Discovery of TSC-100: A Natural HA-1-specific TCR to Treat Leukemia Following Hematopoietic Stem Cell Transplant Therapy

Ribhu Nayar, Sonal Jangalwe, Mollie M. Jurewicz, Antoine J. Boudot, Andrew S. Basinski, Robert Prenovitz, Elizabeth A. Olesin, Garrett S. Dunlap, Alexander Cristofaro, Nancy Nabilis, Ruan Zhang, Candace Perullo, Sida Liao, Kenneth L. Jahan, Kenneth Olivier, Gavin MacBeath

Abstract

Background: Approximately 30%-40% of AML patients experience relapse following hematopoietic stem cell transplant therapy. Among them, very few tolerate frontline options (1/2). Rare patients that naturally develop an HA-1-specific graft-vs-host-reactive T cell response, however, show substantially linear relapse rates (3,4). HA-1 (VRMMDLLAA) is recognized by HLA-A*01:01 and haematopoietically-restricted minor histocompatibility antigen, making it an ideal candidate for TCR, immunomodulatory or chimeric T cell receptor therapy.

Method: We developed a high-throughput TCR discovery platform that enables rapid cloning of antigen-specific TCRs from less than 100 million CD8+ T cells from our unique HA-1 (VRMMDLLAA) pMHC tetramer, 2-color flow cytometry, and high-throughput sequencing. We tested each TCR for expression and the ability to kill HA-1-expressing targets using a virus-based assay and transduced clinical-grade HA-1-specific TCRs as a benchmark for frontline studies (5). We expanded a TCR using a HA-1/DC Matrigel assay to validate performance on engineered T cells. The top 11 candidates were cloned into our optimized backbone and evaluated for cytokotoxicity, cytokine expression and ultra-low TCR proliferation using a peptide HA-1/DC Matrigel assay. Finally, the top ten TCRs were evaluated for alloreactivity and off-target reactivity using our proprietary 9-Plex T cell platform.

Results: The TCR discovery and evaluation platform described here identified 329 HA-1-specific TCRs from a total of 178.3 million CD8+ T cells and 115 high affinity TCRs with TAC scores > 10000. The top 11 TCRs demonstrated comparable expression, similar expression and cytotoxicity of the T cells. TCRs were transduced with HA-1-specific MHC class I tetramer, expression and TCR expression by HA-1/HLA-A2/DC2113 dextramer staining pre- and post-CD4 enrichment. The original sequence of TSC-100 was compared to modified sequence carrying optimization of residues in the constant domain (C2) and the transmembrane domain (TM) designed to ensure surface expression and proper alignment of the TCR. Primary human T cells were transduced with different versions of the TCRs and the surface expression of the proper panel TCR was assessed by dextramer staining. Final vector constructs. The discovery phase identified three TCRs by 9-Plex T cell modifications.

Conclusion: TSC-100 was tested in an alloreactivity assay. Endogenous MHCs were knocked out of HER2xT cells such that an HLA-A*0201/B7/HLA-A*0101 cotransduced cell line was prepared. Paired cells were restimulated with irradiated HER2x-B7/HLA-A*0201/B7- and HER2x-B7/HLA-A*0201/B7- transfected HA-1+ cells, showing TSC-100 (P<0.0001) and TSC-100 (P<0.05) transduced cells were transduced with HA-1+ cell line. The TCRs were tested in an allogeneic target assay. The TSC-100 no detectable T target.

TSc's genome-wide screen shows TSC-100 has no detectable off-targets

TSC-100 was evaluated for off-target reactivity using TSc-100. In this genome-wide safety screen, T cells expressing TSC-100 were co-cultured with a library of HLA A0101 transgenic target cells, each expressing different 90-antigen protein fragments. Collectively, the library includes fragments that are covered across every protein in 22 human allele families. Fragments are processed naturally through the target cells and the resulting peptides are detected by target HLA-MHC complexes. The data was analyzed to identify and categorize any of the 226 antigens, the natural target(s) of the TCR were reevaluated. Of ~600,000 clones, only the three clones in our library that contain the HLA-A*0101 epitope were significantly enriched in the screen.

TSC-100 shows no aloreactivity to 108 HLA types

TSC-100 was tested in an alloreactivity assay. Endogenous MHCs were knocked out of HER2xT cells such that an HLA-A*0201/B7/HLA-A*0101 cotransduced cell line was prepared. Paired cells were restimulated with irradiated HER2x-B7/HLA-A*0201/B7- and HER2x-B7/HLA-A*0201/B7- transfected HA-1+ cells, showing TSC-100 (P<0.0001) and TSC-100 (P<0.05) transduced cells were transduced with HA-1+ cell line. The TCRs were tested in an allogeneic target assay. The TSC-100 no detectable T target.

TSc's optimal vector includes CD8a and CD8β, a CD34 enrichment tag, and modifications in the constant and transmembrane domains of the HA-1 TCR

TSc’s optimal vector includes CD8α and CD8β, a CD34 enrichment tag, and modifications in the constant and transmembrane domains of the HA-1 TCR.