Discovery of a MAGE-A4-specific TCR-T therapy candidate for multiplex treatment of solid tumors

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Abstract # 375

Introduction

Background: While T cell receptor (TCR)-engineered T cell (TCR-T) therapy has transformed the landscape of cancer immunotherapy, efficacy and durability of response are often limited by tumor heterogeneity, antigenic escape, and loss of HLA heterozygosity. Treatment of solid tumors with multiple TCR-Ts specific for different antigens and restricted to several human leukocyte antigens (HLAs) is a promising strategy to overcome cancer immune evasion, and potentially to increase the efficacy of TCR-T therapy. MAGE-A4 is expressed in multiple malignancies, including non-small cell lung cancer, colon cancer, and melanoma, with expression in healthy tissue limited to immune-privileged sites [1]. Expression of MAGE-A4 has been additionally associated with poor prognosis in multiple indications [2,3]. In combination with TCR-T candidates currently in TScan's clinical pipeline, development of MAGE-A4-specific TCR-Ts is expected to further advance the effort of treating patients with multiplex TCR-T therapy.

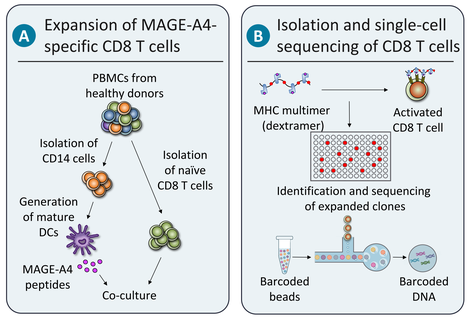
Methods: Using TScan's ReceptorScan platform, we discovered TCRs specific for two A*02:01-restricted MAGE-A4-derived epitopes. The high-throughput screening assay ActivScan was used to select high-expressing and functional TCRs from libraries of MAGE-A4-specific clonotypes, and TCRs were then comprehensively examined for their cytotoxic function using cancer cell lines expressing varying levels of MAGE-A4. Alloreactivity was evaluated by examining reactivity to 110 HLA class I allotypes, and off-target reactivity of lead TCRs was evaluated using our proprietary SafetyScan platform to assess TCR peptide specificity. Safety was additionally confirmed by co-culturing engineered T cells with normal primary human cells. Finally, TCR efficacy was also assessed by transferring engineered T cells into mice implanted with MAGE-A4-expressing xenografts.

Results: ReceptorScan identified 2100+ TCRs specific for two MAGE-A4 epitopes by screening naïve CD8+ T cells from 10 unique healthy donors. Functionally potent TCRs with high avidity were selected using the ActivScan platform. Multiple TCRs displayed efficacy and potency in cytotoxicity assays, as well as in their ability to release cytokine and proliferate in response to antigen. Safety assessment demonstrated on-target peptide specificity by the lead TCR, and no alloreactivity was observed to the 110 allotypes tested or to normal primary human cells. MAGE-A4-specific TCR-T cells also displayed *in vivo* efficacy in reducing tumor burden in xenograft mouse models.

Conclusions: The autologous MAGE-A4-specific TCR-T therapy candidate TSC-202-A0201 has been advanced to IND-enabling studies to be added to TScan's Immunobank of TCRs, with the ultimate goal of being used in a multiplex approach known as T-Plex as a best-in-class immunotherapy strategy for treating patients with solid tumors.

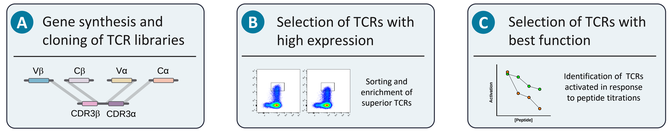
1. Weon JL and Potts PR. The MAGE protein family and cancer. *Curr Opin Cell Biol.* 2015; 37: 1-8.
2. Yoshida N, Abe H, Ohkuri T, Wakita D, Sato M, Noguchi D, *et al*. Expression of the MAGE-A4 and NY-ESO-1 cancer-testis antigens and T cell infiltration in non-small cell lung carcinoma and their prognostic significance. Int J Oncol. 2006; 28(5); 1089-98.
3. Poojary M, Jidhnu PV, Kabekkodu SP. Prognostic Value of Melanoma-Associated Antigen-A (MAGE-A) Gene Expression in Various Human Cancers: A Systematic Review and Meta-analysis of 7428 Patients and 44 Studies. Mol Diagn Ther. 2020; 24(5); 537-555.

