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Preclinical Evaluation of TC-110: CD19-Specific T Cell Receptor (TCR) Fusion Construct (TRuC™) T Cells for the Treatment of Hematologic Malignancy

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Background

Several CD19 targeting T cell therapies have been approved for treatment of hematologic malignancies. These second generation CAR T cell therapies consist of donor T cells engineered to express an extracellular CD19-targeting domain attached to an intracellular signaling domain such as 41BBζ (Kymriah) or CD28 (Yescarta). In the present research, we evaluate a novel **T** Cell **R**eceptor F**u**sion Construct, TRuC™, in which the CD19 targeting domain is fused to the T cell receptor (TCR) at the CD3-ε subunit. While second generation CARs act in isolation from the TCR through an independent signaling domain, TRuCs employ the full signaling machinery of the TCR complex. Fusion of the tumor antigen binder to the TCR may yield more efficient signaling upon binding of the T cell to the tumor antigen. In the present study, we evaluate TC110 TRuC in which a CD19-targeting domain is fused to the CD3-e subunit of the TCR. In mouse models of human leukemia and lymphoma, TC-110 treatment led to faster tumor regression compared to CD19 CAR with minimal cytokine release.

Material and Methods

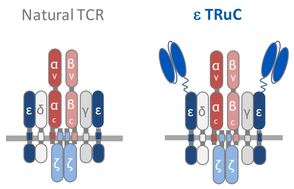
T cell generation: CD19 TRuC, TC-110, was generated by recombinantly fusing FMC63, CD19-specific single chain Fv (ScFv), to the CD3ε subunit. CD19-specific CARs (CD19-CAR) were generated by fusing FMC63 to the 2nd generation CAR with 4-1BB or CD28 intracellular domain. The constructs were introduced 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 of TC-110 and CD19-CAR T cells was tested in NSG mouse models of acute lymphoblastic leukemia (ALL, NALM-6) and Burkitt's lymphoma (Raji). Blood samples were collected at different time points and the presence of TC-110 and CD19-CAR T cells was detected by flow cytometry analysis.

Luminex assay: The levels of human cytokines in mouse serum was measured using the Luminex-based Cytokine Magnetic Bead Panel Multiplex Assay (Millipore-Sigma). The mouse serum was collected on days 1, 2, 4, 7, and 10 days post T cell injection and stored at -80C until sample analysis.

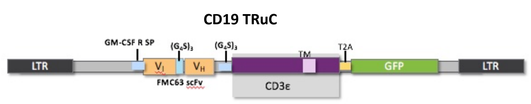
Platform Overview

T Cell Receptor Fusion Construct (TRuC™)

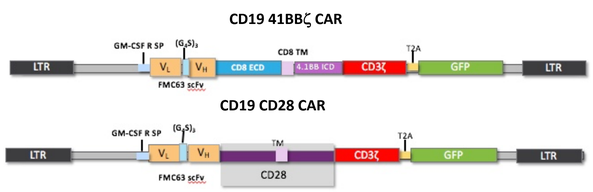


Features:

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



CD19 CAR Constructs for Comparative Studies



Collaborators

MASSACHUSETTS GENERAL HOSPITAL CANCER CENTER

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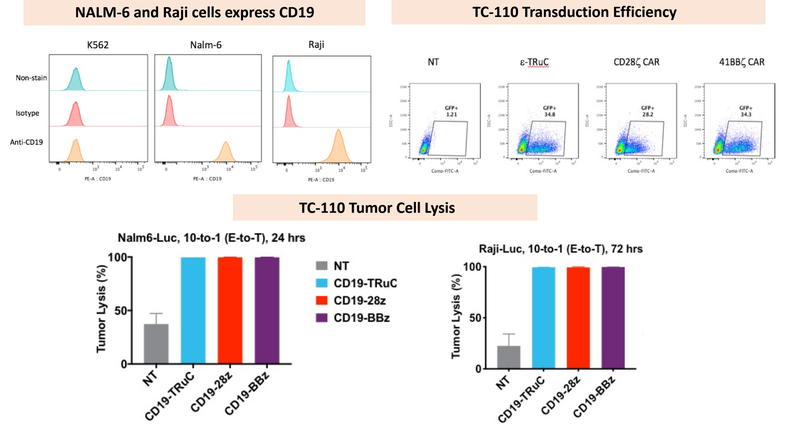
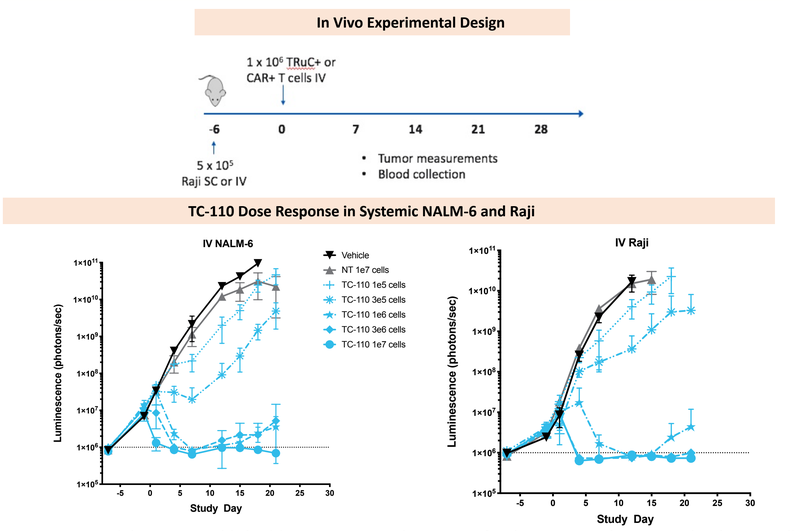
Results

