Introduction

Approximately 50% of AML patients relapse following autologous 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 (KSFVQVSV, genotype RS_61739531 C/C or T/C) is an HLA-A*0201- and haematopoietically-restricted minor histocompatibility antigen derived from the TCR I myeloid leukemia antigen TSC-1HLG(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 identified 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*0201 and MHC01. 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 TSC-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 HLA-As 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 studies to prepare for first-in-human testing in 2022. As described in ASH poster RS863, HA-2-negative 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.

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


TSanT Therapeutics, Wolfsburg, MA

TScan’s proprietary ReceptorScan and DexScan platforms

As previously described for TScan’s TSC-100 clinical candidate, TCR-101 was introduced in a transposon/transposable vector for genetic engineering. The resulting TCR-T cell therapy product, TSC-101, is introduced into 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 CD4+ helper T cells, thus promoting T cell health and persistence. The CD4+ (Q) tag enables removal of non-engineered cells, thereby reducing risk of GVDH.

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

Pan T cells (natural mixture of CD4+ and CD8+ T cells) were transduced to express HA-2-specific TCRs (A) and assessed for functional responses to target cells. (A) TSC-101 shows efficient expression of TCR101 (HA-2+ dextramer) and 2 specific TCRs (HLA A0201). (B) TSC-101 shows strong cytotoxicity in HA-2+ cancer cell lines, the HA-2+ tumor cell line (HLA-A0201) is significantly more sensitive to TCR101 (HLA-A0201) than control (HLA-A0201/HLA-A0201/HLA-A0201). (C) TSC-101 exhibits HA-2-specific CD8+ and CD4+ T cell proliferation when co-cultured with HA-2+ Th1 cells. (D) TCR101 efficiently severs cytokines when co-cultured with TH1 cells. Different HA-2specific TCRs were compared by one-way ANOVA followed by Tukey’s multiple comparisons. In 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.

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

(TCR-101 expresses 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*0202 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 identify specificity of TCRs by overexpressing 90-a protein fragments, which are more efficiently processed than full-length proteins.

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

(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 EC50 values for HA-2+ REF peptide, HA-2+ SNIP peptide, putative off-target MYO3A/B and MALT1 peptides are shown. Support titration did not enable the calculation of the EC50 values for MTA1, KLHL30 and HUWE1.

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

TSC-101 and nonengeneered control (NC) T cells were co-cultured for 20 h with HLA-A0201-positive PBMCs or BMDCs 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 (EC50 = 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-A0201-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 RS863).

References