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A chimeric PD1-CD28 switch receptor enhances the activity of TRuC-T cells

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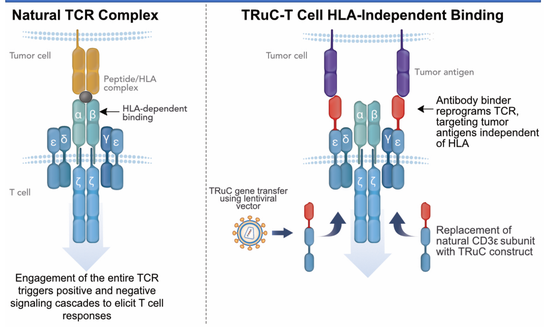
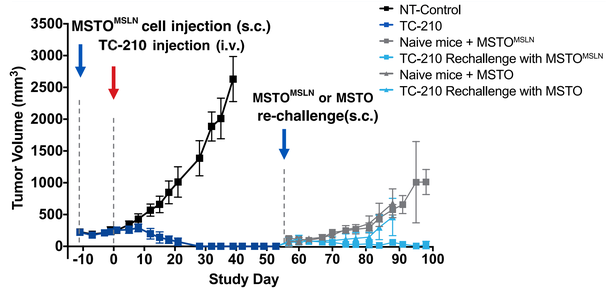
Abstract

Cluster of differentiation 28 (CD28) and programmed death receptor 1 (PD-1) are members of the CD28 superfamily of co-receptors that have critical roles in the regulation of T cell-mediated immunity and inflammation. Upon ligand binding, CD28 signaling synergizes with T cell receptor (TCR) signaling to enhance T-cell activation through the PI3K-Akt pathway, while PD-1 signaling upon binding to its ligands (PD-L1/L2) sequesters critical mediators of signaling from the TCR complex, thereby shunting T-cell activation and effector function. Thus, the expression of PD-L1/L2 in solid tumors may pose a significant barrier to anti-tumor immunity and the efficacy of adoptive T cell therapies (ACT). We have recently described a novel class of engineered T cells that integrate a T cell receptor fusion construct (TRuC®) into the natural TCR complex, thereby reprogramming the specificity of the T cell to recognize tumor surface antigen in a human leukocyte antigen (HLA)-independent fashion. TC-210 T cells expressing mesothelin (MSLN) specific TRuCs demonstrate robust anti-tumor immunity in preclinical models of mesothelioma, protecting mice from tumor re-challenge while inducing lower levels of inflammatory cytokine release when compared to a 2nd generation MSLN-targeted CAR T cell. Here, we show that co-expression of a PD-1:CD28 switch receptor comprising the PD-1 extracellular and transmembrane domains fused to the CD28 intracellular domain, enhances the activity of TC-210 T cells. When compared to TC-210 expressing only the TRuC, co-expression of the PD1:CD28 receptor showed increased Phospho-Erk signaling and maintained effector cytokine production in the presence of PD-L1. In a repetitive stimulation assay, TRuC T cells bearing the PD-1:CD28 receptor demonstrated a competitive advantage in expansion and survival over time, and this enhanced fitness was dependent on the costimulatory domain of the chimeric PD-1 receptor. *In vivo* and further mechanistic studies are currently underway.

TRuC-T Cell Platform

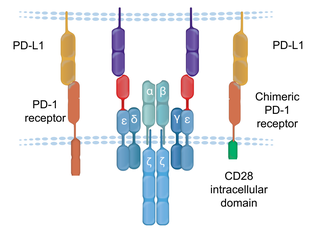


TC-210 Efficacy in Mesothelioma Xenograft Model



Functional persistence of TC-210 was tested in MSTO model by injecting new tumor cells to TC-210 treated NSG mice that had cleared the primary MSTO tumors (tumor-free for 30 days at the time of re-challenge). T cell dose: 2x10⁶ TRuC+ cells.