ReceptorScan platform identifies over 2100 novel MAGE-A4-specific TCRs



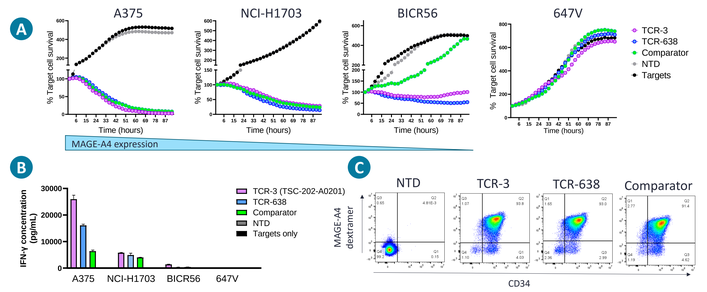
Schematic of the ReceptorScan platform for identification of antigen-specific T cells. **(A)** MAGE-A4-specific T cells were expanded from the naïve CD8+ T cell population of A*02:01-positive healthy donors. Co-culture and expansion of CD8 T cells was performed with autologous mature DCs pulsed with 2 MAGE-A-derived A*02:01-restricted epitopes. **(B)** To isolate antigen-specific CD8 T cells, cocultures were stained with A*02:01 MAGE-A4-specific dextramers and sorted, and single-cell sequencing of dextramer-positive CD8 T cells was performed using the 10X Genomics platform.

ActivScan platform identifies 98 TCRs with high expression and affinity



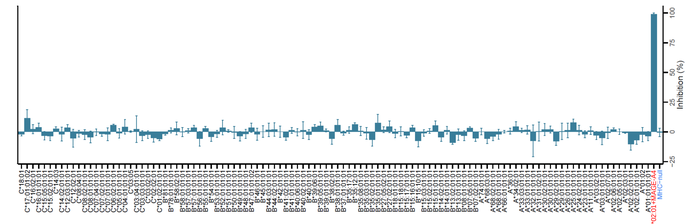
(A) MAGE-A4-specific TCRs identified by the ReceptorScan platform were synthesized using TScan's proprietary PISTACHIO cloning method to generate TCR libraries. **(B)** Selection of TCRs with high expression was performed by lentivirally transducing pan T cells with PISTACHIO-cloned TCR libraries, followed by isolation of dextramer-bound cells. **(C)** Identification of TCRs with high affinity was performed by sorting cells activated by titrations of cognate peptide.

MAGE-A4 TCRs identified by ReceptorScan are specific and potent



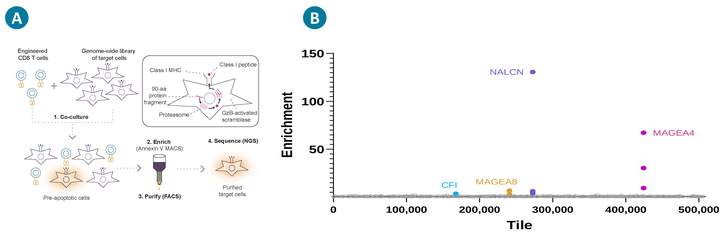
Pan T cells isolated from three HLA-A*02:01-positive healthy donor PBMCs were transduced to express MAGE-A4-specific TCRs, as well as the comparator TCR, and T cells were assessed for functional responses against target cells. **(A)** Cytotoxicity of MAGE-A4 TCRs to HLA-A*02:01$^+$ MAGE-A4$^+$ target cell lines A375, NCI-H1703, and BICR56, and to the HLA-A*02:01$^+$ MAGE-A4-negative control cell line 647V, are shown at an E:T of 2.5:1. **(B)** Production of IFN-γ was measured in co-culture supernatants at 24 h (E:T 1:1). **(C)** Dot plots depict TCR expression, as assessed by A*02:01-restricted MAGE-A4 dextramer staining following lentiviral transduction and TCR enrichment.

No alloreactivity of TSC-202-A0201 TCR to 110 HLA-I allotypes was observed



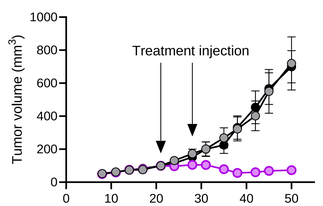
TSC-202-A0201 T cells or untransduced T cells were cocultured with MHC-null HEK293T cells expressing the 110 most frequent Class I MHCs in the US population, with inhibition of target cell growth measured as a readout of alloreactivity. The TSC-202-A0201 TCR displayed ~100% growth inhibition of the A*02:01 MAGE-A4-expressing positive control HEK293T line, while no reactivity to all 110 Class I MHCs was observed.

