Multiplexed TCR-T Therapy: A Strategy to Enhance the Efficacy of Engineered Adoptive Cell Therapy

Yifan Wang, Jenny Tadros, Nancy Nabilsi, Andrew Ferretti, Karl Beutner*, Ariane Lozachmeur*, Qidi Yang*, Sonal Jangalwe, Dalena Nguyen, Mollie Jurewicz, Ribhu Nayar, Amy Virbasius, Qikai Xu, Shrikanta Chattopadhyay, Cagan Gurer, Gavin MacBeath

TScan Therapeutics, Waltham, MA

***Tempus, Chicago, IL**

Abstract

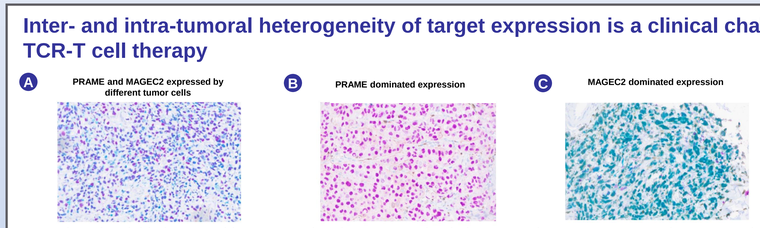
Introduction Adoptive Cell Transfer with genetically engineered T cells holds great promise for treating solid tumors. To date, clinical investigations of TCR-T cell therapies have targeted one antigen at a time and have produced encouraging response rates ranging from 30-50%. Unfortunately, complete responses have been rare, and responses are often short-lived. We submit that there are two main challenges associated with single-antigen TCR-T, both related to solid tumor heterogeneity.

Solid tumor heterogeneity First, expression of most cancer associated antigens is heterogeneous. We performed multiplexed IHC with MAGE-C2 and PRAME, two cancer germline antigens, and observed considerable heterogeneity across samples from different solid tumor types. Additionally, heterogenous antigen expression was observed at the single cell level. This indicates that a single TCR would not be sufficient to eliminate all cancer cells within a tumor, thereby allowing the cells lacking the treated antigen to escape and drive relapse. Second, single agent TCR-T targets only a single HLA allele, which is subject to loss through commonly-observed loss-of-heterozygosity (LOH) mechanisms. In a set of several hundred non-small cell lung cancer samples, we observed clonal LOH of HLA A*02:01 in ~15% of samples and subclonal LOH in an additional ~25% of samples.

Multiplexed TCR-T Multiplexed TCR-T mimics the natural oligoclonal T cell response to cancer and provides a way to address solid tumor heterogeneity. To test this concept experimentally, we first tested multiplexing two high-affinity TCR-Ts, one targeting an HLA-A*02:01-restricted epitope of HPV16-E7 and the second targeting an HLA-C*07:02-restricted epitope of MAGE-A1. Target cells were a mixture of two cell lines, each expressing only one of the two antigens. Whereas individual TCR-Ts caused ~50-60% cell killing at 72 h, a 1:1 mix of the two TCR-Ts resulted in ~80% cell killing at the same overall effector to target (E:T) ratio, indicating a synergistic effect. We next tested multiplexing a high-affinity TCR-T for MAGE-A1 with a low-affinity TCR-T for MAGE-C2. The target cells were a mixture of MAGE-A1- and MAGE-C2-expressing cells. Although the MAGE-C2 TCR-T alone displayed partial killing of MAGE-C2-positive cells, addition of the MAGE-A1 TCR-T enhanced the activity of the MAGE-C2 TCR-T. Using a transwell culturing system, we found that cytokines secreted by the MAGE-A1 TCR-Ts strongly enhanced T cell activation of the MAGE-C2 TCR-T cells. These findings support the hypothesis that multiplexed TCR-T has the potential to overcome antigen heterogeneity not only through independent targeting of different cancer cell populations, but also by cytokine-mediated T cell enhancement.

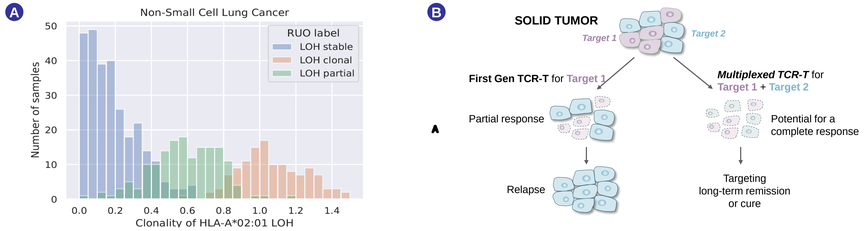
Clinical application To address solid tumor heterogeneity in the clinic, we have designed a screening strategy to test patient tumors for antigen positivity and HLA LOH. We are also building an ImmunoBank of therapeutic TCRs that recognize different targets presented on different HLA alleles. We submit 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.

Inter- and intra-tumoral heterogeneity of target expression is a clinical challenge for TCR-T cell therapy



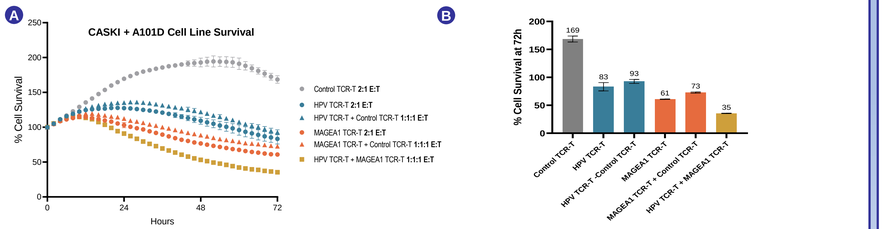
Examples of variable inter- and intra-tumoral antigen expression in human melanoma tumor samples. Immunohistochemistry was performed on human melanoma tumor microarrays using PRAME- (pink) and MAGEC2- (blue) specific antibodies. Heterogenous antigen expression within the tumor was observed in multiple sections as represented in **(A)** as well as sections dominated by the presence of a single antigen **(B and C)** with variable degrees of expression.

