CD45 as a universal target for adjuvant TCR-T cell therapy following allogeneic hematopoietic cell transplantation

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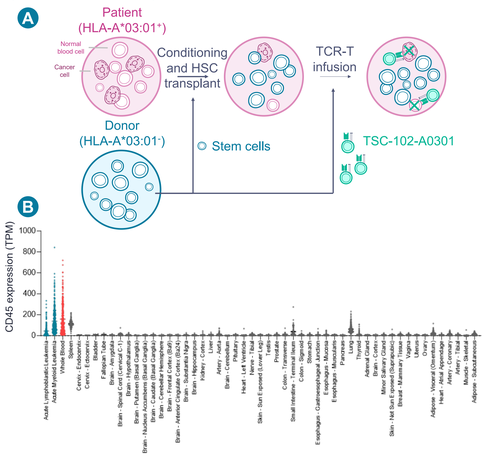
Abstract # 753

Abstract

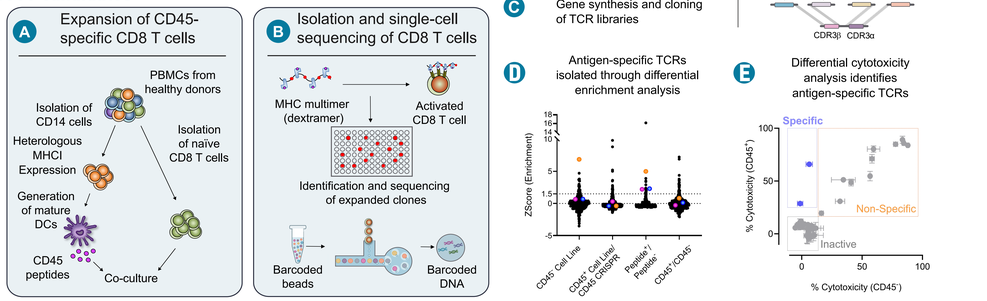
Engineered cell therapies have transformed the treatment of B-cell malignancies, but myeloid malignancies like AML, MDS, and some forms of ALL remain a high unmet need. To date, allogeneic hematopoietic cell transplantation (HCT) remains the only curative option for patients with these malignancies, but up to 40% of patients relapse post-HCT, with >80% mortality within 2 years of relapse. One way to prevent relapse is to administer donor-derived TCR-engineered T cells (TCR-T cells) immediately following transplantation that target antigens presented on patient-derived, but not donor-derived, hematopoietic cells. This provides a way to eliminate residual malignant and pre-malignant hematopoietic cells while sparing normal, donor-derived cells. Early data from the ALLOHA trial suggest that TCR-T cells targeting HA-1 or HA-2, antigens presented on HLA-A*02:01, reduce relapse rates in patients with acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL) undergoing allogeneic HCT with reduced intensity conditioning (Al Malki, ASH 2024). To expand this trial to include TCR-Ts targeting additional HLA types, we report a novel strategy in which TCR-Ts are developed that target antigens derived from the universal heme-restricted protein CD45 (PTPRC). We demonstrate preclinical proof-of-concept studies using a lead, HLA-A*03:01-restricted, CD45-targeted TCR identified using novel in vitro stimulation and screening platforms. We show that the TCR-T candidate is target-specific, with good safety and efficacy profiles, sparing HLA-A*03:01-negative hematopoietic cells and HLA-A*03:01-positive non-hematopoietic cells, while eradicating HLA-A*03:01-positive, CD45-positive hematopoietic cells of normal and transformed origin. This TCR-T candidate has now been advanced to IND-enabling studies.

Development of an A*03:01-restricted TCR-T product targeting a universal antigen in heme malignancies (TSC-102-A0301)



