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# Preclinical Models for T-Plex, a Customized Multiplexed TCR-T Cell Therapy Addressing Intra-Tumor Antigen and HLA Heterogeneity

TSCAN THERAPEUTICS

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Abstract #359

## Introduction

**Background:** Intratumor heterogeneity represents a major challenge to cancer therapies, including TCR-T therapy. Not every cell in a tumor expresses a given tumor-associated protein. Tumor cells lacking the targeted protein will be resistant to a single TCR-T therapy and may inevitably contribute to relapse. Further, loss of heterozygosity at the human leukocyte antigen (HLA) locus can render cells resistant to a TCR-T therapy restricted to the affected HLA.

To address these challenges, TScan is building an ImmunoBank of therapeutic TCRs recognizing different target antigens presented on diverse HLA alleles. Two to three TCRs targeting relevant antigens and intact HLA alleles in patients are selected to build customized T-Plex products addressing target and HLA heterogeneity.

**Method:** TScan's growing ImmunoBank currently contains TCRs targeting epitopes derived from the cancer testis antigens MAGE-A1, PRAME, MAGE-C2, or the oncoviral HPV E7 protein and can address multiple HLAs including HLA-A*02:01, HLA-A*01:01, HLA-B*07:02, and HLA-C*07:02.

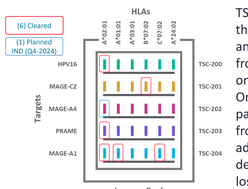
To demonstrate the benefit of T-Plex, we conducted both *in vitro* and *in vivo* studies where mixtures of cancer cell lines served as models of heterogeneous cancers. The efficacy of TCR-T singleplex or multiplex therapies were evaluated to test whether two therapeutic TCRs in T-Plex products exhibit additive or synergistic activities. Multiple scenarios of T-Plex products were evaluated to target a given antigen on different HLAs, two antigens on a shared HLA, or two antigens presented on distinct HLAs.

**Results:** Singleplex TCR-T cell products only addressed the subset of cancer cells presenting the targeted peptide/HLAs. On the other hand, T-Plex products were consistently able to broaden the cytotoxic activity by targeting all tumor cells.

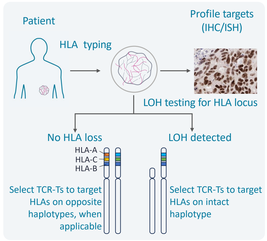
While each TCR-T component was selectively targeting its relevant target cancer subset, synergistic anti-tumor activity between the two components of T-Plex was also evident. For example, a "bystander" cytotoxic activity was often observed when the viability of cancer cell was affected by the T cell-mediated killing of a neighboring cancer cells. Further, the target-dependent activity of a TCR-T component within T-Plex could support the function of the other TCR-T component against the tumor. This phenomenon was largely cytokine-mediated and included the target-independent expansion of the second TCR-T component.

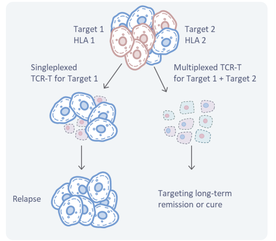
**Conclusion**: Multiplexed TCR-T mimics the natural polyclonal T cell response to cancer and addresses solid tumor heterogeneity. As TScan's ImmunoBank grows, more customized T-Plex products can be built to overcome multiple intratumor heterogeneity scenarios with respect to cancer-associated proteins and/or HLA expression to address each individual patients' needs

## ImmunoBank of TCRs Enables T-Plex, a Multiplexed TCR-T Cell Therapy for solid tumors




TScan Therapeutics is building an ImmunoBank of therapeutic TCRs recognizing a broad range of tumor antigens, consisting of peptide epitopes derived from tumor-associated proteins (targets), presented on diverse human leukocyte antigens (HLAs) class I. Once target and HLA expression are determined in a patient's tumor, TCR-T components are selected from the ImmunoBank to manufacture and administer customized multiplexed TCR-T therapy designed to address intratumor heterogeneity and loss of heterozygosity (LOH) at the HLA locus.



