Nonclinical development of T-Plex component TSC-204-A0101: a natural TCR-T cell therapy for the treatment of MAGE-A1- and HLA-A*01:01-positive cancers

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Abstract # 834

Introduction

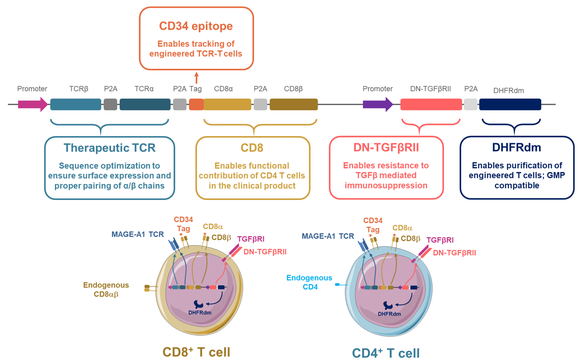
Background: T-Plex is an autologous TCR-T cell therapy product for the potential treatment of solid tumors comprising customized combinations of two to three TCR-T cell components that recognize different tumor antigens presented on specific HLA class I molecules. T-Plex cell therapy products are engineered 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-204-A0101 is intended for the treatment of MAGE-A1- and HLA-A*01:01-positive cancers. The TCR-T cells recognize the cancer-testis antigen MAGE-A1, which is highly expressed in solid tumors but is virtually absent in healthy tissues except testis.

Methods: The TSC-204-A0101 TCR is a naturally occurring TCR discovered using TScan's proprietary ReceptorScan platform. TSC-204-A0101 TCR-T cells engineered using a process representative workflow for the planned manufacturing process were used to investigate the *in vitro* pharmacology and toxicology of TSC-204-A0101. TSC-204-A0101 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 measuring resistance to TGFβ-mediated induction of pSMAD2 of TCR-T cells. Further, TSC-204-A0101 was assessed for risk of alloreactivity and off-target recognition using TScan's SafetyScan screen. Furthermore, to assess the risk of off-target/off-tumor activity, TSC-204-A0101 TCR-T cells were tested for their reactivity to an extensive panel of 53 healthy human primary cells, including those expressing high levels of the putative off-targets of TSC-204-A0101. Finally, to demonstrate the benefit of combining individual TCR-T cell components as part of the T-Plex product for treatment of heterogenous solid tumors, target cancer cell populations were produced to simulate target and/or HLA loss.

Results: TSC-204-A0101 displayed high avidity for the cognate peptide and target-dependent secretion of proinflammatory cytokines, cytotoxicity, and proliferation of both engineered CD4+ and CD8+ T cells and successfully controlled the growth of HLA-A*01:01+ MAGE-A1+ tumors in mice. TGFβ-mediated pSMAD2 induction was strongly decreased in TSC-204-A0101 TCR-T cells demonstrating the resistance of TCR-T cells to immune-suppressive effects of TGFβ. Further, TSC-204-A0101 displayed no alloreactivity to the 110 most common class I HLAs in the U.S. population. Although putative off-targets were identified in the SafetyScan screen, TSC-204-A0101 showed no reactivity to normal primary cells. Lastly, when facing a target cell population simulating heterogeneous target expression, TSC-204-A0101 combined with a second TCR-T cell component in a defined T-Plex product led to the efficient killing of both cell types.

Conclusions: TSC-204-A0101 exhibits high specificity and potency against MAGE-A1- and HLA-A*01:01-positive tumor cells with no projected allo- or off-tumor reactivity. TSC-204-A0101 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 tag, DN-TGFβRII and DHFRdm



Schematic illustration of the TSC-204-A0101 clinical vector. The TCR-T drug product comprises both cytotoxic and helper T cells which have been engineered to express a therapeutic TCR recognizing MAGE-A1+ HLA-A*01:01+ cells. In addition, the TCR-T cells express exogenous CD8 co-receptor, a CD34 tag, DN-TGFβRII and DHFRdm

Additional TScan presentations:

Oral Presentation:

#419: Discovery of Tumor Reactive TCRs and Their Cognate Antigenic Targets via High-Throughput Functional Screening Sat, May 11, 11:15 AM

