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Engineering Off-the-Shelf Anti Mesothelin T-cell Receptor Fusion Construct (TRuC™) T-cells

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Abstract

We have previously described the generation of autologous **T** **C**ell Receptor F**u**sion **C**onstruct (TRuC™) T cells which are engineered to express a fusion protein comprised of an antibody-derived binder tethered to the extracellular domain of the CD3ε signaling subunit. Upon integration of the TRuC into the T cell receptor (TCR), it targets specific tumor surface antigens independent of HLA and uses the complete receptor complex to trigger a comprehensive T cell response. Here, we report about engineering of off-the-shelf TRuC-T cells directed against mesothelin (MSLN). To eliminate the alloreactivity of α/β T cells and reduce the risk of graft-versus-host-disease (GvHD), the TRAC locus is knocked-out. To enable the re-assembly of the TCR, the endogenous TCRα and β subunits are replaced with fusion proteins comprised of antibody-derived binders fused to the TCRγ and δ constant domains. Allogeneic anti-MSLN TRuC-T cells upregulate activation markers, secrete robust cytokines, and lyse tumor cells in an antigen-specific manner without allo-reactivity. In comparison with control autologous TRuC-T cells targeting mesothelin (TC-210), allogeneic TRuC-Ts demonstrate extended tumor clearance in NSG xenograft models due to their enhanced expansion and persistence. To reduce host rejection and further boost the persistence of the allogeneic TRuC-T cells, we eliminated MHC class I surface expression by B2M gene knockout and over-expressed membrane-bound IL15 tethered to its receptor alpha. In summary we have engineered persistence enhanced, allogeneic TRuC-T cells that maintain the signaling properties of the TCR complex with improved efficacy compared to donor-matched autologous TRuC-T cells.

Allogeneic TRuC-T cell Platform

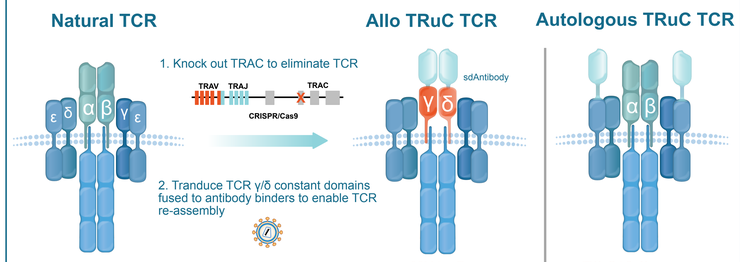

FIGURE 1: Comparison of the natural T cell receptor (TCR), our Allogeneic TRuC TCR and our Autologous TRuC TCR
To generate allogeneic TRuC-T cells, the T Cell receptor Alpha Constant (TRAC) gene is targeted using CRIPSR/CAS9 to eliminate cell surface expression of the TCR. To re-assemble a non-alloreactive TCR, we use lentivirus to transduce primary T-cells with Allo TRuC transgenes. Allo TRuC transgenes targeting Mesothelin (MSLN) are comprised of a single domain antibody (VHH - Binder named 'MH1') fused to the TCR gamma and delta constant domains. In contrast, our autologous TRuC-T cell platform is generated by introducing a TRuC transgene comprised of a single domain antibody (MH1) fused to the extracellular domain of CD epsilon. Because our autologous TRuC-T cells maintain their endogenous TCR alpha and beta subunits, they will react against 'non-self' recipient HLA-I molecules causing GvHD.

Allogeneic TRuC-T cells targeting MSLN are functional *in vitro*

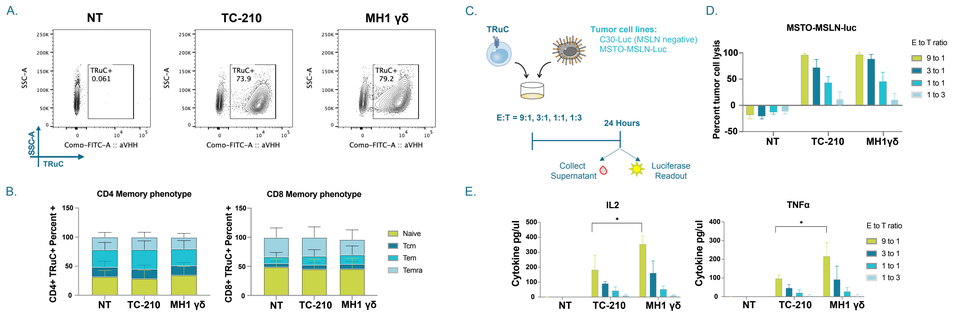

FIGURE 2: Allogeneic TRuC- T cells targeting mesothelin demonstrate similar *in vitro* characteristics to TC-210
A: Flow cytometry staining of TRuC transduction efficiency comparing TC-210 to MH1 γδ Allo TRuC using an anti-VHH antibody. B. Stacked bar plot displaying memory phenotype percentages comparing TC-210 to MH1 γδ. Naive cells= CD45RA+/CCR7+; Tcm (central memory) = CD45RA-/CCR7+; Tem (effector memory) = CD45RA-/CCR7-; Temra = CD45RA+/CCR7- (n = 5 donors). C-E. Schematic representation of TRuC-T cell cytotoxicity assay (n = 2 donors), results (D) and cytokine secretion (n = 2 donors) (E) post 24-hour co-culture of MSTO-MSLN-Luc cells and TRuC-T cells.

