Deep Immunomics Pipeline for Discovery and Validation of Novel Cancer-specific T cell receptors

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Abstract No. 8

KEY POINTS

- There is a paucity of therapeutically relevant TCRs eligible for use in cellular therapies
- Our Deep Immunomics pipeline enables high throughput discovery of low frequency, natural TCRs of therapeutic interest
- TCRs elucidated from this pipeline cover a range of therapeutically relevant cancer-related antigen targets presented on six common HLA molecules (HLA-A*01:01, A*02:01, A*03:01, A*11:01, A*24:02, and B*07:02)

BACKGROUND

T cell receptor-engineered T cell (TCR-T) therapy has been studied as a high potential approach for cancer treatment. Beyond cell-surface tumor antigens, TCR-Ts can recognize HLA-presented intracellular epitopes which allows them to address a wide range of cancer-specific targets. Existing TCR-T therapies focus on a limited number of epitopes, many of which are restricted to HLA allele HLA-A*02:01. Identification of novel anti-tumor TCRs covering a broad range of HLA alleles is necessary to address unmet clinical needs. Natural T cells undergo thymic selection and present a favorable safety profile for mining such anti-tumor TCRs for therapeutic development. However, discovery of natural anti-tumor TCRs has been challenging due to i) the absence of high-throughput methods for identifying tumor-reactive TCRs in limited quantity human samples and ii) lack of sensitivity in detecting low frequency, naturally occurring tumor-reactive T cells.

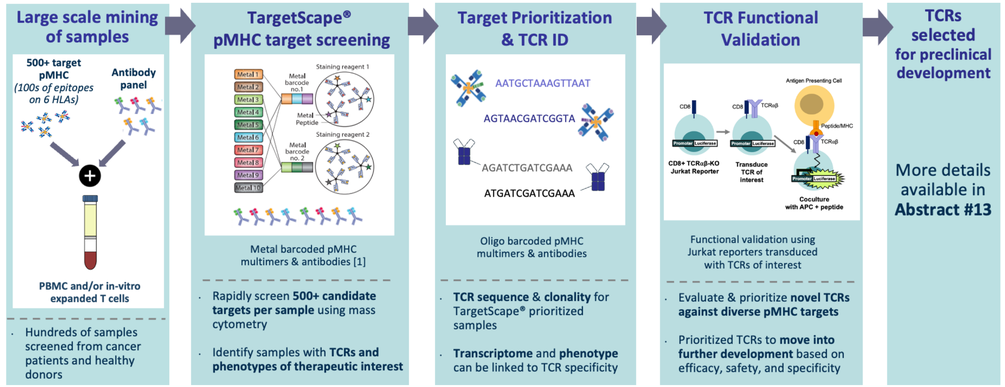
METHODS

TargetScape® was developed as a part of our Deep Immunomics platform for efficient discovery of natural tumor-specific TCRs in healthy donors and cancer patients. Cells from blood and tissue samples were stained with 500+ metal-barcoded peptide-MHC tetramers, including tumor-associated or tumor-specific antigens presented on six common HLA molecules, alongside metal-conjugated antibodies, allowing rapid identification and high dimensional phenotypic analysis of CD8+ T cells at the millions of cells scale by mass cytometry. Single-cell sequencing revealed paired TCR sequences. Selected TCRs were introduced into luminescent Jurkat reporter cells for validation screening with peptide-pulsed targets and the relevant HLA restriction. TCRs of interest were further integrated into primary T cells and evaluated for their effector function.

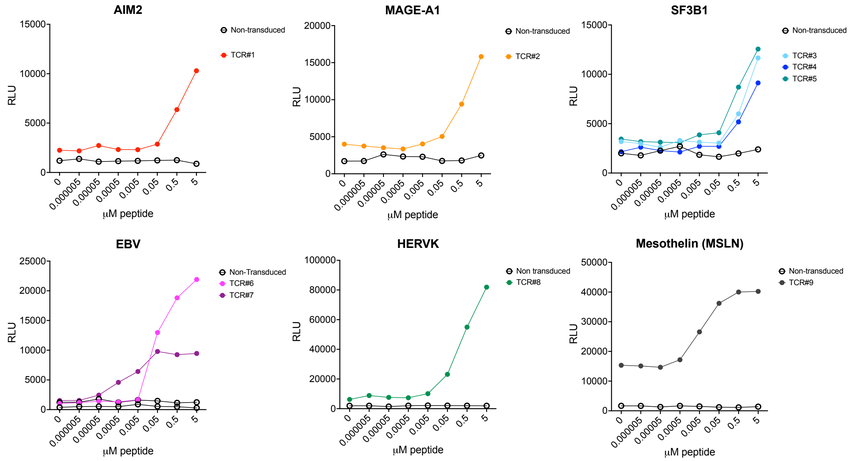
RESULTS

We utilize our sensitive, high-throughput TargetScape® platform to detect and characterize putative cancer-specific TCRs from PBMC and/or in-vitro expanded T cells from healthy or cancer patients at low frequencies within total CD8+ cells. We have functionally validated over 100 TargetScape® derived TCRs with luminescent Jurkat reporters. Validated TCRs cover high prevalence HLA alleles and target a broad range of cancer antigen classes, including tumor associated antigens (TAA), Human endogenous retroviruses (HERVs), shared splice variants, and frameshift mutations. Several natural TCRs demonstrate recognition of low concentrations of peptide, indicating high functional avidity. Further evaluations of specificity and activity of TCRs of interest transduced into primary T cells are currently in progress.

