167 HIGH-EFFICIENCY CAPTURE OF ANTI-TUMOR NEOANTIGEN-REACTIVE T CELL RECEPTORS FROM TUMOR DIGEST

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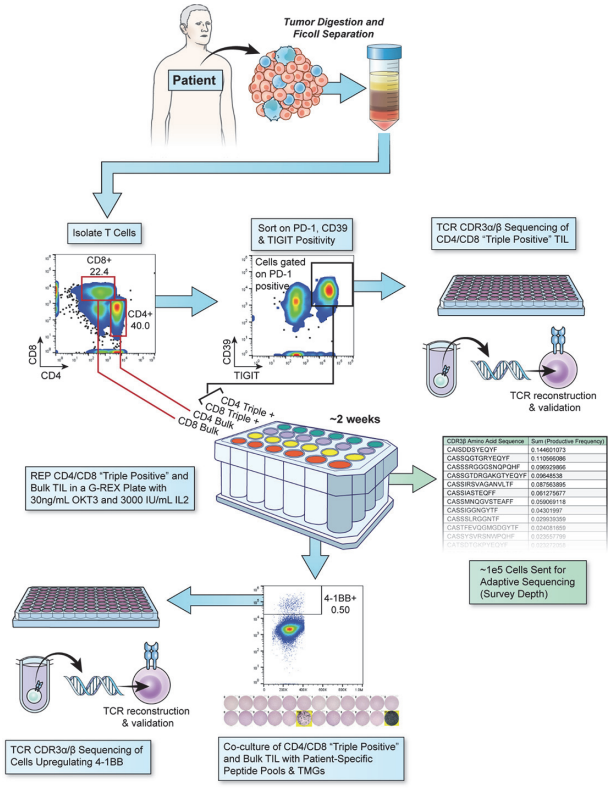
Background As cellular immunotherapies utilizing genetically engineered T cells become a more significant focus of clinical investigation, identification of patient-specific neoantigen-reactive T cell receptors (TCRs) in a practical and efficient manner is a top priority. Using high-dimensional single-cell analysis, we recently identified a common gene expression signature in anti-tumor, neoantigen-reactive tumor-infiltrating lymphocytes (TIL) from patients with metastatic cancer, which included high gene expression of cell-surface markers of exhaustion.[1] Furthermore, analyses of intra-tumoral T cell populations have shown that neoantigen-specific TIL are enriched in subsets defined by cell-surface markers of exhaustion, likely secondary to oligoclonal expansion that occurs upon tumor antigen recognition in vivo.[2–5] In this study we describe an efficient method to prospectively capture and reconstruct neoantigen-reactive T cell receptors from tumor digest based on co-expression of CD39, PD-1, and TIGIT.

Methods We evaluated the ability of PD-1+, CD39+, and TIGIT+ TIL (TIL-TP) to enrich for neoantigen-reactivity by sorting, sequencing, and reconstructing high-frequency TCR alpha/beta pairs from tumor digest in 5 patients with metastatic epithelial cancers. We then tested the ability of PD-1/CD39/TIGIT co-expressing TILs to sustain reactivity to patient-specific tumor neoantigens following in vitro expansion under similar conditions to our current clinical trial protocols (figure 1).

Results We prospectively reconstructed TIL-TP TCRs to identify additional novel TCRs, with 35% of prospectively screened TCRs being neoantigen- or tumor-reactive. Including both previously known and newly predicted TCRs, TIL-TP demonstrated enrichment for neoantigen-reactivity in 4 of 5 patients, with a median of at least 26.8% of sequenced TIL-TP cells being neoantigen-reactive (range: 11.9 – 88.4%). TIL-TP TCR isolation demonstrated a high degree of correlation with single-cell transcriptomic approaches to identification of neoantigen-reactive TCRs, though TIL-TP TCRs represent a more cost-effective and widely available approach compared to those utilizing more advanced technologies. However, despite their substantial enrichment for neoantigen-reactive TCR clonotypes, the majority of TIL-TP populations failed to demonstrate neoantigen-reactivity following in vitro expansion and exhibited loss of neoantigen-reactive clones as well as functional impairment.

Conclusions TIL-TP serve as a highly efficient and reliable source of tumor-reactive TCRs. While direct utilization of these TIL-TP as a source for cellular therapy presents significant challenges, sorting for TIL-TP offers a streamlined approach using readily available and affordable technology to identify neoantigen-reactive TCRs that may be used to design TCR engineered cellular immunotherapies.



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