Discovery and Development of T Cell Receptors Targeting MAGE Family Antigens For Adoptive T Cell Therapy Against Solid Tumors

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Background

T cell receptors (TCRs) recognize epitopes from intracellular and cell surface antigens, enabling targeting of diverse tumor antigen classes including overexpressed, differentiation, cancer-testis antigens, as well as those from driver mutations, frameshift mutations, splice variants, and human endogenous retroviral elements (HERV). Melanoma-associated antigens (MAGE) are highly expressed in various solid tumor types, including melanoma, non-small cell lung cancers (NSCLC), bladder, and head and neck cancers, representing promising targets for TCR-T therapy. However, discovering robust and specific MAGE-reactive TCRs remains challenging due to limited throughput of TCR screening methodologies, and the inherently low frequency of tumor-reactive T cells in tumor samples and peripheral blood.

Materials and Methods

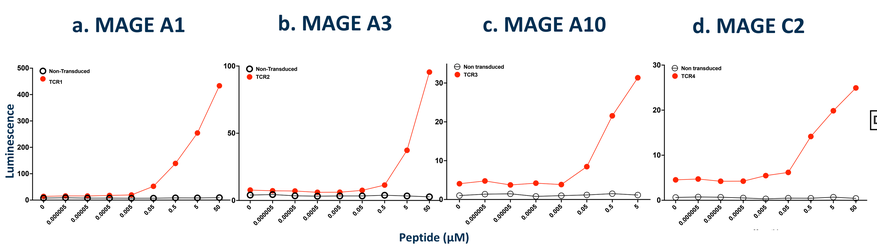
We employ a proprietary mass-cytometry-based multiplexed tetramer staining approach, TargetScape®, to screen and characterize CD8+ T cells in healthy donors and patients' samples. Our screening panels contain multiple peptides for MAGE family antigens including A1, C2, and A10. Subsequent single cell sequencing or single cell PCR (scPCR) of identified tumor antigen-specific T cells with high sensitivity enables comprehensive analysis of T cell phenotype, transcriptome, TCR specificity, and paired TCR sequence. TCRs identified through this method are transduced for expression in Jurkat reporter cell lines and their reactivity to corresponding peptides is assessed. Selected TCR candidates are then transduced into primary T cells and evaluated for activation and effector function using a variety of cancer cell lines expressing the corresponding endogenous antigens. Specificity assessment is performed through alanine scans and identification of potential off-target peptides and screening for reactivity.

Results

We utilized this approach to discover natural TCRs that target several MAGE family antigens across different restrictions. Through Jurkat reporter screening, we observed specific activation of multiple TCRs in the presence of antigen presenting cells and the peptide initially employed to identify the TCRs. Primary T-cells expressing promising TCR candidates exhibited killing activities and cytokine release when confronted with multiple solid tumor cell lines expressing endogenous levels of the MAGE antigen. Specificity assessment is currently in progress, with preliminary data indicating overall favorable specificity profiles for selected TCRs.

Figure 1: Isolation and confirmation of TCR activity against four different MAGE family epitopes

A subset of four different TCRs targeting three different MAGE proteins with confirmed activation when exposed to peptide-pulsed target cells are displayed in the following figure:

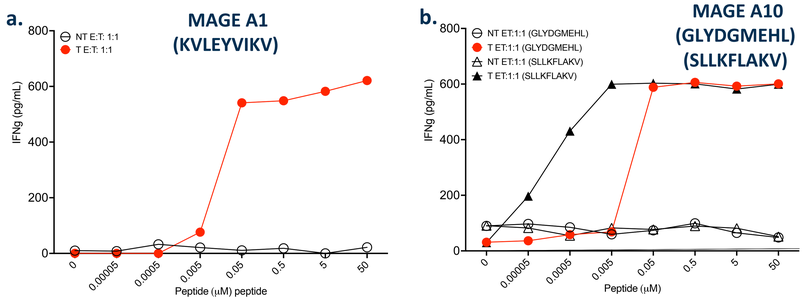


Data represents examples of TCRs isolated using our Deep Immunomics platform for TCR Discovery. Data shows reactivity of TCR-expressing Jurkat reporter cells to target cells expressing the appropriate HLA type and pulsed with the peptide used to identify and isolate the respective TCR; y-axis = luminescence units, x1000.

Results

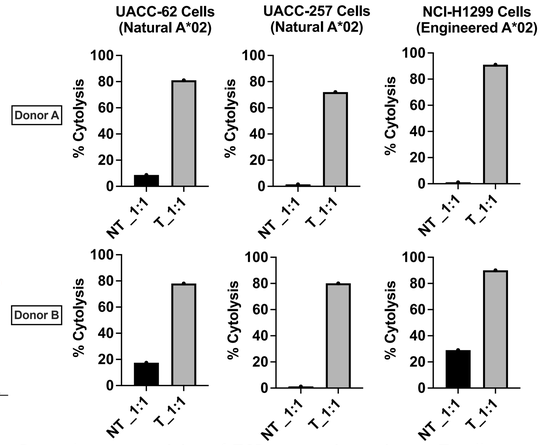
Figure 2: Three TCRs isolated from three different lung cancer patients are highly active and potent against three different MAGE epitopes, two of them targeting the same protein

