Non-clinical development of T-Plex component TSC-200-A0201: A natural HPV16 E7-specific TCR-T cell therapy for the treatment of HPV16-positive solid tumors

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Abstract # 364

Introduction

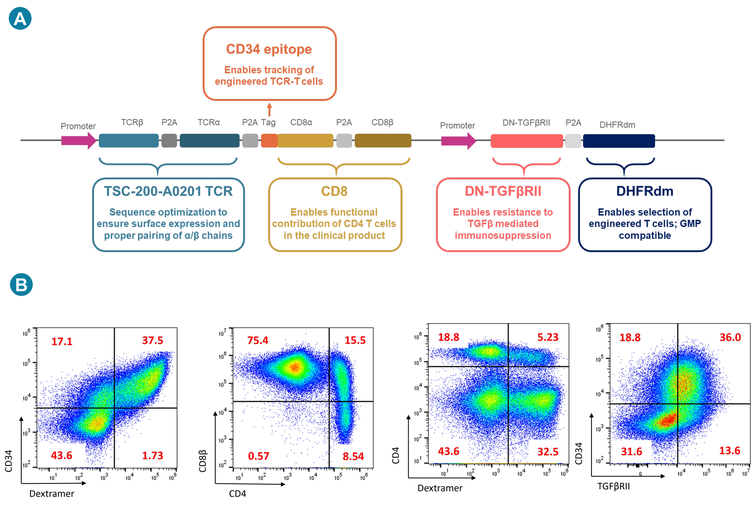
Background: T-Plex is an autologous TCR-T cell therapy product comprising customized combinations of 2-3 TCR-T cell components that recognize different tumor antigens presented on specific HLA class I molecules. Each component of T-Plex is engineered using a transposon-based vector encoding the therapeutic TCR, CD8α and CD8β co-receptors, a CD34 epitope tag, a dominant-negative TGFβRII (DN-TGFβRII), and a mutated form of dihydrofolate reductase (DHFRdm). TSC-200-A0201 is intended for the treatment of HPV16+ HLA-A*02:01+ cancers. HPV16 is an oncogenic virus responsible for ~57% of cervical cancers and ~21% of head and neck squamous cell carcinomas. HPV16 E7 oncoprotein drives oncogenic transformation of infected cells and is not expressed by healthy tissues, making it a compelling immunotherapeutic target.

Methods: The TSC-200-A0201 TCR is a naturally occurring TCR discovered using TScan's proprietary *ReceptorScan* platform. TSC-200-A0201 TCR-T cells engineered using a full-scale representative workflow for the planned manufacturing process were used to investigate the *in vitro* pharmacology and toxicology of TSC-200-A0201. TSC-200-A0201 was evaluated for avidity and target-dependent cytotoxicity, proliferation, and cytokine secretion *in vitro* as well as for anti-tumor efficacy *in vivo*. The contribution of DN-TGFβRII was assessed by testing the ability of TSC-200-A0201 TCR-T cells to resist the immuno-suppressive effects of TGFβ. Further, TSC-200-A0201 was assessed for risk of alloreactivity and off-target recognition using TScan's *SafetyScan* screen. Finally, to assess the risk of off-target/off-tumor activity, TSC-200-A0201 TCR-T cells were tested for their reactivity to an extensive panel of 76 healthy human primary and iPSC-derived cells from tissues that are traditionally assessed in toxicology studies, including those expressing high levels of the putative off-targets of TSC-200-A0201.

Results: TSC-200-A0201 displayed high avidity for the cognate peptide (IC_{50} of ~4.2 pg/mL) and target-dependent secretion of inflammatory cytokines, killing of target cells, and proliferation of both engineered CD4+ and CD8+ T cells. Moreover, TSC-200-A0201 successfully controlled the growth of HLA-A*02:01+ HPV16+ tumors in mice. Target-dependent IFN-γ production and proliferation of TSC-200-A0201 was maintained in the presence of physiological levels of TGFβ. Further, TSC-200-A0201 displayed no alloreactivity to the 110 most common class I HLAs in the US population. Although a few putative off-targets were identified in the *SafetyScan* screen, TSC-200-A0201 showed no reactivity to normal primary or iPSC-derived cells.

Conclusions: TSC-200-A0201 exhibits high specificity and potency. Based on these results, TSC-200-A0201 has been cleared by the U.S. FDA for clinical development and has been incorporated in the T-Plex Phase 1 clinical trial master protocol.

