Discovery of TSC-101: A First-in-Class Natural HA-2-specific TCR to Treat Leukemia Following Hematopoietic Stem Cell Transplant Therapy

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#1704

Introduction

Approximately 50% of AML patients relapse following allogeneic hematopoietic cell transplant therapy, leaving them with very few treatment options (1). Rare patients who naturally develop a minor antigen-specific graft-versus-leukemia T cell response show substantially lower relapse rates (2,3). HA-2 (YIGEVLVSV, genotype RS_61739531 C/C or T/C) is an HLA-A*02:01- and haematopoietically-restricted minor histocompatibility antigen derived from the class I myosin protein, MYO1G (4). Patients receiving donor lymphocyte infusion from HA-2-mismatched donors who develop HA-2-specific T cells show a graft vs leukemia response and often experience long-term remission (2), making HA-2 an ideal candidate for TCR-engineered T cell immunotherapy for liquid tumors.

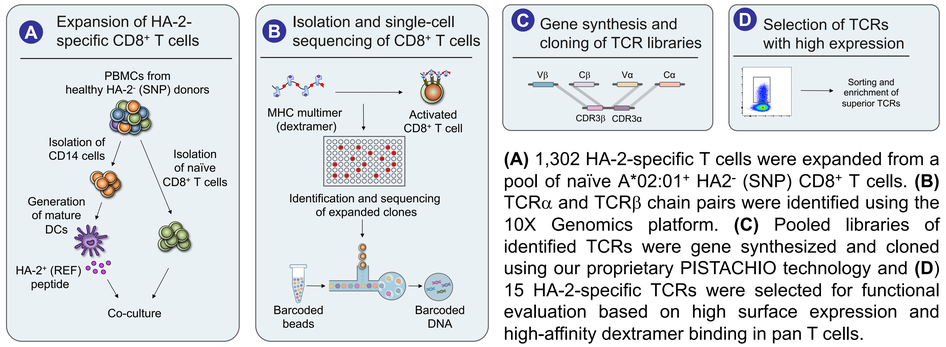
Methods

Using TScan's proprietary *ReceptorScan* platform, we discovered 1,302 HA-2-specific TCRs by screening 237 million naïve CD8$^+$ T cells from 5 healthy HA-2-negative donors. We evaluated these TCRs using our proprietary *DexScan* platform to select the 15 TCRs with the highest surface expression and greatest affinity for the HA-2 peptide when transferred into primary human T cells. We further tested each TCR individually in our clinical vector backbone for surface expression, selective cytotoxicity, cytokine production, and proliferation using a panel of cell lines that express varying levels of HLA-A*02:01 and MYO1G. Finally, the top 5 TCRs were evaluated for alloreactivity using an array-based screen assessing 110 MHC-I molecules individually, and for off-target cross-reactivity using our proprietary genome-wide *TargetScan* platform. A lead TCR with limited alloreactivity and a narrow off-target profile was selected as our lead TCR for TSC-101. The avidity of TCR-101 for its putative off-targets was further measured in peptide-pulsed experiments to better appreciate the toxicity risks associated with our lead clinical candidate.

Results and Conclusion

Of the 1,302 HA-2-specific TCRs identified by our ReceptorScan platform, we identified TCR-101 as the most active TCR. TCR-101 displayed no alloreactivity to 109/110 HLAs tested and limited off-target recognition in a genome-wide screen. TCR-101 displayed extremely weak avidities for all putative off-targets. Based on these results, TCR-101 has been advanced to IND-enabling activities to prepare for first-in-human testing in 2022. As described in ASH poster #3863, HA-2$^+$ patients undergoing HCT will be dosed with HA-2$^-$ donor T cells that have been engineered with TCR-101, with the goal of preventing relapse in these patients.