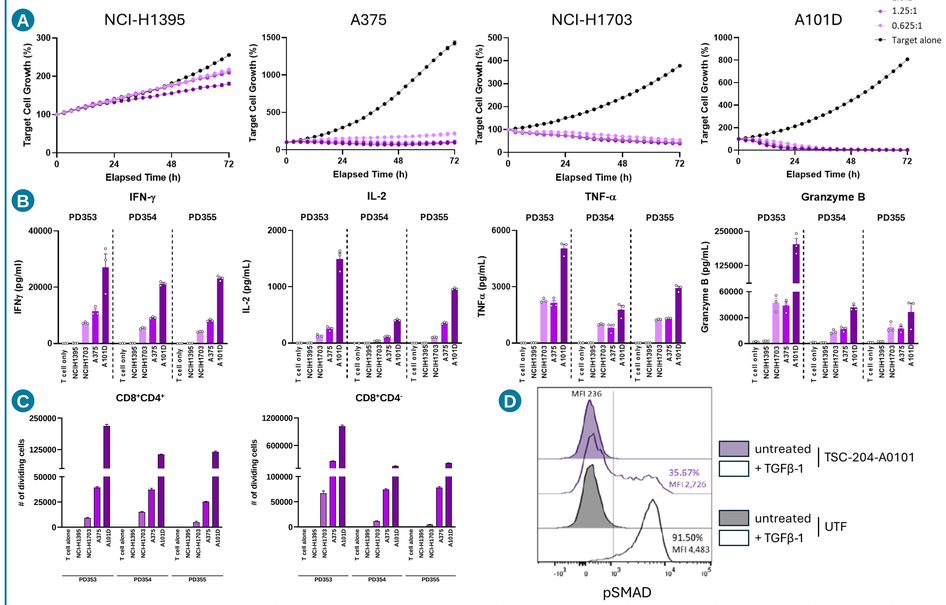
Poster Presentation:

#835 Non-Clinical Development of T-Plex Component TSC-201-B0702: a TCR-T Cell Therapy Directed to a Novel HLA-B*07:02-Restricted MAGE-C2 Epitope for the Treatment of Solid Tumors

#1900: Trial in Progress: A Phase 1, First-in-human Clinical Trial for T-Plex, a Multiplex, Enhanced T Cell Receptor-engineered T Cell therapy (TCR-T) for solid tumors

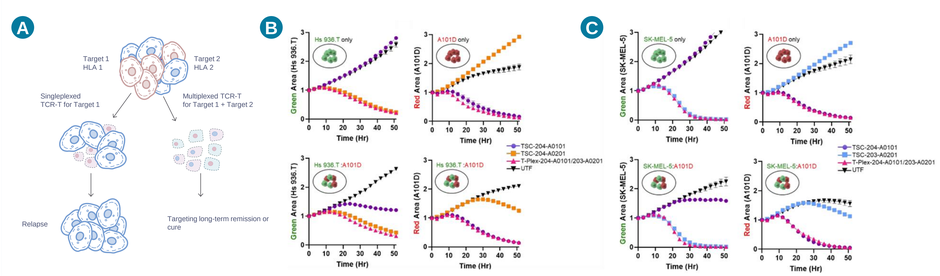
#1901: Trial in Progress: A Phase 1 Trial of TSC-100 and TSC-101, Engineered T Cell Therapies That Target Minor Histocompatibility Antigens to Eliminate Residual Disease After Hematopoietic Cell Transplantation