TScan's vector co-delivers TCRα/β, CD8α/β, CD34 (Q) purification tag, DN-TGFβRII and DHFRdm

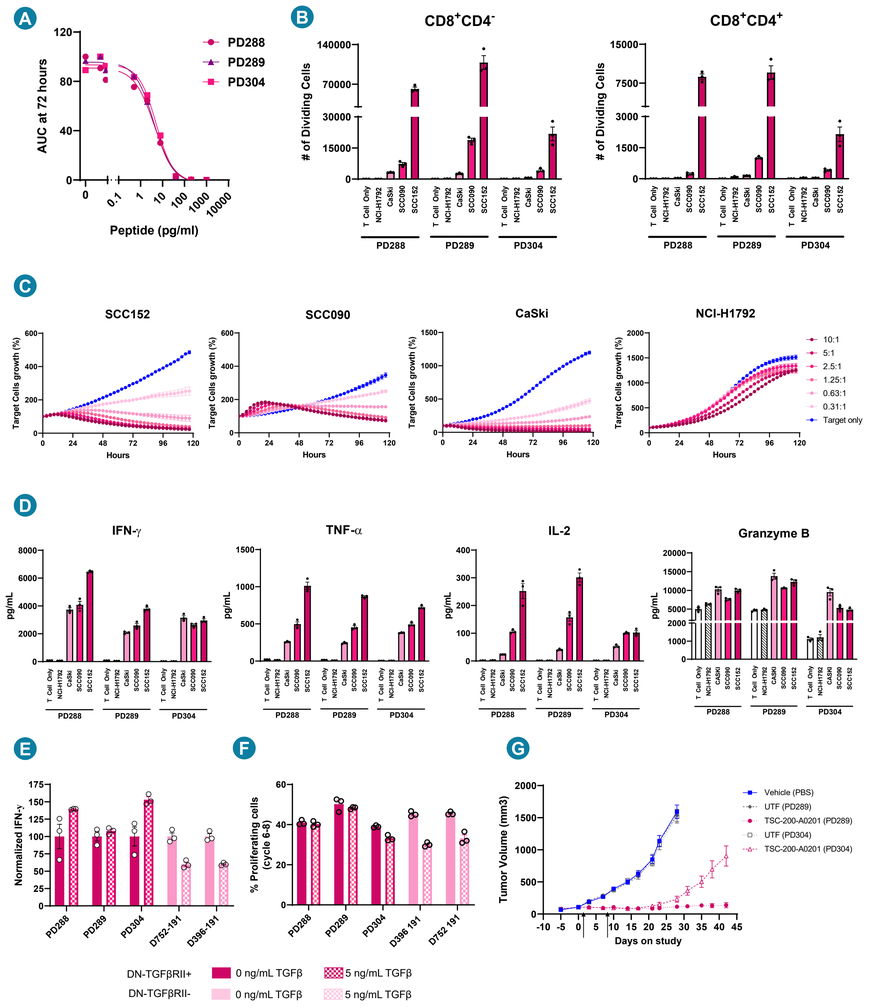


(A) Schematic of the TSC-200-A0201 clinical vector. The therapeutic TCR in TSC-200-A0201 was introduced into a transposon vector. The resulting TCR-T therapy product is a mixture of cytotoxic and helper T cells, both of which are reprogrammed to recognize HPV16+ HLA-A*02:01+ cells. In addition, the TCR-T cells express a CD34 (Q) tag, DN-TGFβRII and DHFRdm. **(B)** Three batches of process-representative TSC-200-A0201 TCR-T cells (PD288, PD289 and PD304) were generated and characterized for expression of CD34, CD4, CD8β, TGFβRII and binding to the HPV16 $E7_{11-19}$ HLA-A*02:01 dextramer. Representative flow cytometry plots are shown for TCR-T cells from batch PD289.

For more information on TScan's ImmunoBank or TScan's clinical trial design and dose-escalation scheme:

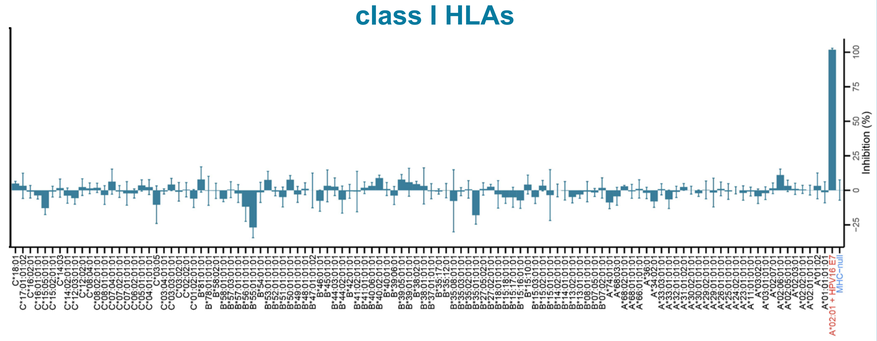
 # 357: Discovery of a novel MAGEC2 epitope for TCR-T adoptive cell therapy from expanded T cell clones of TIL therapy products
 # 390: Discovery of MAGE-A1-specific TCR-T cell therapy candidates to expand multiplex therapy of solid tumors
 # 376: Overcoming tumor heterogeneity – Clinical trial assays to prospectively assign patients customized multiplexed TCR-T cell therapy in Phase 1
 # 682: Phase 1 trial of TSC-100 and TSC-101, Engineered T-Cell Therapies Targeting Minor Histocompatibility Antigens to Eliminate Residual Disease after Hematopoietic Cell Transplantation
 # 709: Product Characteristics and Clinical Trial Design for T-Plex, a Multiplexed, Enhanced T cell Receptor-Engineered T cell Therapy for Solid Tumors

Functional characterization of TSC-200-A0201 TCR-T cells



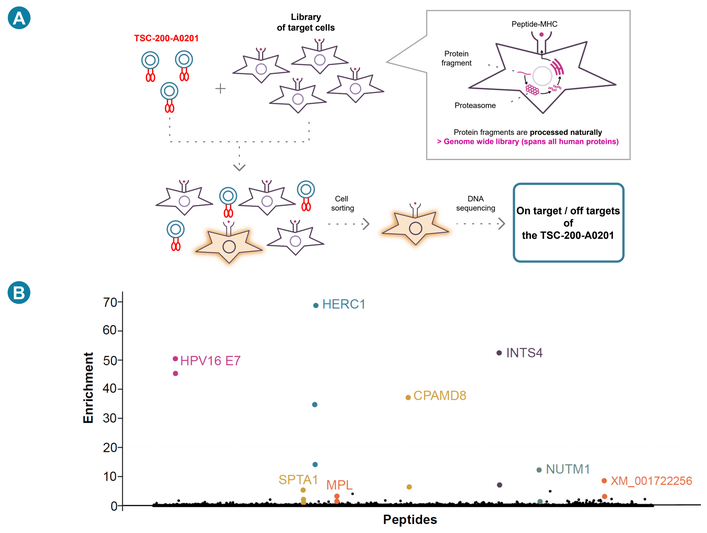
Three batches of process-representative TSC-200-A0201TCR-T cells (PD288, PD289 and PD304) were assessed for functional responses to target cells. **(A)** Area under curve (AUC) for the growth of peptide-pulsed T2 cells cocultured with TSC-200-A0201 TCR-T cells at an E:T of 5:1 over 72 h as a function of the HPV16 E7 peptide concentration. Data were normalized to the growth of the cells in the unpulsed (no peptide) condition. **(B)** Proliferation of TCR^+CD34^+ cytotoxic and helper TSC-200-A0201 TCR-T cells in response to HLA-A*02:01$^+$HPV16$^+$ cell lines CaSki, SCC090 and SCC152, and HLA-A*02:01$^+$HPV16$^-$ cell line NCI-H1792 over 4 days. **(C)** Cytotoxicity of TSC-200-A0201 TCR-T cells from batch PD304 against indicated target cancer cell lines at indicated E:Ts. **(D)** Cytokine production by TSC-200-A0201 TCR-T cells in response to indicated target cancer cell lines after 24h of coculture. (**E-F**) Three batches of TSC-200-A0201 process-representative TCR-T cells expressing DN-TGFβRII (PD288, PD289 and PD304) and two batches of process-similar TSC-200-A0201 TCR-T cells lacking DN-TGFβRII (D752-191 and D396-191) were cocultured with T2 cells pulsed with HPV16 E7 peptide in the presence or absence of TGFβ. Cocultures were assessed for IFN-γ production and T cell proliferation. **(E)** Levels of secreted IFN-γ were quantified in the supernatant of 24h cocultures and normalized to the 0 ng/mL TGFβ condition. **(F)** Percentage of cells undergoing 6 or more cycles of cell division in co-cultures with or without TGFβ were plotted for total CD34+ TCR-T cells. **(G)** Two batches of process-representative TSC-200-A0201 TCR-T cells (PD289 and PD304) were assessed for anti-tumor efficacy in a subcutaneous xenograft model of SCC152 in NCG mice. Mice were inoculated with 1E6 SCC152 cells and injected with 2E7 $CD34^+$ TSC-200-A0201 TCR-T or UTF T cells or PBS on day 1 and day 8. Mean tumor volume of each treatment group of mice (n=12) over time is shown.