TScan's proprietary ReceptorScan and DexScan platforms

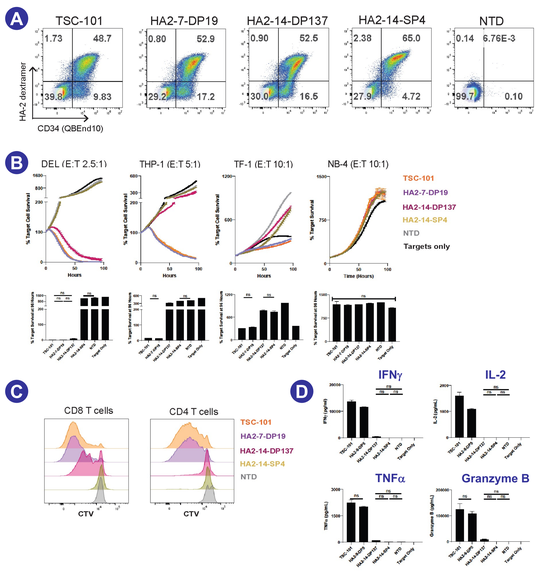


(A) 1,302 HA-2-specific T cells were expanded from a pool of naïve A*02:01$^+$ HA2$^-$ (SNP) CD8$^+$ T cells. **(B)** TCRα and TCRβ chain pairs were identified using the 10X Genomics platform. **(C)** Pooled libraries of identified TCRs were gene synthesized and cloned using our proprietary PISTACHIO technology and **(D)** 15 HA-2-specific TCRs were selected for functional evaluation based on high surface expression and high-affinity dextramer binding in pan T cells.

TScan's vector co-delivers TCRα/β, CD8α/β and CD34 (Q) purification tag

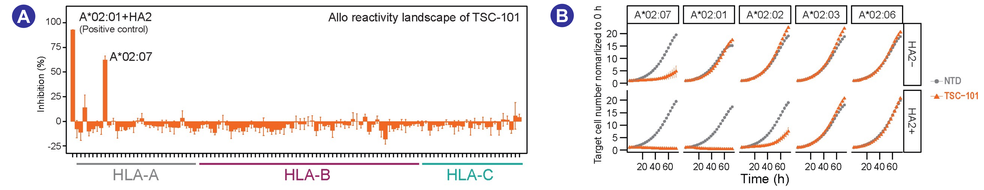
As previously described for TScan's TSC-100 clinical candidate, TCR-101 was introduced into a transposon/transposase vector for genetic engineering. The resulting TCR-T cell therapy product, TSC-101, is a mixture of cytotoxic and helper T cells, both of which are reprogrammed to recognize HA-2$^+$ normal and malignant blood cells. The co-introduction of CD8α/β ensures active engagement of CD4$^+$ helper T cells, thus promoting T cell health and persistence. The CD34 (Q) tag enables removal of non-engineered cells, thereby reducing risk of GvHD.

TSC-101 shows specificity and high functionality against target cell lines



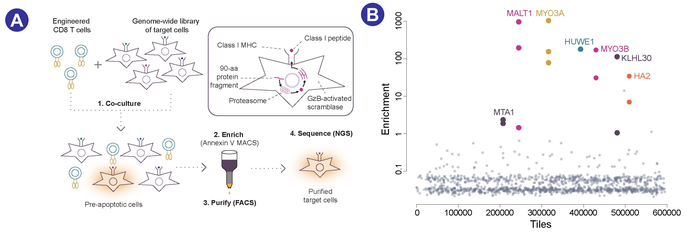
Pan T cells (natural mixture of CD4$^+$ and CD8$^+$ T cells) were transduced to express HA-2-specific TCRs and assessed for functional responses to target cells. **(A)** TSC-101 shows efficient expression of TCR-101 (HA-2$^+$ dextramer) and Q-tagged CD8α (QBend10). **(B)** TSC-101 shows strong cytotoxicity in HA-2$^+$ cancer cell lines. DEL (HLA-A*02:01^{high}/HA-2^{high}), THP-1 (HLA-A*02:01^{high}/HA-2^{low}), TF-1 (HLA-A*02:01^{low}/HA-2^{low}), NB4 (HLA-A*02:01^{negative}). **(C)** TSC-101 exhibits HA-2-specific CD8$^+$ and CD4$^+$ T cell proliferation when co-cultured with HA-2$^+$ THP-1 cells. Histograms show dilution of Cell Trace Violet dye used to label T cells. **(D)** TSC-101 efficiently secretes cytokines when co-cultured with THP-1 cells. Different HA-2-specific TCRs were compared by one-way ANOVA followed by Tukey's multiple comparisons test, in which each TCR was compared to every other TCR. Differences that were non-significant (ns) are shown; all other differences were significant with P<0.05. Data are representative of 2-3 unique donors.

