Discovery of MAGE-A1-specific TCR-T cell therapy candidates to expand multiplex therapy of solid tumors

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Introduction

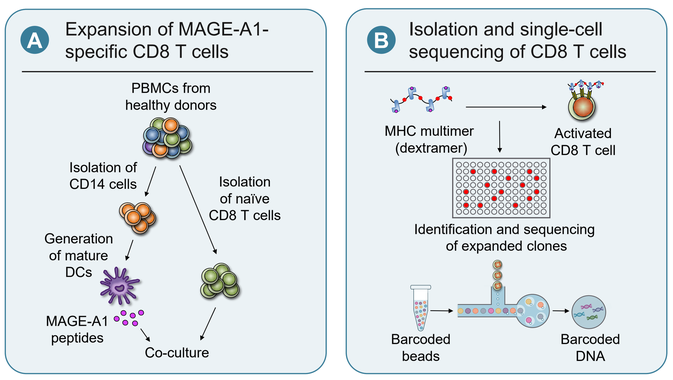
Background: Engineered T cell therapy holds great promise for treating solid tumors. To date, clinical investigations of TCR-T cell therapies have targeted one antigen/HLA at a time and have produced encouraging but partial response rates with limited durations. While heterogeneity of antigen expression is appreciated as a likely driver of patient relapse, the contribution of HLA loss of heterozygosity (LOH), occurring in up to 40% of tumors[1], is only now gaining attention. To address both antigen heterogeneity and HLA LOH requires a collection of TCRs recognizing multiple targets presented on multiple HLAs. MAGE-A1 is a cancer-testes antigen previously identified as the target of expanded tumor infiltrating T-cells using TScan's screening technology[2]. Currently, TScan has two MAGE-A1-TCR-T product candidates, recognizing epitopes on A*02:01 and C*07:02 cleared for clinical development. Here we report discovery and lead selection of a MAGE-A1 TCR recognizing an epitope on A*01:01 (~24% population frequency in the United States).

Methods: We discovered TCRs specific for an A*01:01- restricted MAGE-A1-derived epitope using TScan's proprietary ReceptorScan platform. Using an activation-based screening technology termed ActivScan, we screened a library of MAGE-A1-specific TCRs to select for greatest avidity and expression. These TCRs were functionally characterized using a panel of MAGE-A1 expressing A*01:01-positive cell lines and a xenograft mouse model. Lead TCRs were assessed for potential off-target reactivity using our proprietary SafetyScan platform, which evaluates recognition of antigens from all proteins that comprise the human proteome. Safety was further evaluated by examining alloreactivity to high-frequency Class I HLAs and by testing TCR reactivity to normal primary human cells and cell lines.

Results: ReceptorScan identified 1181 TCRs specific for the MAGE-A1 A*01:01 epitope. Following selection of high-expressing and high avidity MAGE-A1-specific TCRs in ActivScan, 14 TCRs were evaluated for their cytotoxic function, and 5 TCRs compared favorably to a clinical-stage benchmark TCR for cytotoxicity and cytokine release. Safety assessment demonstrated that few putative off-target peptides were recognized, no alloreactivity was observed to 110 allotypes tested, and no reactivity to target-negative cell lines was observed.

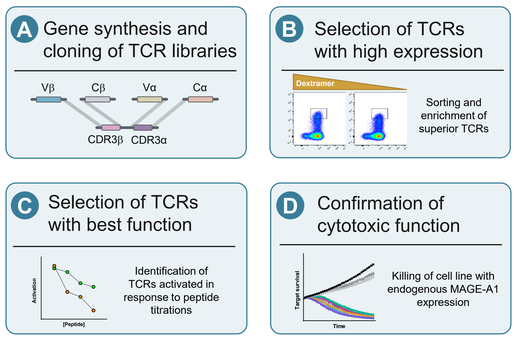
Conclusions: A novel HLA-A*01:01 restricted TCR-T cell therapy candidate has advanced to pre-clinical studies. Addition of this product to TScan's ImmunoBank (repository of therapeutic TCRs) could extend MAGE-A1 TCR-T therapy for solid tumors on three different HLA alleles, potentially expanding therapeutic application to up to ~70% of the patient population (with MAGE-A1+ tumors). Importantly, this creates a unique opportunity to simultaneously target tumor antigens presented on HLA alleles on *different* chromosomes, thus circumventing tumor evasion by HLA LOH with the goal of improving patient responses.

