TCR² THERAPEUTICS

AACR American Association for Cancer Research ANNUAL MEETING 2019 ATLANTA — MARCH 29-APRIL 3 GEORGIA WORLD CONFERENCE CENTER

Abstract number: 2307

Preclinical Evaluation of TC-210, A Mesothelin-Specific T Cell Receptor (TCR) Fusion Construct (TRuC™) T Cells for the Treatment of Solid Tumors

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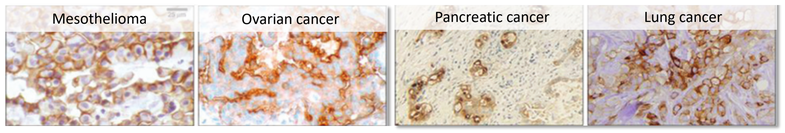
Background

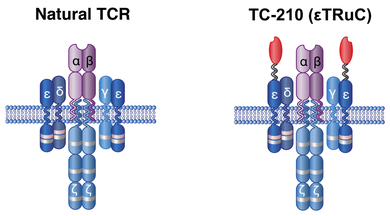
Mesothelin Is A Commonly Expressed Tumor Antigen



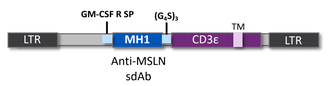
Mesothelin, a GPI-anchored glycoprotein, is expressed on numerous tumor types including mesothelioma, ovarian cancer, pancreatic cancer and lung cancer.
Pastan et al., Cancer Res, 2014, Hassan et al., JCO, 2016; Argani et al., Clinical Cancer Research, 2001.

Despite success in treating hematological malignancies, T cells expressing chimeric antigen receptors (CARs) have shown poor efficacy in solid tumor indications. The failure to initiate and elicit a complete TCR response is arguably a primary underlying factor preventing CAR-T cell success in solid tumor indications. Here, we present a novel T cell engineering platform: T Cell Receptor Fusion Constructs (TRuC™s), which target tumors independent of MHC. Unlike CARs, the constructs integrate into the TCR complex, harnessing the full potential of natural T cell activation, effector function and regulation. Here, we describe preclinical evidence underscoring the efficacy of TRuC™-T cells re-programed to target the solid tumor antigen mesothelin (TC-210).

T Cell Receptor Fusion Constructs (TRuC™)



TC-210: MSLN-targeting CD3ε–TRuC



Features:
- Integration into the complete TCR
- No HLA restriction
- Targeting of tumor surface antigens
- No need for additional signaling domains

Material and Methods

T cell generation: TC-210 were generated by recombinantly fusing MH1, a MSLN-specific single-domain antibody (sdAb), to the CD3ε subunit. MSLN-specific CAR (MSLN-CAR) were generated by fusing MH1 to the 2nd generation CAR construct with a 4-1BB intracellular domain. The construct was introduced into primary human T cells via lentiviral transduction. After standard stimulation and expansion, TRuC™ surface expression and T cell activation were analyzed by flow cytometry.

In vivo efficacy: The anti-tumor efficacy and persistence of TC-210 and MSLN-CAR T cells were tested in NSG mouse models of mesothelioma (MSTO), NSCLC (A549) and ovarian carcinoma (OVCAR3). Blood samples were collected at different time points and the presence of TC-210 and MSLN-CAR T cells was detected by flow cytometry analysis. Plasma cytokine levels were measured by Luminex with HCD8MAG-15K MILLIPLEX MAP Human CD8+ T Cell Magnetic Bead Panel - Immunology Multiplex Assay (Millipore-Sigma).

In vivo migration: TC-210 and MSLN-CAR T cells over-expressing Renilla Luciferase were injected into NSG mice bearing MSTO tumors. Migration of T cells was monitored by bioluminescence imaging. Expression of CXCR3 by T cells after 24 hours co-culture with MSLN+ tumor cells was determined by flow cytometric analysis.

