DEVELOPMENT OF GUCY2C-TAC T CELLS FOR THE TREATMENT OF COLORECTAL CANCER

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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. Here we present results from a new TAC T product targeting guanylyl cyclase 2C (GUCY2C). GUCY2C belongs to a family of membrane-bound mucosal guanylate cyclase receptors which are normally expressed on the apical brush border of intestinal epithelia, a site inaccessible to T cells. In cancer, however, GUCY2C is frequently overexpressed in primary and metastatic colorectal carcinomas, designating it a favorable antigen for specific targeting of tumor cells via TAC T cells. Using both in vitro and in vivo assays, we selected the top 2 GUCY2C-TAC performers out of 34 candidates, which demonstrated strong and specific activity of GUCY2C-targeted TAC T cells against GUCY2C-expressing tumor models.

Materials and Methods

The top 2 GUCY2C-TAC constructs were modified to improve efficacy by mutation of the CD3 binding domain and humanization of the nanobody, antigen binding domain. These new GUCY2C-TACs were functionally characterized using various in vitro and in vivo assays. In vitro assays included proliferation as well as cytotoxicity via real-time microscopy co-culture assays. In vivo studies examined the anti-tumor effect of these GUCY2C-TACs in both liquid and solid tumor models.

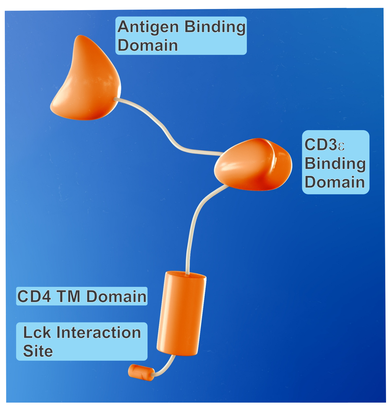
Results

The GUCY2C-TAC T cells showed strong specific activation when co-cultured with a variety of cancer cells expressing GUCY2C in vitro. Proliferation of the GUCY2C-TAC T cells was induced upon co-culture with naturally expressing GUCY2C target cell lines as well as GUCY2C-engineered cell lines. In vitro cytotoxicity assay demonstrated a strong anti-GUCY2C response and killing of GUCY2C-expressing target cell lines. Intravenous administration of GUCY2C-TAC T cells in mice carrying GUCY2C-positive tumor xenografts led to a favorable anti-tumor response.

Conclusions

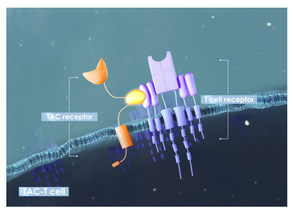
The in vitro and in vivo data confirm strong and specific activity of humanized nanobody GUCY2C-targeted TAC T cells against GUCY2C-expressing tumor cells.

TAC SCIENCE

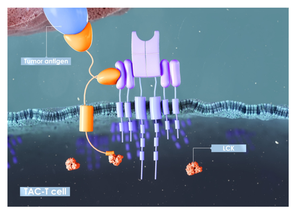


Key features of TAC technology:

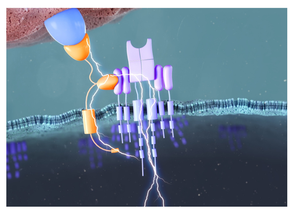
- TAC functions independently of MHC
- TAC activates T cells via the endogenous TCR
- TAC incorporates the co-receptor and recruits the TCR complex, mimicking natural TCR activation



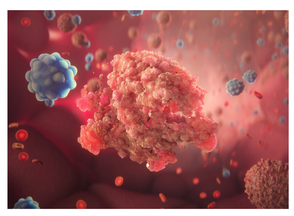
The membrane-bound TAC receptor interacts directly with the TCR-CD3 epsilon domain and...



...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.