ReceptorScan platform identified 1181 novel MAGE-A1 TCRs



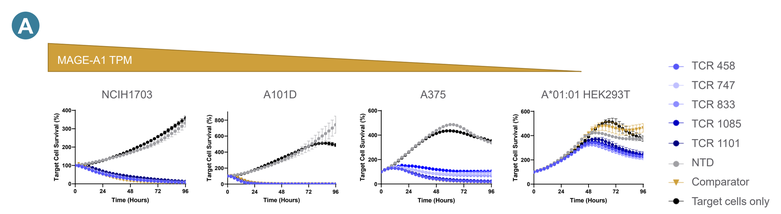
Schematic representation of the ReceptorScan platform for identification and selection of antigen-specific T cells. **(A)** MAGE-A1-specific T cells were expanded from the naïve CD8 T cell population of A*01:01-positive healthy donors. Co-culture and expansion of naïve CD8 T cells was performed with autologous mature dendritic cells (DCs) pulsed with a MAGE-A1-derived A*01:01-restricted epitope. **(B)** To isolate antigen-specific CD8 T cells, co-cultures were stained with DNA-barcoded A*01:01 MAGE-A1-specific dextramers and sorted, and single-cell sequencing of dextramer-positive CD8 T cells was performed using the 10X Genomics platform.

ActivScan identified 14 TCRs with high expression and affinity



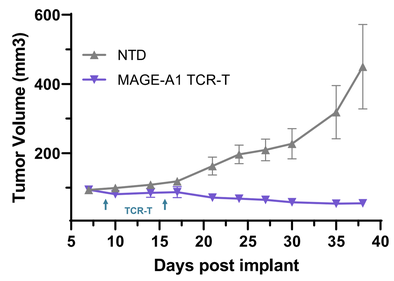
(A) Libraries for MAGE-A1-specific TCRs identified by the ReceptorScan platform were synthesized using TScan's proprietary PISTACHIO cloning method. **(B)** Selection of TCRs with high expression was performed by transducing pan T cells with viruses encoding PISTACHIO-cloned TCR libraries, followed by isolation of dextramer-bound cells. **(C)** Identification of TCRs with high affinity was performed using ActivScan by sorting cells that responded to titrations of peptide. **(D)** 14 TCRs displayed cytotoxicity towards a cell line endogenously expressing MAGE-A1, similar to a comparator TCR.

MAGE-A1 TCRs show specificity and functionality on target cells



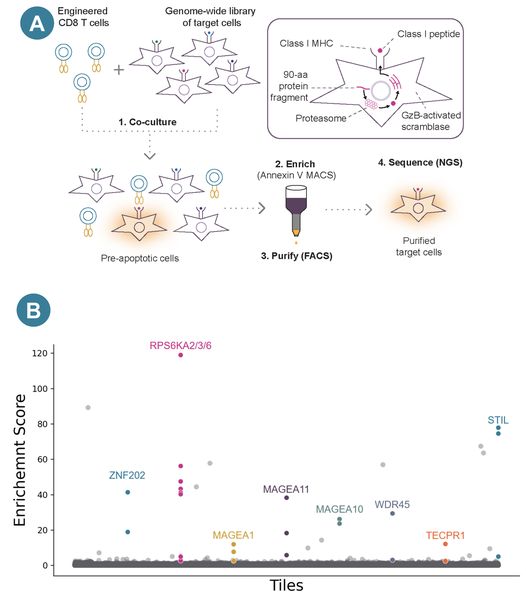
Pan T cells isolated from healthy donor PBMCs were transduced to express MAGE-A1$_{161-169}$-specific TCRs, as well as the comparator TCR, and T cells were assessed for functional responses against target cells. Results for the top 5 candidate TCRs are shown. **(A)** Cytotoxicity of MAGEA1 TCRs to HLA-A*01:01$^+$ MAGE-A1$^+$ target cell lines NCIH1703, A101D, A375 and to the HLA-A*01:01$^+$ HEK293T MAGEA1-negative control cell line (E:T 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*01:01-restricted MAGE-A1$_{161-169}$ dextramer staining. Comparator TCR recognizes MAGE-A1 on a different HLA.

MAGE-A1 TCR demonstrates anti-tumor efficacy *In Vivo*



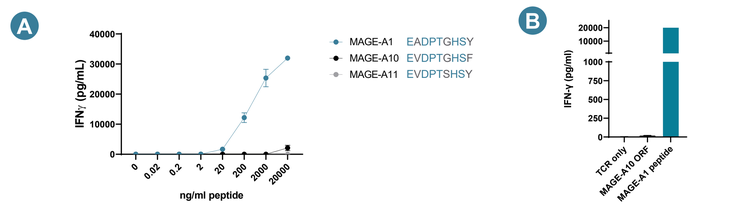
Immunodeficient NCG mice were implanted subcutaneously with 2x10^6 NCI H1703 cells in the right flank (n=7 mice/group). Tumor volume was monitored from day 7 to day 38 post implantation. On day 9 and 16, mice were injected with 2x10^7 MAGE-A1 TCR expressing T cells or with non-transduced donor control T-cells (NTD).

