Development of a target agnostic platform to assess the reactivity of T cell receptor (TCR)-engineered T cell (TCR-T) therapies to primary human tissues

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Abstract # 384

Introduction

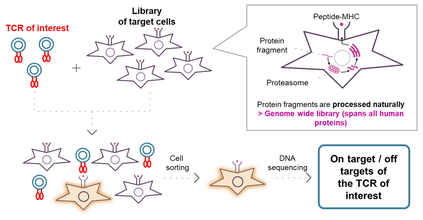
Background: The ability to identify problematic off-targets is critical for TCR-T therapies as TCRs that recognize off-targets expressed at high levels in critical organs could cause toxicities. TCR-T therapies require a fully human system to appropriately de-risk their use in clinical studies. The paucity of relevant animal models to detect potential off-target activity of TCRs has led to reliance on predictive computational algorithms guided by positional scanning mutagenesis. These methods fail to screen against potential off-targets with low sequence homology to target epitopes. To overcome these limitations, TScan has developed SafetyScan- an unbiased, genome-wide, high-throughput platform to eliminate TCR candidates that cross-react with primary human tissues.

Methods: T cells expressing a candidate TCR are co-cultured with target cells expressing a genome-wide library of protein fragments. Target cells recognized by the TCR are sequenced to reveal the natural target(s) of the TCR as well as putative off-targets, even if they have low sequence homology to the target epitope. To determine if any of these putative off-targets represent bona fide off-targets of the TCR, T cells expressing the candidate TCR are co-cultured with an array of primary and induced pluripotent stem cells (iPSC)-derived cells derived from epithelial, mesenchymal, endothelial, fibroblastic and muscle cells from vital and non-vital organs, from male and female donors that endogenously express the cognate human leukocyte antigen (HLA) and putative off-targets. Levels of IFN-γ in the culture supernatants are used as a measure of T cell reactivity. Bulk RNA sequencing quantifies the expression of putative off-targets and HLA in the primary cells.

Results: In a proof of capability study, an affinity-enhanced TCR from a different sponsor that led to clinical toxicity due to off-target reactivity with cardiac muscle protein titin was evaluated [1, 2]. The genome-wide screen identified multiple putative off-targets, including titin. Co-cultures of T cells expressing the affinity-enhanced TCR with iPSC-derived cardiomyocytes displayed significant reactivity. RNA-seq confirmed that titin was expressed in these cells, albeit at lower levels than expected for cardiac tissue. The platform was applied to assess and de-risk putative off-targets of the six TCRs currently in TScan's ImmunoBank and being studied in the clinic, restricted to HLA-A*02:01, HLA-A*01:01, HLA-B*07:02 and HLA-C*07:02 and targeting HPV16 E7, PRAME, MAGE-A1 and MAGE-C2.

Conclusions: These data demonstrate the sensitivity of comprehensive genome-wide screening for identifying putative off-targets and characterization of primary human cell panel using RNA-sequencing, to help select appropriate and physiologically-relevant primary human cells to test for off-target reactivity.

Schematic of TScan's proprietary Genome-wide SafetyScan screen to identify putative off-targets of any TCR



Overview of TScan's proprietary genome-wide *SafetyScan* screen. TCRs are screened against >600,000 protein fragments spanning every protein in the entire human proteome to identify possible reactivities, including reactivities with low sequence homology to the natural target.

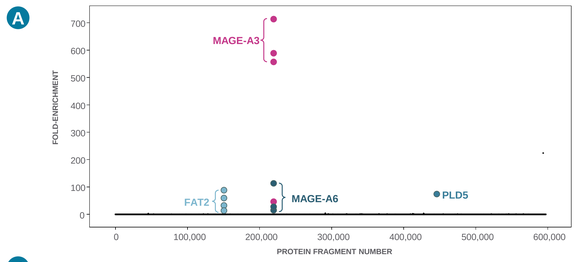
Additional TScan presentations:

#Poster Presentation:

#375: Discovery of a MAGE-A4-specific TCR-T Therapy Candidate for Multiplex Treatment of Solid Tumors

#359: Preclinical Models for T-Plex, a Customized Multiplexed TCR-T Cell Therapy Addressing Intra-Tumor Antigen and HLA Heterogeneity

