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TAC-T CELLS PERSIST AND REMAIN FUNCTIONAL DURING AND AFTER REPEATED TUMOR EXPOSURE IN VITRO AND IN VIVO

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ABSTRACT

Background

T cell antigen coupler (TAC) is a chimeric receptor that redirects T cells (TAC-T) towards surface-expressed tumor antigens to create safe and durable anti-cancer immune responses. The TAC receptor activates T cells by co-opting the endogenous T cell receptor machinery via a CD3ε-specific binding motif and a cytoplasmic co-receptor tail. TAC01-HER2, a first-in-class TAC-T product targeting HER2 (ERBB2), has entered a phase I/II clinical trial. Here, we show that TAC-T cells retain their cytotoxicity capacity during and after repeated tumor challenges in vitro and in vivo.

Materials and Methods

The robustness of anti-tumor T cell responses were assessed in vitro in a recursive killing assay by repeatedly exposing HER2-specific TAC-T cells to HER2-expressing tumor cells for 11 successive rounds (39 days). T cells were characterized by flow cytometry to correlate T cell phenotypes with anti-tumor activity. In vivo, ongoing tumor control established by a single infusion of TAC-T cells was assessed in a tumor rechallenge experiment. MHC I/II-deficient NSG mice were engrafted subcutaneously with HER2$^+$ tumor cells and rechallenged with the same tumor cell line 28 days later. TAC-T cells were isolated from mice at various time points for phenotypic and functional characterization.

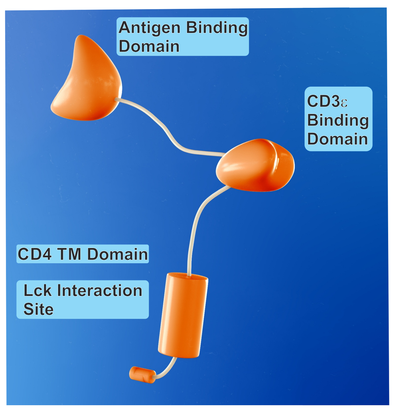
Results

TAC-T products controlled tumor cell growth through 11 rounds of tumor cell challenge in vitro. Signs of reduced functionality were observed at round 11, which coincided with the emergence of a dysfunctional phenotype. During in vivo tumor rechallenge experiments, a single infusion of TAC-T cells led to complete clearance of the solid tumor xenograft and protected mice from a second tumor challenge 28 days after adoptive T cell transfer. TAC-T cells isolated from tumor sites at various time points exhibited phenotypic markers of activation, whereas TAC-T cells isolated from blood and spleen appeared to be antigen-experienced cells but lacked markers indicative of chronic activation and exhaustion. TAC-T cells isolated from spleens before and after the rechallenge were able to proliferate and kill tumor cells ex vivo.

Conclusions

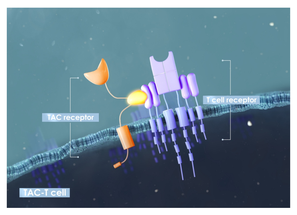
Here we report evidence that TAC-T cells controlled tumor cell growth through 11 rounds of repeated tumor rechallenge in vitro, protected mice against tumor rechallenge, and demonstrated long-term ex vivo proliferative and cytotoxic capabilities. These data indicate long-lasting T cell persistence and functionality against solid tumors.

TAC SCIENCE



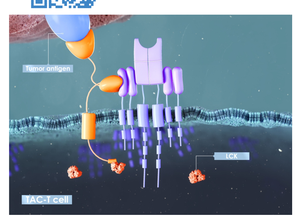
Key features of TAC technology:

- TAC functions independently of MHC
- TAC activates T cells via the endogenous TCR
- TAC incorporates the co-receptor and recruits the TCR complex, mimicking natural TCR activation

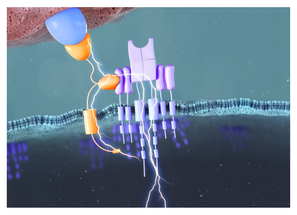


The membrane-bound TAC receptor interacts directly with the TCR-CD3 epsilon domain and...

