PRECLINICAL CHARACTERIZATION AND MODELLING OF ALLOGENEIC Vγ9Vδ2 TAC T CELLS FOR THE TREATMENT OF SOLID TUMORS

400

Stacey X. Xu, Suzanna L. Prosser, Ling Wang, Ritu R. Randhawa, Sailaja Pirati,
Deepika Bhemarasetty, Kyle MacDonald, Seung Mi Yoo, Miyoung E. Jung, Laurentia A. Gheorghiu, Chris L. Ayers,
Sadhak Sengupta, Christopher W. Helsen, and Andreas G. Bader
Triumvira Immunologics, 9433 Bee Caves Rd Building 1, Suite 240, Austin, TX 78733, USA (Headquarters),
270 Longwood Road South, Hamilton, Ontario L8P 0A6, Canada (Research Division)

ABSTRACT

Background

The T cell antigen coupler (TAC) is a novel, proprietary chimeric receptor that facilitates the redirection of T cells to tumor cells and activates T cells by co-opting the endogenous T cell receptor complex with the goal to elicit safe and durable anti-tumor responses. TAC01-HER2, a first-in-class, autologous TAC T cell product targeting HER2 (ERBB2), has entered a phase I/II clinical trial in patients with HER2-positive solid tumors. Vγ9Vδ2 (γδ) T cells belong to a subset of T cells that recognize target cells in an HLA-independent manner. Consequently, γδ T cells are not expected to cause GvHD and, thus, have the potential for allogeneic cell therapy applications. Here, we present preclinical data of an allogeneic HER2-TAC γδ T cell product based on Vγ9Vδ2 T cells.

Materials and Methods

The potency and safety of HER2-TAC γδ T cells generated from multiple donors was evaluated using a variety of in vitro and in vivo assays. Flow cytometric analysis was used to determine cellular phenotypes, intracellular cytokines, CD69 upregulation, and T cell proliferation in response to target antigen. Cytotoxicity was assessed via both luciferase-based killing and real-time microscopy-based co-culture assays. To assess HER2-TAC γδ T cell responses to HLA mismatches between unrelated donors, mixed lymphocyte reactions (MLR) were performed using dendritic cells representing the major North American HLA subtypes. In vivo studies examined the anti-tumor effects of HER2-TAC γδ T cells against established HER2-expressing solid tumors.

Results

HER2-TAC γδ T cells selectively reacted to HER2-expressing tumor cells in vitro, as measured by CD69 upregulation, intracellular cytokine production, proliferation, and cytotoxicity. In contrast, HER2-TAC γδ T cells failed to show proliferative activity in MLR assays, indicating that HER2-TAC γδ T cells are likely free of GvH reactivity. In addition, HER2-TAC γδ T cells showed strong anti-tumor efficacy in HER2-positive tumor xenograft models without signs of toxicity. Similar anti-tumor efficacy was observed in both NSG mice bearing the human IL-15 transgene (Tg-hIL-15) and NSG mice supplemented with exogenous IL-15. Comparison of serum IL-15 levels between Tg-hIL15 mice and NSG mice with cytokine supplementation revealed significantly lower levels in Tg-hIL15 mice, suggesting that Tg-hIL15 mice may be a more physiologically relevant model to study γδ T cells in vivo.

Conclusions

HER2-targeted TAC γδ T cells display strong and specific activity against HER2-expressing tumor models in vitro and in vivo. This highlights the versatility of the TAC platform and its potential in the development of an allogeneic product for therapeutic applications in solid tumors.

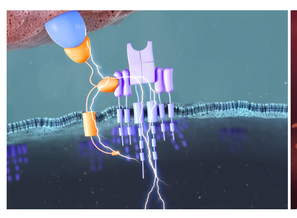
TAC SCIENCE

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

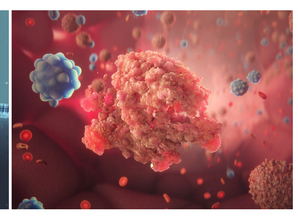
...binds directly to the targeted tumor antigen. Clustering of TAC-TCR complexes leads to recruitment of kinases (Lck) via the cytoplasmic co-receptor domain and...

