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Expression of an IL-15 Receptor Fusion Protein Enhances the Persistence of TRuC-T Cells

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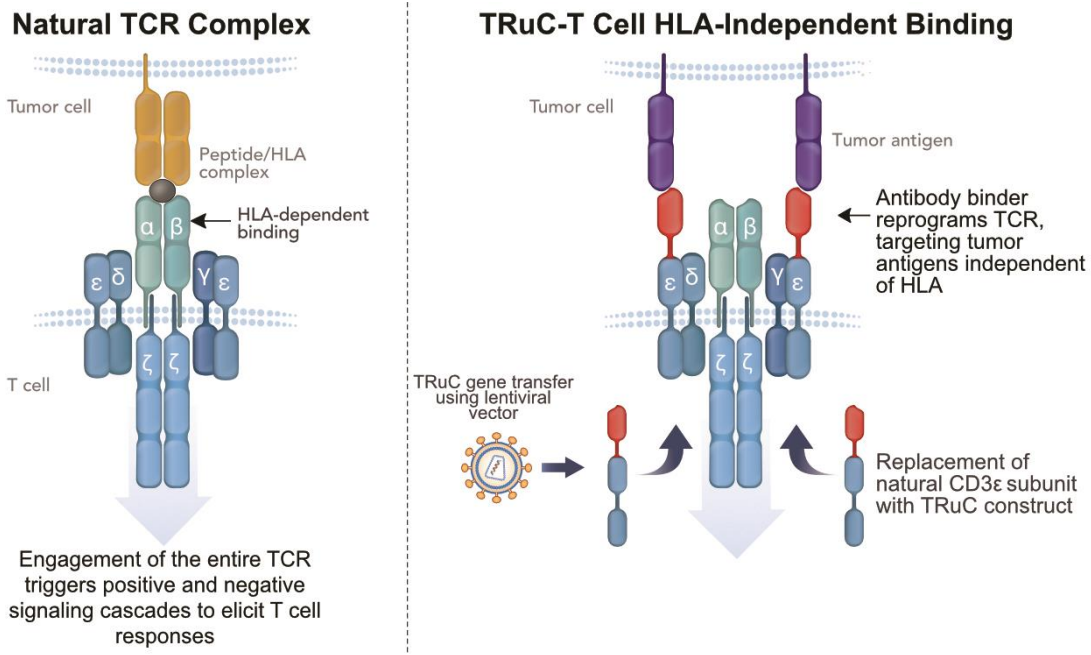
Disclaimer

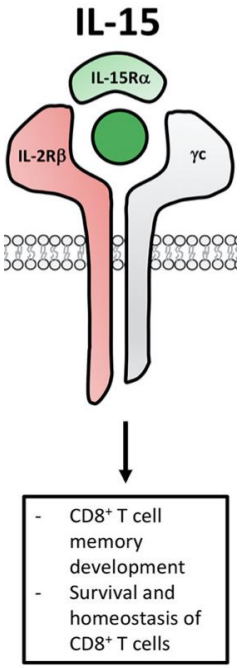
- I am an employee of TCR^2 Therapeutics.

TRuC-T Cell Platform

- TRuC-T cells have an engineered T cell receptor that utilizes all TCR signaling subunits and recognizes tumor-associated antigens independent of HLA.



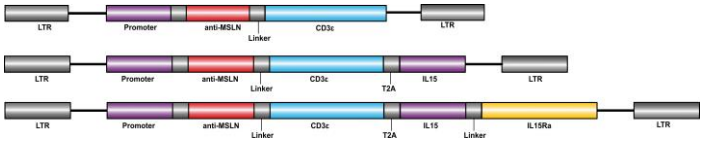
IL-15 as an Enhancement for T cell Function

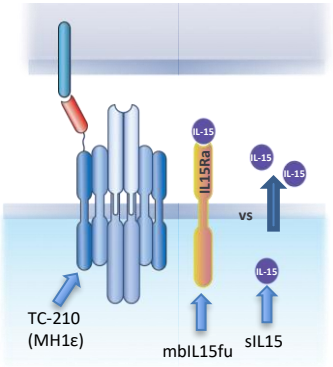


Dwyer et. al. *Frontiers in Immunology* 2019.