Genome-wide safety screen identified putative off-targets of lead MAGE-A1 TCR



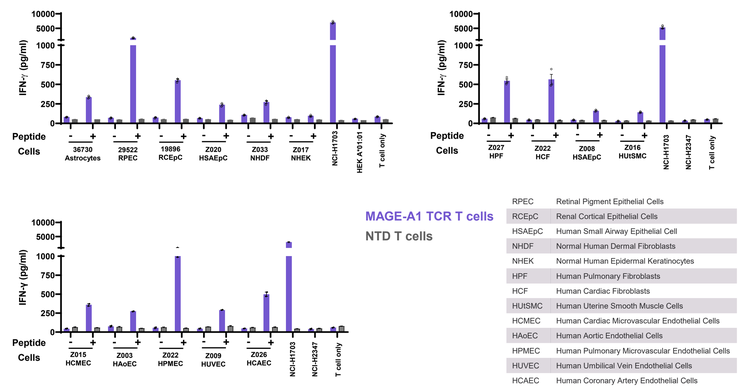
(A) Overview of TScan's proprietary genome-wide *SafetyScan* screen. TCRs are screened against >500,000 protein fragments spanning every protein in the entire human proteome to identify possible reactivities, including reactivities with low sequence homology to the natural target. **(B)** *SafetyScan* of TScan's lead MAGE-A1 TCR identifies 9 proteins other than MAGEA1 that, when overexpressed as 90-amino acid long fragments, are recognized by the TCR on multiple tiles. The physiological relevance of these 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.

MAGE-A1 TCR does not recognize MAGE-A10 or MAGE-A11



(A) Indicated peptides were pulsed at a dilution series in A*01:01 HEK293T reporter cells or **(B)** The full-length MAGEA10 ORF was expressed in A*01:01 HEK293T cells. IFN-γ was measured from the supernatants after 24h of co-culture with T cells expressing the MAGE-A1 TCR.

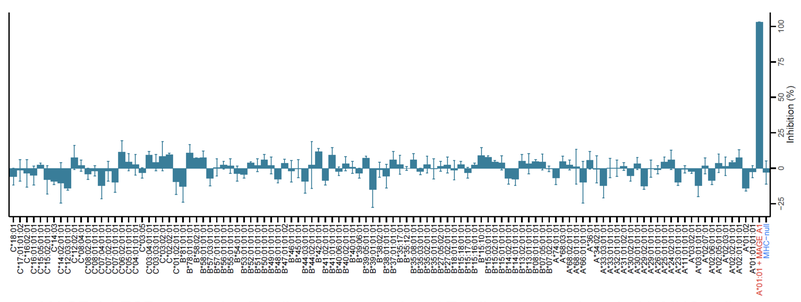
MAGE-A1 TCR showed no reactivity to healthy primary cells



RPEC	Retinal Pigment Epithelial Cells
RCEpC	Renal Cortical Epithelial Cells
HSAEpC	Human Small Airway Epithelial Cell
NHDF	Normal Human Dermal Fibroblasts
NHEK	Normal Human Epidermal Keratinocytes
HPF	Human Pulmonary Fibroblasts
HCF	Human Cardiac Fibroblasts
HUISMC	Human Uterine Smooth Muscle Cells
HCMEC	Human Cardiac Microvascular Endothelial Cells
HAoEC	Human Aortic Endothelial Cells
HPMEC	Human Pulmonary Microvascular Endothelial Cells
HUVEC	Human Umbilical Vein Endothelial Cells
HCAEC	Human Coronary Artery Endothelial Cells

The MAGE-A1 TCR was tested for reactivity to primary cells derived from healthy HLA-A*01:01+ tissues that naturally express the putative off-targets identified in the safety screen. Target cells were pulsed with the MAGE-A1 (EADPTGHSY) peptide or left unpulsed, and co-cultured with MAGE-A1 TCR-expressing or non-transduced (NTD) pan-T cells. IFN-γ was measured in co-culture supernatants after 24 h (E:T ~2:1). NCI-H1703 and A*01:01 HEK cells were used as positive and negative controls respectively.

MAGE-A1 TCR showed no alloreactivity to 110/110 HLA types



MAGE-A1 TCR-expressing T cells or non-transduced T cells were cocultured with MHC-null HEK293T cells engineered to express the 110 most frequently-encountered Class I MHCs in the US population; inhibition of target cell growth was measured as a readout of alloreactivity.

References

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 # 357: Discovery of a novel MAGEC2 epitope for TCR-T adoptive cell therapy from expanded T cell clones of TIL therapy products

 # 364: 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

 # 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