EVIDENCE FOR DURABLE ANTI-TUMOR RESPONSES BY TAC-T CELLS IN PRECLINICAL MODELS OF SOLID TUMORS

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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 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 have characterized the fate of TAC-T cells during anti-tumor responses in vitro and in vivo.

Materials and Methods

In vitro, HER2-specific TAC-T products were challenged with HER2-expressing and HER2-negative tumors cells. Kinetics of T cell proliferation, degranulation, activation, differentiation, and memory generation was assessed by flow cytometry. TAC-T products were subjected to multiple rounds of tumor cell exposure in vitro to test the durability of the T-cell-mediated immune response. Bioinformatic clustering analysis of flow cytometry data was performed to identify T cell populations and track them over time.T cell expansion in blood, tumor, bone marrow, and spleen were evaluated in vivo after primary xenograft tumor treatment and secondary tumor rechallenge. Tumor- and spleen-infiltrating or circulating T cells were phenotyped by flow cytometry after treatment with TAC-T cells.

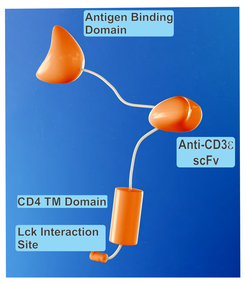
Results

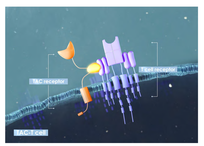
Co-culture studies revealed that TAC-T products become rapidly activated and degranulate upon contact with HER2-expressing, but not HER2-negative, cell lines. Activation coincided with rapid downregulation of the TAC receptor. A large proportion of the T cells expressed activation markers, and a majority of these also expressed degranulation markers, indicating ongoing cytotoxicity. In vitro and in vivo studies demonstrated a CD8-biased response characterized by a considerable expansion in the activated CD8 population enriched at the tumor site. Later, activation and differentiation markers returned to baseline concurrently with the re-emergence of surface TAC expression, initiating T cell proliferation. Importantly, central memory T cells were expanded, and stem-like cells were maintained, suggesting strong self-renewal potential. In vitro serial cytotoxicity assays showed that TAC-T products could repeatedly kill tumor cells up to 12 rounds over 40 days. In tumor rechallenge experiments, a single dose of TAC-T cells expanded to clear solid tumor xenografts and protected mice from a second tumor challenge 30 days post initial tumor clearance, indicating long-lasting T cell persistence.

Conclusion

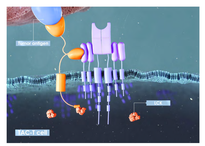
The TAC-T product mounts an effective anti-tumor response in multiple preclinical models, comprising activated TAC-T cells that do not become terminally exhausted but are dominated by an activated CD8 response and supported by the expansion of a memory population, indicating robust self-renewal capacity.

TAC Science

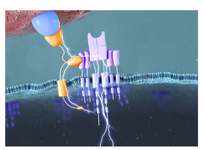




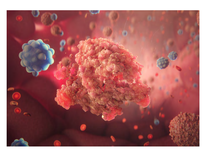
The membrane-bound TAC receptor interacts directly with the TCR-CD3 epsilon domain and...



...binds directly 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.

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

Watch a short animation to understand the TAC mechanism

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Cocoon® T cell manufacturing enhances memory profile

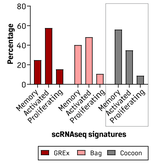


Figure 1: (Left) Lonza Cocoon® Platform; (Right) Bar graph representing memory (TCF7, SELL/CD62L, CCR7, CD27, LRRN3), activated (IFNG, S100A4, KLRK1, TTGB1, TNFRSF4), and proliferating (MKI67, TOP2A, HMGB2, HMGB3) gene signatures of TAC-T cells manufactured by different technologies, analyzed by scRNAseq studies.

Antigen-driven T cell activation in the absence of terminal T cell exhaustion

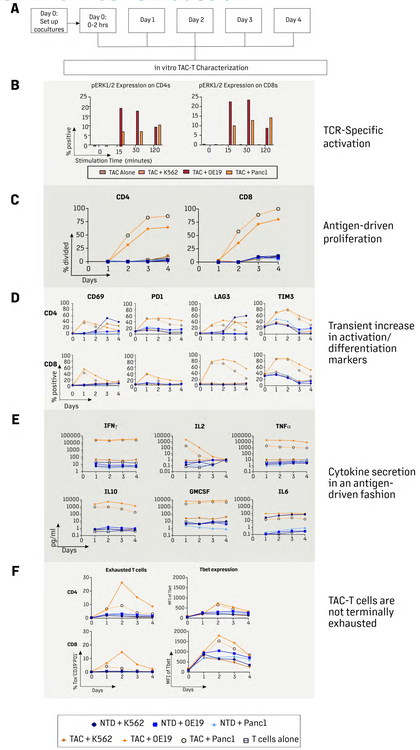


Figure 2: Antigen-driven T cell activation in absence of terminal T cell exhaustion. (**A**) Design of Experiment: Flow cytometric analysis of T cells after 0 minutes to 4 days of culture with tumor and control cells; (**B**) bar graph representing pERK1/2 levels in T cells; Line graphs representing (**C**) antigen-driven T cell proliferation measured by CTV® dilution, (**D**) surface expression of T cell activation markers. (**E**) Intracellular cytokine levels, and (**F**) terminal exhaustion markers and intracellular T-bet levels.