- γ chain cytokine
- Important for the development and homeostasis of NK cells and CD8+ T cells
- Promotes the survival and proliferation of naïve and central memory CD8+ T cells
- Inhibits IL-2 activation induced cell death (AICD)
- Based on these properties, IL-15 signaling is expected to enhance TRuC-T cell persistence and improve efficacy against solid tumors

IL-15 constructs expressed in tandem with anti-mesothelin TRuC





T-cells Coexpressing an anti-mesothelin TRuC and IL-15 Enhancements Possess a Favorable Phenotype

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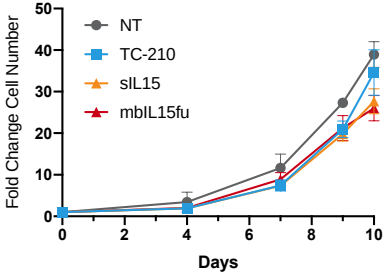
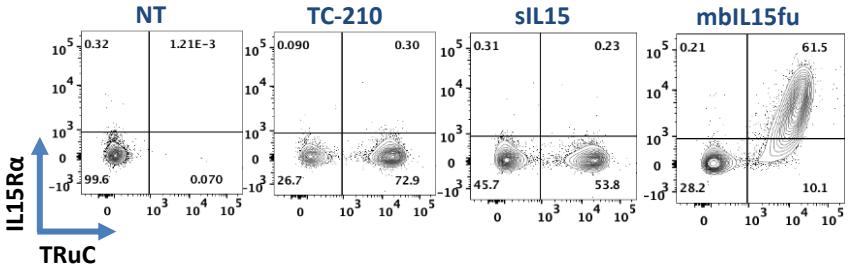
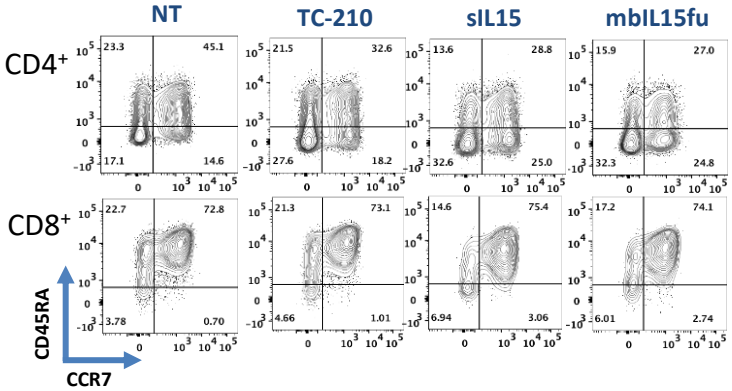
A. T-cells expand well during manufacturing process

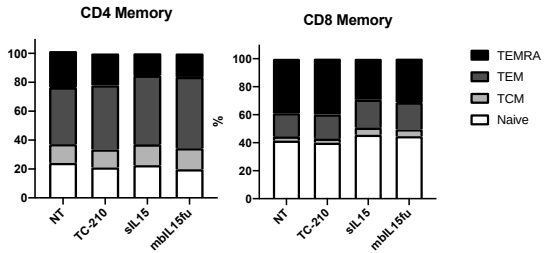


B. TRuC and mbIL-15fu proteins show high coexpression



C. IL-15 expression increases proportion of CD8⁺ naïve/TCM cells within T cell products





A. Fold change in T cell numbers over a 10-day manufacturing process. **B-C.** At the end of expansion, transduced T cells were phenotyped by flow cytometry to characterize expression of the MSLN TRuC, IL-15rα expression (B), and memory phenotype (C) (n=7 donors).