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



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

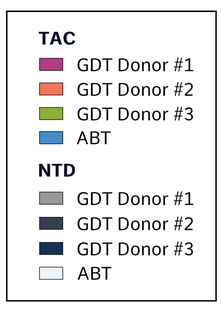


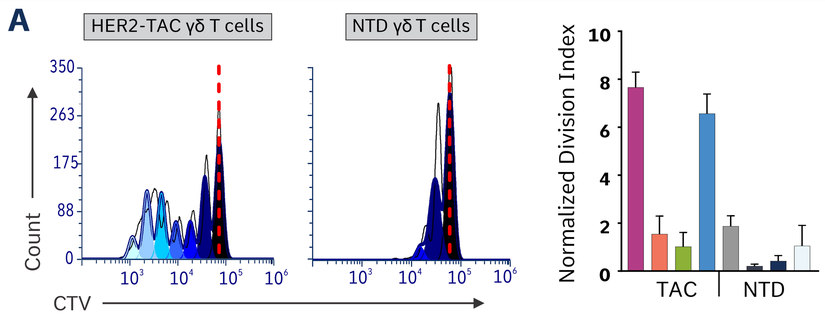
This results in effective cell lysis of multiple tumor cells during multiple killing events.

Watch a short animation to understand the TAC mechanism

TAC Activation Requires Binding to Both CD3 and Antigen

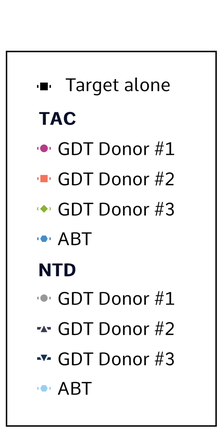
γδ T cells were engineered using either the full length TAC, or mutant TAC missing either the antigen-binding domain (ΔBinder) or missing the CD3-binding domain (ΔUCHT1). Engineered T cells were co-cultured with antigen-positive or -negative target cells, or stimulated with a T cell activator as a positive control. **A**. Early activation as measured by CD69 upregulation. **B.** Intracellular cytokines.