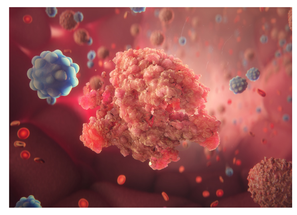
Watch a short animation to understand the TAC mechanism



...binds directly to the targeted tumor antigen. Clustering of TAC-TCR complexes leads to recruitment of kinases (Lck) via the cytoplasmic co-receptor domain and...



...initiates T cell activation via the endogenous CD3-TCR complex.



This results in effective cell lysis of multiple tumor cells during multiple killing events.

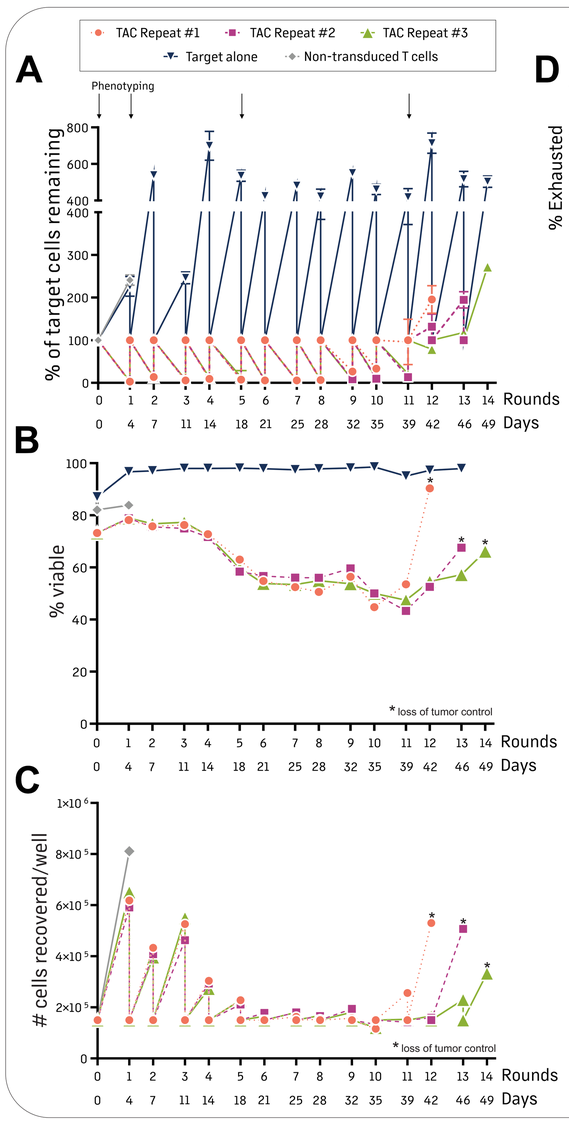


Figure 1: HER2-TAC T cells control tumor cells over 11 rounds of successive rechallenge

T cells were cocultured with HER2$^+$GFP$^+$ Nalm6 target cells at a 3:1 T cell to target cell ratio for alternating rounds of 3 or 4 days. At the end of each round, cells were collected, counted, and reseeded with fresh target cells. **A:** At the end of each round, the proportion of GFP$^+$ tumor cells remaining compared to the beginning of the round was quantified. **B,C:** At the end of each round, cells were assessed for viability (B) and the number of cells recovered was quantified (C). **D:** At rounds 0, 1, 5, and 11, cells were stained for inhibitory markers, and the proportion of cells expressing an exhausted phenotype (CD39$^+$TIM3$^+$ LAG3$^+$CD69$^+$) was quantified.