Figure 1. In Vitro Characterization of TC-110



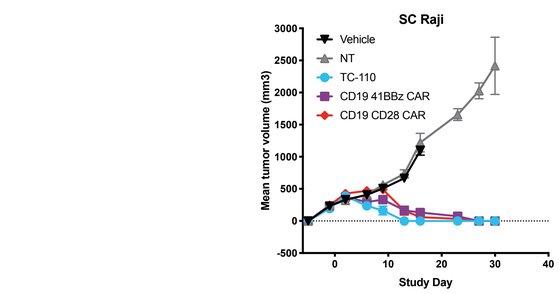
Surface expression of CD19 was demonstrated on NALM-6 and Raji tumor cells. The transduction efficiency of the TRuC and CARs on T cells was detected using an anti-GFP antibody in flow cytometry. Tumor cell lysis of luciferase-expressing NALM-6 (24 hrs) and Raji tumor cells (72 hrs) was performed at an effector to target ratio of 10:1.

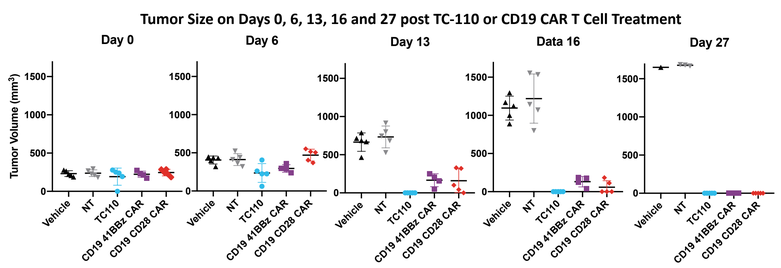
Figure 2. TC-110 Dose Response in NALM-6 and Raji



Tumor models were established by i.v. or s.c implantation of either NALM-6-luc or Raji-luc ($5x10^5$ cells per mouse) cells in 7-week-old female NSG mice. T cells were injected 6 days after tumor implantation at doses ranging from $1x10^5$ to $1x10^7$ (TRuC+ T cells) . Tumor growth was monitored by IVIS imaging.

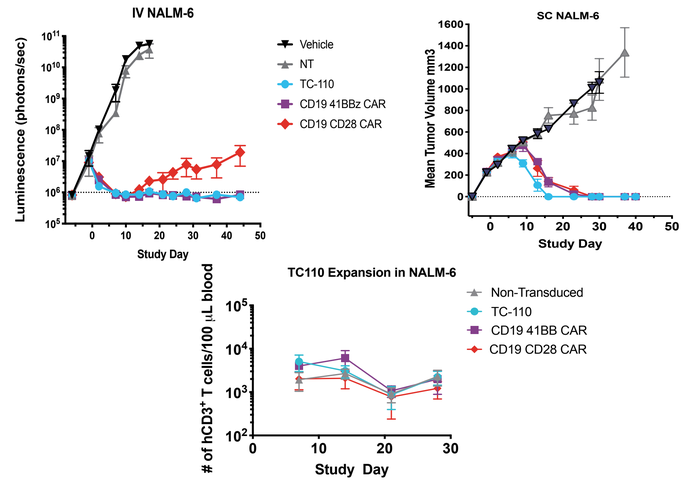
Figure 3. Faster Tumor Regression for TC-110 versus CD19 CAR in SC Raji