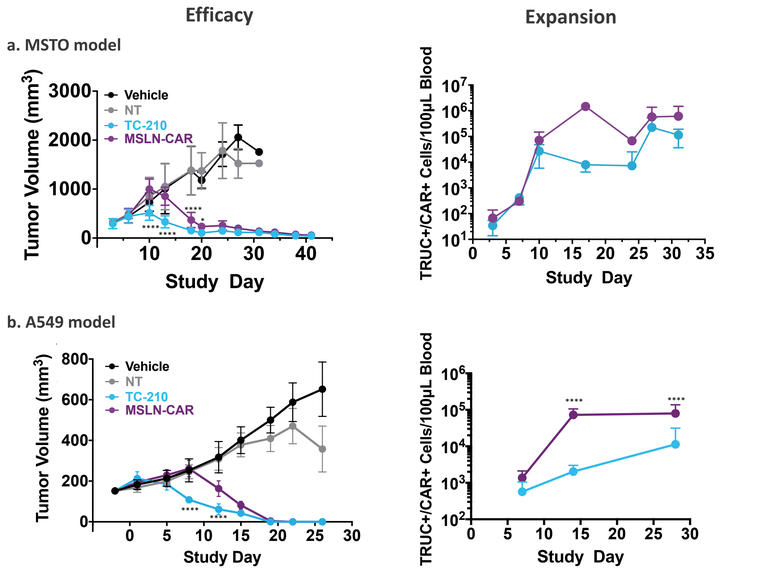
Metabolic profile: T cell metabolic output was measured by Seahorse technology for TC-210 and MSLN-CAR T cells. T cells were plated on Cell-Tak coated Seahorse culture plates and analyzed using a Seahorse XFe96 (Agilent). Extracellular acidification (ECAR) and oxygen consumption rates (OCR) were recorded to monitor the glycolysis, oxidative phosphorylation and respiratory capacity.

Collaborators

UPMC HILLMAN CANCER CENTER · THE UNIVERSITY OF SYDNEY · MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER · UNI FREIBURG

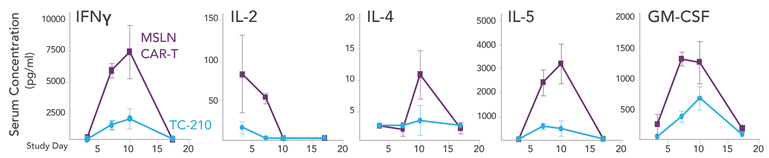
Results

Fig. 1 Robust Tumor Clearance by TC-210, Faster than MSLN-CAR Despite Less *In Vivo* Expansion, in Various MSLN+ Tumor Models



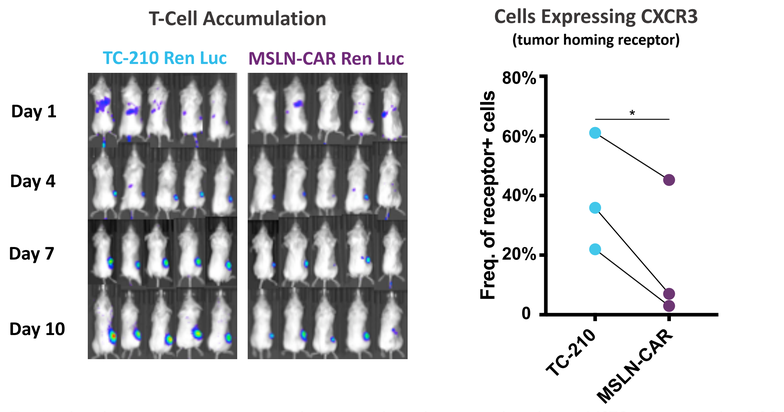
Tumor models were established by s.c. inoculation of MSTO (a, 1x10⁶ cells per mouse) or A549 (b, 1x10⁶ cells per mouse) cells overexpressing human MSLN, or i.p. injection of OVCAR3 cells (c, 1x10⁷ cells per mouse) to 6-week-old female NSG mice. T cells were injected 12 days (a and b) or 6 days (c) after tumor inoculation. T cell dose for MSTO model (a): TC-210 group, 4x10⁶ TRuC+ cells; MSLN-CAR group, 5x10⁶ CAR+ cells. A549 model (b): TC-210 group, 2x10⁶ TRuC+ cells; MSLN-CAR group, 2x10⁶ CAR+ cells. OVCAR3 model (c): TC-210 group, 4x10⁶ TRuC+ cells.

