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 apportioned as a likely driver of patient response, the contribution of HLA loss of heterozygosity (LOH), occurring in up to 40% of tumors, is only now gaining attention. To address both antigen heterogeneity and HLA LOH, a collection of TCRs recognizing multiple targets presented on multipleHLAs. MAGE-A1 is a cancer-testes antigen previously identified as the target of expanded tumor infiltrating T-cells using TScan’s screening technology. Currently, TScan has two MAGE-A1 TCR-T product candidates on an N=101/02 phase 1 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-deipded 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. SafetyScan 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 specificity and functionality.

Conclusions: A novel HLA-A*01:01 restricted TCR-T cell therapy candidate has advanced to preclinical studies. Addition of this product to TScan’s ImmunoBank (repository of therapeutic TCRs) could extend MAGE-A1 TCR therapy for solid tumors on three different HLA alleles, potentially expanding therapeutic application up to ~24% of the patient population (with MAGE-A1+ tumors). Importantly, this creates a unique opportunity to simultaneously target tumor antigens presented on HLAs across different chromosomes, thus circumventing tumor evasion by HLA LOH with the goal of improving patient responses.

References

#337: Discovery of a novel MAGE2 epitope for TCR-T adoptive cell therapy from expanded T cell clones of TIL therapy products

#354: Non-clinical development of T-Plan component TSC-200-A0201 - A novel 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 matched TCR-T cell therapy in Phase

#632: Phase 1 trial of TSC-102 and TSC-101, engineered T cell therapies targeting minor histocompatibility antigens to eliminate residual disease after hematopoietic cell transplantation

#793: Product characteristics and clinical trial design for T-Plan, a multiplexed, enhanced T cell receptor-engineered T cell therapy for solid tumors


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MAGE-A1 TCRs show specificity and functionality on target cells

Pan T cells isolated from healthy donor PBMCs were transduced to express MAGE-A1-specific, A*01:01 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 MAGE-A1 TCRs to HLA-A*01:01-MAGE-A1 target cell line NCI-H1703, A101D, A375 and to the HLA-A*01:01 HEK290T MAGE-A1-negative control cell line (ET-5:1). (B) Production of IFNγ was measured in co-culture supernatants after 24 h (ET-1:1). (C) Dot plots depict TCR expression, as assessed by A*01:01-restricted MAGE-A1 TCR staining. Comparator TCR recognizes MAGE-A1 on a different HLA.

MAGE-A1 TCR showed no reactivity to healthy primary 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. IFNγ was measured in co-culture supernatants after 24 h (ET ~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

Inhibition of target cell growth was measured as a readout of alloreactivity. MAGE-A1 TCR-expressing T cells or non-transduced T cells were cocultured with MHC-null HEK290T 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.

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 negative off-targets. This is then followed by co-culturing the TCR-T cells with primary cells that naturally express the full-length proteins at normal levels.