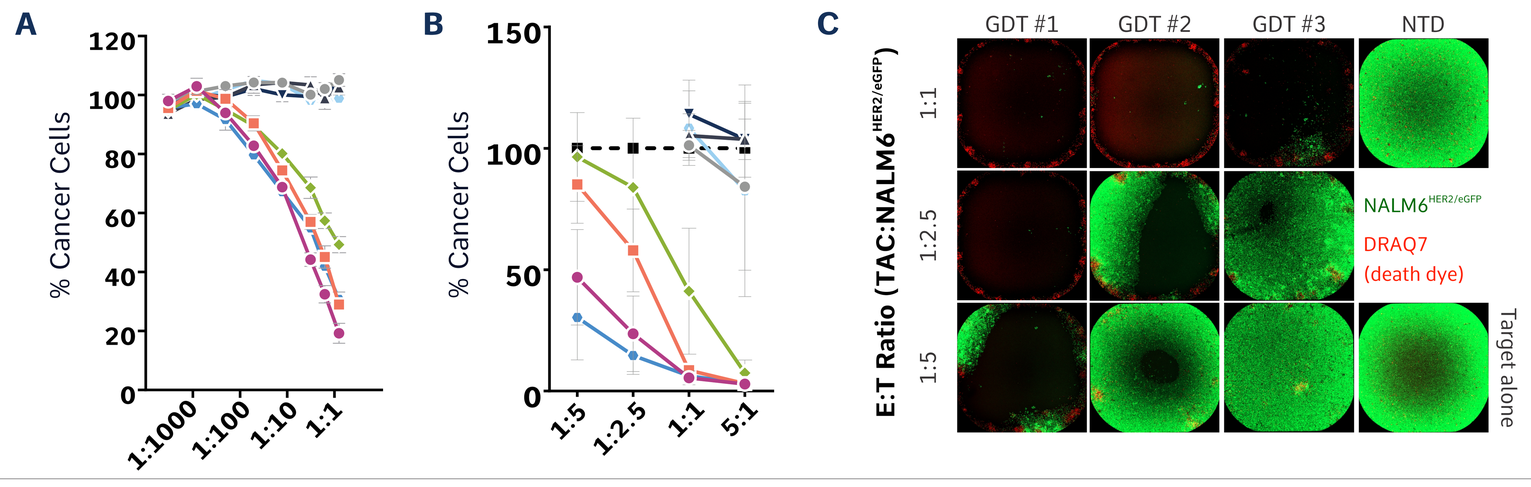




Stimulation with Target Antigen Elicits a Strong HER2-TAC γδ T Cell Response

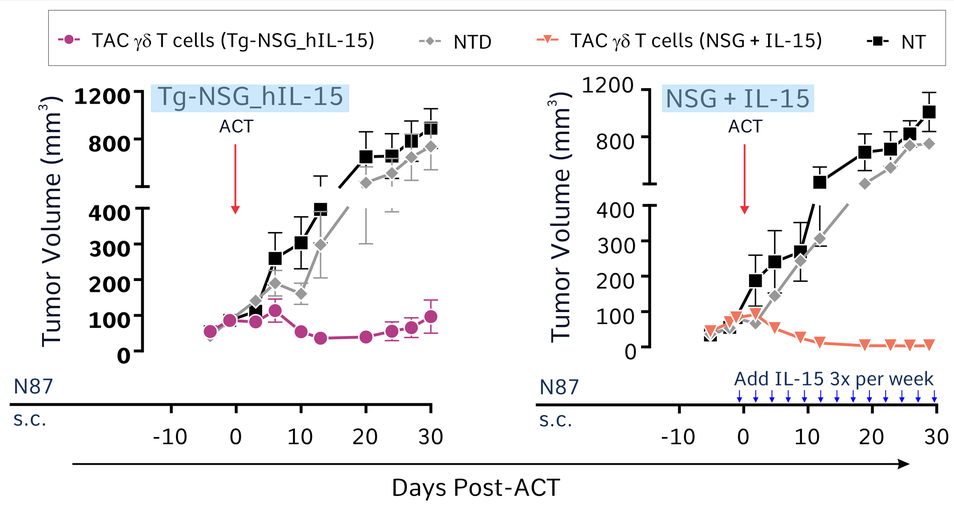
HER2-TAC γδT cells engineered from 3 healthy donors were tested in a range of in vitro assays. TAC01-HER2 αβ T cells (ABT) served as a positive control.
Proliferation: **A.** 4-day co-culture with HT1080 target cells. Early Activation: **B.** 4-hour co-culture with N87 target cells. Intracellular Cytokines: **C.** 4-hour co-culture with N87 target cells. All data shown as mean ± SD.





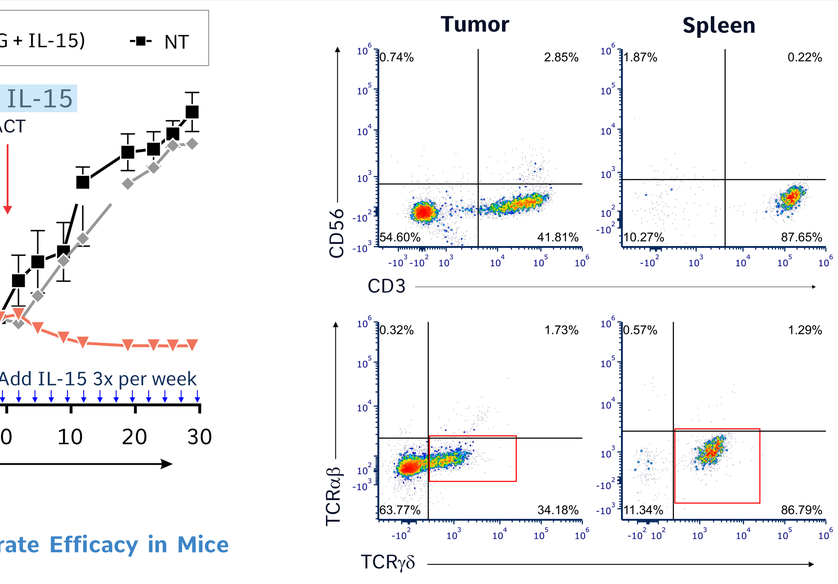
HER2-TAC γδ T Cells Effectively Kill Tumor Cells In Vitro