Expression of certain proteins (restricted to immune-privileged tissues) during cancer development has been reported in multiple cancers including lung cancer. Furthermore, expression of these proteins in peripheral blood has served as a poor prognostic marker for patients with lung cancer and low overall survival [1,2,3]. After screening hundreds of samples, we have successfully isolated and validated multiple TCRs from patients with different types of cancer including Colorectal, Cervix and Lung. Three TCRs isolated from three different lung cancer patients demonstrated activity when exposed to target cells expressing the right HLA (A*02) and pulsed with the peptide used to identify and isolate the respective TCRs.



a. and b. Data shows reactivity of TCR-expressing primary T cells to peptide-pulsed HLA-A*02(+) target cells after overnight co-culture; Interferon Gamma (IFN-γ) quantified by ELISA. Both graphs show three different TCRs targeting two different proteins and three different epitopes. NT (Non-Transduced), T (Transduced). E:T: effector to target ratio

Figure 3: A MAGE A1 targeting TCR is active in transduced primary T cells and exhibits cytotoxicity against HLA A*02 engineered or naturally expressing target cells presenting its cognate peptide

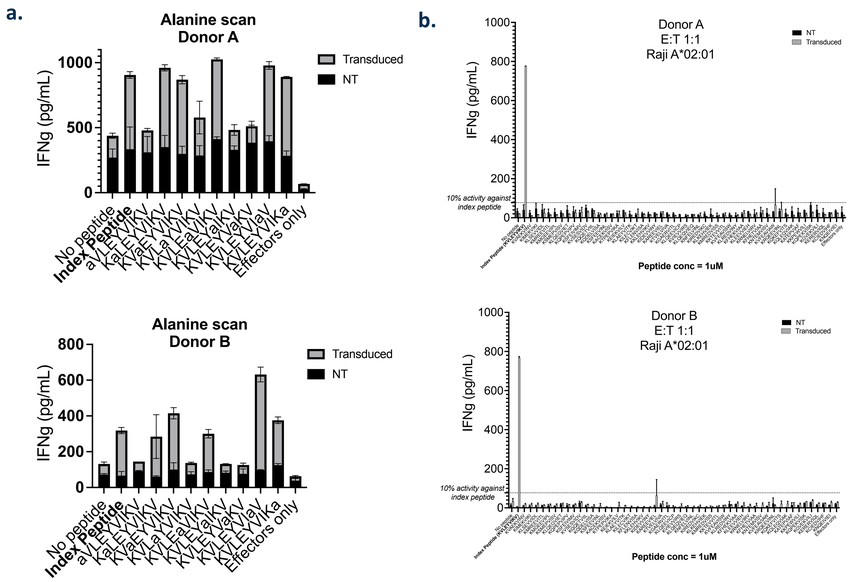


Here, we have confirmed that the TCR from Figure 2a. is functional when transduced into primary T cells exposed to target cells reported to express the antigen of interest and can kill cancer cells presenting endogenous levels of the target MAGE-A1 epitope. Furthermore, the same TCR was able to target a different indication (Melanoma) from the one that was isolated from (Lung Cancer).

Data shows reactivity of TCR-expressing primary T cells to endogenous peptide presentation in engineered (NCI-H1299) or naturally expressing HLA-A*02 target cells (UACC-62 and UACC-257) after overnight co-culture and detected in a real-time cell analyzer (Agilent xCelligence). Target cell index, measuring survival and growth of adherent target cells over time was used to calculate cytotoxicity of target cells using standard protocols and xCelligence Immunotherapy Software. Effectors were added at an effector to target ratio of 1:1. Timepoint indicate time elapsed after adding effectors (24 hrs).

Figure 4: A MAGE A1 targeting TCR showed high specificity and lack of cross-reactivity against top 50 ranked human peptides predicted to bind to HLA-A*02:01

Finally, initial safety screenings replacing each amino acid of index peptide (KVLEYVIKV) for an alanine ("alanine scan"), evidenced 5 tolerated amino acids (a) which informed in silico search for peptides from human proteome that shared the TCR recognition motif[4]. One hundred seventy-five unique potential cross-reactive peptides were identified and ranked (with NetMHCPan4.1) depending on the likelihood to be presented by HLA A*02:01. Top 50 predicted binders were tested, and no cross reactivity was detected (b).



a. Alanine scan for target peptide of respective TCR. Each amino acid of peptide was replaced in turn by an alanine (a) and individual peptides were used to pulse Raji-A*02:01 target cells in the presence of primary effector cells obtained from two different donors (A and B) and transduced with TCR of interest. Each graph shows data for one donor (upper graph for donor A, lower graph for donor B. Bars show means ± SD of duplicates. b. Binding of TCR of interest to human proteins containing a peptide with the motif of interest. Top 50 peptides predicted to bind to HLA-A*02:01 were tested using TCR-transduced primary T cells from two different donors as effectors. IFNγ production was used as a readout. X-axis indicates peptides that were tested.

Conclusion

Our Deep Immunomics platform enables us to detect, characterize, and validate highly specific TCR candidates against clinically relevant shared cancer antigens. Leveraging this platform, we identified various natural tumor specific TCRs exhibiting activities against a range of solid tumor cell lines expressing MAGE antigens. Promising candidates are currently undergoing in vivo efficacy studies.

References

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