Allo TRuCs have prolonged tumor clearance compared to TC-210

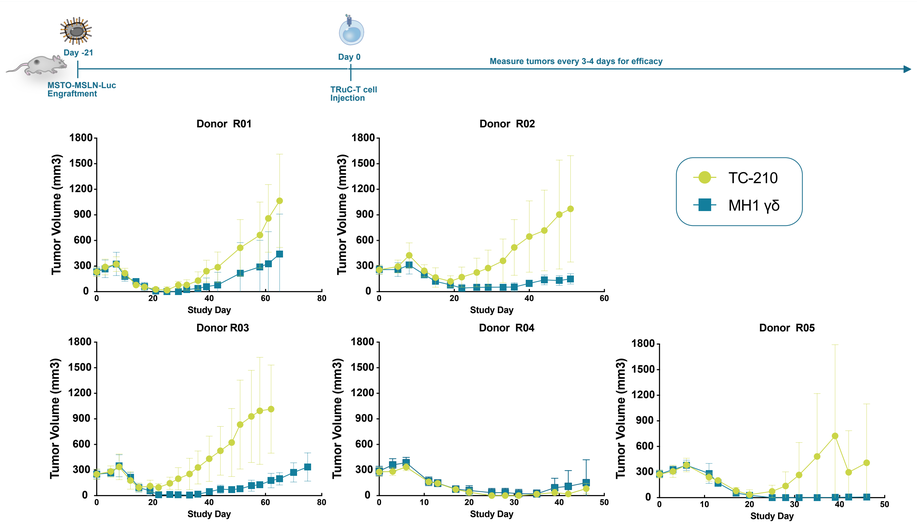

Figure 3: Allo TRuCs targeting MSLN demonstrate prolonged tumor clearance compared to TC-210 *in vivo*
Graphical representation of Allo TRuC-T cell *in vivo* study design. MSTO-MSLN cells (1e6) mixed with matrigel are injected subcutaneously into the right flank of NSG mice. When tumors reach 250-350mm^3 (21 days post tumor engraftment), 2e6 TRuC-T cells are injected intravenously and tumors are measured every 3-4 days. Tumor growth curves of 5 distinct studies (and donors) comparing TC-210 to MH1 γδ Allo TRuCs.

Allo TRuCs have enhanced persistence *in vivo*

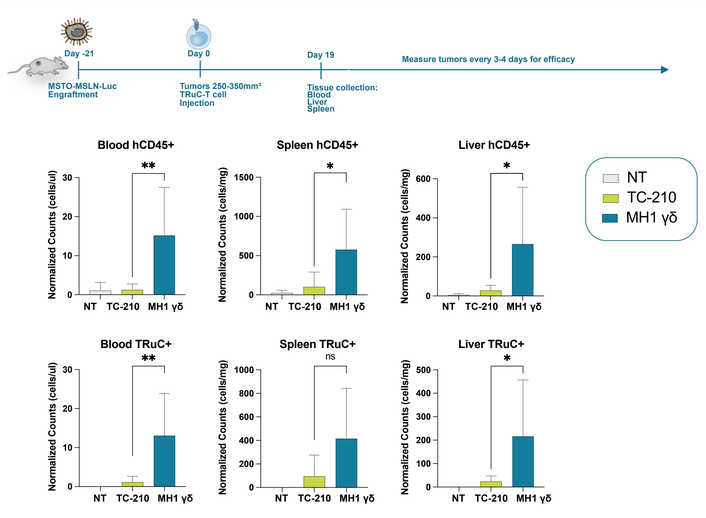

Figure 4: MH1 Allo TRuCs are detected *in vivo* longer than TC-210
On Day 19 post TRuC-T cell injection, tissues (blood, spleen, liver) were harvested, and analyzed for TRuC-T cell expansion and proliferation. Graphs shows normalized counts of TRuC-T cells analyzed by human CD45 detection and subsequent VHH (TRuC) detection. Results are combined from three different donors of TRuC-T cells (n = 9).