TSCM cell fraction persists after stimulation

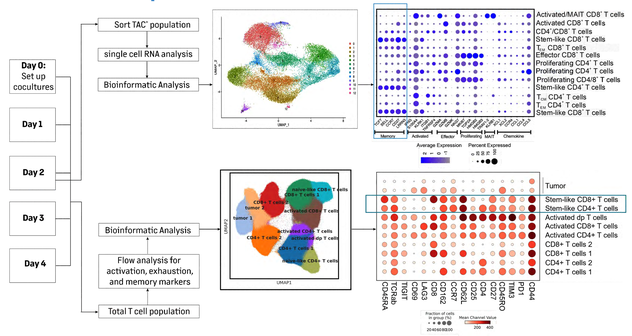
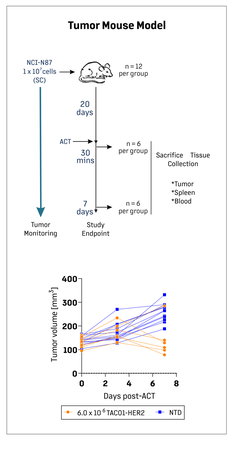


Figure 3: Persistent TSCM fraction during TAC-T target engagement. (Top) Bioinformatic analysis of TAC-T scRNA signature clusters and dot plots from 48 hr co-cultures with tumor cells. (Bottom) T cell phenotype clusters and dot plot from 17-color complex flow cytometric analysis of cells after 0-4 days of coculture with tumor cells.

Tumor-specific activation and persistent circulating TSCM cells in xenograft tumor model



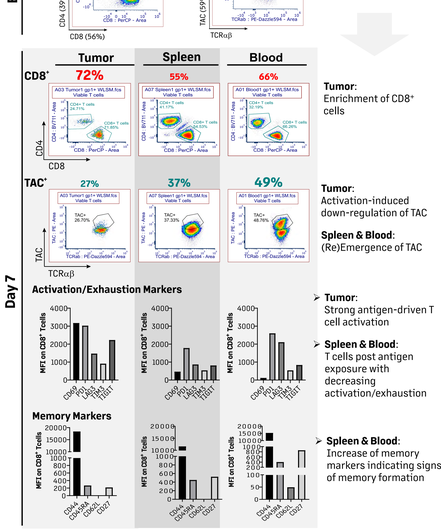


Figure 4: Tumor-specific activation and persistent circulating TSCM fraction during in vivo tumor rejection. (Left) Design of in vivo experiment and line graph representing in vivo tumor growth. (Right) Key in vivo T cell phenotype in descending order: baseline CD4/CD8 ratio and surface TAC expression; CD4/CD8 ratio and surface TAC expression on T cells harvested from tumor, spleen, and blood 7 days after adoptive cell therapy (ACT); bar graph representing activation/exhaustion markers; bar graph representing memory markers.

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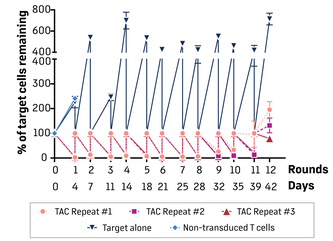
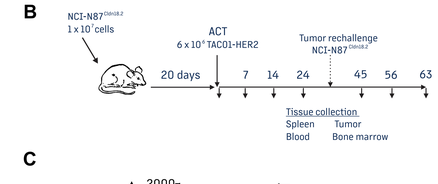
Durable anti-tumor responses





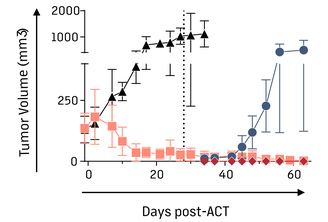


Figure 5: TAC-T cells elicit durable anti-tumor responses. (**A**) Line graph depicting the survival curve of tumor cells following exposure to TAC-T cells in an in vitro repeat killing assay; (**B**) Design of in vivo tumor rechallenge experiment in MHC I/II-DKO xenograft model; (**C**) Line graph representing in vivo tumor growth.

Summary

- TAC-T products are marked by a significant fraction of **stem-like memory cells (TSCM), enhanced by Cocoon® technology**
- **TSCM cells persist** during TAC-T cell target engagement
- Tumor-specific TAC-T activation leads to **persistent TSCM cell fraction** during tumor rejection in vivo
- TAC-T cells elicit **durable anti-tumor responses** in vitro and in vivo