Comprehensive Identification of Tumor-reactive TCRs and Cognate Targets for Novel TCR T-cell Cancer Therapies

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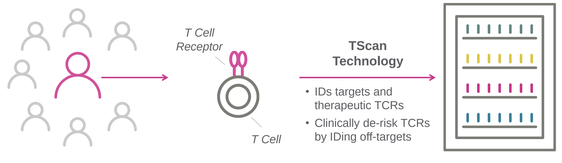
#1161

Abstract

TCR-engineered T-cell therapy is one of the most promising approaches to cancer therapy but is currently limited by a lack of diverse targets and by an inability to comprehensively identify off-target interactions. These limitations are addressed by “TScan”, a genome-wide screen that enables the unbiased identification of the natural targets of T cell receptors[1]. Here, we demonstrate application of the TScan technology to therapeutic development in two ways. First, TScan was used to identify two previously unknown off-targets of a MAGE-A3-specific TCR, neither of which were obvious based on sequence similarity. Second, TScan was used to discover a novel TCR target using TILs from a colorectal cancer tumor. Based on these results, a high-throughput discovery platform was developed to identify tumor-reactive TCRs that recognize novel shared-antigen targets from patient TILs. Using this platform, we identified several novel targets that are currently being evaluated for further development. Collectively, this platform enables construction of a repository of therapeutic TCRs with diverse targets and HLA restrictions, providing a way to develop multiplexed TCR therapy tailored for each patient.

Learning from patients that are winning their fight against cancer …to treat those that are not



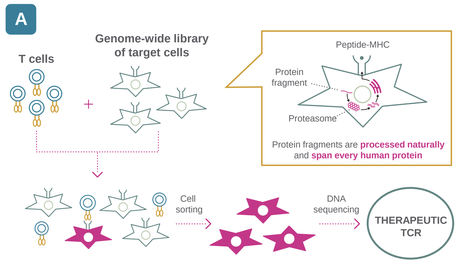
TScan Technology

- IDs targets and therapeutic TCRs
- Clinically de-risk TCRs by IDing off-targets

When a patient responds to a check-point inhibitor, it is their T cells that are driving their response. The central hypothesis at TScan is that, if we can isolate TILs that drive clinical response and identify the TCRs and targets of those TILs, we can develop effective TCR therapies for a broad range of cancer patients whose tumors express the same antigens.

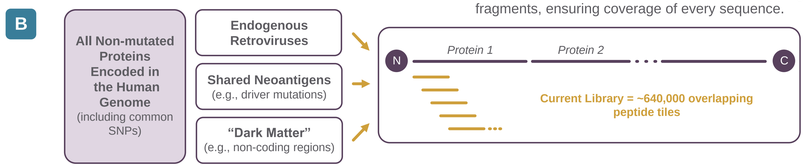
TScan technology

Unbiased, genome-wide screen for targets of TCRs



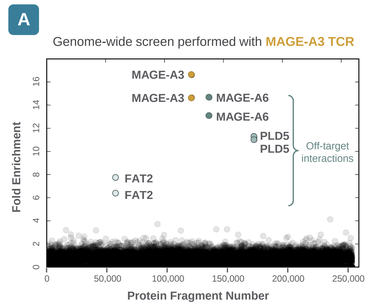
(A) T cells expressing a TCR of interest are mixed with a genome-wide library of target cells, each expressing a different protein fragment. Fragments are processed naturally by the target cells and the resulting peptides are displayed on cell-surface MHCs. If a T cell recognizes its target, it attempts to kill the target cell, thereby activating a fluorescent reporter. By isolating fluorescent cells and sequencing their expression cassettes, the natural target(s) of the T cell are revealed.

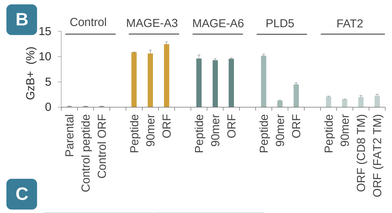
(B) To ensure broad target coverage, TScan’s libraries comprise hundreds of thousands of protein fragments spanning every human protein and all common SNPs. They also include elements unique to cancer cells: all common driver mutations, endogenous retroviral elements, and a large collection of non-coding sequences. The libraries are constructed using a tiling pattern of overlapping fragments, ensuring coverage of every sequence.

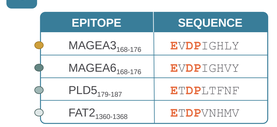


Safety screen

Comprehensive identification of the off-targets of TCRs





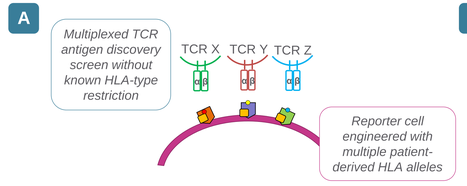


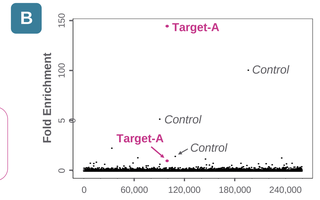
TScan screen reveals off-targets **not predicted using bioinformatics**

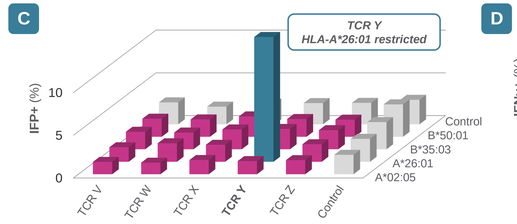
As proof-of-concept, the TScan screen was used to identify all the targets of a MAGE-A3-specific TCR[1]. (A) In addition to the known target, the screen identified three “off-targets”: MAGE-A6, PLD5, and FAT2. (B) All four targets were subsequently verified. (C) The peptide sequences of PLD5 and FAT2 share little sequence similarity with MAGE-A3, demonstrating the value of using an experimental approach to identify off-target interactions, rather than bioinformatics.

