T Cell Receptor Diversity Analysis of *in vitro*-Expanded T Cells Against IDO1 and PD-L1-Derived Peptides

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Abstract #1029

Abstract:

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 against IDO1+ and PD-L1+ cells, respectively. In this study we explore TCR diversity analysis as a potential platform to characterize vaccine-expanded T cells.

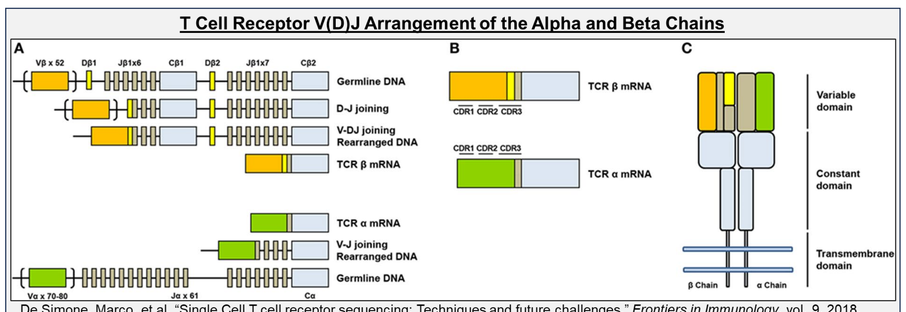
Methods:
Human PBMCs from healthy subjects were cultured with IO102 (IDO1 peptide) or IO103 (PD-L1 peptide). Samples were collected 6 days following culture, and 24 hours following restimulation with peptide at day 8. Control samples included PBMCs prior to culture, samples cultured without peptide, 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 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 TRAV and TRBV, with limited expansion of TRGV and TRDV. 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.

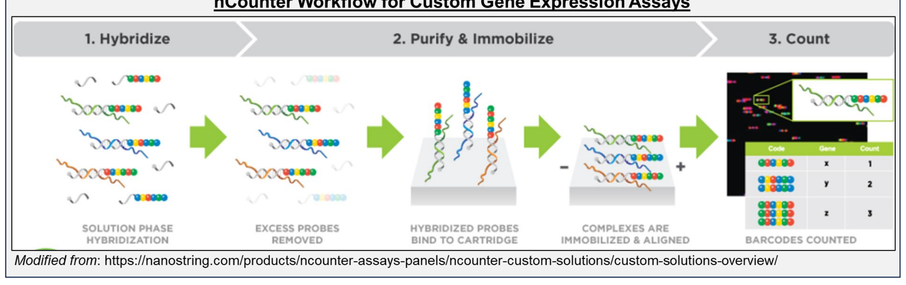
Background:

T Cell Receptor V(D)J Arrangement of the Alpha and Beta Chains



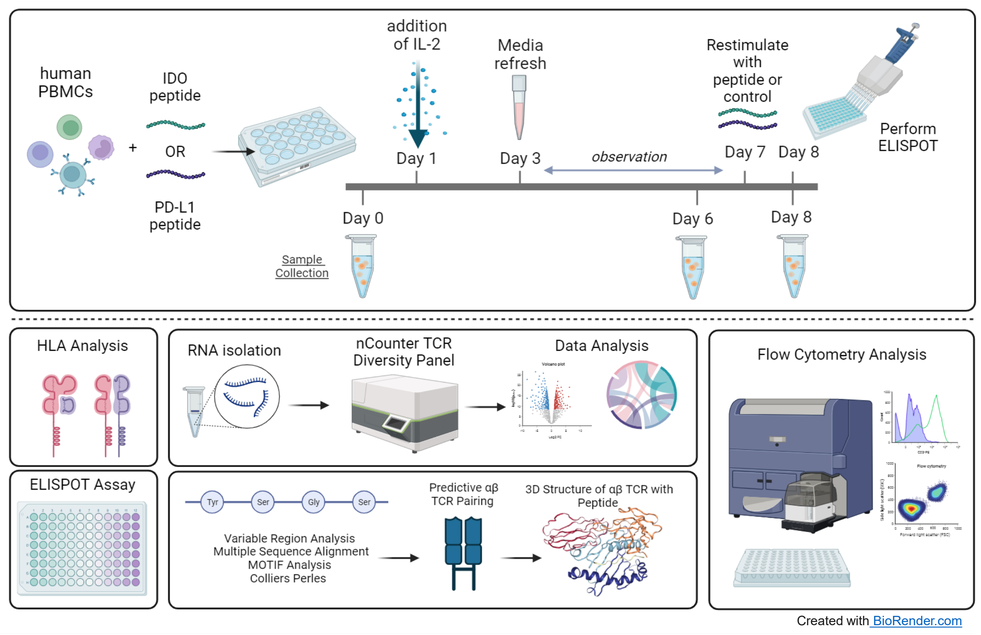
De Simone, Marco, et al. "Single Cell T cell receptor sequencing: Techniques and future challenges." *Frontiers in Immunology*, vol. 9, 2018, https://doi.org/10.3389/fimmu.2018.01638.

