# 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

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Abstract # 835

## Introduction

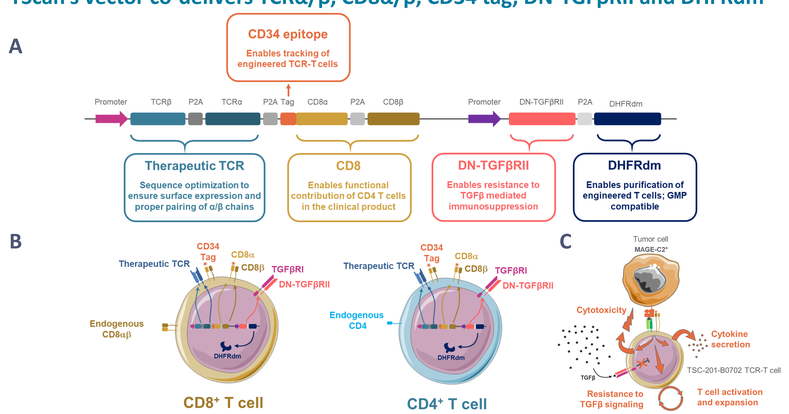
**Background:** T-Plex is an autologous TCR-T cell therapy product comprised of two to three TCR-T cell components for the potential treatment of solid tumors from TScan's ImmunoBank, a repository of therapeutic TCRs each recognizing a different antigen presented by an HLA class I molecule. By combining components from the ImmunoBank, a multiplex product is customized to match the target and HLA expression pattern of a patient's tumor. 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-201-B0702 is a new component of the ImmunoBank designed to recognize an HLA-B*07:02 epitope derived from the cancer-testis antigen MAGE-C2, which is frequently overexpressed in solid tumors but is absent in healthy tissues except testis.

**Methods:** A novel HLA-B*07:02-restricted T cell epitope of MAGE-C2 was discovered with TScan's proprietary TargetScan platform and TScan's ReceptorScan platform was used to identify a potent naturally occurring TCR recognizing this epitope. The MAGE-C2 specific TCR was then used to build TSC-201-B0702. TSC-201-B0702 TCR-T cells were engineered using a full-scale representative workflow for the planned clinical manufacturing process and were used to investigate the *in vitro* pharmacology and safety of TSC-201-B0702. The specificity of TSC-201-B0702 for the HLA-B*07:02 restricted MAGE-C2 epitope was tested, and target-dependent cytotoxicity, proliferation and cytokine secretion was evaluated *in vitro* and *in vivo*. Resistance to TGFβ—conferred by expression of DN-TGFβRII—was evaluated by assessing TGFβ-mediated induction of phospho-SMAD2, and resistance to suppression of IFN-γ secretion. Further, TScan's SafetyScan was used to investigate allo- and off-target reactivity. In addition, the reactivity of TSC-201-B0702 TCR-T cells to a panel of 54 healthy HLA-B*07:02-positive human primary and iPSC-derived cells isolated from tissues that are traditionally assessed in toxicology studies was evaluated.

**Results:** TSC-201-B0702 TCR-T cells recognized their cognate MAGE-C2 peptide in a dose dependent manner and displayed potent target-dependent secretion of inflammatory cytokines, cytotoxicity, and proliferation of both engineered CD4+ and CD8+ T cells. Moreover, TSC-201-B0702 TCR-T cells displayed anti-tumor activity against A101D xenografts in mice. Target-dependent IFN-γ production was maintained in the presence of physiological levels of TGFβ, and TGFβ-mediated phospho-SMAD2 induction was strongly reduced. Further, TSC-201-B0702 displayed no alloreactivity to the 110 most common class I HLAs in the US population. Although two putative off-targets were identified in the SafetyScan screen, TSC-201-B0702 showed no off-tumor reactivity to normal primary or iPSC-derived cells.

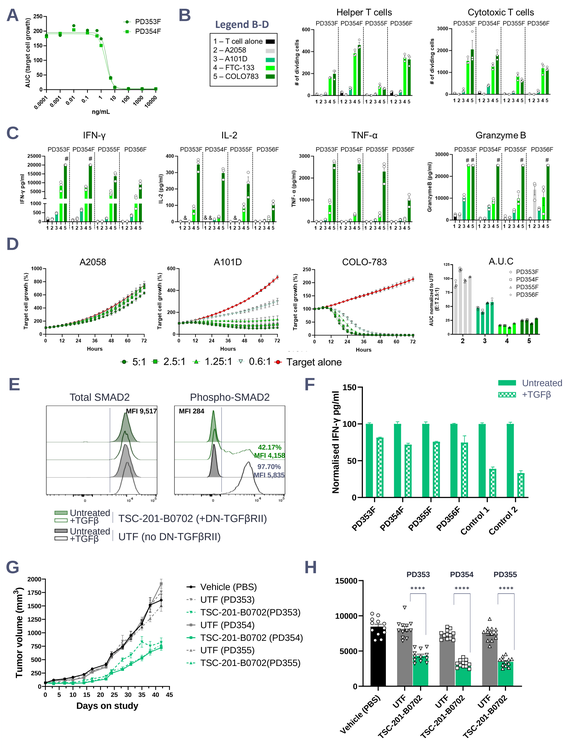
**Conclusions**: TSC-201-B0702 exhibits high specificity and potency against MAGE-C2-positive, HLA-B*07:02 tumor cells with no projected allo- or off-tumor reactivity. TSC-201-B0702 has been cleared by the U.S. FDA for clinical development and has been incorporated in the T-Plex Phase 1 clinical trial master protocol.