IL-15 Expressing TRuC-T cells Upregulate Stemness Markers and Have Enhanced Persistence *In Vitro*

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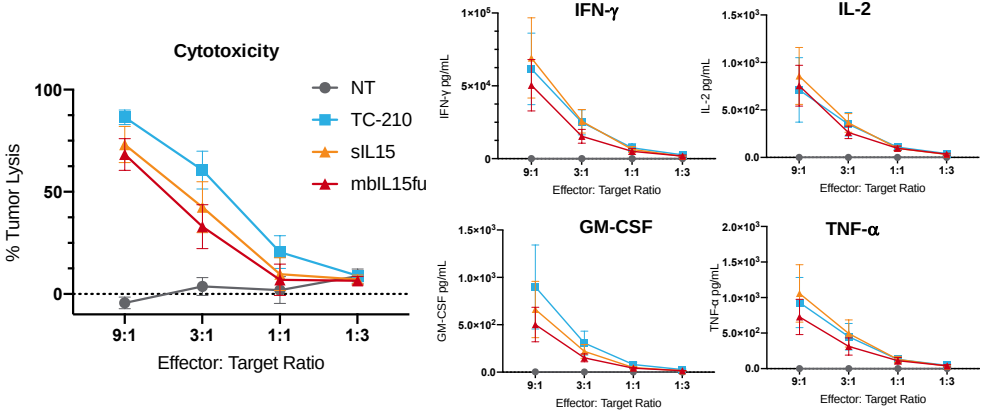
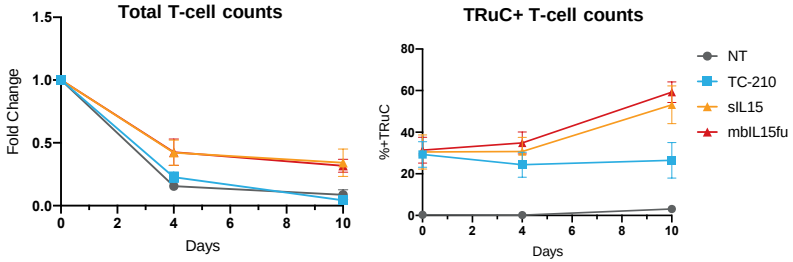
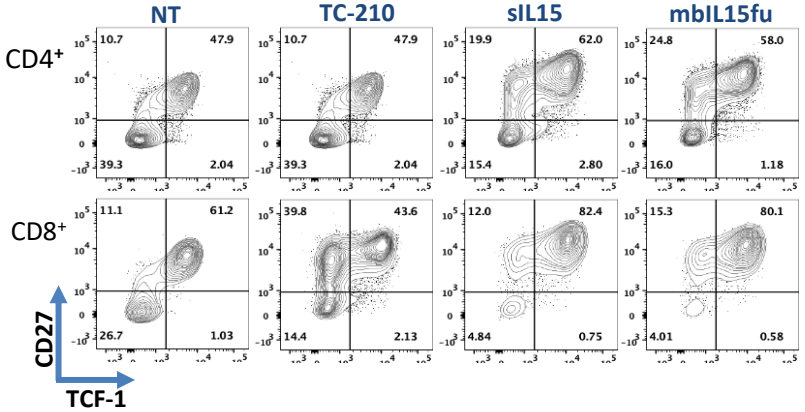
A. IL-15 enhanced TRuC T-cells show good killing activity and cytokine production



B. Increased persistence under cytokine-free culture conditions



C. Upregulation of stemness markers following T-cell activation



A. Cytotoxicity by bioluminescence assay and cytokine production by MSD ELISA were measured after 24 hours of T-cell/MSTO-MSLN cell coculture (n=6)
B. T-cells were cocultured with MSTO-MSLN cells for 96 hours and then stained for TCF-1 and CD27 and analyzed by flow cytometry.
C. T-cells were cultured in vitro for 10 days in cytokine-free media and cell numbers were quantified on indicated days (n=4).