nCounter Workflow for Custom Gene Expression Assays



Modified from: https://nanostring.com/products/ncounter-assays-panels/ncounter-custom-solutions/custom-solutions-overview/

Methods:



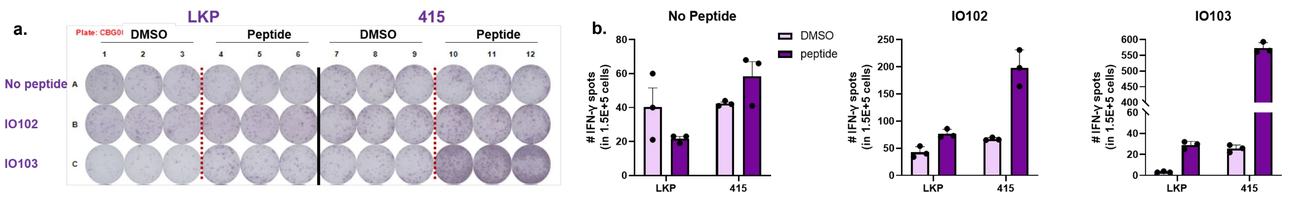
Created with BioRender.com

Data:

Human Leukocyte Antigen (HLA) Genes for Study PBMC Donors

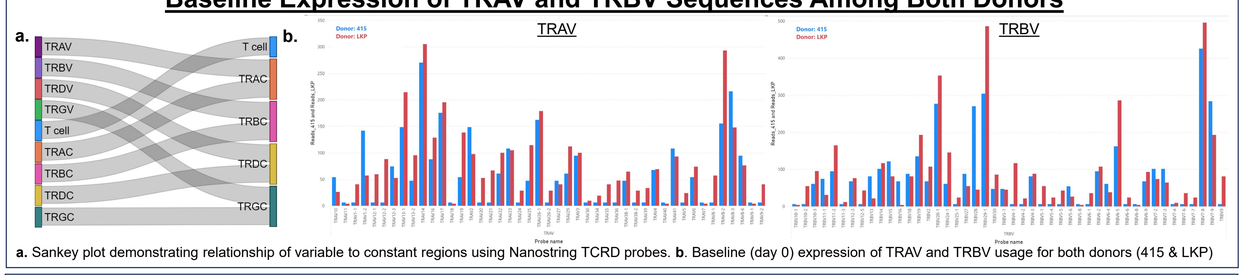
	HLA-A Allele 1	HLA-A Allele 2	HLA-B Allele 1	HLA-B Allele 2	HLA-C Allele 1	HLA-C Allele 2	HLA-DPA1 Allele 1	HLA-DPA1 Allele 2	HLA-DPB1 Allele 1	HLA-DPB1 Allele 2	HLA-DQA1 Allele 1	HLA-DQA1 Allele 2	HLA-DQB1 Allele 1	HLA-DQB1 Allele 2	HLA-DRB1 Allele 1	HLA-DRB1 Allele 2	HLA-DRB345 Allele 1	HLA-DRB345 Allele 2
LKP	A*68:02:01	A*74:01:01	B*15:03:01	B*53:01:01	C*02:10:01	C*04:01:01	DPA1*02:01:08	DPA1*02:01:08	DPB1*01:01:01	DPB1*01:01:01	DQA1*01:02:01	DQA1*05:05:01	DQB1*03:01:01	DQB1*05:01:01	DRB1*13:02:01	DRB1*13:03:01	DRB3*01:01:02	DRB3*03:01:01
415	A*11:01:01	A*24:02:01	B*40:01:02	B*40:01:02	C*03:04:01	C*03:04:01	DPA1*01:03:01	DPA1*02:06	DPB1*03:01:01	DPB1*05:01:01	DQA1*01:02:01	DQA1*04:01:01	DQB1*04:02:01	DQB1*06:04:01	DRB1*08:01:01	DRB1*13:02:01	DRB3*03:01:01	DRB345*Not_Present

