Multiplexed TCR-T Cell Therapy Targeting MAGE-A1 and PRAME Enhances The Activity of Adoptive T Cell Therapy in Pre-Clinical Models

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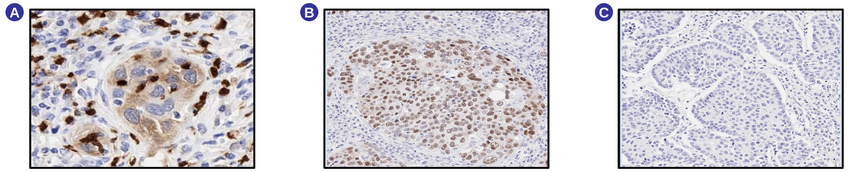
Background Adoptive Cell Transfer with genetically engineered T cells holds great promise for treating solid tumors. To date, clinical investigations of TCR-T cell therapies have targeted one antigen at a time and have produced encouraging response rates ranging from 30-50%. Unfortunately, complete responses have been rare, and responses are often short-lived. One possible reason why patients rapidly relapse is that their tumors exhibit substantial heterogeneity of antigen expression: not every cancer cell within a tumor expresses the target of a mono TCR therapy and, even when they do, the target is expressed at variable levels among the individual tumor cells. This suggests that TCR-T targeting one antigen could allow the cells lacking the treated antigen to escape and drive relapse.

TScan Approach To address antigen heterogeneity, we are developing multiplexed TCR-T cell therapy in which a patient is treated with multiple TCR-T cell products, chosen from a collection of highly active TCRs and matched to the patient's tumor antigens and HLA type. One of these antigens, MAGE-A1, was identified as the target of expanded tumor infiltrating T-cells from a head & neck cancer patient using TScan's screening technology. The other one, PRAME, is highly expressed in a variety of cancers. Using our ReceptorScan platform, we developed two high affinity TCRs that recognize HLA-A*02:01-restricted epitopes from MAGE-A1 and PRAME and assessed the benefits of combining these two TCR-T cell products using a variety of pre-clinical models.

Results Multiplexed TCR-T mimics the natural oligoclonal T cell response to cancer and provides a way to address solid tumor heterogeneity. Individually, both TCRs show strong cytotoxic activity in vitro when co-cultured with HLA-matched cancer cell lines expressing endogenous MAGE-A1 and PRAME. Additionally, in xenograft mouse models, each TCR was able to control the growth of tumors expressing their cognate antigens. To test whether the two TCRs exhibit additive or synergistic activity, a mixture of two different cell lines expressing either MAGE-A1 or PRAME were grown as xenograft tumors in mice, mimicking the observed heterogeneity of these targets in human tumors. Notably, when treated with multiplexed MAGE-A1/PRAME TCR-T, the mice achieved longer lasting tumor control compared to either singleplexed treatment alone. These findings support the hypothesis that multiplexed TCR-T has the potential to overcome antigen heterogeneity, which may contribute to the observed lack of durability in clinical trials of singleplexed TCR-T therapy.

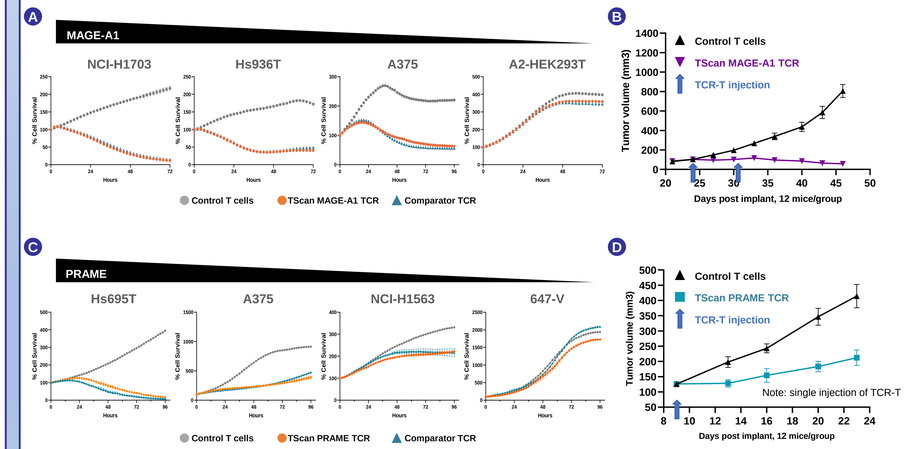
Clinical application To address solid tumor heterogeneity in the clinic, we are building an ImmunoBank of therapeutic TCRs that recognize different targets presented on different HLA alleles. We submit that selecting multiplexed TCR-Ts that target intact antigens and HLA alleles in patient tumors should synergistically overcome solid tumor heterogeneity, and we are designing trials to test this hypothesis clinically.