Fig. 2 TC-210 T Cells Released Less Inflammatory Cytokines than CAR T Cells *in vivo*



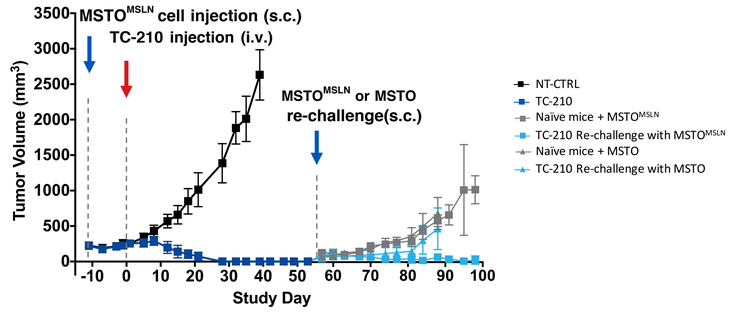
Blood samples were collected at different time points and the cytokine levels in the plasma were determined by Luminex. During tumor clearance, TC-210 treatment was associated with less inflammatory cytokines in circulation than CAR T cells, despite of more efficient tumor clearance.

Fig. 3 Faster Migration to Tumor for TC-210 than CAR-T Cells Correlates with Higher Level of Tumor Homing Receptor Expression



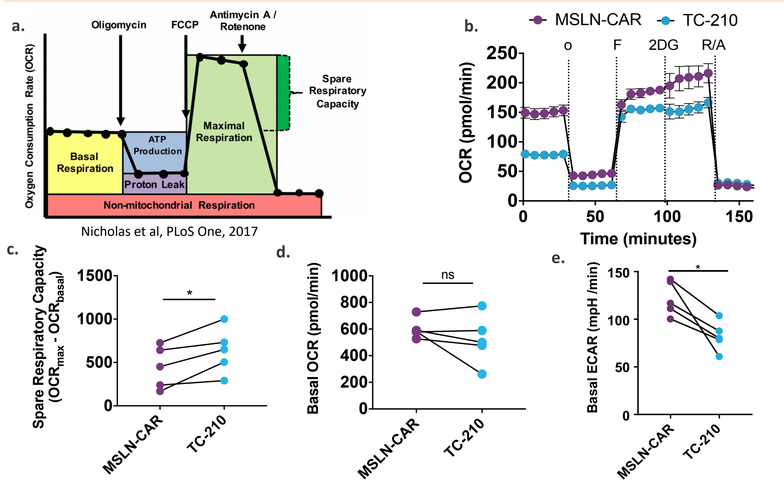
T cell migration to the tumor was monitored by bioluminescence imaging of MSTO tumor bearing NSG mice injected with Renilla Luciferase (Ren Luc) expressing TC-210 (1x10⁶ TRuC+ cells per mouse) or MSLN-CAR (1x10⁶ CAR+ cells per mouse) (left panel). Expression of tumor homing receptor, CXCR3, was determined for TC-210 and MSLN-CAR T cells after 24 hours co-culture with MSLN+ tumor cells. Each set of linked dots represent T cells generated from an individual healthy donor.

Fig.4 TC-210 T Cells Persist and Remain Potent Upon Re-Challenge



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. µ

Fig.5 TRuC Signaling Resulted in Oxidative Phosphorylation in TC-210 T Cells, Correlated with *in vivo* Persistence



Nicholas et al, PLoS One, 2017

(a) A schematic of the mitochondrial stress test using the extracellular flux analyzer. (b) Representative OCR trace of TC-210 (TRUC) and MSLN-CAR (CAR) T cells activated with MSLN protein for 4 days. (c-e) Tubulated spare respiratory capacity (c), basal OCR (d) and basal ECAR (e) of TRUC and CAR T cells. Each set of linked dots represent T cells generated from an individual healthy donor.

Conclusions

- TC-210 T cells demonstrated potent *in vivo* activity in mesothelioma, NSCLC and ovarian carcinoma models.
- TC-210 T cells demonstrated faster migration to tumor, faster kill kinetics, but lower cytokine release compared to CAR T cells.
- TC-210 T cells demonstrated functional persistence in mesothelioma re-challenge model, correlated with metabolic characteristic.