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 has 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 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 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 syngeneic 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 a range of antigens across multiple cancers. Using this approach, we select 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.

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

In vitro
Multiplexing two TCR-Ts, one targeting MAGE-A1 and another targeting PRAME, allowed prolonged killing of both tumor cell lines when compared to singleplexed MAGE-A1 or PRAME TCR-Ts. 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 syngeneic 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.

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