Genome-wide SafetyScan screen identifies clinically relevant off-targets

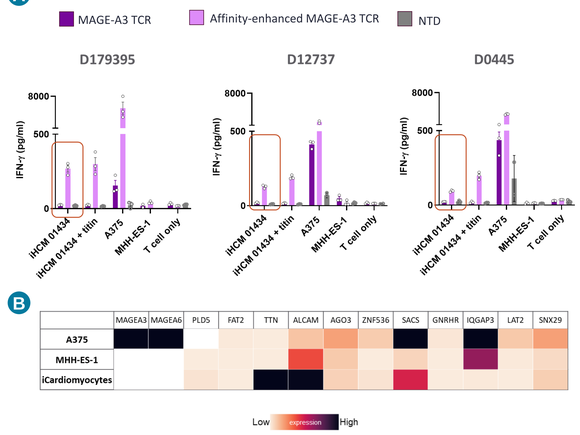


Graphical representation of the results of the SafetyScan screens testing the MAGE-A3 and affinity-enhanced MAGE-A3 TCR. Plotted is the enrichment score for each of ~590,000 tiles/peptides in the screen calculated from 8 technical replicates, measured relative to the input. Tiles that are enriched above background are highlighted in colors, with overlapping tiles displayed in matching colors; the corresponding protein names are indicated on the graph

(A) SafetyScan of MAGE-A3-specific TCR identified MAGE-A3 and 3 putative off-targets: FAT2, MAGE-A6 and PLD5.

(B) SafetyScan of affinity-enhanced MAGE-A3-specific TCR identified MAGE-A3 and multiple putative off-targets: FAT2, MAGE-A6, PLD5, TTN, ALCAM, AGO3, ZNF536, SACS, GNRHR, IQGAP3, LAT2, SNX29 and XM_001717344.

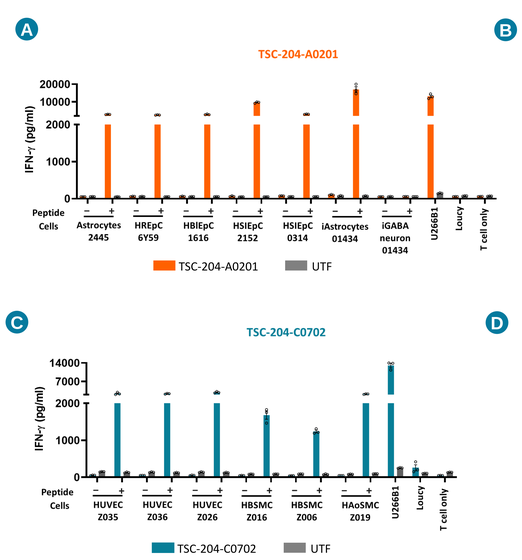
SafetyScan displays reactivity of affinity-enhanced MAGE-A3 TCR to iCardiomyocytes



(A) Three batches of MAGE-A3 and affinity-enhanced MAGE-A3 TCR-T cells (D179395, D12737 and D0445) along with donor matched NTDs (non-transduced T cells) were tested for reactivity against HLA-A*01:01-transduced iCardiomyocytes. IFN-γ production was used as a readout of the assay. Cells pulsed with the titin peptide served as positive control. A375 cells were used as a positive control target cell line and MHH-ES-1 as negative control target cell line.

(B) Expression of the putative off-targets of the affinity-enhanced MAGE-A3 TCR in the iCardiomyocytes and control cancer cell lines tested was determined by RNAseq.

Representative data from SafetyScan shows no apparent reactivity of TSC-204-A0201, TSC-204-C0702 and TSC-201-B0702 TCR-T cells to healthy human primary cells



(A) (C) (E) Representative results of TCR-T cells and control UTFs (untransfected T cells) being tested for reactivity to primary cells. IFN-γ was assessed as a measure of TCR-T cell reactivity. **(B) (D) (F)** Expression of the putative off-targets of the therapeutic TCR used in TSC-204-A0201 **(B)**, TSC-204-C0702 **(D)** and TSC-201-B0702 **(F)** in the various cell types tested was determined by RNAseq. **(A)** Three batches of process-representative TSC-204-A0201 TCR-T cells were assessed for risk of off-tumor reactivity against 70 HLA-A*02:01-positive healthy primary human cells and iPSC-derived cells. Cells pulsed with the MAGE-A1 peptide served as positive control. U266B1 cells were used as a positive control target cell line and Loucy as negative control target cell line. **(C)** Three batches of process-representative TSC-204-C0702 TCR-T cells were assessed for risk of off-tumor reactivity against 49 HLA-C*07:02-positive healthy primary human and iPSC-derived cells. Cells pulsed with the MAGE-A1 peptide served as positive control. U266B1 cells were used as a positive control target cell line and Loucy as negative control target cell line. **(E)** Three batches of process-representative TSC-201-B0702 TCR-T cells were assessed for risk of off-tumor reactivity against 54 HLA-B*07:02-positive healthy primary human and iPSC-derived cells. Cells pulsed with the MAGE-C2 peptide served as positive control. COLO-783 cells were used as a positive control target cell line and CaSki as negative control target cell line.

1) Cameron, Brian J et al. "Identification of a Titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3-directed T cells." Science translational medicine vol. 5,197 (2013): 197ra103.
2) Linette, Gerald P et al. "Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma." Blood vol. 122,6 (2013): 863-71.