HLA Loss of Heterozygosity (LOH) is common, highlights need for multiplexed TCR-T



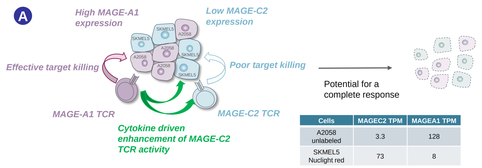
(A) HLA loss of heterozygosity (LOH) analysis of non-small cell lung cancer samples shows the wide prevalence of clonal and partial LOH of the HLA-A*02:01 allele. **(B)** Monotherapy TCR-T frequently leads to partial responses and rapid relapse, partly due to target antigen or HLA heterogeneity. To address this, we developed a multiplexing approach to improve long-term remission.

TScan's multiplexing approach has the potential to overcome target heterogeneity

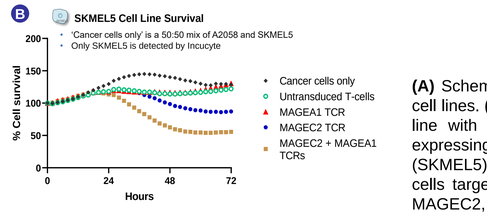


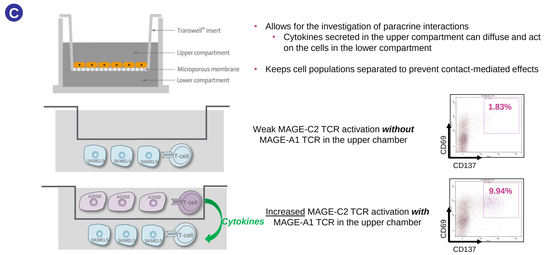
(A) Nuclight Red-labeled HPV+ (Caski) and MAGEA1+ (A101D) cell lines were grown in the presence of HPV16 E7-TCR-T, MAGEA1-TCR-T, or a combination of both TCR-Ts. Cell growth was assessed using Incucyte® over a period of three days. **(B)** Synergistic cytotoxicity was observed between the two TCRs as calculated by % Cell Survival at 72 hours.

Multiplexing TCR-Ts has synergistic anti-tumor activity due to cytokine-mediated enhancement



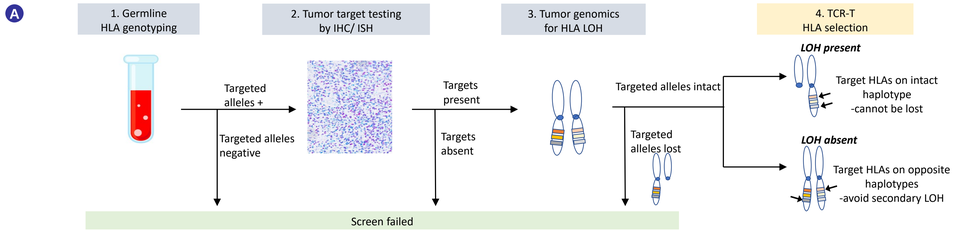
Cells	MAGEC2 TPM	MAGEA1 TPM
A2058 unlabeled	3.3	128
SKMEL5 Nuclight red	73	8

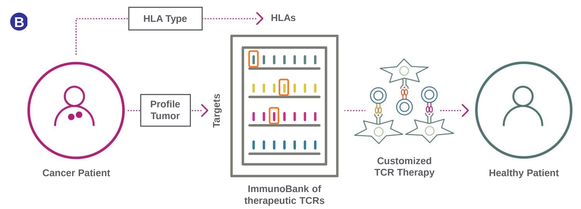




(A) Schematic of modeling intra-tumor target expression variability using two different cell lines. **(B)** Co-culture of T-cells expressing a MAGEA1-specific TCR and a target cell line with high MAGEA1 expression (A2058) enhances the cytotoxicity of T cells expressing a MAGEC2 TCR against a cell line with moderate MAGEC2 expression (SKMEL5). **(C)** The increase in cytotoxicity is driven by soluble factors secreted by the T cells targeting MAGEA1, which leads to increased activation of the T cells targeting MAGEC2, as shown in the Transwell experiment.

Screening strategy to select patients and TCR-Ts; ImmunoBank enables customized multiplexing of TCR-Ts





(A) Screening strategy to select patients and TCR-Ts for multiplexed TCR-T therapy. Following germline HLA genotyping, patient tumors are assessed for target expression by immunohistochemistry (IHC) or RNA in situ hybridization (ISH). Tumor samples are also assessed for HLA LOH by genomic sequencing. If LOH is observed, TCR-Ts are chosen that target 2 different HLAs on the intact chromosome arm. If LOH is not observed, TCR-Ts are chosen that target HLAs on opposite chromosomes. **(B)** Customized TCR-T therapies for individual cancer patients require the building of an ImmunoBank of therapeutic TCRs recognizing different targets (in rows) presented on different HLA alleles (in columns). By multiplexing across both targets and HLAs, this strategy is designed to prevent resistance arising from either target loss or HLA LOH.