Methods We developed a high-throughput TCR discovery platform that enables rapid cloning of antigen-specific TCRs from healthy donors. We then used this platform to screen 178.3 million naïve CD8+ T cells from six unique HA-1-(VLRDDLLEA, genotype RS_1801284 G/G) donors, identifying 329 HA-1-specific TCRs. We tested each TCR for expression and the ability to kill HA-1+ target cells, using a previously published, clinical-stage HA-1-specific TCR as a benchmark for these studies. In parallel, we tested TCR constant region modifications to promote expression and proper pairing of exogenous TCR alpha and beta chains and designed a lentiviral vector to co-deliver CD8 coreceptors as well as a CD34 enrichment tag to enable purification of engineered T cells. The top 11 candidates were cloned into our optimized backbone and evaluated for cytotoxicity, cytokine production, and T cell proliferation using a panel of HLAA*02:01+ HA-1+ cell lines. Finally, the top two TCRs were evaluated for allo-reactivity and off-target cross-reactivity using our proprietary genome-wide T-Scan platform.

Results The TCR discovery and evaluation platform described here identified 329 HA-1-specific TCRs from a total of 178.3 million naïve T cells, and TSC-100 as the most active TCR. Defined mutations in the constant region of TSC-100 enhanced its surface expression while decreasing expression of endogenous TCRs, and co-introduction of CD8 enabled efficient engagement and function of engineered CD4 cells. Overall, TSC-100 exhibited comparable activity to a clinical-stage benchmark TCR when challenged with cell lines expressing moderate to high levels of HA-1, and superior activity when incubated with cell lines expressing low levels of both HA-1 and MHC-I. In addition, TSC-100 exhibited no detectable allo-reactivity to 108 different HLA types tested, and minimal off-target effects when challenged with a genome-wide library expressing peptides derived from human proteins.

Conclusions TSC-100 exhibits comparable or superior activity to a clinical-stage therapeutic TCR, with minimal allo-reactivity or off-target effects. Based on these results, TSC-100 has been advanced to IND-enabling activities to prepare for first-in-human testing in 2021.

Ethics Approval All clinical samples used in the study were collected by STEMCELL Technologies, StemExpress and HemaCare using their IRB approved protocols.

REFERENCES

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157 A CRITICAL ROLE OF CD40 AND CD70 SIGNALING IN CDC15 IN EXPANSION AND ANTITUMOR EFFICACY OF ADOPTIVELY TRANSFERRED TUMOR-SPECIFIC T CELLS
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Background Adoptive cell therapy (ACT) with antigen-specific CD8+ T cells is a promising approach for treating patients with various solid malignancies including melanoma. In vivo expansion of adoptively transferred T cells is one of the major determinants of successful ACT. On the other hand, a frequently overlooked consideration is that the host antigen-presenting cells affect the antitumor efficacy of ACT. Accumulating evidence suggests that tumor-residing Batf3-dependent conventional type I dendritic cells (cDC1s) play an important role in trafficking of adoptively transferred T cells into the tumor by producing chemokines such as CXCL10, and improve antitumor efficacy of ACT. However, a role of cDC1s in expansion of adoptively transferred T cells remains unclear.

Methods We utilized Pmel-1 T cell receptor transgenic T cells in the B16 melanoma model to investigate the role of cDC1s in expansion of adoptively transferred tumor-specific T cells.

Results While adoptive transfer of in vitro-activated Pmel-1 T cells with vaccination of cognate antigen, hlg100, agonistic anti-CD40 monoclonal antibody (mAb), and Toll-like receptor 7 (TLR7) agonist delayed the tumor growth and survival in wild type C57BL/6 mice (WT), antitumor efficacy of ACT was completely abrogated in Batf3–/– mice. Flow cytometric analysis of peripheral blood showed expansion of adoptively transferred Pmel-1 T cells was significantly compromised in WT mice but not in in Batf3–/– mice. Mechanistically, loss-of-function studies using mixed bone marrow chimera reconstituted with Batf3–/– and CD40–/– (Batf3–/–/CD40–/–), Batf3–/– and CD70–/– (Batf3–/–/CD70–/–), or Batf3–/– and CD80/86–/– (Batf3–/–/CD80/86–/–) revealed CD40-CD70 axis but not CD80/86 signaling in host cDC1s plays an important role in expansion of adoptively transferred T cells. Accordingly, overall survival of Batf3–/–/CD70–/– mixed chimeric was significantly shorter than that of Batf3–/–/WT mice, while survival of Batf3–/–/CD80/86–/– mice was similar to that of Batf3–/–/WT mice. Furthermore, induction of cDC1s by administration of Fms-like tyrosine kinase 3 receptor ligand (gain-of-function) demonstrated significantly enhanced in vivo expansion of adoptively transferred Pmel-1 T cells associated with improved tumor control and survival.

Conclusions These findings elucidate a role of host cDC1s in expansion of adoptively transferred in vivo restimulated tumor-specific T cells, and identify CD40 and CD70 as key molecules.

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158 INHIBITION OF PI3Kζ IMPROVES TUMOR SPECIFIC T CELL IMMUNITY AND METABOLIC FITNESS
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Background Durable responses have been observed with adoptive T cell therapy (ACT) in some patients. However, current...