## TScan's vector co-delivers TCRα/β, CD8α/β, CD34 tag, DN-TGFβRII and DHFRdm



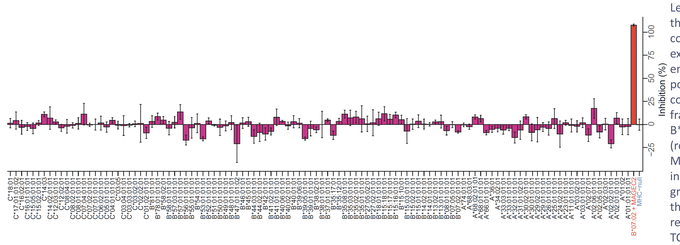

**A** Schematic illustration of the TSC-201-B0702 clinical vector. **B** Graphic depicting the expression of vector elements in TSC-201-B0702 TCR-T cells. The TCR-T drug product comprises both cytotoxic and helper T cells which have been engineered to express the therapeutic TCR recognizing MAGE-C2+ HLA-B*01:01+ cells. In addition, the TCR-T cells express DHFRdm, which confers resistance to methotrexate and enables enrichment of engineered TCR-T cells, as well as an exogenous CD8 co-receptor, a CD34 epitope tag and DN-TGFβRII. **C** Graphic depicting mechanism of action of TSC-201-B0702 TCR-T cells. The transgenic TCR and the CD8αβ co-receptor engage the cognate peptide-MHC, i.e. MAGE-C2-derived peptide presented on HLA-B*07:02, inducing proliferation, cytokine secretion and cytotoxicity in the TCR-T cell. In addition, expression of a DN-TGFβRII confers resistance to TGFβ-mediated immunosuppression.

## Functional characterization of process-representative TSC-201-B0702 TCR-T cells



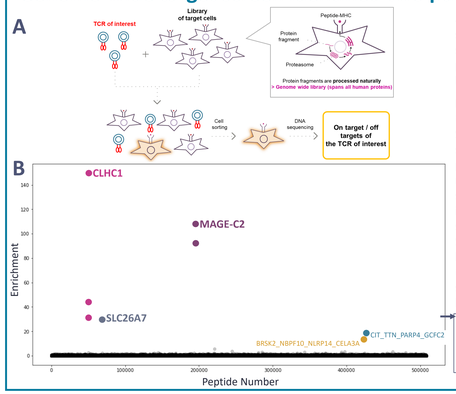

*In vitro* and *in vivo* assays were conducted to evaluate the function of two to four batches of process representative TSC-201-B0702 TCR-T cells (PD353F, PD354F, PD355F, PD356F), i.e., TCR-T cells engineered using a full-scale representative workflow for the planned clinical manufacturing process. **A** TSC-201-B0702 TCR-T cells exhibit peptide dose-dependent cytotoxicity towards T2 cells engineered to express HLA-B*07:02 and pulsed with the cognate peptide (cytotoxicity evaluated over three days with IncuCyte). **B-D** Cancer cells expressing HLA-B*07:02 and MAGE-C2 (A101D, FTC-133, COLO-783) elicited robust target dependent proliferation (**B**), cytokine and Granzyme B secretion (**C**) as well as cytotoxicity (**D**) in process-representative TSC-201-B0702 TCR-T cells. No reactivity was observed to A2058, a cell line reported to be MAGE-C2 negative, HLA*B07:02 positive, but later revealed to express low levels of MAGE-C2 by in-house RNAseq. Donor matched untransfected (UTF) control T cells showed no reactivity to any of the cell lines (data not shown). **B** TCR-T cells were labeled with cell trace violet (CTV) dye and the number of proliferating engineered helper (CD4+) or cytotoxic (CD4-) TCR-T cells (TCRαβ+CD34+CTVdim) was evaluated by flow cytometry after four days of co-culture (E:T 1:1). **C** After 24h coculture with cancer cells (E:T 1:1), cytokine and Granzyme B secretion were evaluated with automated ELISA (ELLA). &, not detected; #, values out of range. **D** IncuCyte-based cytotoxicity assay. Left panels show representative growth curves of cancer cells cocultured for three days with TSC-201-B0702 TCR-T cells at an E:T of 5:1 – 0.6:1; right panel shows area under the curve (A.U.C) normalized to the A.U.C of donor matched UTF control cells (E:T 2.5:1). **E-F** TSC-201-B0702 TCR-T cells are resistant to TGFβ signaling. **E** Total SMAD2 and phospho-SMAD2 levels were evaluated by flow cytometry after stimulation with TGFβ (10 ng/mL). Compared to donor-matched untransfected (UTF) T cells, TSC-201-B0702 TCR-T cells displayed reduced induction of phospho-SMAD2. **F** TCR-T cells were cocultured with two consecutive rounds (24h each) of peptide-pulsed T2 cells +/- TGFβ (5 ng/mL), and IFN-γ secretion was evaluated after the second round of co-culture by ELLA (first round serving to deplete premade IFN-γ mRNA). Compared to control TCR-T cells lacking DN-TGFβRII, TSC-201-B0702 TCR-T cells were less susceptible to TGFβ-mediated inhibition of IFN-γ secretion. **G,H** TSC-201-B0702 TCR-T cells control A101D β2M (stably expressing exogenous β2M protein) tumors in NCG tumor model. Injection of two doses (20E7 each) of TSC-201-B0702 TCR-T cells, but not vehicle or donor-matched UTF T-cells, resulted in slower tumor growth (**G**) and reduced tumor volume (**H**).

