Product Characteristics and Multi-Arm Clinical Trial Design for TSC-100 and TSC-101, TCR-T Cells That Target Leukemia Following Hematopoietic Cell Transplantation

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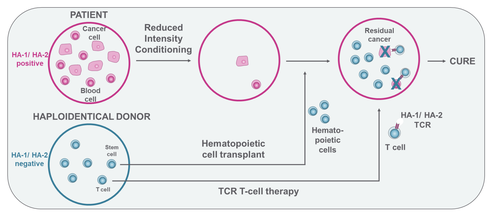
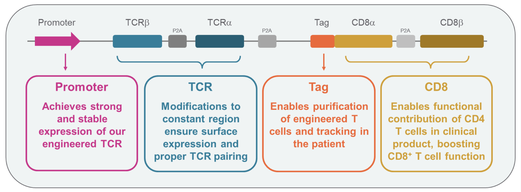
Introduction

While CAR-T therapies have transformed the treatment of lymphoid malignancies, there are currently no approved adoptive cell therapies for myeloid malignancies. T cells expressing T cell Receptors (TCRs) for HLA-A*02:01-restricted minor histocompatibility antigens HA-1 and HA-2 clonally expand after hematopoietic cell transplantation (HCT) in donor-recipient pairs mismatched for these antigens and are associated with significantly lower relapse rates[1,2] indicating a specific graft versus leukemia effect. TCR-T cells engineered to target HA-1 have demonstrated preliminary safety and anti-leukemic activity in relapsed leukemia after HCT[3]. We have developed engineered TCR-T cell products, TSC-100 and TSC-101, that target HA-1 and HA-2 to prevent leukemia relapse after HCT.

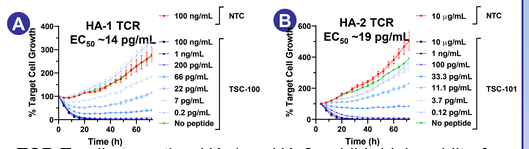


Methods and Results

To minimize potential safety risks, process variability, and costs associated with lentiviruses, our proprietary T-Integrate manufacturing platform uses a transposon/ transposase system delivered into pan T cells. This enables the introduction of larger vectors with an increased number of functional elements. Our transposon vector encodes both TCRα/β and CD8α/β under control of a strong promoter.

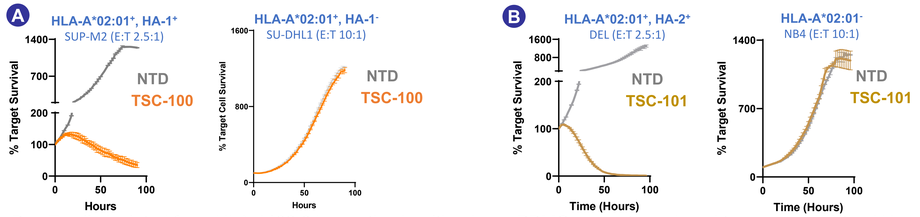


Potent TCRs identified for HA-1 and HA-2



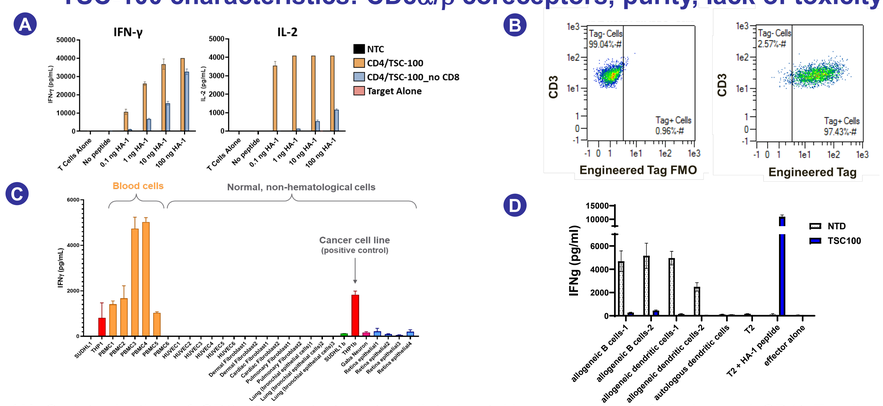
TCR-T cells targeting HA-1 or HA-2 exhibit high avidity for peptide-pulsed T2 cells (EC_{50} values of 14 and 19 pg/mL).

TSC-100 and TSC-101 selectively target HA-1/ HA-2 positive cancer cells



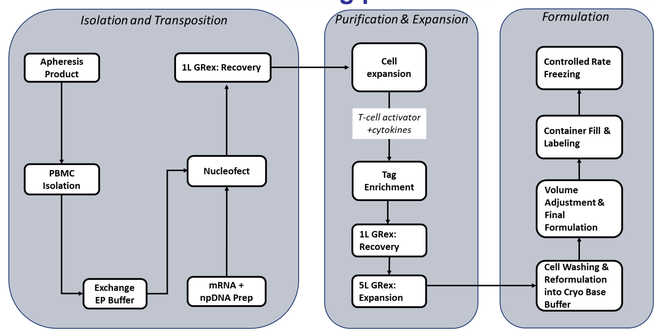
Pan T cells isolated from HLA-A*02:01-negative healthy donor PBMCs were transduced to express **(A)** the HA-1-specific TCR (TSC-100) or **(B)** the HA-2-specific TCR (TSC-101). NTD indicates nontransduced control. TCR-T cells were co-cultured with target cell-lines expressing HA-1 or HA-2 (left panels) or with target cells negative for HA-1 or HA-2 (right panels). Cytotoxicity was measured at indicated effector to target cell ratios.