Watch a short animation to understand the TAC mechanism

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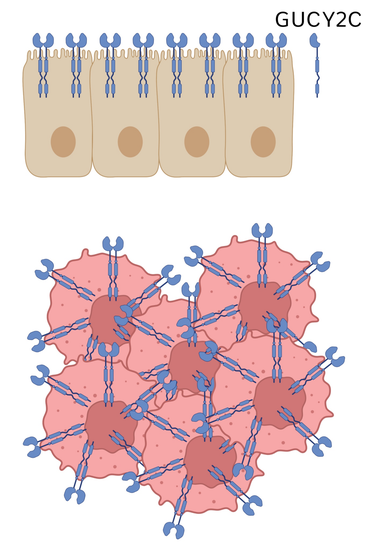
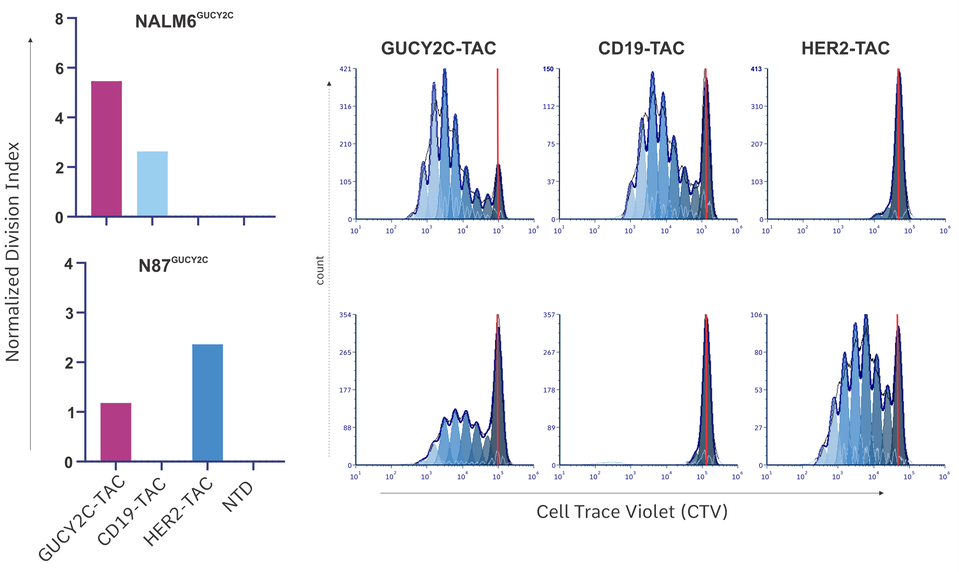


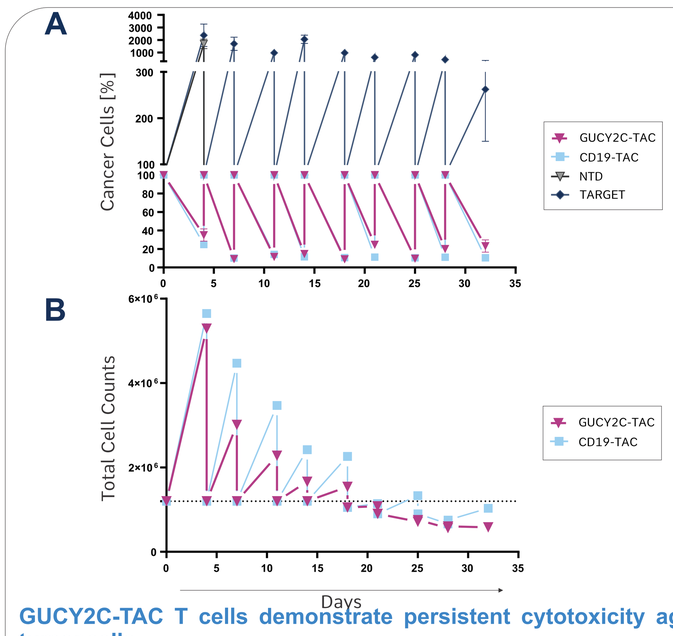
Guanylate cyclase 2C

Guanylate cyclase 2C (GUCY2C) is essential for intestinal fluid and ion homeostasis. In healthy, polarized epithelial cells, GUCY2C is only exposed to the intestinal lumen and inaccessible to immune cells. In a vast majority of colorectal cancers and several gastric and pancreatic cancers, GUCY2C is overexpressed and no longer restricted to the lumen, thus becoming a specific target for immune cell therapy. Figure created in BioRender.com.



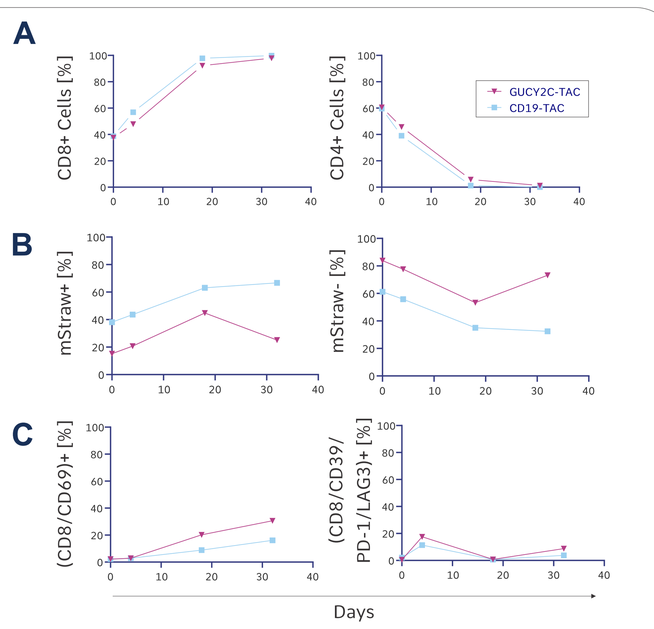
Proliferation of GUCY2C-TAC T cells against GUCY2C-positive cell lines