Results of ELISPOT Assay Following Culture with IO102 or IO103 Peptide



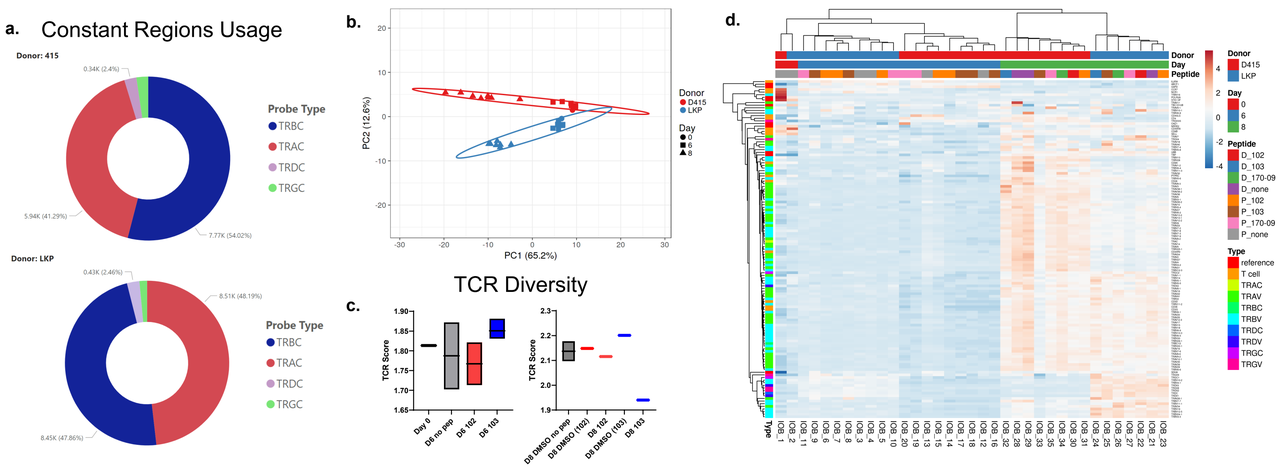
Stimulation and culture conditions along with scientific procedure for ELISPOT assay are detailed under "Methods." **a.** IFN-γ producing spots generated by PBMCs from 2 donors (LKP or 415) cultured with no peptide, IO102, or IO103 at day 8, 24 hours following restimulation **b**. Counts of IFN-γ producing spots corresponding to culture conditions, peptide, and donor

Baseline Expression of TRAV and TRBV Sequences Among Both Donors



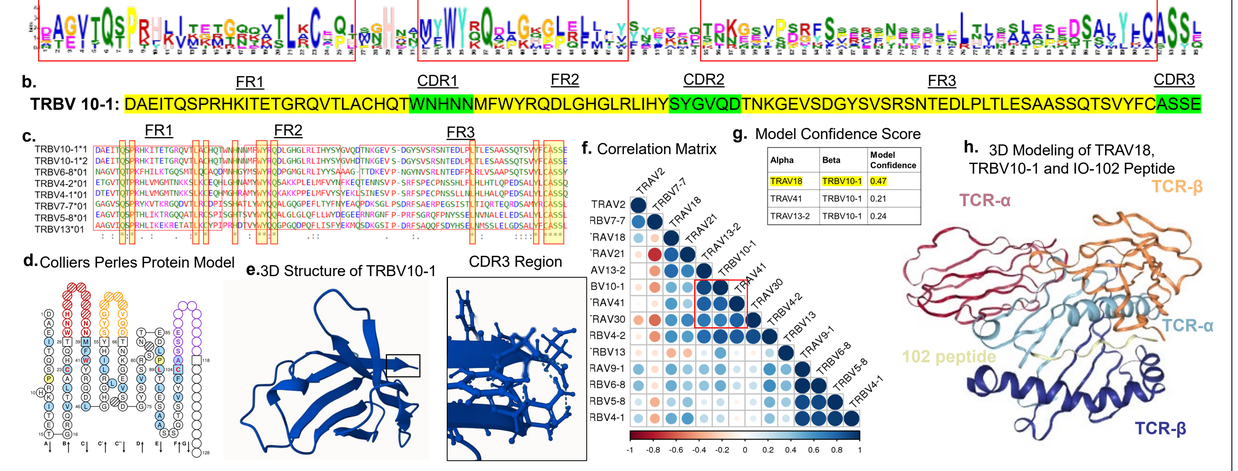
a. Sankey plot demonstrating relationship of variable to constant regions using Nanostring TCRD probes. **b**. Baseline (day 0) expression of TRAV and TRBV usage for both donors (415 & LKP)