Heterogeneity of target expression is a clinical challenge for TCR-T cell therapy



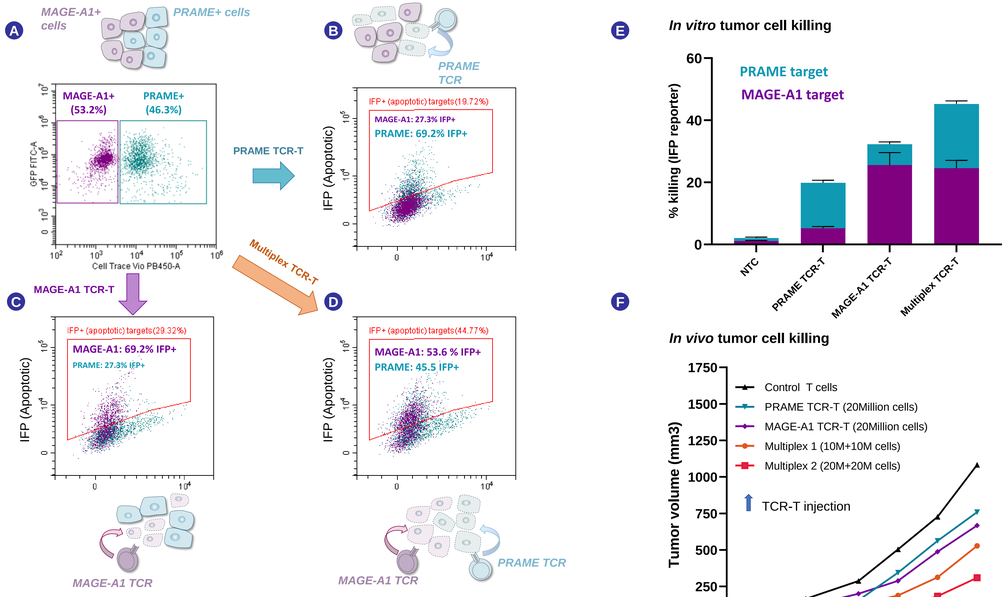
Examples of variable antigen expression in human NSCLC tumor samples. Immunohistochemistry was performed on human NSCLC tumor microarrays using MAGE-A1- and PRAME-specific antibodies. Heterogenous antigen expression within the tumor was observed in multiple sections as represented for MAGE-A1 **(A)** and PRAME **(B)** with variable degrees of expression. Unstained tumor control shown in **(C)**.

TScan's HLA-A*02:01-restricted MAGE-A1- and PRAME-specific TCRs control tumor cell growth

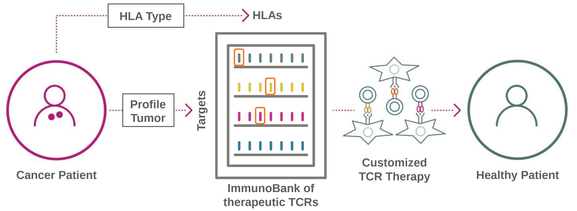


Nuclight Red-labeled HLA-A*02:01$^+$ cancer cell lines with varying levels of endogenous MAGE-A1 **(A)** and PRAME **(C)** were grown in the presence of TScan's MAGE-A1- and PRAME-specific TCR-T cells, respectively. TCR-T-mediated cytotoxicity was assessed using Incucyte® over a period of three days. NCG immunodeficient mice (Charles River) were implanted subcutaneously with human HLA-A*02:01$^+$ tumor cell lines with endogenous expression of MAGE-A1 (U266B1) **(B)** or PRAME (Hs695T) **(D)**. Once the tumors reached 100 mm^3, 20 million cognate TCR-T cells were administered twice for MAGE-A1 and once for PRAME on the indicated days.

Multiplexing with two TCR-Ts, *in vitro* and *in vivo*, reduces tumor cell evasion driven by antigen heterogeneity



(A) Modeling intra-tumor target heterogeneity using two different HLA-A*02:01$^+$ HEK293T cell lines. 1:1 ratio of GFP-labeled MAGE-A1+ cells and GFP/CTV double-labeled PRAME+ cells were incubated with T cells expressing a PRAME-specific TCR **(B)**, a MAGE-A1-specific TCR **(C)**, or multiplexed TCR-T targeting both antigens **(D)**. Target cell death was measured using a Granzyme-activated IFP reporter and summarized in **(E)**. **(F)** NCG immunodeficient mice (Charles River) were implanted with the same tumor cell combination as shown in **(A)** and treated with either singleplex or multiplex TCR-T cells.



TScan is building **customized, multiplexed TCR-T cell therapies** for solid tumors. For each patient, two or three therapeutic TCRs will be selected from the ImmunoBank that best match the targets and HLAs expressed in their tumors. By **multiplexing** across both targets and HLAs, this strategy is designed to **prevent resistance** arising from either target loss or HLA loss.