Functional characterization of TSC-204-A0101 TCR-T cells



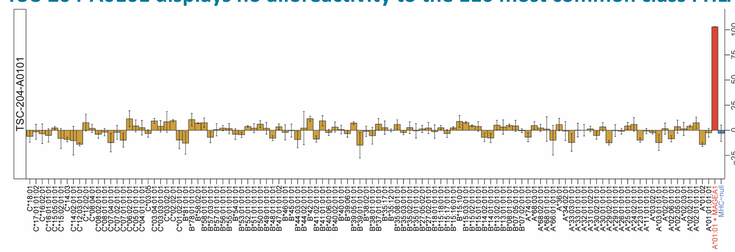
Three batches of process-representative TSC-204-A0101 TCR-T cells (PD353, PD354 and PD355) were assessed for their functionality. **(A)** Cytotoxicity of TSC-204-A0101 TCR-T cells from batch PD355 against indicated target cancer cell lines at indicated E:Ts. **(B)** Cytokine production by TSC-204-A0101 TCR-T cells in response to indicated target cancer cell lines after 24h of coculture. **(C)** Proliferation of TCR+CD34+ cytotoxic and helper TSC-204-A0101 TCR-T cells in response to MAGE-A1+HLA-A*01:01+ cell lines NCI-H1703, A375 and A101D, and MAGE-A1-HLA-A*01:01+ cell line NCI-H1395 over four days. **(D)** TSC-204-A0101 TCR-T cells and their respective donor-matched UTFs were treated with TGFβ-1 to assess for the functionality of the DN-TGFβRII when expressed in TCR-T cells. Histogram analysis overlays consist of phospho-SMAD2 (pSMAD) in untreated CD34 positive-TCR-T cells (dark purple, filled), TGFβ-1 treated CD34 positive-TCR-T cells (dark purple, open), untreated CD34 negative-UTF (black, filled) and TGFβ-1 treated CD34 negative-UTF (black, open).

Combining T-Plex components can overcome target and HLA heterogeneity in solid tumors



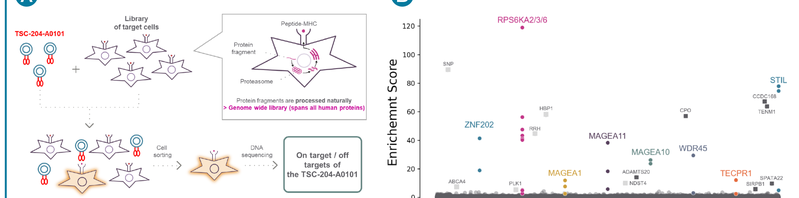
(A) Schematic rationale of addressing heterogeneity with multiplex TCR-T therapy (T-Plex). **(B)** Cytotoxicity of TSC-204-A0101 individually or in combination with TSC-204-A0201 against target cells expressing the same targets (MAGE-A1) on different HLA alleles, HLA-A*01:01 for A101D and HLA-A*02:01 for Hs936T. **(C)** Cytotoxicity of TSC-204-A0101 individually or in combination with TSC-203-A0201 against target cells expressing different targets, MAGE-A1 for A101D and PRAME for SK-MEL-5.

TSC-204-A0101 displays no alloreactivity to the 110 most common class I HLAs



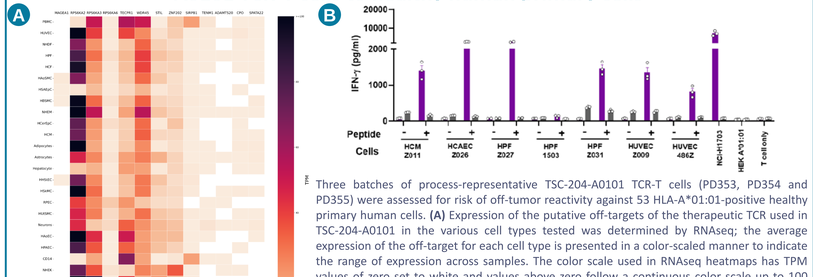
Lentiviral engineered CD8+ T cells expressing the recombinant TSC-204-A0101 TCR were co-cultured with MHC-null HEK293T cells re-expressing one of the 110 most frequently encountered Class I HLAs in the U.S. population for 48hr. A positive control consisting of HEK293T cells expressing a fragment of MAGE-A1 containing the HLA-A*01:01 and HLA-A*01:01 (red) and a negative control consisting of MHC-null 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-204-A0101



(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-204-A0101 identified 12 putative off-targets.

Representative data from SafetyScan shows no apparent reactivity of TSC-204-A0101 TCR-T cells to healthy human primary cells



Three batches of process-representative TSC-204-A0101 TCR-T cells (PD353, PD354 and PD355) were assessed for risk of off-tumor reactivity against 53 HLA-A*01:01-positive healthy primary human cells. **(A)** Expression of the putative off-targets of the therapeutic TCR used in TSC-204-A0101 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. **(B)** Representative results of TSC-204-A0101 TCR-T cells and control UTFs being tested for reactivity to primary cells. IFN-γ was assessed as a measure of TCR-T cell reactivity. Cells pulsed with the MAGE-A1 peptide served as positive control. NCI-H1703 cells were used as a positive control target cell line and HEK A*01:01 as negative control target cell line.