Engineering Off-the-Shelf T Cell Receptor Fusion Construct (TRuC™) T cells

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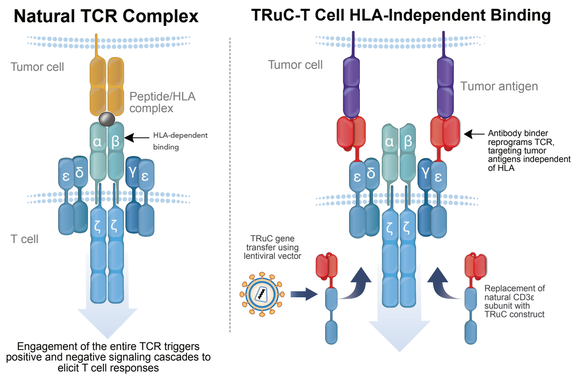
Abstract

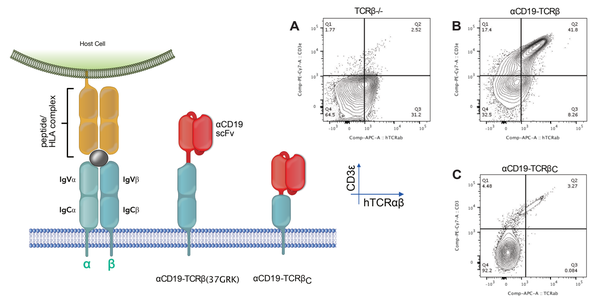
The T-Cell Receptor (TCR) can be functionally split into antigen recognition mediated by the TCRα/β heterodimer and signal transduction triggered by the CD3 complex comprising CD3ε/γ, CD3ε/δ and CD3ζ/ζ dimers. Unlike antibodies, the TCR recognizes its cognate peptide antigen only when presented on human leukocyte antigen (HLA) molecules. Recently, we reported that tethering an antibody-derived binder to one of the TCR subunits redirects T cells to specifically kill tumor cells independent of HLA. Different from CAR-T cells, the T cell receptor fusion constructs (TRuC™) are integrated into the natural TCR and require all TCR subunits for receptor translocation to the cell surface. The development of off-the-shelf TRuC-T cells is desirable to shorten the vein-to-vein production time and reduce manufacturing costs. Here, we describe the generation of TRuC-T cells expressing a fully functional TRuC TCR without alloreactivity. Inactivation of the endogenous TRAC gene disrupts natural TCR formation. Yet replacing the endogenous TCRα subunit with a human TCRα constant region without variable domain to avoid alloreactivity is insufficient for TRuC TCR expression. However, a functional TRuC TCR can be created by substituting the following constructs for the inactivated TRAC gene: (i) murine TCRα and β constant domains without variable domains, (ii) chimeric human/murine TCRα and β constant regions or (iii) TCRγ and δ constant domains. Off-the-shelf TRuC-T cells upregulate activation markers, secrete cytokines, and kill tumor cells in an antigen-specific manner. Importantly, the genome-engineered TRuC-T cells lack alloreactivity as demonstrated in mixed lymphocyte reactions and show efficacy in an NSG xenograft models without signs of graft versus host disease (GvHD). Our findings warrant further development of allogeneic TRuC-T cells for cancer therapy.

TRuC-T cell platform



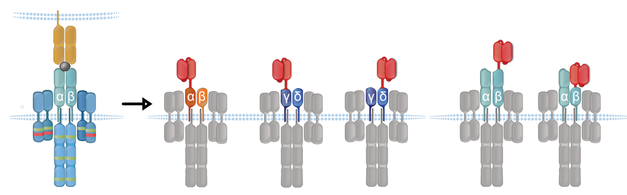
Engagement of the entire TCR triggers positive and negative signaling cascades to elicit T cell responses

Restoration of TCR in KO cells



(**A**) TCRβ knockout Jurkat cells were generated by inactivation of TCRβ gene with CRISPR/Cas9. (**B**) Restoration of cell surface TCR was observed after transduction with full-length TCRβ TRuC. (**C**) However, TCR restoration was not obtained with transduction of a construct with the TCRβ constant region only (TCRβC).

