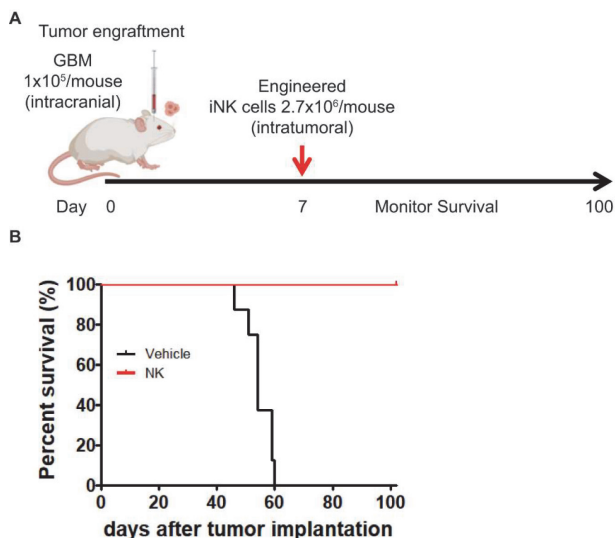


NK cell adoptive transfer has shown clinical benefit in patients with advanced cancer.^{4–7} However, limitations of this approach include relatively low numbers of donor NK cells that can be isolated during an apheresis and variability in the quality of NK cells between donors. To overcome these limitations, we have developed a GMP manufacturing strategy to mass produce NK cells from induced pluripotent stem cells (iPSCs) as an approach to off-the-shelf cancer immunotherapy. We refer to these cells as 'iNK' (iPSC-derived NK) cells. Here, we provide preclinical data demonstrating the efficacy of iNK cells for immunotherapy against glioblastoma.



Abstract 155 Figure 1 Engineered iNK cells exhibit highly effective antitumor function in a xenogeneic model of glioblastoma. (A) Schematic of the experimental design to test iNK cell function against glioblastoma in vivo. (B) Kaplan Meier plots showing survival for groups of mice that received either vehicle alone or iNK cells after tumor engraftment (n=5 mice/group)

Methods We generated iNK cells using previously published methods.^{8–10} iNK cells were used as effectors against an array of patient-derived glioblastoma lines in 2-dimensional live imaging IncuCyte assays where iNK cell-mediated killing was observed over the course of 48 hours. To investigate iNK cell infiltration and cytotoxicity in a more physiological context that accounts for the 3-dimensional architecture of the tumor, we also performed live imaging IncuCyte assays using iNK cells as effectors against glioblastoma spheroids. To test the anti-tumor function of iNK cells in vivo, we implanted patient-derived glioblastoma cells into mice via intracranial injection. Seven days later, 5 mice received intratumoral injections of iNK cells, and 5 mice received vehicle alone (as a control; figure 1A). All mice were monitored for weight and survival over 100 days.

Results iNK cells exhibited strong and sustained cytotoxicity against 6 primary patient-derived mesenchymal glioblastoma lines in 2-dimensional IncuCyte assays and complete infiltration and destruction of glioblastoma spheroids in 3-dimensional IncuCyte assays. In xenogeneic adoptive transfer experiments, all mice receiving intratumoral injections of iNK cells survived out to day 100, while all mice in the vehicle group became moribund and had to be sacrificed by day 60 (figure 1B).

Conclusions iNK cells are highly cytotoxic against glioblastoma cells, and our preclinical in vivo data provides proof-of-concept for future clinical trials.

Ethics Approval This project has been approved by the University of Minnesota IACUC. Approval ID: 1812-36595A

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DISCOVERY OF TSC-100: A NATURAL HA-1-SPECIFIC TCR TO TREAT LEUKEMIA FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANT THERAPY

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Background Approximately 30–40% of AML patients relapse following allogeneic hematopoietic stem cell transplant therapy, leaving them with very few treatment options.^{1 2} Rare patients that naturally develop an HA-1-specific graft-versus-leukemia T cell response, however, show substantially lower relapse rates.^{3 4} HA-1 (VLHDDLLEA, genotype RS_1801284 A/G or A/A) is an HLA-A*02:01-and hematopoietically restricted minor histocompatibility antigen, making it an ideal candidate for TCR immunotherapy for liquid tumors.⁵

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.⁶ 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 HLA-A*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.⁶ 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.

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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, hgp100, 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 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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INHIBITION OF PI3Kδ 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