Discovery of TSC-200-A02: A natural HPV16 E7-specific TCR-T cell therapy candidate for the treatment of HPV-positive solid tumors

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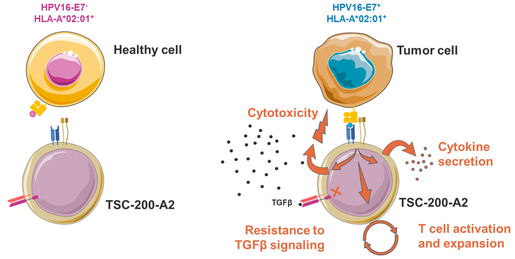
Introduction

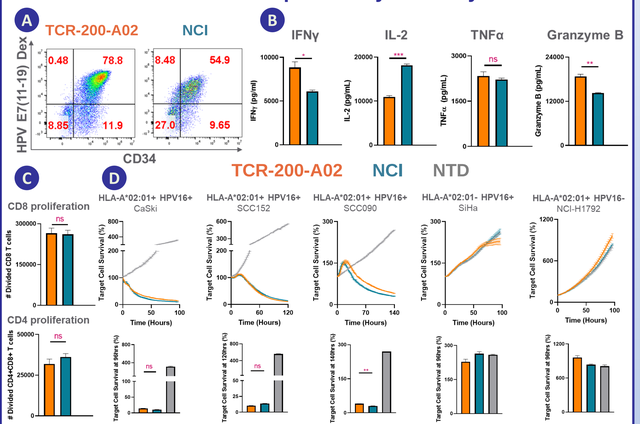
Human papilloma virus (HPV) is an oncogenic virus responsible for over 90% of cervical and anal cancers and over 25% of head and neck cancers, which are typically incurable upon metastasis. HPV E7 oncoprotein is a compelling target for TCR-engineered T cell therapy as it is homogenously expressed in every tumor cell, essential for tumor cell survival, and not expressed by healthy tissues. Notably, a recent clinical trial of an E7-directed TCR-T cell therapy conducted at the National Cancer Institute (NCI) showed a 50% objective response rate in heavily pre-treated patients with HPV+ cancers (1).

Using TScan's proprietary *ReceptorScan* platform, we discovered 453 putative HPV16 $E7_{11-19}$-specific TCRs by screening 681 million naïve CD8+ T cells from 15 unique healthy donors. We tested each TCR for expression in primary T cells and for its ability to kill T2 cells pulsed with the $E7_{11-19}$ peptide, using the NCI TCR as a benchmark for these studies (2). The top 3 TCRs from this screen were evaluated in depth for cytotoxicity, cytokine production, and T cell proliferation in response to a panel of HPV16+ cancer cell lines expressing varying levels of HLA-A*02:01 and E7. A lead TCR was identified that showed comparable activity relative to the NCI TCR. The lead TCR was evaluated for allo-reactivity using an array-based screen and for off-target reactivity using our proprietary *SafetyScan* platform, which is a highly sensitive screen for off-target recognition based on supraphysiologic expression of protein fragments that span the entire human proteome. No alloreactivity was observed to 108/110 HLAs tested, and only a few putative off-targets were identified. The TCR-T cells showed no reactivity to a panel of normal primary human cells, including cells that naturally express the putative off-targets identified in the *SafetyScan* screen. In vivo studies in immunocompromised mice showed efficient control of xenogeneic tumor cell growth by TCR-200-A02 cells in two independent tumor models.

To further enhance the activity of our T cells, we designed a transposon-based vector that delivers the TCR gene, along with the genes for CD8 α/β and a dominant-negative form of TGFβRII, into both CD4+ and CD8+ T cells. We have advanced the resulting autologous TCR-T cell therapy candidate, TSC-200-A02, to IND-enabling studies. These results validate the use of *ReceptorScan*, in conjunction with *SafetyScan*, as a way to rapidly identify naturally occurring, high affinity TCRs that are suitable for clinical development.