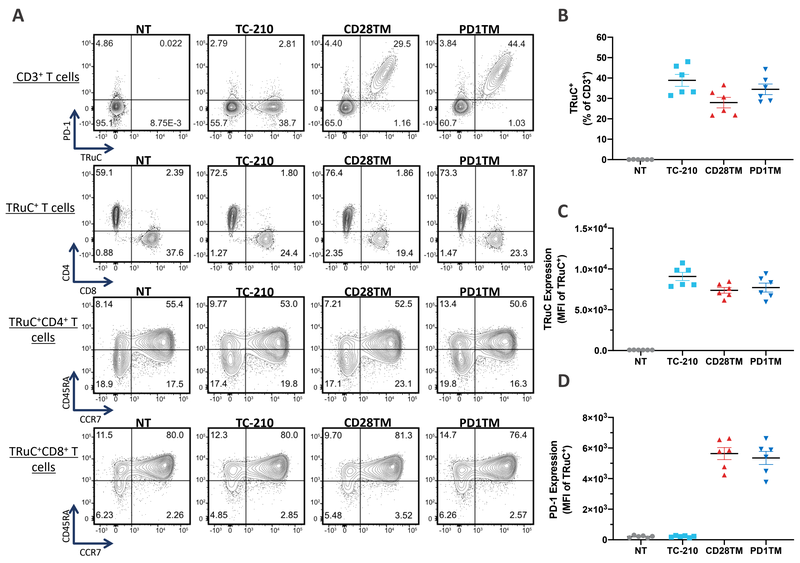
A Chimeric PD-1 Receptor



Ligation of PD-L1 by PD-1 during TCR stimulation mediates inhibition of T-cell function in the TME.

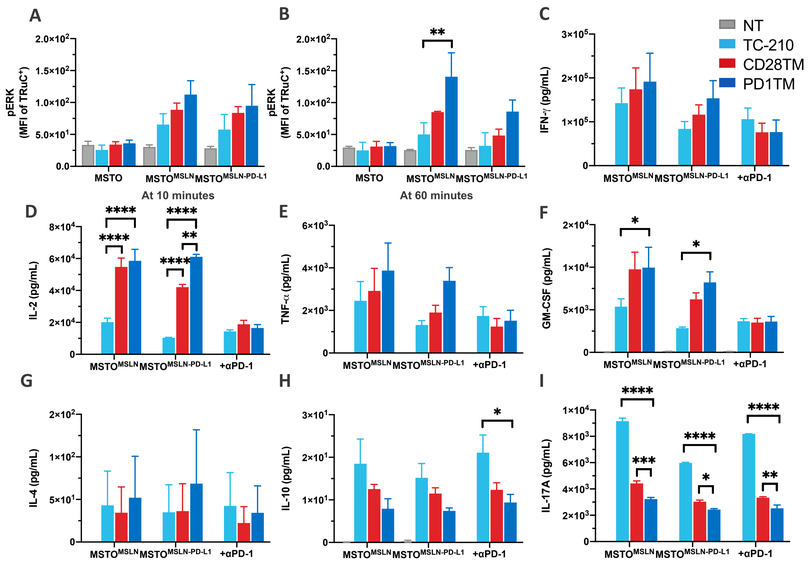
Fusion of the CD28 intracellular domain to the PD-1 receptor can convert PD-1 signaling into a positive costimulatory signal.