18-hour Cytotoxicity: **A.** Luciferase-based killing assay following co-culture with HT1080eLuc target cells.
5-day Cytotoxicity: (**B,C**) HER2-TAC γδ T cells were co-cultured with NALM6HER2/eGFP target cells at different E:T ratios. Tumor cell growth was monitored by GFP fluorescence. Data is normalized to target cells alone. **B.** Mean percentage tumor cell survival is shown ± SD. **C.** Example images of 5-day co-cultures.



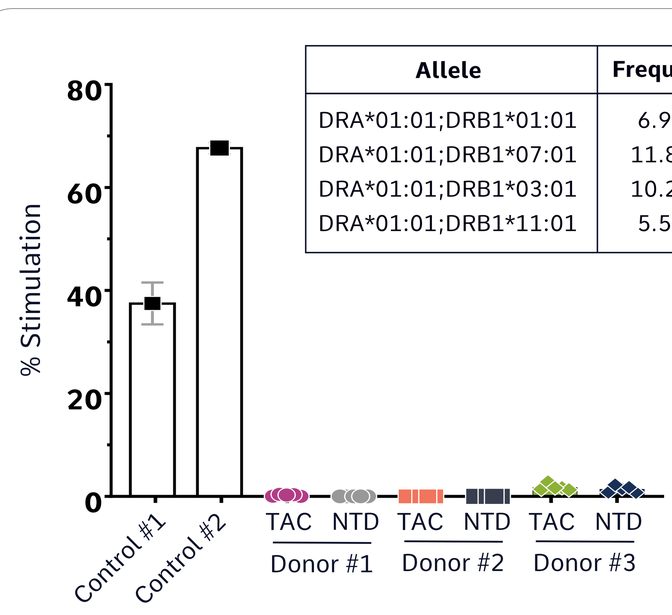
HER2-TAC γδ T Cells Demonstrate Efficacy in Mice Expressing Human IL-15

A. NSG and transgenic Tg-NSG_hIL-15 mice inoculated with N87 tumor cells were treated with 12 x 10⁶ HER2-TAC γδ T cells or controls. NSG mice were supplemented with IL-15 (28 000 IU) starting 1 hour pre-ACT, then 3 times per week thereafter. **B.** IL-15 serum levels were measured in untreated, tumor-bearing NSG mice either 1 hour or 2 days after a single IL-15 injection. Tg-NSG_hIL-15 mice were bled as comparisons and not supplemented with any cytokines.



HER2-TAC γδ T cells persist in vivo without expansion of natural killer and αβ T cells

Tumor and spleen were isolated from a tumor-bearing transgenic Tg-NSG_hIL-15 mouse 36 days after being treated with 12 x 10⁶ TAC cells. Tissues were processed into single cell suspension and positively-enriched for hCD45+ by magnetic beads. Populations were gated on live cells and presence of natural killer (NK) cells was assessed by CD56 and CD3 staining.



Allele	Frequency
DRA*01:01;DRB1*01:01	6.99%
DRA*01:01;DRB1*07:01	11.83%
DRA*01:01;DRB1*03:01	10.24%
DRA*01:01;DRB1*11:01	5.56%

MLR assay shows HER2-TAC γδ T cells do not proliferate when stimulated with different HLA subtypes, indicating lack of alloreactivity

HER2-TAC γδ T cells engineered from 3 healthy donors (GDT #1-3) were tested in a mixed lymphocyte reaction (MLR) assay with dendritic cells derived from 5 different donors covering the most dominant North American HLA subtypes (inset table).

Summary

- **HER2-TAC γδ T cells are selectively activated in the presence of HER2-positive tumor cells**
- **HER2-TAC γδ T cells display strong cytotoxicity towards HER2-positive tumor cells in co-culture assays**
- **HER2-TAC γδ T cells effectively eradicate HER2-expressing solid tumors in vivo**
- **HER2-TAC γδ T cells lack activity in MLR assays, highlighting its potential as an allogeneic cell therapy platform**