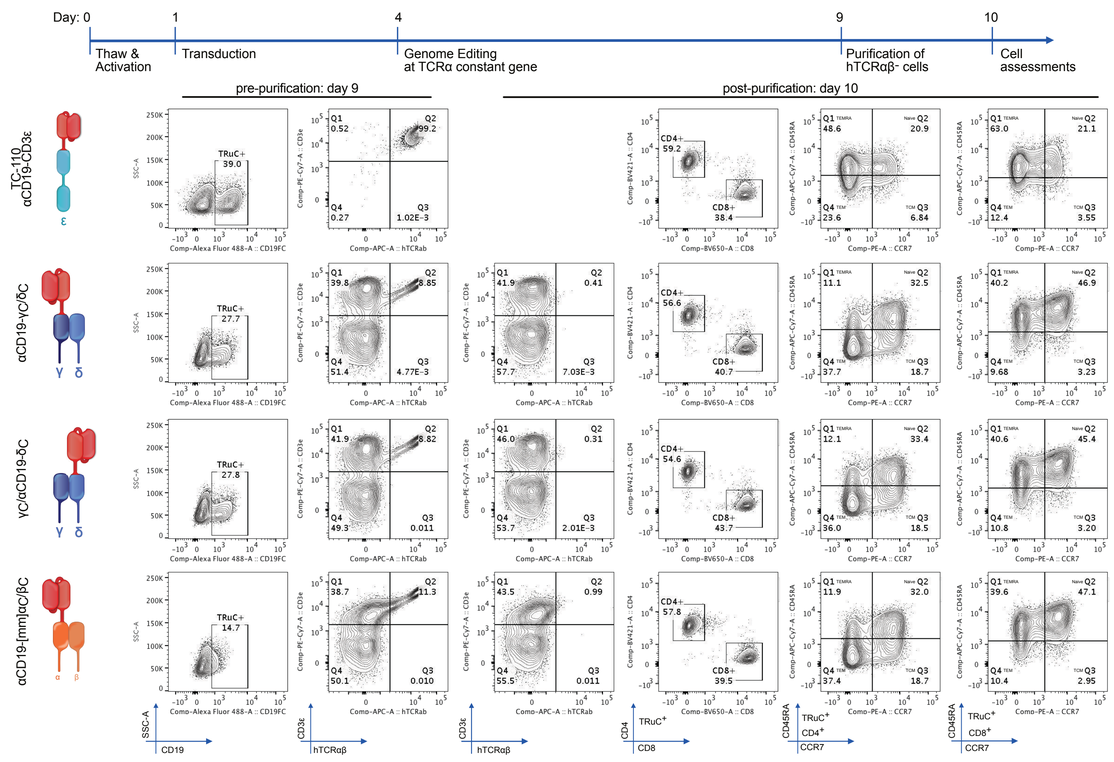
Engineered Allo TRuC-T cell constructs



TCR KO	TRA-/-	TRA-/-	TRA-/-	TRB-/-	TRB-/-
Transgene	αCD19-[mm]αC/βC	αCD19-γC/δC	γC/αCD19-δC	αCD19-TCRβ(37GRK)	αCD19-TCRβC
TCR reconstitution	Yes	Yes	Yes	Yes	No

