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

**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 responses rates ranging from 30-50%. Unfortunately, complete responses have been rare, and responses are often short lived. We submit that there are two major challenges associated with single antigen TCR-T; both related to solid tumor heterogeneity.

Patient heterogeneity

First, expression of most cancer associated antigens is heterogeneous. We performed multiplexed IC with MAGE-A1, and PRAME, two cancer germinant antigens, and observed considerable variation in expression across multiple tumors. Heterogenous antigen expression was observed at the single cell level. This indicates that a single TCR would not be sufficient to eradicate 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 could result in loss of expression due to LOH. In a set of several hundred non-small lung cancer samples, we observed clinical 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 TCRs, one targeting an HLA-A*01:01 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, 1 in 6 mix of the dual TCR-Ts resulted in ~100% cell killing at the same effector to target (E:T) ratio, indicating a synergetic effect. We next tested multiplexing a high-affinity TCR for MAGE-A1 with a low-affinity TCR 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 culture 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-Ts have 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 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 improve therapeutic efficacy against solid tumor heterogeneity, and we are designing trials to test this hypothesis clinically.

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

Examples of variable inter- and intratumor antigen expression in human melanoma tumor samples. Immunohistochemistry was performed on tissue sections from melanoma 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.

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

(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) enhanced the cytotoxicity of T-cells expressing a MAGEC2 TCR against a cell line with moderate MAGEC2 expression (SKMEL). (C) The increase in cytokine secretion is driven by soluble factors secreted by target cells targeting MAGEC1, which leads to increased multiplexing 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 TCRs**

Using the ImmunoBank, we have designed a screening strategy to select patient tumors and TCR-Ts to maximize therapeutic efficacy. First, potential patients are assessed for target expression by immunohistochemistry (IHC) or RNA in situ hybridization (ISH). Tumor samples are assessed for HLA LOH by genomic sequencing. If LOH is observed, TCR-Ts are chosen that target different HLA alleles. If LOH is not observed, TCR-Ts are chosen that target multiple HLA alleles. Clinical trials assess for therapeutic efficacy for individual cancer patients. Results show that the ImmunoBank has enhanced the therapeutic potential of TCR-Ts recognizing different target antigens (on various) presented on different HLA alleles (in columns). By multiplexing both TCRs, we are able to prevent resistance arising from either target loss or HLA LOH.

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

(A) Multiplexed TCRs-MAGEA1 TCR and MAGEC2 TCR in different solid tumors. (B) Cytokine driven T cell therapy contributes to complete tumor regression. (C) Potential for a prolonged effect following treatment with TCR-Ts. (D) Tumor regression is observed even in the absence of complete TCR-T activity.