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1029 T CELL RECEPTOR DIVERSITY ANALYSIS OF *IN VITRO*-EXPANDED T CELLS AGAINST IDO1 AND PD-L1-DERIVED PEPTIDES

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Background Recognition of cognate peptides results in expansion of antigen-specific T cells. Since humans have ~10^{15} TCRs, identification of responding TCRs has remained difficult. The variable (V) region gene segment, which comprises one of the components of the CDR3 loop of the TCRβ chain, determines TCR uniqueness. IO Biotech is pioneering development of a novel immunomodulatory vaccine, with our lead candidate IO102-IO103, that is designed to activate and expand T cells specific for IDO1 and PD-L1, respectively. In this study we explore TCR diversity analysis as a potential platform to characterize vaccine-expanded T cells.

Methods Human PBMCs were cultured with IO102 (IDO1 peptide) or IO103 (PD-L1 peptide). Samples were collected 6 days following culture, and 24 hours following restimulation with peptide at day 8. Control samples included PBMCs prior to culture, samples cultured without peptide, and restimulation without peptide. ELISPOT assay was conducted in tandem. Following extraction of RNA, samples were analyzed on the Nanostring nCounter Analysis System using the TCR diversity panel kit. Using the nSolver Analysis Software, data were quality controlled and normalized prior to analysis of TCR variable regions. Rosalind Software was used to calculate TCR Score to assess diversity. Changes detected in T cell phenotype detected by NanoString were validated using flow cytometry.

Results Exposure to and restimulation with IO102 or IO103 peptides in short-term *in vitro* cultures resulted in expansion of a specific and narrow set of TCR variable regions. Expansion of TCRV genes were mostly represented by TCRAV and TCRBV, with limited expansion of TCRGV and TCRDV. Six-day culture with peptide resulted in expansion of certain TCRVs that were distinctly and further expanded following restimulation with the same peptide. Uniqueness of TCRV usage was determined between peptides and donors. Expansion of peptide-responsive T cells further impacted TCR clonality and diversity. Expanded TCRV regions were correlated to T cell phenotypes and associated with markers of activation and memory.

Conclusions Our results demonstrate that exposure to IO102 or IO103 in short-term *in vitro* cultures results in a discernable impact to the TCR repertoire. Further, we were able to identify TCRV segments that are responsive to either IO102 or IO103 peptides in a donor-specific manner. These studies will allow us to further characterize peptide-specific T cell responses that arise following vaccination. We are working to pair TCRV expansion with functional cytokine assays and spatial transcriptomics in tumor microenvironments for personalizing monitoring of peptide vaccine responsiveness.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.1029