Deep Immunomics Platform:
Large scale mining of patient and donor samples for therapeutically relevant TCRs

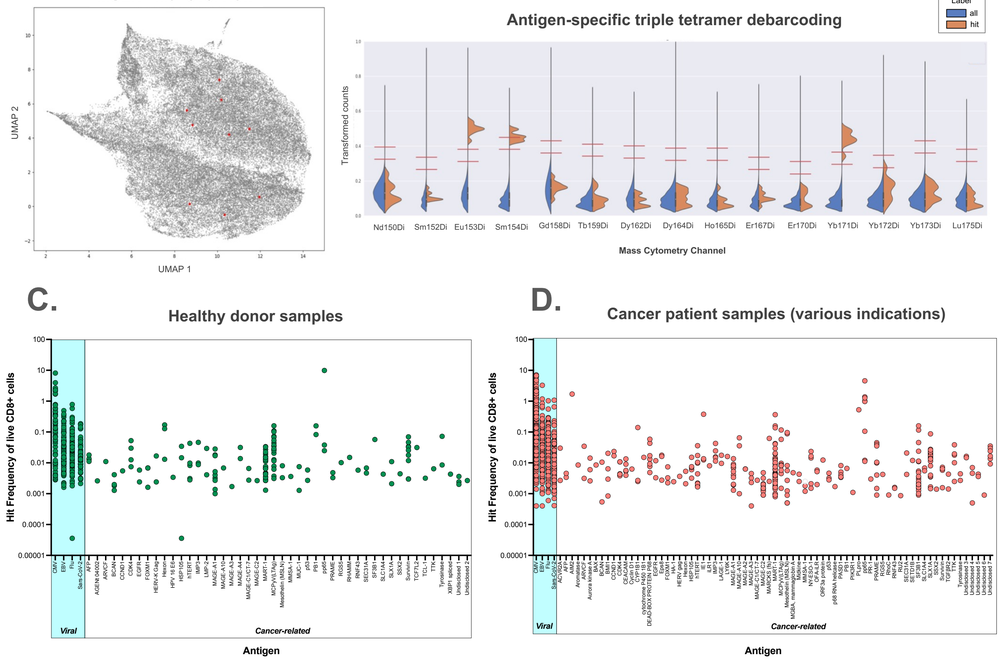


TCR hits of interest undergo functional validation



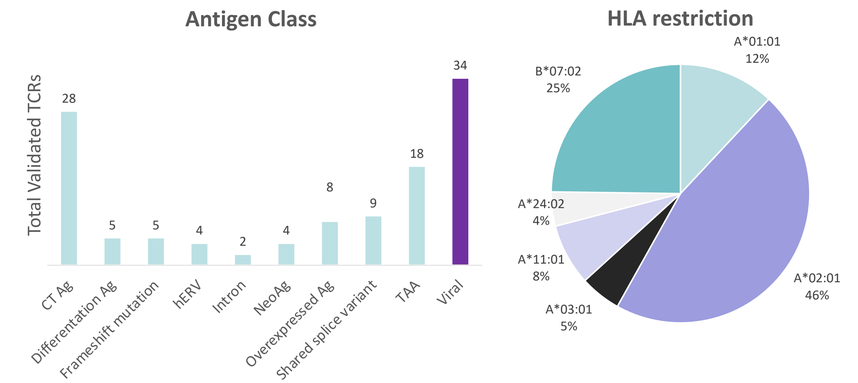
Jurkat reporter functional validation of TargetScape® hit-derived TCRs. Representative functionally validated TCRs from bioluminescent assay readout of endogenous TCR KO CD8+ Jurkat reporters transduced or not-transduced with TCR of interest and plated with titrated peptide epitope and target cells engineered to express the relevant HLA restriction. Results measured in raw luminescence units (RLU).

A high-throughput and sensitive workflow enables detection of low frequency natural TCR hits



Identification of putative viral- and cancer-specific T cell identification (TargetScape® "hit" calling) TCR using TargetScape® and TAP workflows. PBMC from lung cancer patient D3037 were *in vitro* expanded, processed with TargetScape® workflow, and tetramer-barcoded cells were evaluated for hit veracity, including A*11:01 MSLN epitope, used here for representative hit calling. A) Collective UMAP representation of tetramer barcoded A*11:01 MSLN epitope hits (red dots) in bulk sample acquired on CyTOF (gray dots). B) Antigen-specific triple tetramer positive cells (hits) were identified based on an automated peptide-MHC gating method. Hit calling was performed using CYTOGRAPHER®, ImmunoScape's cloud based analytical software. C-D) Frequency of viral- and cancer-specific TCRs of therapeutic interest in total CD8+ cells in C) 129 healthy donor and D) 281 cancer patient PBMC samples of various indications.

>115 validated TCRs cover a range of cancer-related antigen classes and six common HLA alleles



CONCLUSIONS

ImmunoScape's Deep Immunomics platform allows us to identify and confirm activity of novel, naturally-derived TCR candidates against therapeutically relevant cancer antigens presented on six common HLA alleles. These findings validate our discovery workflow leading to a portfolio of potential clinically relevant candidates that are currently under preclinical development.

References

[1] E.W. Newell, and M.M. Davis. "Beyond model antigens: high-dimensional methods for the analysis of antigen-specific T cells". Nature Biotechnology; 32 (2) 149-157, Feb. 2014.