Allo TRuCs detect lower MSLN antigen density

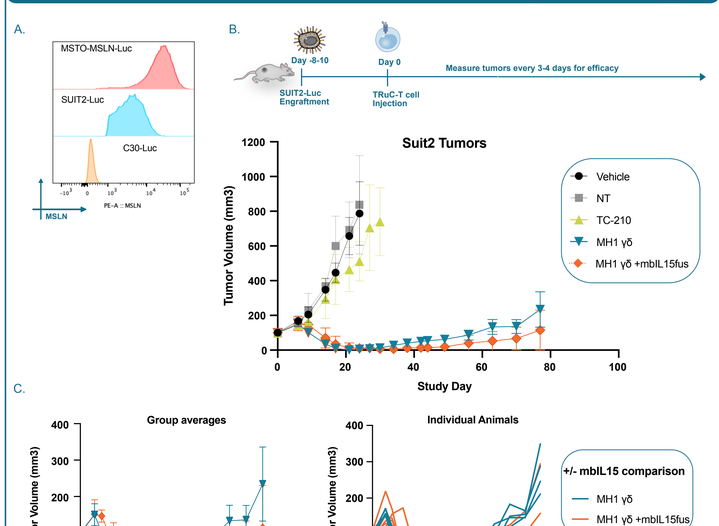

Figure 6. MH1 Allo TRuC-T cells clear tumors in a low MSLN tumor model where TC-210 has no efficacy
A. Surface staining of MSLN on MSTO-MSLN-Luc tumor cells compared to SUIT2-Luc cells using C30-Luc cells as a negative control. B. Graphical representation of the *in vivo* efficacy study challenging TRuC-T cells against SUIT2 cells. C. MH1 γδ Allo TRuCs were enhanced with membrane-bound IL15 receptor alpha, IL15 fusion protein to increase persistence. The left plot shows the averages on the animals within the two Allo groups and the righthand plot show the individual animals within those two groups. 3 animals in the MH1 γδ +mbIL15fus group resist tumor regrowth longer than MH1 γδ alone, with one animal maintaining tumor clearance throughout the duration of the study. Study needs to be repeated in additional donors.

Allo TRuC-T cells maintain potency with TRAC/B2M multiplexed knock out conditions

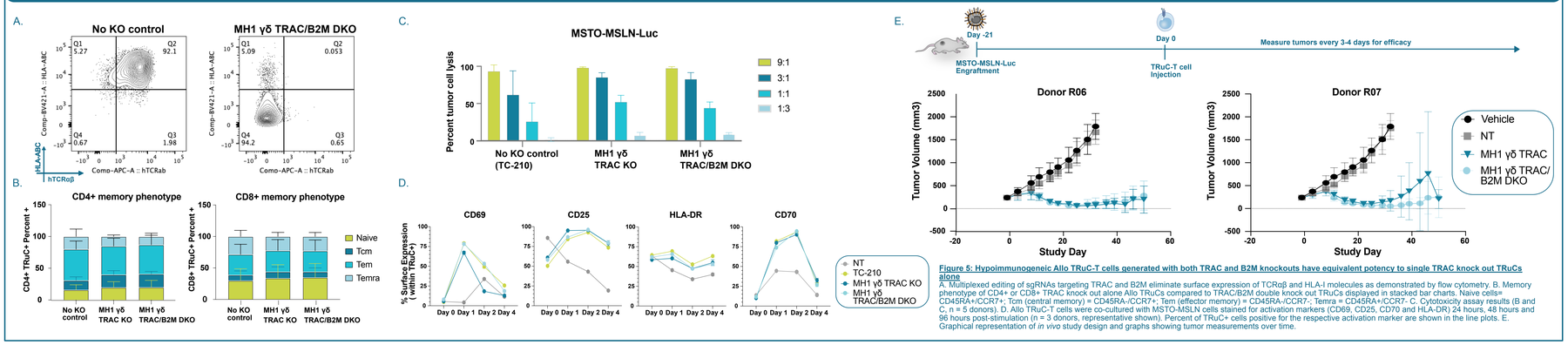

Figure 5: Hypoimmunogeneic Allo TRuC-T cells generated with both TRAC and B2M knockouts have equivalent potency to single TRAC knock out TRuCs alone
A. Multiplexed editing of sgRNAs targeting TRAC and B2M eliminate surface expression of TCRαβ and HLA-I molecules as demonstrated by flow cytometry. B. Memory phenotype of CD4+ or CD8+ TRAC knock out alone Allo TRuCs compared to TRAC/B2M double knock out TRuCs displayed in stacked bar charts. Naive cells= CD45RA+/CCR7+; Tcm (central memory) = CD45RA-/CCR7+; Tem (effector memory) = CD45RA-/CCR7-; Temra = CD45RA+/CCR7- C. Cytotoxicity assay results (B and C, n = 5 donors). D. Allo TRuC-T cells were co-cultured with MSTO-MSLN cells stained for activation markers (CD69, CD25, CD70 and HLA-DR) 24 hours, 48 hours and 96 hours post-stimulation (n = 3 donors, representative shown). Percent of TRuC+ cells positive for the respective activation marker are shown in the line plots. E. Graphical representation of *in vivo* study design and graphs showing tumor measurements over time.

Conclusions

- MH1 γδ Allo TRuC-T cells show similar *in vitro* activity to TC-210, but have improved *in vivo* efficacy against well-established MSTO-MSLN tumors
- MH1 γδ Allo TRuC-T cells show prolonged persistence *in vivo* compared to TC-210
- In contrast to TC-210, MH1 γδ Allo TRuC-T cells show *in vivo* efficacy against SUIT2 tumors with low MSLN expression
- MH1 γδ Allo TRuC-T cells generated with a TRAC and B2M multiplexed knock out retain full potency