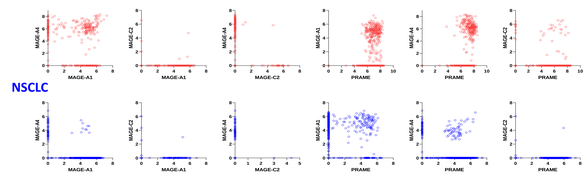




## Targets of T-Plex Are Found Co-Expressed in Solid Tumors and are Typically Presenting Intra-Tumor Heterogeneity

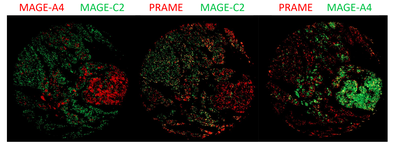
A

| INDICATION | Both PRAME and MAGE-C2 | Both MAGE-A1 and MAGE-C2 | Both PRAME and MAGE-A1 | Both MAGE-A4 and PRAME | Both MAGE-A4 and MAGE-A1 | Both MAGE-A4 and MAGE-C2 |
|---|---|---|---|---|---|---|
| Skin Cutaneous Melanoma (N=457) | 40.9% | 18.4% | 27.1% | 21.7% | 15.8% | 14.9% |
| Lung Squamous Cell Carcinoma (N=427) | 11.0% | 7.0% | 33.7% | 58.5% | 27.2% | 8.9% |
| Ovarian Serous Cystadenocarcinoma (N=413) | 7.7% | 1.2% | 5.6% | 27.1% | 3.9% | 2.7% |
| Head & Neck Squamous Cell Carcinoma (N=268) | 6.3% | 4.5% | 19.8% | 42.5% | 19.0% | 7.5% |
| Cervical & Endocervical Cancer (N=122) | 3.3% | 1.6% | 11.5% | 19.7% | 10.7% | 2.5% |