In Vivo, IL-15 Enhanced TRuC-T cells have Increased Persistence that Protects from Tumor Rechallenge

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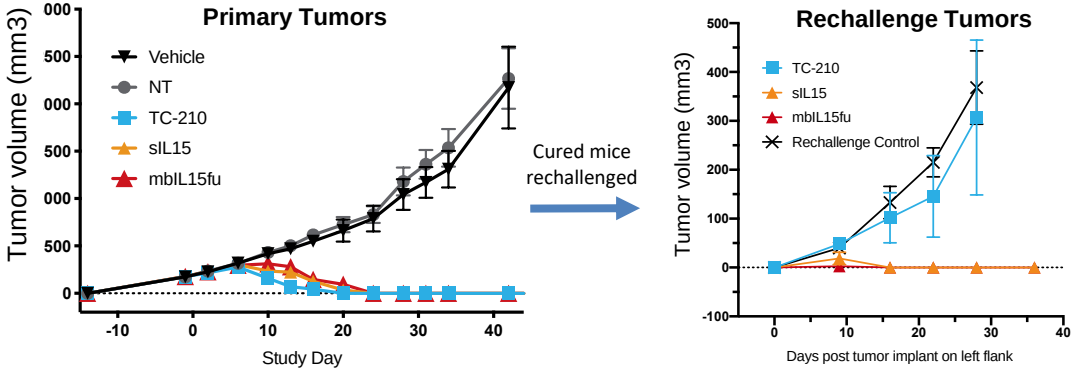
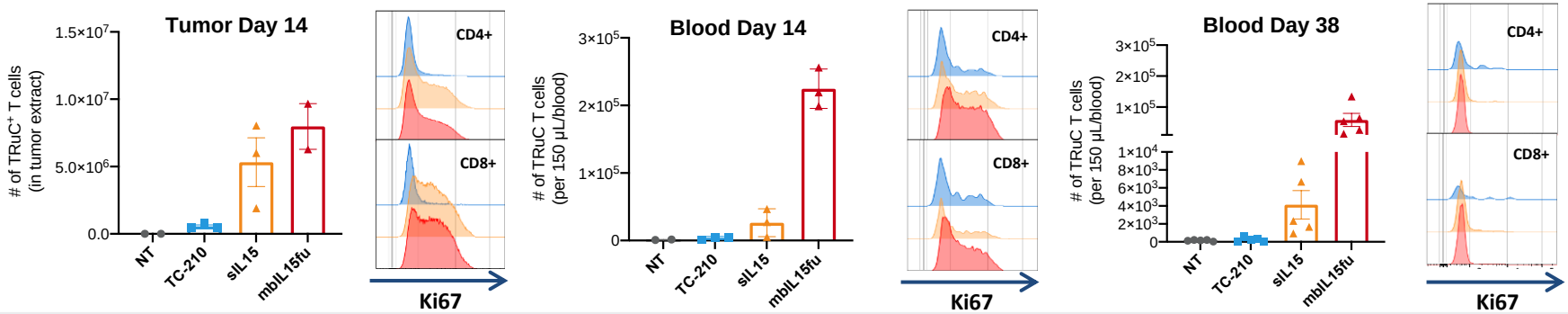
A. IL-15 enhanced TRuC-T cells protect cured mice from tumor rechallenge



A. MSTO-MSLN tumor cells were implanted into NSG MHC class I/II$^{-/-}$ mice and 14 days later the mice were infused with TRuC-T cells. 44 days after T cell infusion, cured mice were rechallenged with MSTO-MSLN tumors on the opposing flank.
B. 14 or 38 days after T cell infusion, tumor and blood samples were collected and subjected to flow cytometric analysis of TRuC and Ki67 expression. TRuC$^+$ cells were enumerated using counting beads.

B. Higher tumor infiltration by IL-15 enhancements is associated with increased T-cell proliferation, expansion, and persistence



Summary and Next Steps

- T-cells engineered with an anti-mesothelin TRuC and IL-15 enhancements show high transduction efficiency and good *in vitro* potency
- IL-15 expressing TRuC-T cells have a favorable phenotype enriched for CD8+ naïve/Tcm cells and show enhanced stemness following activation
- IL-15 enhancements autonomously increase TRuC-T cell persistence *in vitro* and *in vivo* in the absence of external, activating stimuli
- TRuC-T cells bearing IL-15 enhancements display increased expansion & persistence *in vivo* that fully protects mice from tumor rechallenge
- These IL-15 enhancements have the potential to increase TRuC-T cell persistence in cancer patients for improved efficacy against solid tumors