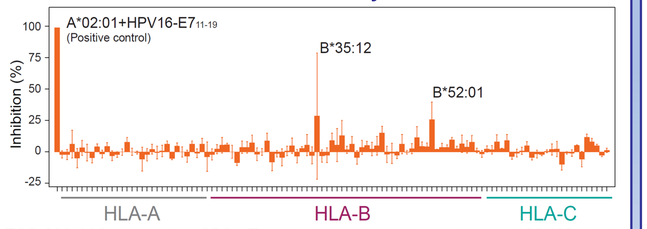


TCR-200-A02 shows comparable cytotoxicity to NCI TCR



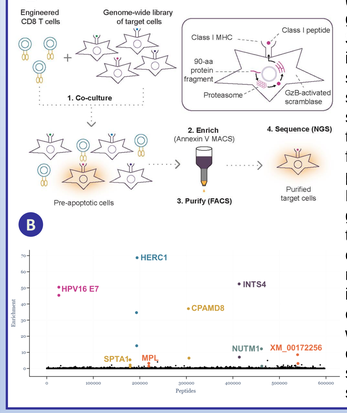
Pan T cells (natural mixture of CD4+ and CD8+ T cells) were transduced to express HPV16 $E7_{11-19}$-specific TCRs and assessed for functional responses to target cells. **(A)** TCR-200-A02 shows efficient surface expression of TCR-200 (HPV16 $E7_{11-19}$-specific dextramer) and Q-tagged CD8α (QBEnd/10). **(B)** TCR-200-A02 efficiently secretes cytokines when co-cultured with SCC152 cells. **(C)** TCR-200-A02 exhibits HPV16-specific CD8+ and CD4+ T cell proliferation when co-cultured with SCC152 cells. **(D)** TCR-200-A02 shows strong cytotoxicity in HLA-A*02:01+ HPV16+ target cell lines CaSki, SCC152, SCC090, and no reactivity to HLA-A*02:01- HPV16+ cell line SiHa or the HLA-A*02:01+ HPV16- cell line NCI-H1792. TCRs were compared by one-way ANOVA followed by Dunnett's multiple comparison test. Differences that were non-significant (ns) are shown; all other differences were significant with P<0.05. Data are representative of 3 unique donors.

TCR-200-A02 shows no alloreactivity to 108/110 HLAs tested



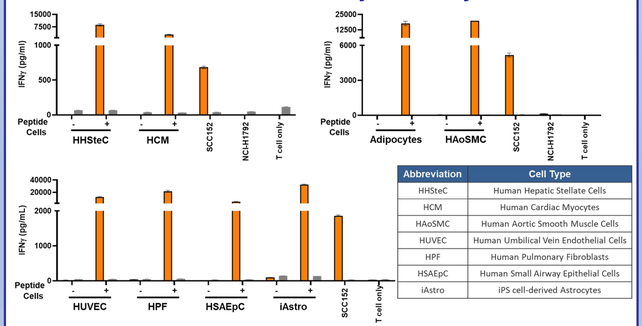
TCR-200-A02-expressing CD3+ T cells showed no alloreactivity to 108/110 of the most frequent class I MHCs in the US population.

TScan's genome-wide *SafetyScan* screen identifies putative off-targets for TCR-200-A02



(A) Overview of TScan's proprietary genome-wide *SafetyScan* screen. **(B)** *SafetyScan* screen of TCR-200-A02 identifies seven potential off-targets in a screen of >600,000 protein fragments spanning every w.t. human protein. The screen is designed to overpredict off-targets by overexpressing 90-aa protein fragments, which are more efficiently processed than full-length proteins. Putative off-targets are identified by gene names. XM_0017722256 maps to the heterochromatic centromere region of chromosome 20 and has been removed from the RefSeq annotation indicating a lack of evidence for its expression. No expression of this gene was detected using RNA-seq analysis of 51 samples including normal tissue samples, cancer cell lines and tumor samples.

