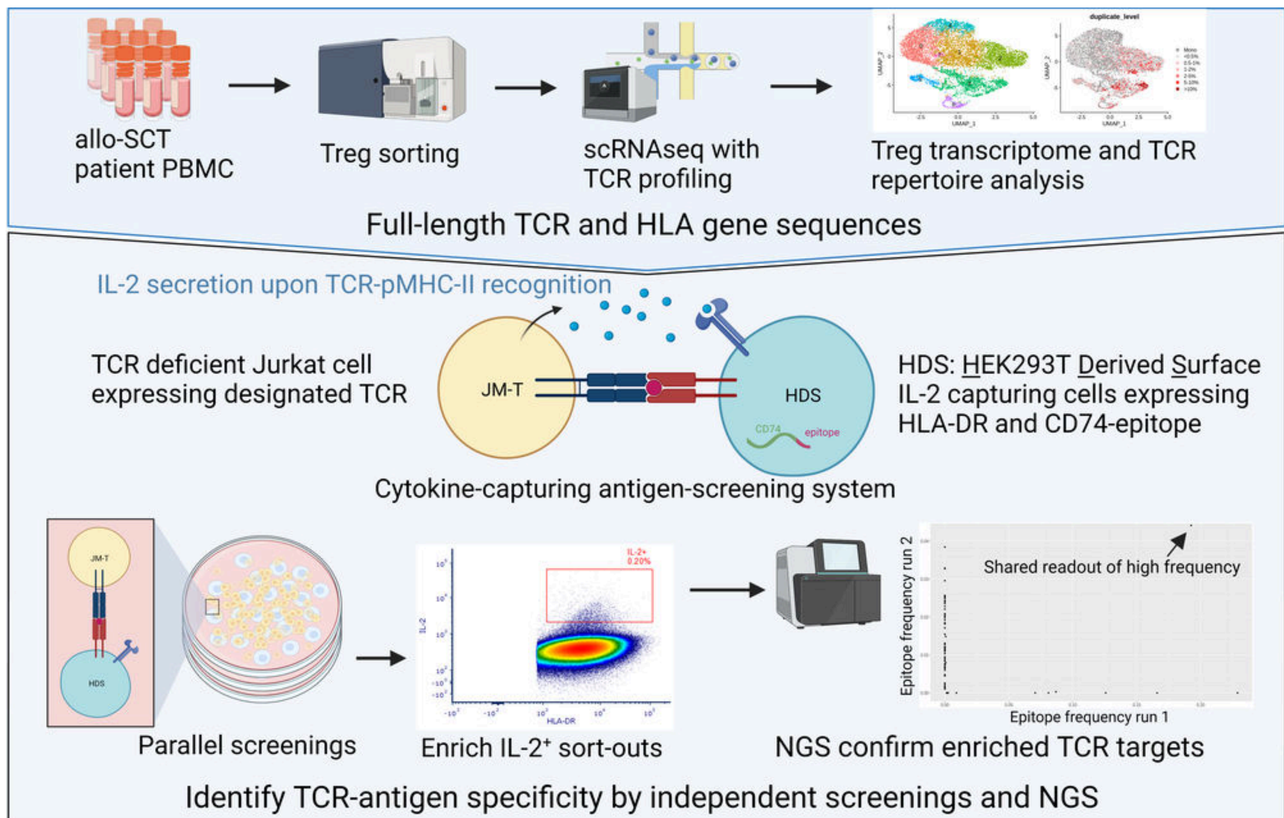

Figure 1

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