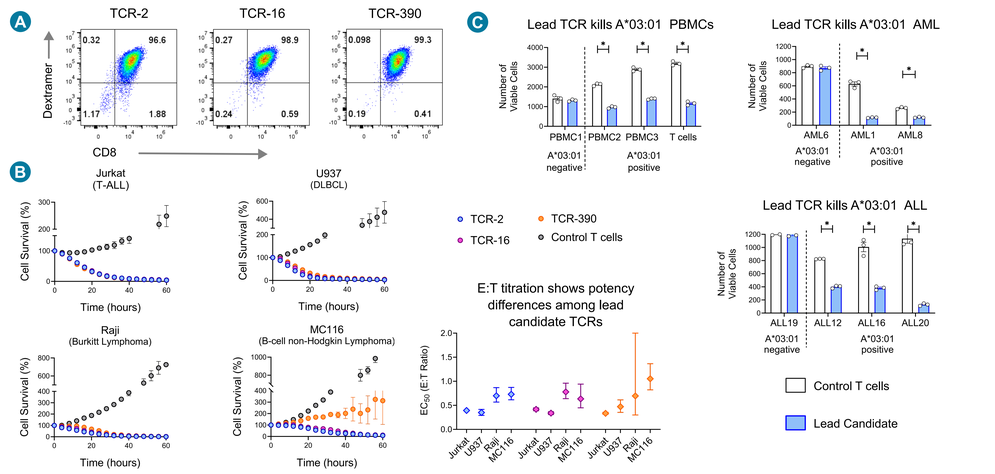
(A) A*03:01-positive AML, MDS, and ALL patients undergoing reduced intensity myeloablative conditioning followed by HCT could be eligible to receive the TSC-102-A0301 TCR-T product targeting CD45, also known as leukocyte common antigen. Infusion of TSC-102-A0301 would occur after count recovery and engraftment post-HCT, with CD45 targeting aimed at selectively eliminating residual leukemic cells as well as the patient's native blood cells in order to promote complete donor chimerism and reduce relapse rates. **(B)** The receptor protein tyrosine phosphatase CD45 (PTPRC) is exclusively expressed on all nucleated cells of the hematopoietic system, and CD45 targeting has been associated with an optimal safety profile in multiple treatment modalities [1,2]. TSC-102-A03 is specific for all isoforms of the CD45 protein to enable universal targeting of hematopoietically-derived cells.

ReceptorScan and ActivScan platforms identify HLA-A*03:01-restricted CD45-specific TCRs from a library of over 1500 candidates



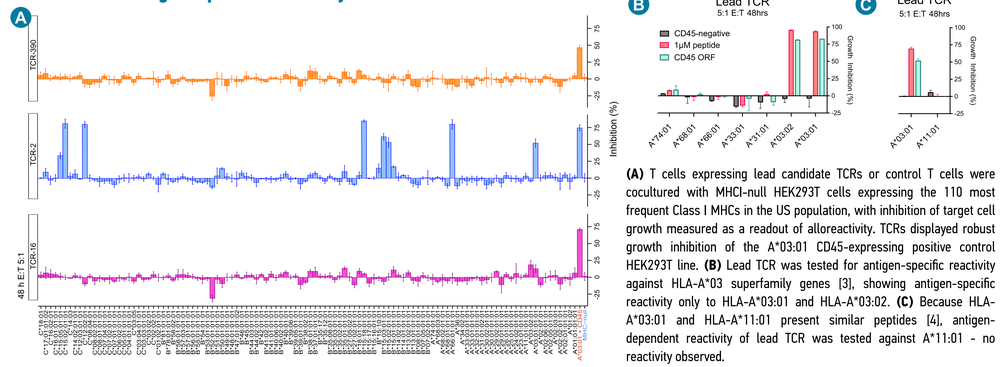
(A) CD45-specific T cells were expanded from the naïve CD8+ T cell population of A*03:01-negative healthy donors. Co-culture and expansion of CD8 T cells was performed with autologous mature DCs expressing exogenous HLA-A*03:01, pulsed with CD45-derived A*03:01-restricted epitopes. **(B)** To isolate antigen-specific CD8 T cells, co-cultures were stained with A*03:01 CD45-specific dextramers and sorted, and single-cell sequencing of dextramer-positive CD8 T cells was performed using the 10X Genomics platform. **(C)** TCRs identified by the ReceptorScan platform were synthesized using TScan's proprietary PISTACHIO cloning method to generate TCR libraries. **(D-E)** ActivScan platform identifies antigen-specific TCRs through functional screening of PISTACHIO-cloned TCR libraries. **(D)** PISTACHIO library was screened for differential, antigen-specific T cell activation by co-culturing library engineered T cells with cell lines with or without antigen, isolating activated T cells by FACS, TCR sequencing and calculating an enrichment score; TCRs with enrichment >1.5σ in at least one condition were evaluated further. The values for each of 3 lead candidate TCRs are colored: TCR-2 in blue, TCR-16 in purple, TCR-390 in yellow. **(E)** Lead candidate TCRs are selected after differential cytotoxicity analysis.

