417 RAPID DISCOVERY OF PERSONALIZED TCRS THROUGH A SINGLE-CELL TRANSCRIPTOMIC SIGNATURE THAT PREDICTS TUMOR REACTIVITY

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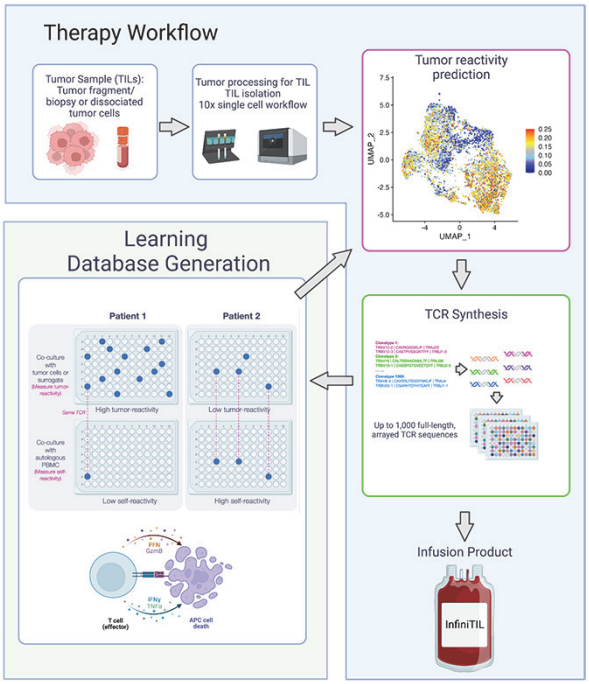
Background Personalized T cell therapies are currently limited by complex manufacturing, unreliable potency, long timelines, and the associated high cost. An ideal personalized workflow would simplify this process by leveraging affordable sequencing and commoditized cell manufacturing, while still resulting in a potent therapeutic without time-consuming and costly experimental validation. The T cell population that mediates response, the cells containing tumor-reactive TCRs, is undefined by traditional phenotypic characterization. To better understand this cryptic population critical for therapy, we devised a workflow that involves scRNA-Seq of thousands of cells, synthesizing 1000s of TCRs per patient, and screening them for reactivity (figure 1). We then applied this workflow across a broad spectrum of cancer types. The synthesis of 100,000s of TCRs to enable this survey is cost-prohibitive using traditional vendors. To solve this problem, we developed a novel technology, named PathFinder DNA Assembly™, that allows parallel synthesis of thousands of TCRs simultaneously for a cost of **under $1 per TCR**. The tumor-reactivity of these TCRs can then be experimentally tested, and their reactivity profile mapped back to high-complexity scRNA-Seq data to better define the population responsible for mediating therapeutic responses.

Methods To date, we've queried 26 patients across 10 different cancer types, synthesized between 350-2,800 TCRs per patient, and identified reactive TCRs against autologous tumor and allogeneic HLA matched cell lines. This database allows us to correlate the reactivity profile of a TCR with the transcriptomic profile of the original, unexpanded tumor infiltrating lymphocytes (TIL). In doing so, we've identified a transcriptomic profile (TR1.0) that enriches for tumor reactivity and is corroborated when we interrogating public scRNA-Seq datasets

Results To demonstrate the utility of our TR1.0 signature, we performed scRNA-Seq on a liver cancer sample, selected TCRs based on TR1.0, synthesized 192 TCRs from more than 4,500 cells encompassing high-scoring CD8 and CD4 populations, as well as TCRs predicted by other published metrics. Reactivity of the synthetic TCRs was established by ELISPOT assays against autologous tumor in a 384-well microplate and the highly reactive TCRs highlighted on the UMAP. The TR1.0 score correctly identifies the small population of T cells containing the reactive TCRs where other published metrics fail to predict the reactive population. This data, in combination with ongoing work, demonstrates the feasibility of a personalized therapy with simplified manufacturing and clinically viable timelines while maintaining a high potency of the product.



Abstract 417 Figure 1 RootPath InfiniTIL Workflow
Overview of the InfiniTIL workflow. Tumor samples are collected from as small amount of material as a biopsy, T cells selected, and single-cell sequenced. TCRs from T cells that score highly by TR1.0 metric are then selected for production. Additionally, each patients TCRs are screened in a companion assay to establish each TCR's reactivity profile. This establishes a database of reactivity information and it's correlation with transcriptomic and cell surface marker expression, that can then be leveraged to improve TR1.0 scoring and enable new therapies.

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