TRuC-T cell phenotype



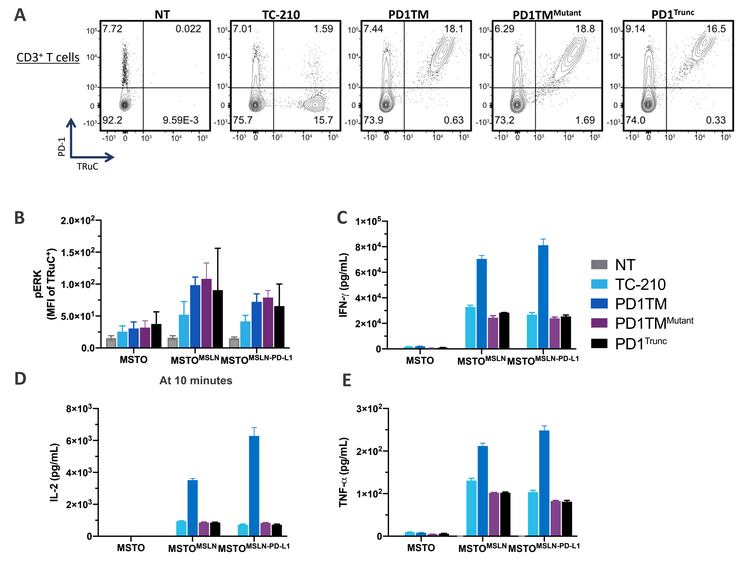
Day 10 TRuC-T cell characterization. (A) Primary T cells were transduced 24 hours after activation with a lentiviral construct containing the MH1 anti-mesothelin TRuC (TC-210), or with either of two bicistronic constructs containing the anti-mesothelin TRuC followed by a sequence encoding the extracellular PD-1 domain and the intracellular signaling domain of CD28. The transmembrane domain of either CD28 (CD28TM) or PD-1 (PD1TM) was used to stabilize expression of the chimeric receptor at the cell membrane. At day 10 of expansion, T cells where characterized for co-expression of PD-1 and the TRuC receptor, CD4 and CD8, and memory phenotype (CD45RA versus CCR7). Data shown are from a single experiment representing 3 normal donors. (**B**) Percent transduction of CD3⁺ T cells as measured by TRuC receptor expression on Day 10 of expansion following activation and transduction (**C**) Mean florescence intensity (MFI) of TRuC receptor expression on TRuC⁺ T cells. (**D**) MFI of PD-1 expression on TRuC⁺ T cells..

The PD-1 switch enhances early TCR signaling and T_H1 polarization



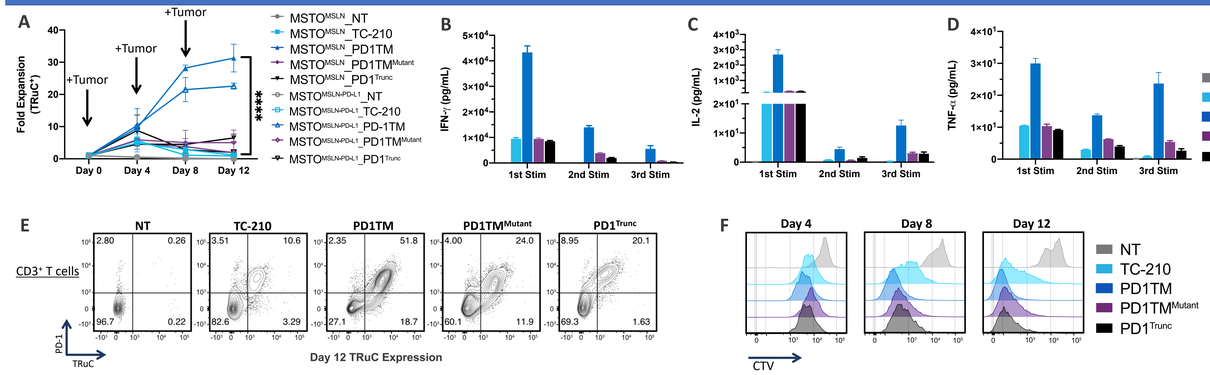
Day 10 TRuC-T cell receptor signaling and polarization. (A-B) TruC-T cells were thawed and co-cultured at a 3:1 ratio with the low-antigen expressing cell line MSTO-211H (MSTO), MSTO overexpressing mesothelin (MSTO^{MSLN}), or MSTO^{MSLN} overexpressing PD-L1 (MSTO$^{MSLN-PD-L1}$). Cultures were harvested at the indicated timepoints, fixed and permeabilized in methanol before staining with antibodies. Graphs depict the mean fluorescence intensity (MFI) of phosphorylated ERK (pERK) at 10 minutes (**D**) and at 60 minutes (**E**) of incubation. Plotted data represent the mean ± SEM of two independent experiments with two separate donors. Data were analyzed for statistical significance by two-way ANOVA.(**C-I**) TRuC T cells were co-cultured with MSTO, MSTO^{MSLN}, MSTO$^{MSLN-PD-L1}$ and MSTO$^{MSLN-PD-L1}$ plus a monoclonal anti-PD-1 antibody (+αPD-1) at a 1:1 effector to tumor ratio for 24 hours. At the end of incubation, the culture supernatants were harvested for cytokine analysis by MSD ELISA. Results shown are from 2 donors and are plotted as mean (± SEM). Data were analyzed for statistical significance by two-way ANOVA..