Lead identification through potency evaluation against CD45+ cell lines or normal and transformed cells



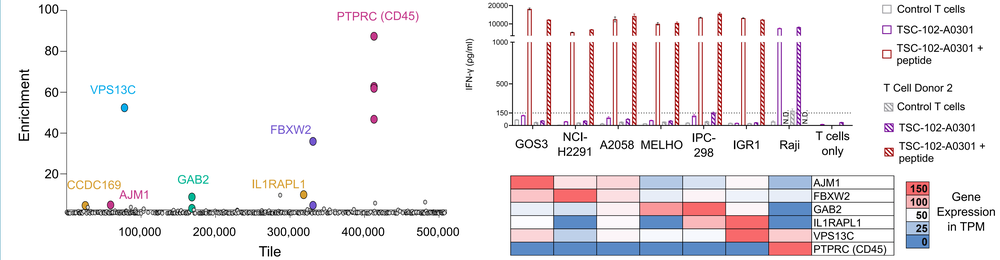
TCR expression, dextramer binding, and cytotoxicity assays were used to evaluate CD45-specific TCRs and nominate a lead candidate. **(A)** Dot plots depict TCR expression and pMHCI engagement of lead candidates, as assessed by A*03:01-restricted, CD45 dextramer staining of lentivirally-transduced TCR-T cells. **(B)** Lead candidate TCRs potently kill various CD45^{+} blood cancer cell lines. Cytotoxicity of lentivirally-transduced TCR-T cell products expressing lead candidate TCRs was assayed microscopically (Incucyte® instrument) at 5:1 effector to target ratios (E:T) against HLA-A*03:01^{+} CD45^{+} blood cancer cell lines from various indications, where the target cells were engineered with the fluorescent NucLight™ Red marker. TCRs show potent killing with EC_{50} <1 E:T at 48hrs. **(C)** Lead TCR specifically and potently kills HLA-A*03:01^{+} non-transformed peripheral blood mononuclear cells (PBMCs), and transformed primary samples sourced from patients diagnosed with AML or with ALL; data are representative of multiple donors tested.

Lead candidate TCRs were assayed for alloreactivity against 110 MHCI allotypes using the AlloScan platform and evaluated for antigen-specific reactivity to HLA-A*11:01



(A) T cells expressing lead candidate TCRs or control T cells were cocultured with MHCI-null HEK293T cells expressing the 110 most frequent Class I MHCs in the US population, with inhibition of target cell growth measured as a readout of alloreactivity. TCRs displayed robust growth inhibition of the A*03:01 CD45-expressing positive control HEK293T line. **(B)** Lead TCR was tested for antigen-specific reactivity against HLA-A*03 superfamily genes [3], showing antigen-specific reactivity only to HLA-A*03:01 and HLA-A*03:02. **(C)** Because HLA-A*03:01 and HLA-A*11:01 present similar peptides [4], antigen-dependent reactivity of lead TCR was tested against A*11:01 - no reactivity observed.

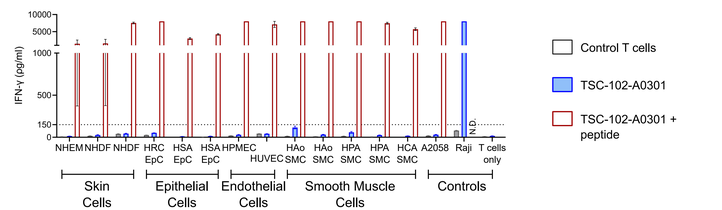
Genome-wide safety screen identifies putative off-targets of TSC-102-A0301 TCR which are de-risked through functional assays



(A) SafetyScan, TScan's proprietary genome-wide TCR target screening platform suggests 6 putative off-targets for TSC-102-A0301 TCR, when overexpressed as 90-amino acid long fragments. **(B)** The physiological relevance of the 5 potential off-targets is assessed in detail by co-culturing the TCR-T cells with CD45-negative, HLA-A*03:01+ cells that naturally express the full-length proteins at normal levels as shown by the heatmap. The TSC-102-A0301 TCR was engineered into T cells from two donors and tested against target cells at 1:1 effector-to-target ratio for 24hrs; secretion of IFN-γ was used as a measure of reactivity. Raji cells (CD45+) and target peptide pulse at 1μM were used as positive controls for reactivity. Gene expression was quantified in-house using Illumina® sequencing and compared to levels in normal tissues. No reactivity was observed against the cell lines tested. CCDC169 is testis-restricted and therefore not tested.

Lack of reactivity to primary cells suggests TSC-102-A0301 is a safe candidate for clinical development

A panel of 20 HLA-A*03:01-positive normal primary or iPSC-derived human cell samples was tested as targets for TSC-102-A0301 to test off-tumor reactivity. The TSC-102-A0301 TCR was engineered into T cells and tested against target cells at 1:1 effector-to-target ratio for 24hrs; secretion of IFN-γ was used as a measure of reactivity. Raji cells (CD45+) and target peptide pulse at 1μM were used as positive controls for reactivity. No reactivity was observed against the cell lines tested.



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