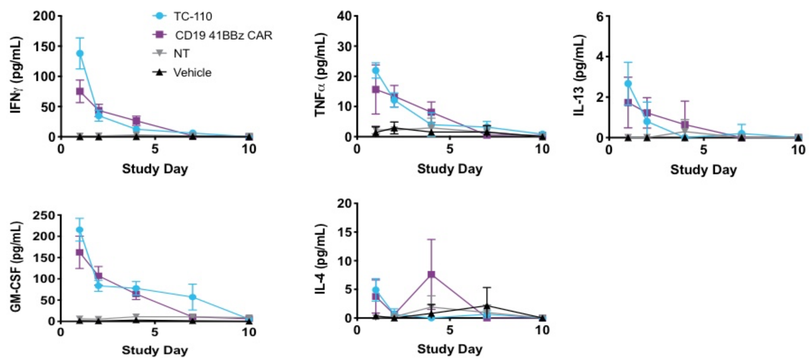
NSG mice were implanted SC with Raji ($5x10^5$ cells per mouse). Six days post tumor implant, mice were injected IV with TC-110 ($1x10^6$ TRuC+ cells per mouse) or CD19 BBz CAR or CD28 CAR ($1x10^6$ CAR+ cells per mouse). Tumor growth was monitored by caliper measurement of SC tumors. Tumors regressed more quickly after TC-110 treatment (top panel). Tumors completely regressed by day 13 for TC-110–treated mice (lower panel). In contrast, complete tumor regression occurred by day 27 for CD19 BBz CAR and CD28 CAR-treated mice.

Figure 4. TC-110 Clears Tumor Faster in IV and SC NALM-6 and TC-110 T Cells Expand at Early Timepoints

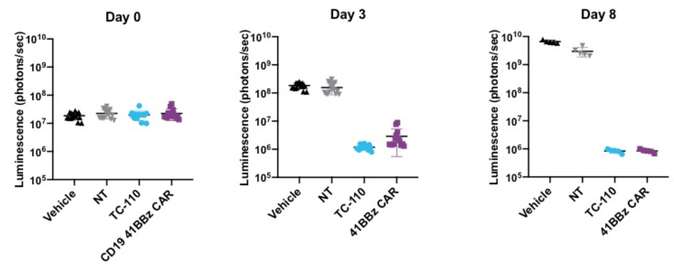


NSG mice were implanted IV or SC with NALM-6 ($5x10^5$ cells per mouse). Six days later, mice were injected IV with $1x10^6$ TRuC+ or CD19 BBz CAR or CD19 CD28 CAR T cells ($1x10^6$ CAR+) on Study Day 0. Blood samples were collected at different time points and the number of T cells in the blood was determined by FACs. During active tumor clearance (days 1-8 for IV model and 1-14 for SC model), TC-110 T cell numbers were highest compared to day 20 (post tumor regression). CD19 41BBz CAR had peak T cell expansion on day 14. CD19 CD28 CAR had minimal T cell expansion. T cell expansion on day 29 was most likely due to GVHD which sometimes occurs in xenogeneic tumor models treated with human T cells.

Figure 5. Serum Cytokines after Treatment with TC-110 in IV NALM-6



Tumor Size on Days 0, 3 and 8 post TC-110 or CD19 CAR T Cell Treatment



Blood was collected on Days 1, 2, 4, 7 and 10 post treatment with TC-110 or CD19 BBz CAR T cells in mice bearing IV NALM-6. Serum was assayed for cytokine levels using Luminex (top panel). For TC-110-treated mice, IFNγ, TNFα, IL-13 and GM-CSF levels were highest on day 1, followed by rapid decline to baseline. IL-4 was high in CD19 BBz CAR-treated mice on day 4. For both TC-110 and CD19 CAR, highest levels of cytokines correlated with times of active tumor regression (days 1-4) and cytokine levels were lowest at time of complete tumor regression (days 8-10).

Conclusions

- TC-110, a CD19-targeting TRuC T cell therapy, demonstrated potent in vivo efficacy in xenogeneic models of acute lymphoblastic leukemia (NALM-6) and Burkitt's lymphoma (Raji).
- Tumor-bearing mice had faster tumor regression after treatment with TC-110 compared to treatment with CD19 BBz CAR and CD19 CD28 CAR in both NALM-6 and Raji tumor models.
- Cytokine release declined rapidly after TC-110 treatment and correlated with time of active tumor regression.
- These results support clinical development of TC-110 for treatment of hematologic malignancies.