TSC-200-A0201 displays no alloreactivity to the 110 most common class I HLAs



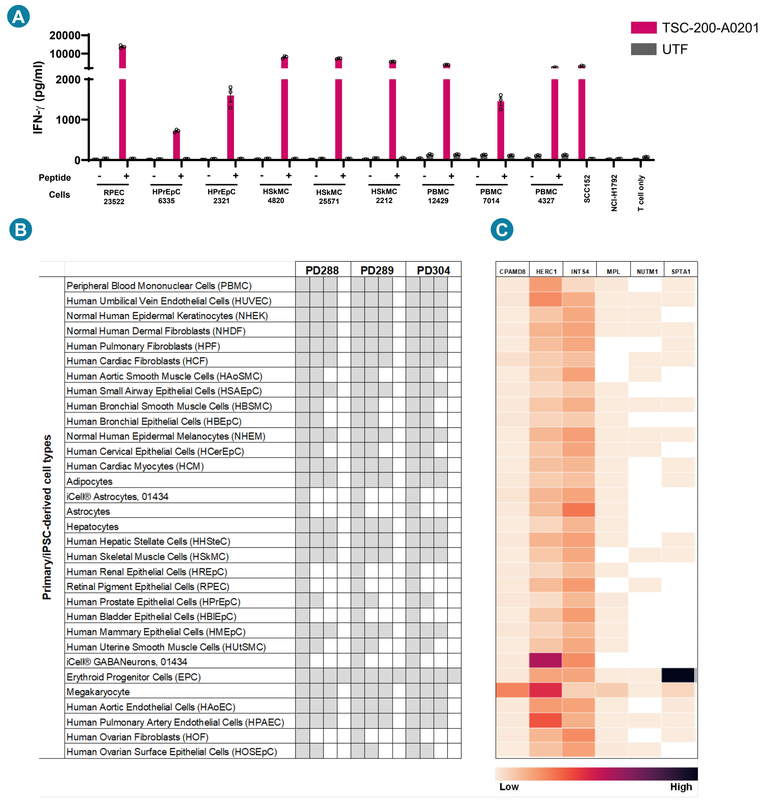
Lentivirally engineered CD8+ T cells expressing the recombinant TSC-200-A0201 TCR were co-cultured with MHC-null HEK293T cells re-expressing one of the 110 most frequently encountered Class I HLAs in the US population for 51hr. A positive control consisting of HEK293T cells expressing both a fragment of HPV16 E7 containing the HLA-A*02:01- restricted epitope and HLA-A*02:01 (red) and a negative control consisting of MHC-/- HEK293T cells (blue) were included in the screen. The inhibition of target cell growth by the TCR-T cells relative to that by the UTF control T cells was measured as a readout of the reactivity of the therapeutic TCR to allogeneic HLA proteins.

Genome-wide *SafetyScan* screen identifies putative off-targets of TSC-200-A0201



(A) 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. **(B)** *SafetyScan* of TSC-200-A0201 identified 7 putative off-targets. XM_001722256 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.

TSC-200-A0201 TCR-T cells display no reactivity to healthy human primary and iPSC-derived cells



Three batches of process-representative TSC-200-A0201 TCR-T cells (PD288, PD289 and PD304) were assessed for risk of off-tumor reactivity. **(A)** TSC-200-A0201 TCR-T cells and control untransfected cells (UTF) were tested for reactivity to primary and iPSC-derived cells with IFN-γ production as a readout of the assay. Cells pulsed with the HPV16 E7 peptide served as positive control for the assay. SCC152 cells were used as a positive control target cell line and NCI-H1792 as negative control target cell line. **(B)** Summary of data from the off-tumor reactivity assay described in Figure (A). A total of 76 primary and iPSC-derived cell lots were tested as targets. Each colored cell in the table illustrates a single lot of cells for the indicated cell type. For each cell type, 1-4 lots of cells were tested, depending on the availability of the primary cells. **(C)** Expression of the putative off-targets of the therapeutic TCR used in TSC-200-A0201 in the various cell types tested was determined by RNAseq; the average expression of the off-target for each cell type is presented in a color-scaled manner to indicate the range of expression across samples. The color scale used in RNAseq heatmaps has TPM values of zero set to white and values above zero follow a continuous color scale up to 100 TPM.