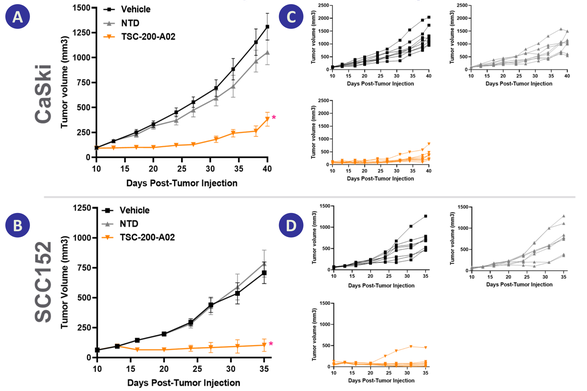
TCR-200-A02 shows no reactivity to healthy human cells



Abbreviation	Cell Type
HHSteC	Human Hepatic Stellate Cells
HCM	Human Cardiac Myocytes
HAoSMC	Human Aortic Smooth Muscle Cells
HUVEC	Human Umbilical Vein Endothelial Cells
HPF	Human Pulmonary Fibroblasts
HSAEpC	Human Small Airway Epithelial Cells
iAstro	iPS cell-derived Astrocytes

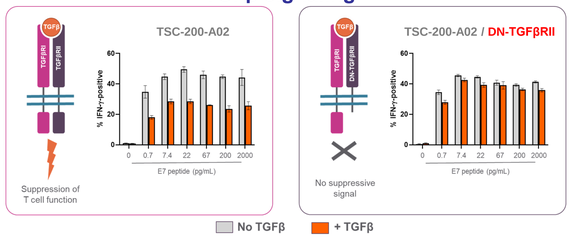
Primary cells or iPSC-derived cells from healthy HLA-A*02:01+ human donors naturally expressing off-targets identified in the genome wide *SafetyScan* screen were co-cultured with TCR-200-A02 or nontransduced (NTD) cells. Cell pulsed with E7 peptide served as positive controls. IFNγ secretion in culture supernatants was used as a read-out of TCR-200-A02 reactivity to target cells. SCC152 cells were used as a positive control and NCI-H1792 cells were used as a negative control.

TCR-200-A02 efficiently controls tumor growth in vivo



NCG mice were subcutaneously inoculated with either $1x10^6$ CaSki or SCC152. When tumors reached 95 ± 15 mm^3 on Day 10, the mice were randomized and treated on Day 11 with $20x10^6$ cells of TCR-200-A02, non-transduced (NTD) cells or vehicle. **(A & B)** Treatment with TCR-200-A02 showed significant inhibition of tumor growth in vivo. **(C & D)** Individual mouse tumor growth per group over time. *p<0.05, One-way ANOVA, Holms-Šidák correction for multiple comparisons test in vivo.

TGFβRII-DN provides resistance to the suppressive effect of TGFβ signaling



Dominant negative TGFβ Receptor II (DN-TGFβRII) renders TSC-200-A02 resistant to TGFβ-mediated suppression. T cells were co-transduced with lentivirus encoding TCR-200-A02 and DN-TGFβRII and were FACS sorted into DN-TGFβRII positive and DN-TGFβRII negative fractions. Intracellular IFNγ within the TSC-200-A02 expressing T cells was quantified after 24 h co-culture with peptide-pulsed T2 cells in the presence or absence of 5 ng/mL TGFβ.

1. Nagarsheth NB, Norberg SM, Sinkoe AL, et al. TCR-engineered T cells targeting E7 for patients with metastatic HPV-associated epithelial cancers. Nat Med. 2021 Mar;27(3):419-425.
2. Jin BY, Campbell TE, Draper LM, et al. Engineered T cells targeting E7 mediate regression of human papillomavirus cancers in a murine model. *JCI Insight*. 2018;3(8): e99488