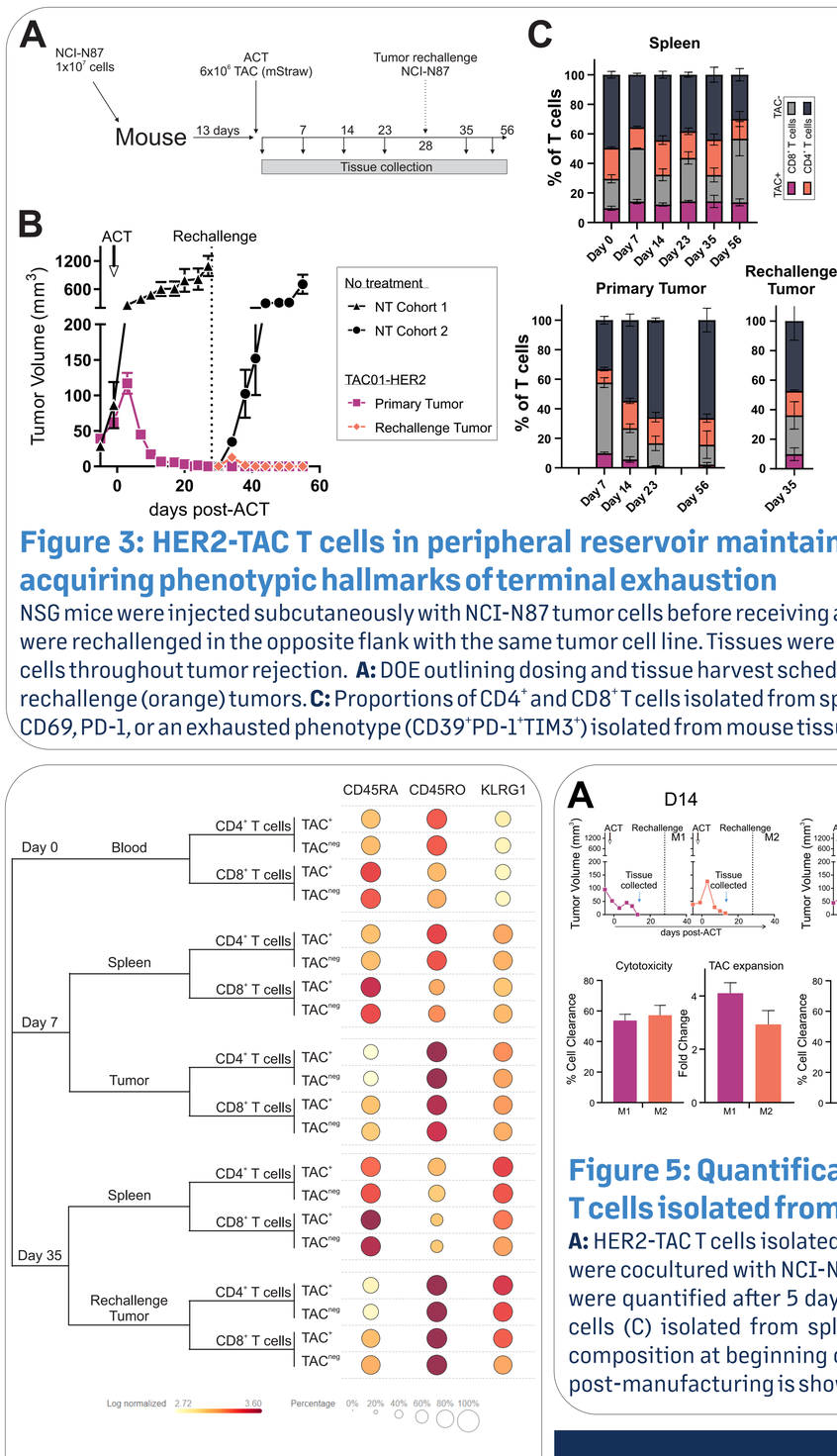


Figure 3: HER2-TAC T cells in peripheral reservoir maintained over 56-day in vivo rechallenge study without acquiring phenotypic hallmarks of terminal exhaustion

NSG mice were injected subcutaneously with NCI-N87 tumor cells before receiving a single ACT dose of HER2-TAC T cells. Twenty-eight days after ACT, mice were rechallenged in the opposite flank with the same tumor cell line. Tissues were harvested at various timepoints to track the phenotype of HER2-TAC T cells throughout tumor rejection. **A:** DOE outlining dosing and tissue harvest schedule for rechallenge assay. **B:** Tumor volumes of primary (magenta) and rechallenge (orange) tumors. **C:** Proportions of CD4$^+$ and CD8$^+$ T cells isolated from spleen, tumor, and rechallenge tumor. **D:** Proportion of T cells expressing CD69, PD-1, or an exhausted phenotype (CD39$^+$PD-1$^+$TIM3$^+$) isolated from mouse tissues.