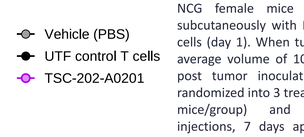
Genome-wide safety screen identifies putative off-targets of TSC-202-A0201 TCR



(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 all possible reactivities, including reactivities with low sequence homology to the natural target. **(B)** *SafetyScan* of TScan's TSC-202-A0201 TCR identifies 4 proteins that, when overexpressed as 90-amino acid long fragments, are recognized by the TCR. The physiological relevance of the 3 potential off-targets is then assessed in detail by co-culturing the TCR-T cells with primary cells that naturally express the full-length proteins at normal levels.

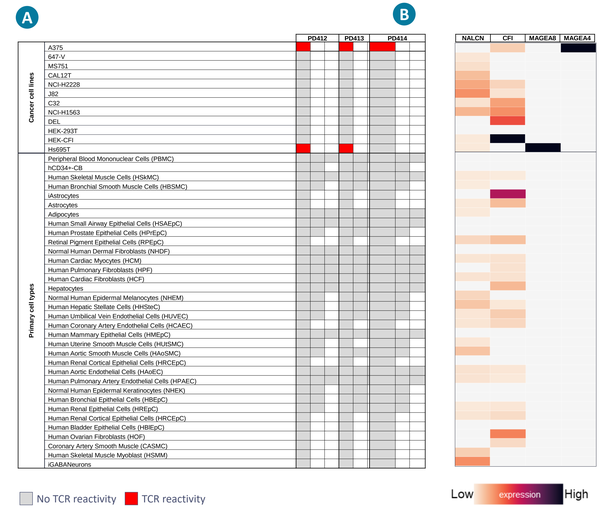
TSC-202-A0201 demonstrates *in vivo* anti-tumor efficacy





NCG female mice were implanted subcutaneously with NCI-H1703 tumor cells (day 1). When tumors reached an average volume of 100 mm3 (21 days post tumor inoculation), mice were randomized into 3 treatment groups (10 mice/group) and received two injections, 7 days apart, of TSC-202-A0201 cells (2e7 viable TCR-T cells), untransfected control T cells from matching donor (UTF), or vehicle (PBS) as a control as indicated by arrow. Tumor volume was monitored biweekly up to day 50 of the study.

TSC-202-A0201 TCR-T cells display no risk of off-tumor reactivity



Three batches of process-representative TSC-202-A0201 TCR-T cells (PD412, PD413, and PD414) were assessed for risk of off-tumor reactivity. **(A)**. **Top:** TSC-202-A0201 TCR-T cells showed no reactivity to a panel of HLA-A*02:01-positive cancer cell lines naturally expressing off-targets, or to HEK293T cells transduced to express physiologically relevant levels of CFI, while reactivity to the MAGE-A8-positive (MAGE-A4-negative) cancer cell line H695T was observed for two TCR-T cell batches. **Bottom:** A panel of 70 HLA-A*02:01-positive normal primary or iPSC-derived human cell samples derived from 34 tissues was tested as targets for TSC-202-A0201 to test off-tumor reactivity. Normal primary human cells included epithelial cells, mesenchymal cells, endothelial cells, fibroblasts, and muscle cells derived from multiple vital and non-vital organs, reproductive and non-reproductive organs, and male and female donors. The data is presented as a tabulated summary of the reactivity of the TCR-T cells as assessed by IFN-γ measurement; each colored cell in the table illustrates a single lot of cells for the indicated cell type. For each cell type, 1-3 lots of cells (i.e. donors) were tested, depending on the availability of the primary cells. Reactivity is indicated in red, lack of reactivity by gray. **(B)** Expression of the putative off-targets of the TSC-202-A0201 therapeutic TCR was determined by RNAseq in the various cell types tested; the average expression of the off-target for each cell type is presented as a heat map to indicate the range of expression across samples. The color scale used in RNAseq heat maps has TPM values of zero set to white, and values above zero follow a continuous color scale up to 100 TPM.

Additional Posters from TScan Therapeutics

 # 359: Preclinical Models for T-Plex, a Customized Multiplexed TCR-T Cell Therapy Addressing Intra-Tumor Antigen and HLA Heterogeneity

 # 384: Development of a Target Agnostic Platform to Assess the Reactivity of T Cell Receptor (TCR)-Engineered T Cell (TCR-T) Therapies to Primary Human Tissues