## The therapeutic TCR used for TSC-201-B0702 exhibits no alloreactivity to the 110 most common HLA class I alleles of the US population




Lentivirally engineered T cells expressing the therapeutic TCR used in TSC-201-B0702 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 two days. A positive control consisting of HEK293T cells expressing both a fragment of MAGE-C2 containing the HLA-B*07:02-restricted epitope and HLA-B*07:02 (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 target screen identified two putative off-targets for TSC-201-B0702

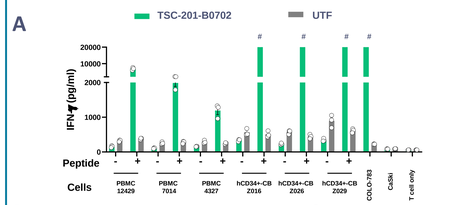


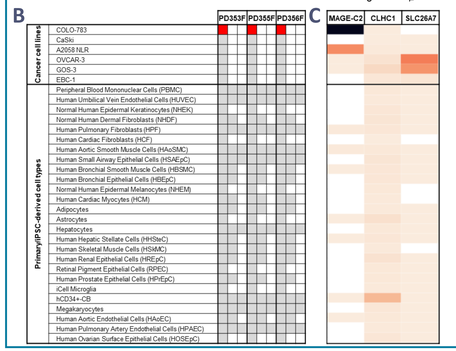

**A** Overview of TScan's proprietary genome-wide SafetyScan screen. TCRs are screened against ~500,000 protein fragments spanning every protein in the entire human proteome to identify possible reactivities, including reactivities with low sequence homology to the natural target.

**B** SafetyScan of TSC-201-B0702 identified two putative off-targets: CLHC1 and SLC26A7. Also detected in the screen are two SNP clones each expressing a unique concatemer of four gene segments that contain a SNP. These clones do not represent potential off- targets for TSC-201-B0702 because each true SNP is represented three times in the peptide library, but only one clone scored in this screen. Further, four separate gene segments are included on one peptide tile generating a unique artificial sequence at the junction of each gene segment. For the enriched SNP tiles, the known peptide derived from MAGE-C2 recognized by TSC-201-B0702 aligns with a region spanning the junction of two gene segments included on the peptide tile. These sequences do not correspond to peptides naturally found in the human proteome.

*Graphical representation of the results of the SafetyScan screens testing the recombinant TCR used in TSC-201-B0702. Plotted is the enrichment score for each of ~500,000 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.*

## TSC-201-B0702 TCR-T cells display no risk of off-tumor reactivity




Three batches of process-representative TSC-201-B0702 TCR-T cells (PD353F, PD355F, and PD356F) were assessed for the risk of off-tumor reactivity.

**A** TSC-201-B0702 TCR-T cells and donor matched untransfected control cells (UTF) were tested for reactivity to various cancer cell lines and primary human cells (shown here: PBMC, CB-CD34+), with IFN-γ production as a readout of the assay. 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 or A2058 as negative control target cell line. #, values > 20000 pg/mL (out of range).

**B** Summary of data from the off-tumor reactivity assay described in panel A. **Top:** TSC-201-B0702 TCR-T cells showed no reactivity to a panel of HLA-B*07:02 positive cancer cell lines expressing the putative off-targets (CHLC1 and SLC26A7). **Bottom:** 54 HLA-B*07:02 positive 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, one to three 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-201-B0702 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.

### 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 Presentations:**

**#834** 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

**#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