TSC-100 characteristics: CD8α/β coreceptors, purity, lack of toxicity



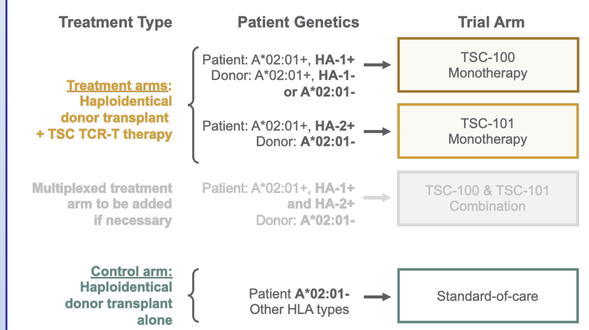
(A) Co-introduction of CD8α/β boosts target-mediated cytokine production of engineered $CD4^+$ T cells. **(B)** Tag-mediated enrichment results in >90% purity of T cell product. **(C)** TCR-T cell product recognizes $HA\text{-}1^+$ hematologic cells but exhibits no recognition of $HLA\text{-}A*02:01^+$ non-hematologic cells. **(D)** In mixed lymphocyte reactions, TSC-100 does not exhibit alloreactivity (indicative of potential GvHD) compared to nontransduced controls.

Manufacturing process for TSC-100/ TSC-101



The manufacturing process for TSC-100/ TSC-101 has 3 parts: (1) isolation of PBMCs from the donor apheresis product and nucleofection with the transposon/ transposase vector (left); (2) purification of tagged cells and cytokine-mediated expansion (middle); and (3) formulation and controlled rate freezing (right) prior to the frozen product shipped back to the treating site.

Overall trial design for TSC-100/ TSC-101 in patients undergoing HCT



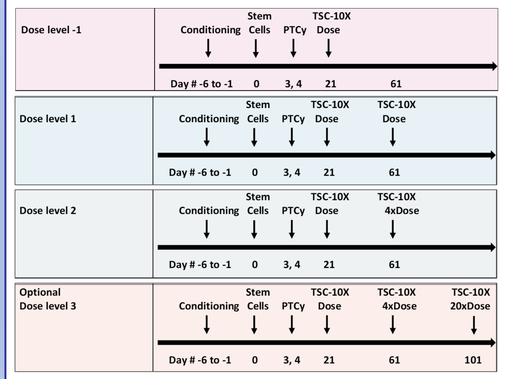
Patients with AML, MDS and ALL planned for HCT with reduced intensity conditioning (RIC) from a haploidentical donor (haplo) will be assigned to treatment or control arm depending on their HLA and minor antigen type. All patients with HLA-A*02:01 (~40% prevalence) will be genotyped to assign treatment arms. If they are HA-1 positive (~60% prevalence), they will receive TSC-100. If HA-1 negative, they will be HA-2 positive (~97% prevalence) and will receive TSC-101. Donors would need to be mismatched for either HLA or minor antigen type. Patients without HLA-A*02:01 (~60%) or without mismatched donors will be assigned to the standard-of-care control arm.

Retrospective analysis of CIBMTR data supports HLA-based 'randomization'

1-year outcomes Percentages (CI)	Disease-free survival	Relapses	Overall survival	Non-relapse mortality	Acute GvHD (II-IV) at 6 months	Chronic GvHD
HLA-A*02:01+ (N=444)	52 (48-57)	32 (28-37)	67 (63-72)	15 (12-19)	30 (25-34)	25 (21-30)
Not HLA-A*02:01 (N=864)	50 (47-54)	34 (30-37)	66 (63-70)	16 (14-19)	29 (26-32)	24 (21-28)

Collaborative analysis with CIBMTR of patients undergoing RIC-HCT from haploidentical donors from 2017-2019 did not find significant differences in outcomes between patients with HLA-A*02:01 and other HLA types. (CI= confidence intervals)

Dose level cohorts and treatment regimen for Phase I trial



Patients will undergo standard RIC conditioning followed by stem cell infusion from haplo donors and post-transplant cyclophosphamide (PTCy). Upon count recovery (around Day 21), they will receive TSC-100 or TSC-101 (TSC-10X). If there is no excessive toxicity, the TSC-10X dose will be repeated after 40 days (around Day 61) at the same dose for Dose level 1. The second dose will be escalated to 4X the starting dose for Dose level 2 patients. An optional Dose level 3 cohort will open if any patient in Dose level 2 has donor chimerism <98% at Day 100, minimal residual disease (MRD+) post HCT or TSC-10X persistence <3% at Day 100. In this cohort, a 3rd dose at 20X starting dose will be administered. Dose escalation will follow interval 3+3 design[4] (i3+3) with 3-12 patients per cohort. Primary endpoints include adverse event profile and dose limiting toxicities. Secondary endpoints include relapse rates at 1 year. Exploratory endpoints include donor chimerism kinetics, MRD+ rates, and TSC-10X persistence.

References

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2. Spierings E., Kim Y., Hendriks M. *et al. Biol Blood Marrow Transplant,* 2013, **19,** 1244-1253.
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4. Liu M., Wang S.J., Ji Y., *et al. J Biopharm Stat*. 2020, **30**, 294-304.