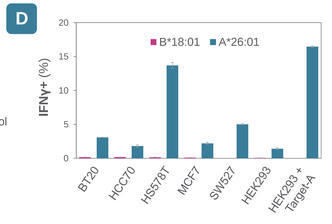
Multiplexed screening

Identification of new targets for TCR T-cell therapy





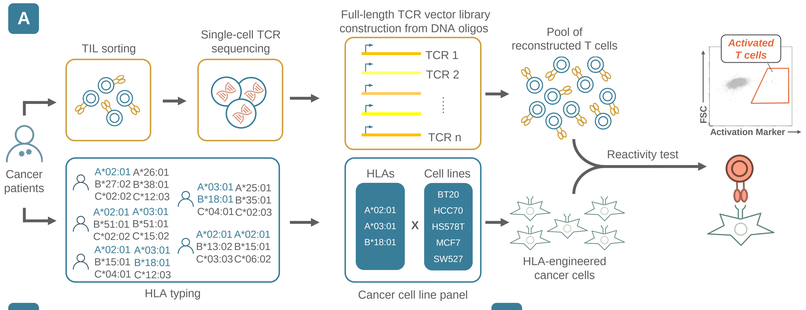


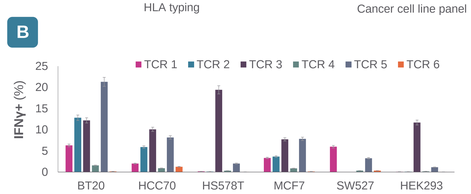


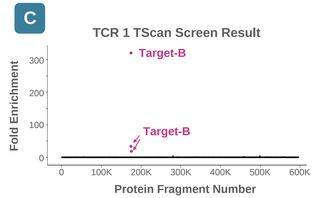
To increase the efficiency of target discovery, methods were developed to screen multiple TCRs simultaneously. (A) Multiplexed screens include several TCRs and require expression of multiple HLA alleles in the target cells. (B) Five published colorectal cancer-(CRC) reactive TCRs[2] along with a positive control TCR were screened with four patient-derived HLA alleles, revealing a novel target (Target-A). (C) Deconvolution revealed the identity of TCR Y and its HLA restriction (HLA A*26:01). (D) In addition to CRC cell lines, the TCR recognizes 2 of 5 breast cancer cell lines expressing the correct HLA.

High-throughput discovery

Screening TILs for new TCR/target pairs



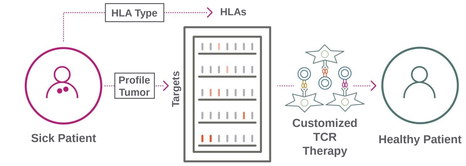




High-throughput methods were developed to discover novel TCR/target pairs from patient tumors. (A) TILs were isolated from 5 patient tumors and their paired α/β TCR sequences determined by single cell sequencing. Thousands of TCRs were then synthesized, cloned, and transduced as a pool into primary T cells. In parallel, HLA types of the patients were determined and a panel of cancer cell lines engineered with the most prevalent HLAs. By co-culturing the T cells with the engineered cell lines, cancer-reactive TCRs recognizing shared antigens were ID’d by isolating and sequencing activated T cells. (B) A variety of cancer-reactive TCRs were ID’d using this method (6 here). (C) Novel targets for TCR therapy were ID’d using TScan’s screen.

Multiplexed therapy

A therapeutic strategy to address tumor heterogeneity



TCR-T multiplexing:

- Overcomes the problem of **tumor heterogeneity**
- Solves **resistance** due to target loss

Because solid tumors are heterogeneous, successful TCR therapy will likely require an oligoclonal mixture of TCRs. Our goal is to provide customized, off-the-shelf, multiplexed therapy by building a repository of safe and effective TCRs that recognize a broad range of targets and HLAs.

Conclusion

Safe and effective TCR therapy requires tools to identify the natural targets of anti-cancer TCRs and to ensure they do not exhibit problematic off-target effects. Here, we showed that a genome-wide screening technology, TScan, correctly identified the known target of a TCR and identified previously unknown off-targets. Based on these results, a high-throughput discovery platform was developed to identify tumor-reactive TCRs from patient TILs. This platform enabled the discovery of several new TCR/target pairs and is currently being used to build a repository of therapeutic TCRs for multiplexed TCR T-cell therapy.

References: 1. Kula, T. et al. Cell, 178(4):1016-1028. 2. Scheper, W et al. Nature Medicine, 25:89-94.