GUCY2C-TAC, CD19-TAC, or HER2-TAC (positive control) T cells or non-transduced (NTD) T cells (negative control) were co-cultured with either NALM6^{GUCY2C} (acute lymphocytic leukemia - top) or N87^{GUCY2C}(gastric carcinoma - bottom) tumor targets for 4 days at 1:3 E:T ratio. Prior to co-culture, T cells were labeled with cell trace violet (CTV) dye, while targets were treated with mitomycin. After 4 days, proliferation was evaluated by measuring the amount of CTV dye that remained in the T cells using flow cytometry. The division index, a measure of proliferation, is shown on the left bar graphs, normalized to T cells alone. GUCY2C-TAC T cells proliferate similarly to positive control TAC T cells in both tumor target cell models.



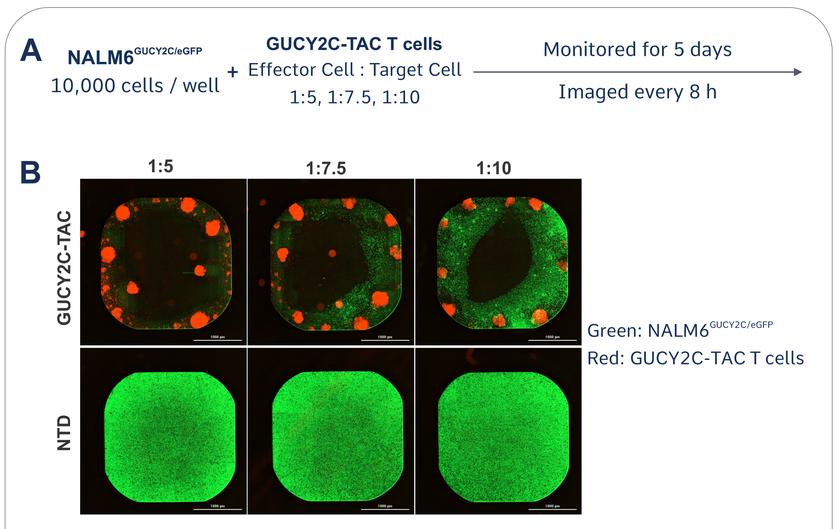
GUCY2C-TAC T cells demonstrate persistent cytotoxicity against tumor cells

GUCY2C-TAC T cells were co-cultured with NALM6$^{GUCY2C/eGFP}$ target cells at a 3:1 E:T ratio. **(A)** Alternating rounds of 3 and 4 days of co-culture were used. At the end of each round, cytotoxicity was evaluated by GFP fluorescence and quantified by calculating the area under each GFP curve. The T cells were carried forward into a new round with fresh target cells at the same 3:1 E:T ratio. The assay was performed in a 96-well plate with initially 8 wells setup per condition. **(B)** The total amount of T cells retrieved after each round was graphed relative to the initial total cells seeded at the start of each round. GUCY2C-TAC T cells killed targets repeatedly up to 9 rounds, similar to CD19-TAC T cell postive controls.



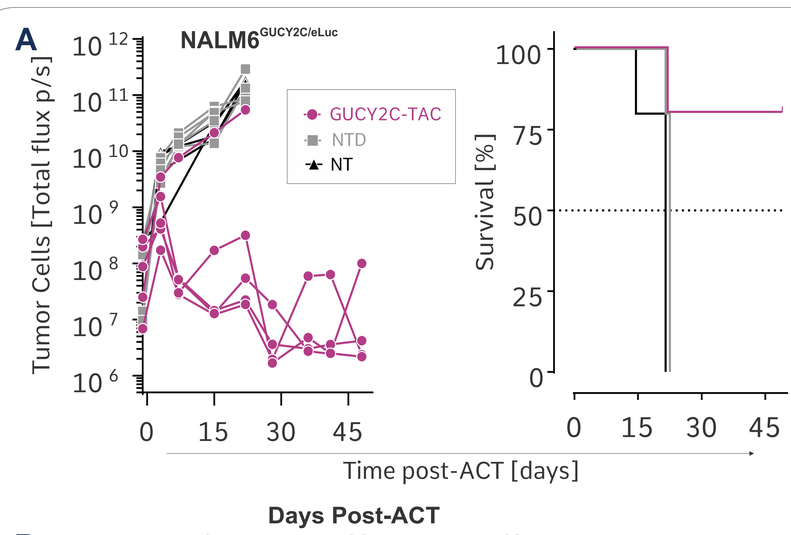
GUCY2C-TAC T cells lack signs of terminal exhaustion