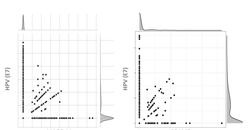
B Melanoma




C Melanoma

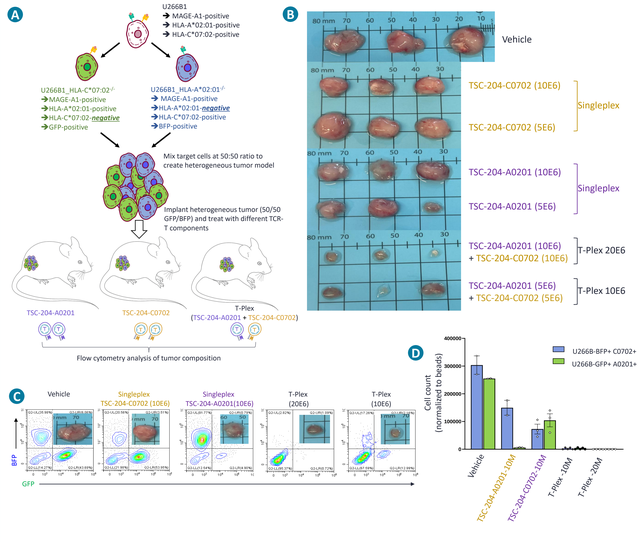



D CERVICAL HEAD AND NECK CANCER



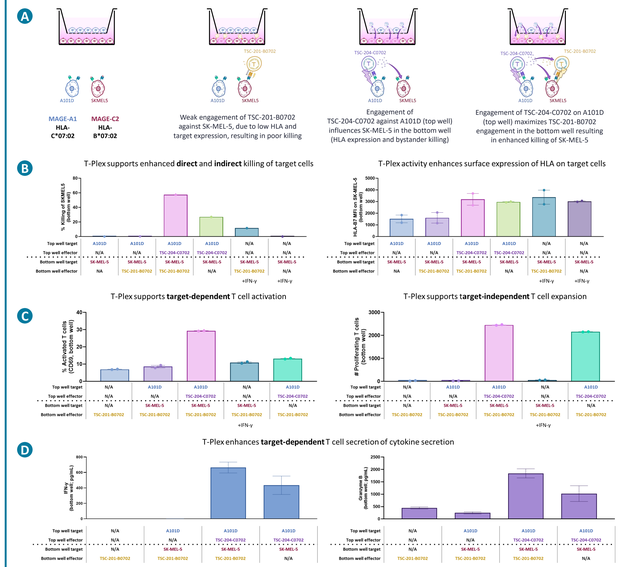

**A.** Publicly available bulk RNA expression data was analyzed for the frequency of co-expression of the targets of the ImmunoBank across different solid tumor indications (source: TCGA). **B.** Publicly available single cell RNAseq data was analyzed for expression of PRAME, MAGE-A1, MAGE-C2, and MAGE-A4 in cells from melanoma and non-small cell lung cancer specimens positive for these targets (source: Curated Cancer Cell Atlas from the Weizmann Institute). **C.** Representative images of immunofluorescence analysis of MAGE-A4, MAGE-C2, and PRAME in a melanoma specimen. **D.** Publicly available single cell RNAseq for HPV16 E7, PRAME, and MAGE-A1 in cervical cancer cells (Li, 2021), HPV+ head and neck cancer cells (Janjic, 2022).

## POC: T-Plex Addresses Intra-Tumor Heterogeneity *In Vivo*




**A.** U266B1 cancer cells (MAGE-A1+, HLA-A*02:01+, HLA-C*07:02+) were engineered by CRISPR knockout to create two versions of the cell line where only one of the two HLAs of interest is intact (knocking out HLA-A*02:01 or HLA-C*07:02). Each target cell subset was labeled with distinct fluorescent dyes (GFP or BFP, respectively) to be tracked in downstream flow cytometry readouts prior to being mixed at a 1:1 ratio to create a tumor model with intratumor heterogeneity and implanted in NCG mice. The efficacy of the MAGE-A1-targeting TSC-204-A0201, or TSC-204-C0702 as Singleplex products (5E6 or 10E6 total T cell injected), or T-Plex (TSC-204-A0201+TSC-204-C0702; 10E6 or 20E6 total TCR-T cell injected) was tested. **B.** Tumors were dissected and photographed to estimate efficacy based on tumor volume. **C**. The tumors were dissociated to analyze their cell composition by flow cytometry. **D.** Change in proportion of the subset of target tumor cells (i.e., BFP+/GFP+) following Singleplex and different T-Plex doses of TCR-T cell treatment is shown.

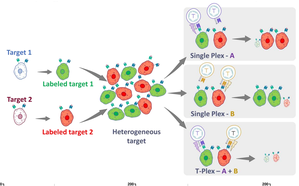
## T-Plex Presents Additive and Synergistic Activities



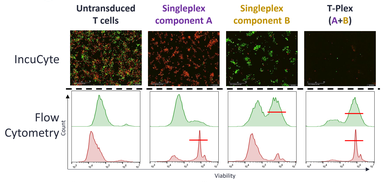

**A.** Experimental design schematic; coculture of T-Plex components was performed in trans-well settings to evaluate the effect of the activity of the TCR-T cells in the top well on the performance of the TCR-T cell in the bottom well. **B.** Activity of TSC-204-C0702 against A101D in the top well supports the target-dependent function of TSC-201-B0702 against SK-MEL-5 in the bottom well; TSC-204-C0702 activity can influence the viability of the SK-MEL-5 cells and promotes their surface expression of HLA-B*07:02, similarly to IFN-γ treatment. **C.** Activity of TSC-204-C0702 against A101D in the top well promotes the target-dependent activation of TSC-201-B0702 (CD69 surface expression) and supports T cell proliferation in the bottom well. **D.** Activity of TSC-204-C0702 against A101D in the top well results in enhanced target-dependent secretion of IFN-γ and granzyme-B by TSC-201-B0702 in the bottom well.