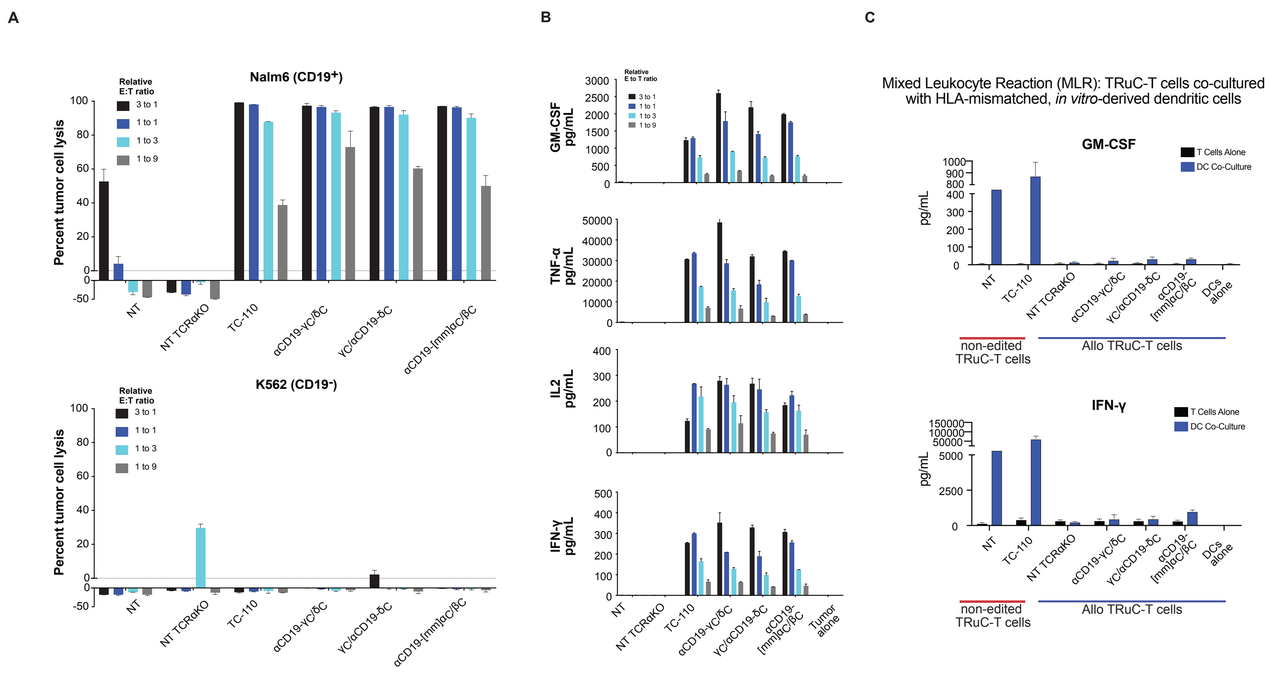
Schematic representation of engineered TCR transgenes assessed to replace the endogenous TCR.