TCR-101 shows no alloreactivity to 109/110 HLA types



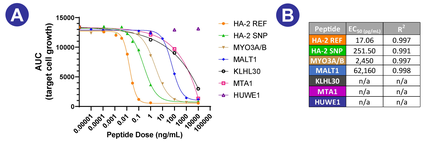
(A) TCR-101-expressing CD8$^+$ T cells showed no allo-reactivity to 109/110 of the most frequent Class I MHCs in the US population. **(B)** TCR-101 recognizes the HA-2$^+$ (REF) peptide presented on 2 of the 5 HLA-A*02 supertypes.

TScan's genome-wide TargetScan screen identifies putative off-targets for TCR-101



(A) Overview of TScan's proprietary genome-wide safety screen. **(B)** TCR-101 recognizes six potential off-targets in a screen of >600,000 protein fragments spanning every w.t. human protein. The screen is designed to overpredict off-targets by overexpressing 90-aa protein fragments, which are more efficiently processed than full-length proteins.

TSC-101 displays >100-3,000-fold lower affinity for putative off-target peptides

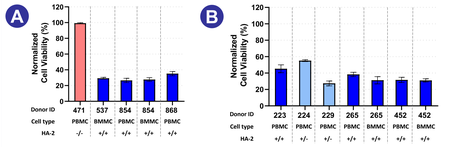


B

Peptide	EC_{50} (pg/mL)	R^2
HA-2 REF	17.06	0.997
HA-2 SNP	251.50	0.991
MYO3A/B	2,450	0.997
MALT1	62,160	0.998
KLHL30	n/a	n/a
MTA1	n/a	n/a
HUWE1	n/a	n/a

(A) The affinity of TCR-101 for on- and off-target peptides was measured by calculating Area under the curve (AUC) for T2 cell growth as a function of peptide dose. **(B)** Individual EC_{50} values for HA-2$^+$ REF peptide, HA-2$^-$ SNP peptide, putative off-target MYO3A/B and MALT1 peptides are shown. 3-point titration did not enable the calculation of the EC_{50} values for MTA1, KLHL30 and HUWE1.

TSC-101 exhibits robust cytotoxicity against primary AML and ALL tumor samples



TSC-101 and nonengineered control (NC) T cells were co-cultured for 20 h with HLA-A*02:01-positive PBMCs or BMMCs from **(A)** AML or **(B)** ALL patients at an E:T of 10:1. The viability of targets upon coculture with TSC-101 T cells was then assessed by flow cytometry and normalized to the viability of targets upon coculture with NC. Data are representative of 3 donors.

Summary and Next Steps

Therapeutic TCRs for TCR-T must exhibit high affinity for their targets but low recognition of off-targets. By screening 237 million naïve T cells from five HA-2-negative donors, we isolated an ultrahigh affinity TCR (EC_{50} = 17 pg/mL) that does not recognize off-targets with appreciable affinity. Using a nonviral vector, we developed an enhanced T cell product comprising both cytotoxic and helper T cells derived from HLA-A*02:01-negative donors. TSC-101 is being advanced to clinical development to prevent relapse in patients with AML, MDS, and ALL undergoing HCT (see ASH 2021 poster #3863).

References

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