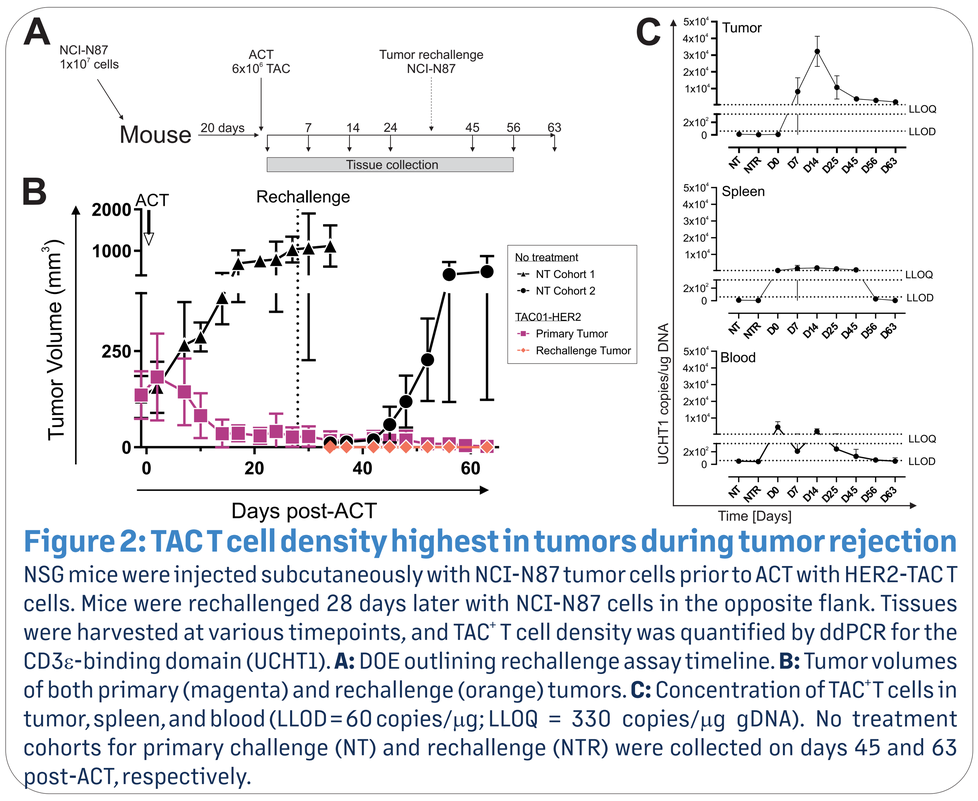


Figure 2: TAC T cell density highest in tumors during tumor rejection

NSG mice were injected subcutaneously with NCI-N87 tumor cells prior to ACT with HER2-TAC T cells. Mice were rechallenged 28 days later with NCI-N87 cells in the opposite flank. Tissues were harvested at various timepoints, and TAC$^+$ T cell density was quantified by ddPCR for the CD3ε-binding domain (UCHT1). **A:** DOE outlining rechallenge assay timeline. **B:** Tumor volumes of both primary (magenta) and rechallenge (orange) tumors. **C:** Concentration of TAC$^+$T cells in tumor, spleen, and blood (LLOD = 60 copies/μg; LLOQ = 330 copies/μg gDNA). No treatment cohorts for primary challenge (NT) and rechallenge (NTR) were collected on days 45 and 63 post-ACT, respectively.