## POC: T-Plex Addresses Intra-Tumor Heterogeneity *In Vitro*

A Developing models to demonstrate the concept of T-Plex:



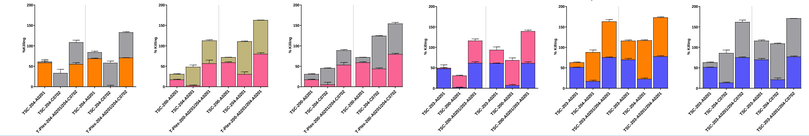

B Measure of residual tumor cells after co-culture:




C Selecting target cells as a function of antigen and HLA:

| Target | HLA | MAGE-A1 | PRAME | MAGE-C2 | MAGE-A4 | HPV |
|---|---|---|---|---|---|---|
| U266B1 | A0201 | ++ | - | +/- | ++ | - |
| U266B1 | C0702 | ++ | - | +/- | ++ | - |
| SCC090 | A0201 | - | +/- | - | - | ++ |
| SW1271 | A0201 C0702 | + | +/- | - | ++ | - |
| SK-MEL-5 | A0201 | - | ++ | + | - | - |
| FTC-133 | A0101 | + | - | ++ | - | - |
| A101D | A0101 | + | + | ++ | - | - |
| HS.936.T | A0201 | ++ | + | - | - | - |

D




**A.** Two target cell lines were combined to simulate a heterogeneous tumor. For tracking purposes, targets were pre-labeled with lentiviral dye NucLight Red (NLR) or NucLight Green (NLR; IncuCyte), or Cell Trace CFSE or Violet (Flow Cytometry) **B**. The efficacy of the T-Plex products against the mixtures of target cells was monitored by either IncuCyte-based or Flow Cytometry-based cytotoxicity assays. IncuCyte enabled tracking the changes in target cell density as assessed by the expression of fluorescent protein NLR or NLG over time. Flow Cytometry enabled end-point (48 or 72hrs) viability measurements of target cells as assessed by 7-AAD live/dead cell-impermeant dye. **C**. Target cell combinations to create heterogeneous targets were based on target antigen and HLA expression. **D.** Percentage killing of each tumor cell subset was determined by taking the area under the curve (AUC) calculated from each culture condition and normalized to the relevant UTF condition (IncuCyte) or by normalizing the dead cell count for each culture condition to the relevant UTF condition (flow cytometry). When facing a heterogeneous target cell population, a Singleplex component is sub-optimal as it only targets a subset of the heterogeneous target cells expressing relevant antigens and HLAs. Combining TCR-T cell components from the ImmunoBank into a customized T-Plex product achieves broad cytotoxic activity against heterogenous cancers.

## Additional Posters from TScan Therapeutics

**# 375: Discovery of a MAGE-A4-specific TCR-T Therapy Candidate for Multiplex Treatment of Solid Tumors**

**# 384: Development of a Target Agnostic Platform to Assess the Reactivity of T Cell Receptor (TCR)-Engineered T Cell (TCR-T) Therapies to Primary Human Tissues**

Janjic BM, Kulkarni A, Ferris RL, Vujanovic L, Vujanovic NL. Human B Cells Mediate Innate Anti-Cancer Cytotoxicity Through Concurrent Engagement of Multiple TNF Superfamily Ligands. Front Immunol. 2022 Mar 22;13:837842. doi: 10.3389/fimmu.2022.837842. PMID: 35392082; PMCID: PMC8983021.

Li C, Guo L, Li S, Hua K. Single-cell transcriptomics reveals the landscape of intra-tumoral heterogeneity and transcriptional activities of ECs in CC. Mol Ther Nucleic Acids. 2021 Apr 2;24:682-694. doi: 10.1016/j.omtn.2021.03.017. PMID: 33996252; PMCID: PMC8099483.