406 HIGH THROUGHPUT IDENTIFICATION OF CANCER-SPECIFIC CD4+ T CELLS FOR TCR T-CELL IMMUNOTHERAPY THROUGH ENGINEERED PROTEINS

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Background Despite advances with single-clone CD8+ T cells in cancer therapy, results often fall short due to immune evasion by cancer cells and the limited lifespan of administered CD8+ T cells.[1–3] To address these challenges, there is growing interest in utilizing cancer-specific CD4+ T cells. However, the diversity of CD4+ TCRs and Class II HLAs presents challenges in identifying antigen-specific CD4+ T cells across diverse patients. Here, we developed a high-throughput platform to screen antigen-specific CD4+ T cells, providing multilayer information on TCR sequences, HLA-antigen targets, and phenotypes.

Methods We engineered Class II pMHC proteins as single-chain trimers (SCTs) to address these challenges.[4] A library of SCTs with common infectious disease antigens was built to identify and capture antigen-specific CD4+ T cells from healthy donors. TCRs were sequenced and validated through tetramer binding and peptide-pulsed activation assays. Subsequently, we constructed a large SCT library encoding the SARS-CoV-2 RBD domain for high-throughput screening of 22 COVID-19 patients at seven timepoints. Antigen-specific CD4+ T cells were sorted for single-cell sequencing of their TCR sequences, transcriptomes, and antigen targets encoded in the SCT multimer barcodes. We analyzed their phenotypes, characterized their immune responses to the virus, and validated the TCRs against their targets. Additionally, we applied this technology to discover HPV-16 specific CD4+ T cells in pre-cancerous patients from an HPV vaccine clinical trial. We developed a library of Class II SCTs targeting the E6 and E7 oncogenic proteins to capture HPV-16 specific CD4+ T cells from patients and analyzed their transcriptome data.

Results The Class II SCT technology successfully identified and captured antigen-specific CD4+ T cells. The technology was highly compatible with single-cell multi-omics analysis, as demonstrated with the SARS-CoV-2 library. We identified over 2000 antigen-specific CD4+ T cells with various phenotypes, including naïve, effector memory, exhausted, Th1, cytotoxic, Th17, Treg, and Tr1. A subset of TCRs from different phenotypes was validated. The technology was also applied to discover HPV-16 specific CD4+ T cells, identifying over 100 HPV-specific CD4+ T cells with various phenotypes. Five HPV-specific CD4 TCRs were validated through tetramer binding, and further pre-clinical assessments are underway for their therapeutic potential.

Conclusions We developed a high-throughput platform for screening antigen-specific CD4+ T cells using engineered Class II SCTs. The phenotypic information provided by this technology can guide the selection of CD4 TCRs with better therapeutic potential. With this approach, we envision covering a broader range of patients for off-the-shelf T cell therapy.

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Ethics Approval The use of patient samples and data was approved by the ISB's Institutional Review Board. Written informed consent was obtained from all patients.

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