Figure 4: Expression of CD45RA, CD45RO, and KLRG1 on T cell populations isolated from mouse tissues during tumor rechallenge

Principal component analysis was done on scaled, batch-normalized flow data. Nearest neighbourhood graph was computed of flow data, and data was subsequently clustered using Leiden network clustering algorithm with a resolution parameter of 0.5. T cell populations were identified by expression of CD4, CD8, and TAC. Each population was further analyzed based on the proportional (size of circle) and the mean cellular expression level (color) of CD45RA, CD45RO, and KLRG1.

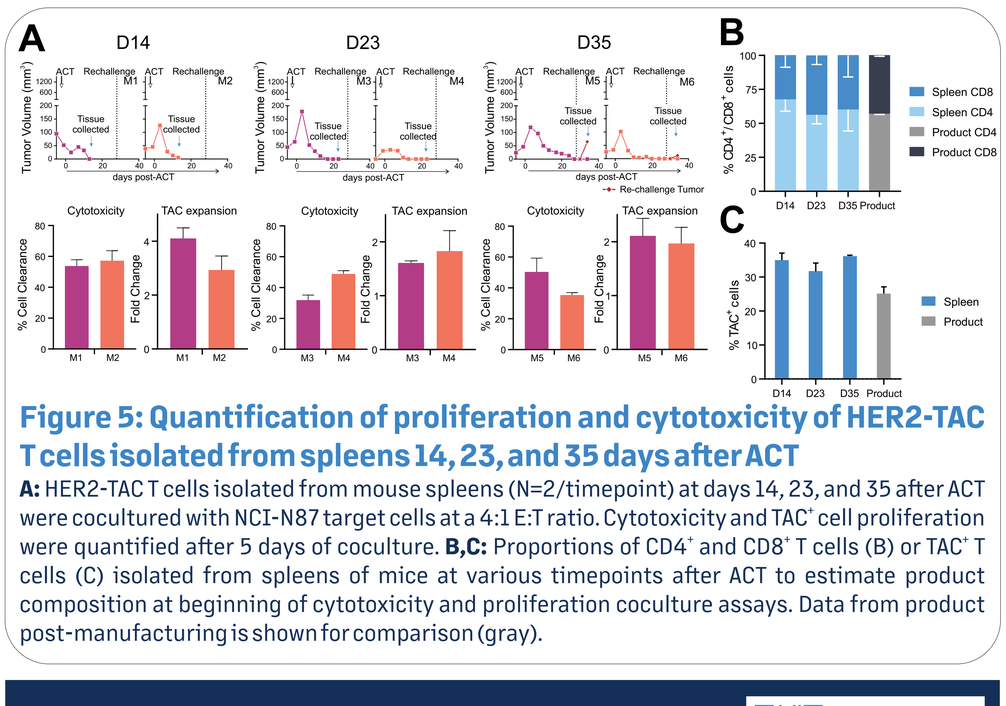


Figure 5: Quantification of proliferation and cytotoxicity of HER2-TAC T cells isolated from spleens 14, 23, and 35 days after ACT

A: HER2-TAC T cells isolated from mouse spleens (N=2/timepoint) at days 14, 23, and 35 after ACT were cocultured with NCI-N87 target cells at a 4:1 E:T ratio. Cytotoxicity and TAC$^+$ cell proliferation were quantified after 5 days of coculture. **B,C:** Proportions of CD4$^+$ and CD8$^+$ T cells (B) or TAC$^+$ T cells (C) isolated from spleens of mice at various timepoints after ACT to estimate product composition at beginning of cytotoxicity and proliferation coculture assays. Data from product post-manufacturing is shown for comparison (gray).

Summary

View a poster of the HER2-TAC T cell activity in vitro
"Evidence for Durable Anti-Tumor Responses by TAC-T Cells In Preclinical Models of Solid Tumors"

- **HER2-TAC T cells controlled tumor cell growth over 11 successive rounds (39 days) of in vitro tumor rechallenge**
- **A single dose of HER2-TAC ACT cleared established primary tumors and provided durable protection against in vivo tumor rechallenge**
- **HER2-TAC T cells isolated from spleens after the 1st (Days 14 & 23) and 2nd (Day 35) tumor challenge demonstrated proliferative and cytotoxic capability in ex vivo coculture experiments**