POOLED T CELL RECEPTOR SCREENING (POTS) PROVIDES UNBIASED, HIGH-THROUGHPUT METHOD FOR TCR DISCOVERY

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Background T Cell Receptor (TCR)-T cell therapies have shown some promising results in cancer clinical trials, however the efficacy of treatment remains suboptimal. Outcomes could potentially be improved by utilizing highly functional TCRs for future trials. Current TCR discovery methods are relatively low throughput and rely on synthesis and screening of individual TCRs based on tetramer binding and peptide specificity, which is costly and labor intensive. We have developed and validated a pooled approach relying on directly cloned TCRs transduced into a fluorescent Jurkat reporter system (figure 1). This approach provides an unbiased, high-throughput method for TCR discovery.

Methods As a model for POTS, T cells specific for a peptide derived adenovirus structural protein were sorted on tetramer and subjected to 10x single cell VDJ analysis. Pools of randomly paired TCR alpha and beta chains were cloned from the 10x cDNA into a lentiviral vector and transduced into a Jurkat reporter cells. Consecutive stimulations with cognate antigen followed by cell sorts were performed to enrich for functional TCRs. Full length TCRAb pools were sequenced by Oxford Nanopore Technologies (ONT) and compared to a 10x dataset to find naturally paired TCRs.

Results Comparison between the ex vivo single cell VDJ sequencing and ONT sequencing of the transduced antigen specific TCRs showed more than 99% of the TCR pairs found in reporter positive Jurkat cells were naturally paired TCRs. The functionality of 8 TCR clonotypes discovered using POTS were compared and clone #2 showed the strongest response. Of the selected clonotypes, clone #2 showed a low frequency of 0.9% in the ex vivo single cell VDJ sequencing. After the first round of stimulation and sequencing, clone #2 takes up of 5% of all reporter-positive clones. The abundance of clone #2 further increased to 17% after another round of stimulation, sorting and sequencing, suggesting this method can retrieve and enrich for highly functional antigen specific TCRs.

Conclusions POTS provides a high-throughput method for discovery of naturally paired, high-avidity T cell receptors. This method mitigates bias introduced by T cell differentiation state by screening TCRs in a clonal reporter system. Additionally, POTS allows for screening of low abundance clones when compared with traditional TCR discovery techniques. Pooled TCRs could also be screened in vivo with primary T cells in a mouse model to screen for the most functional and physiologically fit TCR for cancer treatment.

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