TCR Constant Region Usage, TCR Diversity, and TCR Variable Gene Expression Among LKP and 415 Stimulated with IO102 or IO103



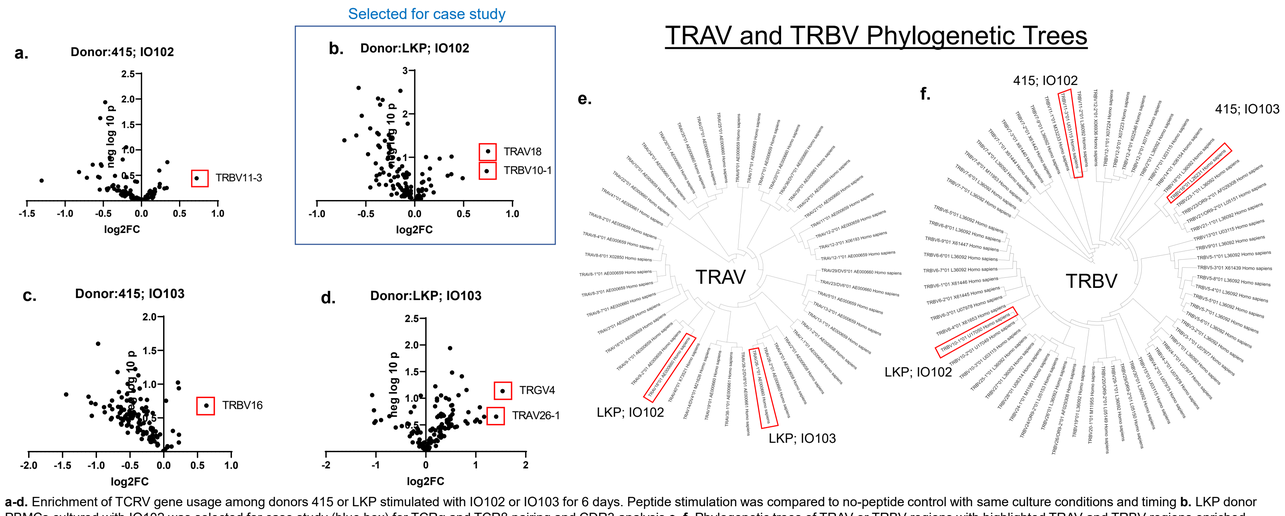
a. Baseline (day 0) constant region usage for both donors (415 and LKP) **b.** Timepoints for sample collection & PBMC culture conditions are detailed under "Methods." PCA plot of all samples for both donors **c.** TCR Score demonstrating TCR Diversity of TCRV usage at day 6 (left) and day 8 (right). **d**. Unsupervised heatmap of T cell phenotype, constant and variable gene usage across time

Case Study of Donor LKP + IO102 Peptide for TCRα and TCR-β Pairing



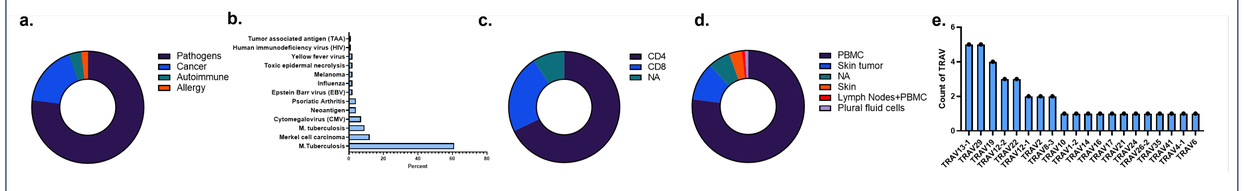
a. MAST (Motif Alignment and Search Tool) and **c**. Multiple sequence alignment (MSA) for top 7 TCRBV regions enriched among LKP donor stimulated with IO102. Framework region (FR) and complementarity determining region (CDR) are annotated and conserved regions are highlighted **b**. Variable region sequence and annotation for TRBV 10-1 **d.** Colliers Perles Protein Model of TRBV 10-1 **e.** 3D structure of TRBV10-1 and detail of the CDR3 region **f.** Correlation matrix of the top 6 TRAV and top 6 TRBV regions enriched among LKP donor stimulated with IO102. Highlight (red box) of predictive pairing of TRBV 10-1 with correlated TRAV regions **g**. Model Confidence Score of top TRAV binding to TRBV 10-1. **h.** 3D structure of TRAV18, TRBV10-1 and IO-102 peptide accounting for donor HLA