A CD28^{Null} switch provides little enhancement to TRuC-T cell function



Importance of the intracellular costimulatory domain of the chimeric PD-1 receptor. (**A**) TRuC-T cell characterization of transduction efficiency by lentiviral constructs encoding anti-mesothelin TRuC (TC-210), the anti-mesothelin TRuC plus PD1TM, and a PD1TM receptor with null CD28 ITAMs (PD1TM^{Mutant}) or a truncated version of the PD-1 receptor lacking an intracellular domain (PD1^{Trunc}). TRuC-T cells were stained for the MH1 TRuC receptor and PD-1 prior to normalizing for transduction efficiency with expanded non-transduced T cells. (**B**) T cells were incubated at a 3:1 ratio with the tumor cell lines for 10 minutes and then fixed and stained for p-ERK. Data are from two experiments where mean and SEM are shown. (**C-E**) T cells normalized for transduction efficiency were plated with tumor cells at a a 1:1 ratio and incubated for 72 hours. At 72 hours, supernatants were collected from the cultures and analyzed by MSD for IFN-γ (**C**), IL-2 (**D**), and TNF-α (**E**). Data shown are from a single donor representing 2 independent experiments.

The chimeric PD-1 receptor confers a competitive advantage to TRuC-T cells in a repetitive stimulation assay



The chimeric PD-1 receptor confers enhanced fitness during repeated stimulation challenge. (**A**) Day 10 TRuC-T cells were normalized for transduction efficiency to 20% and cultured with MSLN-bearing tumor cells at a 1:20 effector to tumor ratio. On day 4, day 8 and day 12 post initiation, cultures were harvested, counted, and stained for analysis by flow cytometry. The data plotted represent the mean (± SEM) fold expansion of TRuC T cells over time in two combined experiments using separate donors. Closed symbols represent cultures of MSTO^{MSLN} while open symbols represent cultures of MSTO$^{MSLN-PD-L1}$. Arrows indicate days on which T cells were challenged with additional tumor cell targets. Data were analyzed for statistical significance by two-way ANOVA. (**B-D**) Supernatants were harvested from MSTO$^{MSLN-PD-L1}$ cultures 72 hours after each challenge (1st stim, 2nd stim, and 3rd stim) and analyzed by MSD ELISA for (**B**) IFN-γ, (**C**) IL-2 and (**D**) TNF-α. (**E**) TC-210 contracts following repeated challenge with PD-L1 expressing targets. FACs plots of PD-1 and TRuC receptor expression on viable CD45⁺CD3⁺ T cells from MSTO$^{MSLN-PD-L1}$ cultures at day 12 of culture. (**F**) TRuC T cell proliferation as measured by CellTraceViolet (CTV) labeling on day 4, day 8, and day 12 of cultures repeatedly challenged with MSTO$^{MSLN-PD-L1}$ targets. T cells were gated on viable CD45⁺CD3⁺TRuC⁺ cells.

Conclusions

In agreement with previously published reports[1,2], the expression of a chimeric PD-1:CD28 receptor significantly enhanced the function of our anti-mesothelin TRuC-T cell when cultured in the presence of cancer cell lines expressing PD-L1 by augmenting TCR signaling and effector cytokine production.

This enhanced responsiveness of TRuC T cells bearing the chimeric receptor may be partially attributed to a small role of the PD-1 switch receptor functioning as a decoy PD-1[3]; however, the major contribution of the PD-1 switch receptor was in providing costimulation to TRuC-T cells encountering PD-L1 as evidenced by the enhanced proliferation and survival that it conferred in a repetitive stimulation assay.

Expression of a chimeric PD-1:CD28 receptor represents a significant enhancement to our TRuC-T cells to counteract the PD-L1/PD-L2-mediated inhibition of T-cell function in solid tumors.

References

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