Allo TRuC-T cell generation and phenotype



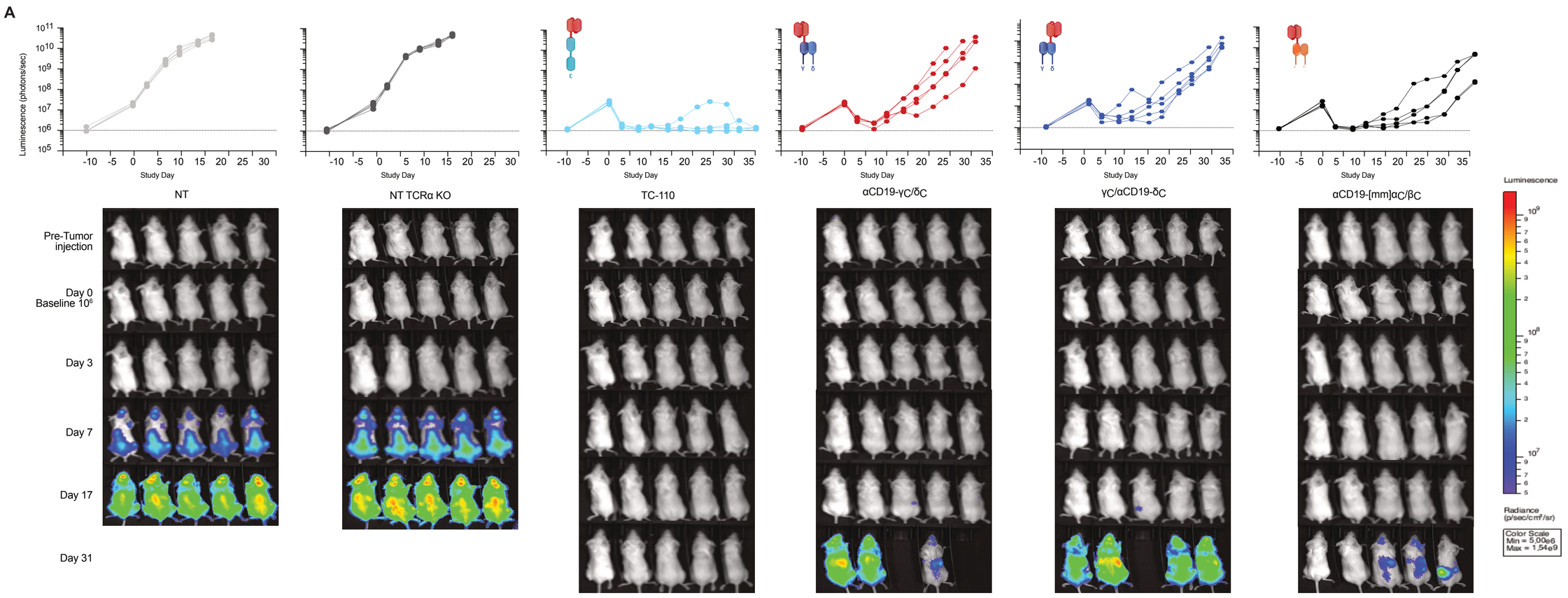
Primary T cells were transduced 24 hours after activation with a lentivirus containing α-CD19 scFv TRuC construct. The endogenous TCR was inactivated with CRISPR/Cas9 72 hours post activation by targeting the TCRα constant region locus. T cells were purified to remove left over T cells that still expressed the endogenous TCR by negative selection. Flow cytometry panel shown was performed at Day 9 and 10 to assess transduction, editing efficiency and T-cell phenotype.

In vitro Allo TRuC-T cells kill target cells, produce cytokines, but are not alloreactive

