

AN UNBIASED SURVEY OF TUMOR REACTIVITY AND TRANSCRIPTIONAL LANDSCAPE OF 1,000 CLONES OF PD-1^{hi} T-CELLS FROM PERIPHERAL BLOOD DURING ICB TREATMENT REVEALS BREATH AND DYNAMICS OF ANTI-TUMOR IMMUNITY

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Background Immune checkpoint blockade (ICB) treatment often results in drastic changes in the TCR repertoire and T-cell phenotypes in the tumor and periphery. While scRNA-Seq allows the sequencing of thousands of T-cells, the high cost of TCR synthesis (>\$100 per TCR) makes it cost-prohibitive to identify which clones are tumor-reactive, particularly in peripheral blood where tumor-reactive cells are rarer. This severely hinders our mechanistic understanding of these treatments and the possibility to develop non-invasive personalized T-cell-based therapies. To solve this problem, we developed a novel technology, named PathFinder DNA AssemblyTM, that allows parallel synthesis and cloning of thousands of TCRs simultaneously for a cost of **under \$1 per TCR**. This enables the high-throughput experimental screening of up to 1000 TCRs for tumor reactivity. We have applied this technology to survey peripheral bulk or PD-1^{hi} T-cells from a melanoma patient before and after ICB treatment, since previous studies have demonstrated that PD-1 may be a biomarker for tumor-reactive T-cells from both tumor-infiltrating and peripheral T-cells.^{1 2}

Methods We obtained PBMCs of a melanoma patient at multiple time points before and after the ICB treatment. Next, we obtained scRNA-Seq data for both bulk CD3⁺ sorted and PD-1^{hi} sorted T-cells. We then synthesized, cloned, and expressed 1,000 TCR sequences in peripheral T-cells from unrelated donors while ensuring each T-cell only expresses a single TCR, creating polyclonal TCR-T-cells, which were then screened for tumor reactivity by co-culturing with autologous tumor or melanoma cell lines expressing the patient-matched HLAs. After co-culture, activated TCR-T-cells were sorted based on an activation marker, sequenced to identify the enriched TCRs, and then validated individually.

Results Both pre- and post-treatment PMBCs harbor T-cell clones that are also found in the tumor, with many emerging after ICB treatment. More than 30 TCRs were identified to be tumor-reactive, many of which emerged during the ICB treatment. Interestingly, 12 out of the 14 tumor-reactive TCRs obtained from screening on 3rdparty cancer cell lines also appeared to be reactive to the autologous tumor.

Conclusions These results not only reveal previously hidden details of ICB treatment but also exemplify the approach to affordably identify tumor-reactive TCRs for each patient without any tumor tissue resection. These TCRs can be used to produce highly potent, autologous, polyclonal TCR-T products that have the potential to be the next generation of adoptive cell therapy.

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