Differential Expression of TCRV Regions 6 Days Following IO102 or IO103 Stimulation Among 2 Donors



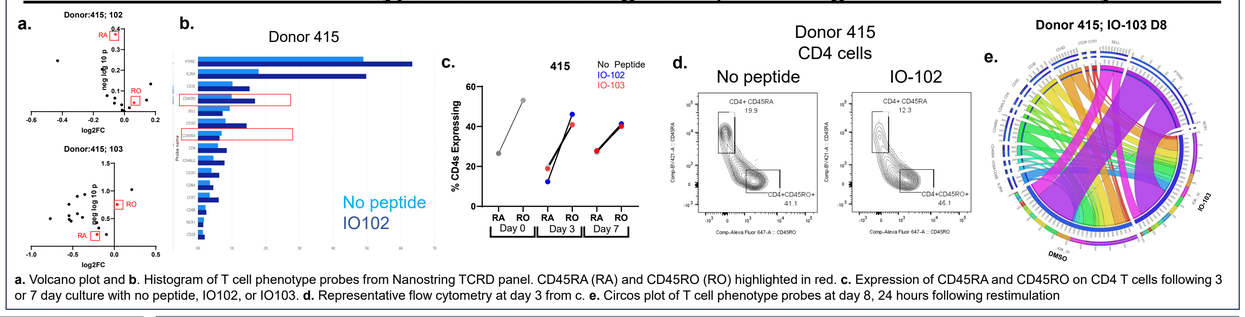
a-d. Enrichment of TCRV gene usage among donors 415 or LKP stimulated with IO102 or IO103 for 6 days. Peptide stimulation was compared to no-peptide control with same culture conditions and timing **b.** LKP donor PBMCs cultured with IO102 was selected for case study (blue box) for TCRα and TCRβ pairing and CDR3 analysis **e, f.** Phylogenetic trees of TRAV or TRBV regions with highlighted TRAV and TRBV regions enriched following either IO102 or IO103 peptide stimulation between donors for assessment of relatedness

Profile of Public Human TCR Clones Expressing TRBV10-1:



Query of "McPAS-TCR: A manually curated catalogue of pathology associated T-cell receptor sequences" for known information about human T cells expressing **TRBV 10-1**. **a.** Association with pathology **b.** Detail of pathologies from a. **c.** Description of associated T cell type **d.** Tissue location of T cells **e.** TRAVs with known pairing

Assessment of T Cell Phenotype from Nanostring TCRD, including Validation of Memory Markers



a. Volcano plot and **b**. Histogram of T cell phenotype probes from Nanostring TCRD panel. CD45RA (RA) and CD45RO (RO) highlighted in red. **c**. Expression of CD45RA and CD45RO on CD4 T cells following 3 or 7 day culture with no peptide, IO102, or IO103. **d.** Representative flow cytometry at day 3 from c. **e.** Circos plot of T cell phenotype probes at day 8, 24 hours following restimulation

Conclusions:

- The nCounter TCR Diversity panel from NanoString provides wide coverage of T cell variable gene usage in healthy human PBMCs
- Among the 2 donors analyzed, exposure to IO102 or IO103 in short-term *in vitro* cultures resulted in discernable changes to the TCR repertoire, as per assessment of variable gene region usage and TCR diversity
- Using control cultures, we are able to identify TCRV segments that are responsive to either IO102 or IO103 peptides in a donor-specific manner
- Relatedness of TRAV and TRBV regions between peptides and donors can be assessed at a phylogenetic level
- Top TRBVs, including TRBV10-1, responding to IO102 peptide have regions of epitopes within their protein sequence that are conserved
- Correlation matrixes of top TRAV and TRBV usage along with enrichment in TCRV gene usage provides scenarios for potential TCRα and TCRβ pairing
- Using 3D modeling, we can predict which TCRα chain potentially pairs with TRBV10-1 given IO102 peptide sequence and donor HLA
- TRBV10-1 appears in public profiles of T cells and its association with pathogenicity, T cell phenotype, tissue, and potential TCRα pairing is noted
- Exposure to antigen shifts T cell phenotype slightly towards a memory phenotype
- Future studies: We are further working on validation of TCRV gene usage among IO102 or IO103 responsive T cells using enriched cultures, qPCR primer probes specific for variable regions, and antibodies targeting specific TRAV and TRBV regions

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