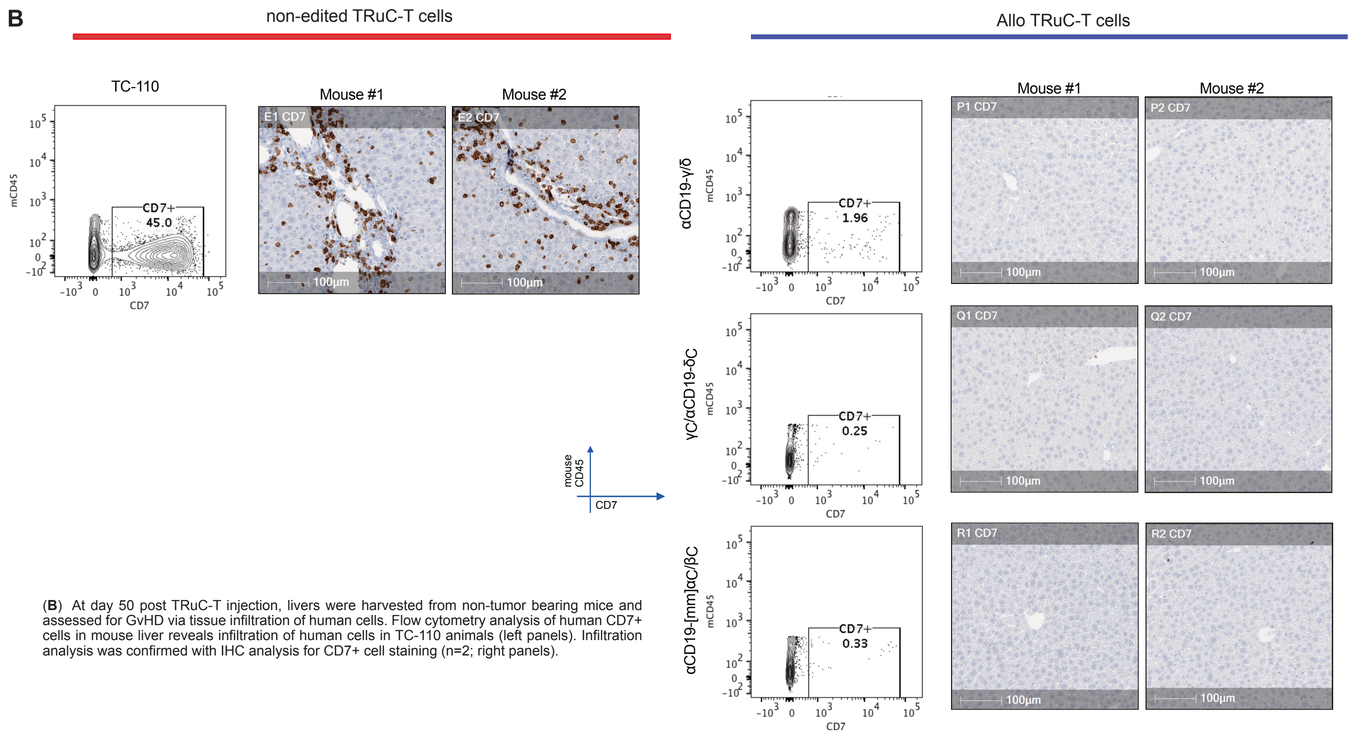


(**A**) TRuC-T cells (E), and CD19+ (Nalm6-luc) and CD19- (K562-luc) Tumor cells (T) cells were co-cultured for 24 hours. Percent tumor cell lysis was measured by a luciferase release-based cytotoxicity assay. (**B**) Cytokine secretion (GM-CSF, IL-2, IFN-γ, TNF-α) into supernatants was analyzed using Meso Scale Discovery (MSD) technology and normalized based on TRuC+ transduction percentage. Percentage of TRuC+ population is displayed on graph to account for differences in TRuC transduction efficiency among constructs. (**C**) TRuC-T cells were co-cultured with HLA-mismatched, *in vitro*-derived dendritic cells at a 1:3 (T:DC) ratio for 72 hours. Secreted cytokines were measured in supernatants by MSD assay. Allo TRuC-T cells secreted significantly less cytokine compared with non-edited TRuC-T cells.

Anti-tumor efficacy of Allo TRuC-T cells and no signs of GvHD in mouse xenograft model



(**A**) *In vivo* efficacy of Allo TRuC-T cells in a Nalm6-luc tumor mouse model. Tumor cells were injected into NSG mice at Day -10. After establishment of the tumors, mice received one injection of 2.2x10^6 non-edited or Allo TRuC-T cells on Day 0. Mice were imaged every 3 days post TRuC-T cell injection to assess tumor load.



(**B**) At day 50 post TRuC-T injection, livers were harvested from non-tumor bearing mice and assessed for GvHD via tissue infiltration of human cells. Flow cytometry analysis of human CD7+ cells in mouse liver reveals infiltration of human cells in TC-110 animals (left panels). Infiltration analysis was confirmed with IHC analysis for CD7+ cell staining (n=2; right panels).

Conclusions

- Allogeneic TRuC-T cells were successfully engineered by replacing the endogenous T cell receptors α and β subunits with TCR transgenes containing the constant regions of murine TCRα and TCRβ or human TCRγ and TCRδ.
- Expression of the human constant TCRα and TCRβ subunits did not restore a functional TCR complex.
- Allogeneic TRuC-T cells with αCD19 scFv lysed CD19+ tumor cells efficiently *in vitro* and produced cytokines at similar levels as non-edited αCD19 TRuC-T cells.
- Allogeneic TRuC-T cells were not alloreactive in a mixed leukocyte reaction with HLA-mismatched dendritic cells .
- *In vivo*, non-edited TRuC-T cells exhibited complete anti-tumor activity, whereas Allo TRuC-T cells showed tumor regression.
- Allo TRuC-T cells showed no signs of GVHD.
- Optimization of the T cell production process is expected to improve anti-tumor activity of Allo TRuC-T cells.