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, CD8 co-receptors, and CD8αβ co-receptors, a CD34 epitope tag, a dominant-negative TGFβRII (DN-TGFβRII), and a stabilized form of dihydrofolate reductase (DHFrdm). TSC-200-A0201 is intended for the treatment of HPV16+ HLA-A*02:01+ cancers. HPV16 is an oncopgenic 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 that are 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. T-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 immune-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 (IC50 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 HLA 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.

Three batches of process-representative TSC-200-A0201 TCR-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 as an E:T ratio 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-DNCD34+ cytotoxic and helper TSC-200-A0201 TCR-T cells in response to HLA-A02:01+HPV16+ cell lines CaSki, SCC090 and SCC152, and HLA-A02:01+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:T ratios. (D) Cytotoxic production by TSC-200-A0201 TCR-T cells in response to indicated target cancer cell lines after 24 hr of coculture. (E) 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 (D752-191-191, D289-191) 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. (F) Levels of secreted IFN-γ were quantified in the supernatant of 24 hr cocultures and normalized to the 0 ng/mL TGFβ condition. (G) 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. (H) 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 169 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.

TScan’s vector co-delivers TCRs/β, CD8αβ, CD34 (Q) purification tag, DN-TGFβRII and DHFrdm

TSC-200-A0201 displays no alloreactivity to the 110 most common class I HLA's

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 controls 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 heatmap has TPM values of zero set to white and values above zero follow a continuous color scale up to 100 TPM.

For more information on TScan’s ImmuneBank or TScan’s clinical trial design and dose-escalation scheme:
- #237: Discovery of a novel MAGE-A1 epitope for TCR-T adoptive cancer therapy from expanded T cell clones of T cell therapy products
- #238: Discovery of MAGE-A1 specific TCR-T cell therapy candidates to expand multiples therapy of solid tumors
- #239: Off-target interactions - clinical trials to prospectively assign patients customized TCR-T cell therapy for Phase 2 trials
- #240: Engineering T Cell Therapies Targeting Minor Homocytotoxicity Antigens to Eliminate Residual Disease after Hematopoietic Cell Transplantation
- #241: Nanoscale TCRs for Highly Efficient T Cell Therapy for Solid Tumors
- #242: Novel Personalized T Cell Therapy Design for T-Plex, a Multiprotein, Enhanced T Cell Receptor-Engineered TCell Therapy for Solid Tumors