GUCY2C-TAC T cells from cytotoxicity assay were phenotyped by flow cytometry at Day 0 and after rounds 1, 5, and 9. GUCY2C-TAC T cells killed targets repeatedly up to 9 rounds, similar to CD19-TAC T cell positive controls. **(A)** Percentage of total CD8+ and CD4+ T cells. **(B)** Percentage of mStrawberry+ (mStraw) (transduction marker - left) and mStraw- (right) T cells. **(C)** Percentage of CD8-gated T cells expressing CD69 (left). Percentage of CD8-gated then CD39-gated T cells expressing PD-1 and LAG3 (right). GUCY2C-TAC T cells gradually increase CD8+ T cells that are mostly mStraw negative. These T cells show minimal exhaustion marker expression by endpoint.



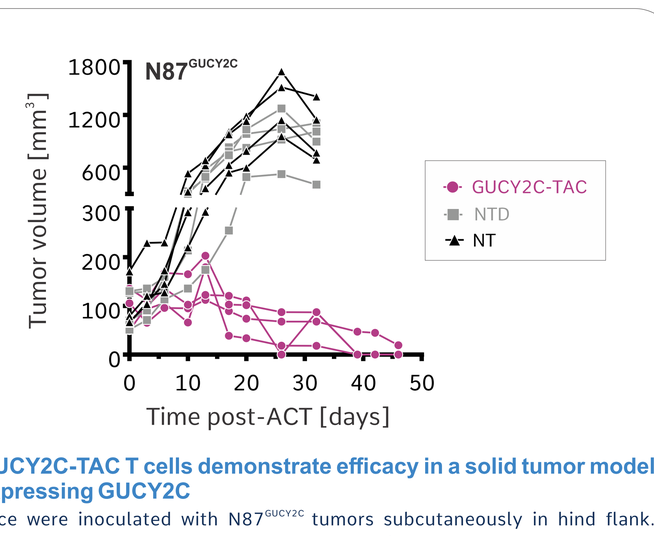
GUCY2C-TAC T cells demonstrate highly potent cytotoxicity

(A) GUCY2C-TAC T cells or non-transduced (NTD) T cells (negative control) were co-cultured with NALM6$^{GUCY2C/eGFP}$target cells for 5 days at different E:T ratios. Each well was imaged every 8 hours. Tumor cell growth was monitored by GFP fluorescence. **(B)** Example final images after 5-day co-culture. **(C)** Quantitative analysis of tumor cell growth as a function of eGFP area at different E:T ratios. GUCY2C-TAC T cells controlled tumor cell growth at all E:T ratios whereas NTD T cells did not.



GUCY2C-TAC T cells demonstrate efficacy at subtherapeutic dose levels in a liquid tumor model expressing GUCY2C

(A) Mice were inoculated with NALM6$^{GUCY2C/eLUC}$ tumors via tail vein injection. Treatment with a subtherapeutic dose of 1x10^6 GUCY2C-TAC T cells/mouse occurred on Day 0 (12 days following tumor seeding). Control animals had either no treatment (NT) or were administered non-transduced (NTD) T cells. Mice were monitored for tumor burden by bioluminescent imaging weekly. **(B)** IVIS imaging of tumor burden in individual mice at different times post-ACT.



GUCY2C-TAC T cells demonstrate efficacy in a solid tumor model expressing GUCY2C

Mice were inoculated with N87^{GUCY2C} tumors subcutaneously in hind flank. Treatment of tumor-bearing mice with 6x10^6 GUCY2C-TAC T cells/mouse occurred on Day 0. Control animals had either no treatment (NT) or were administered non-transduced (NTD) T cells. Tumors were monitored weekly by caliper measurements.

Summary

- GUCY2C-TAC T cells are specifically activated by cells expressing the GUCY2C antigen
- GUCY2C-TAC T cells demonstrate durable anti-tumor activity in 50-day repeat cytotoxicity assay
- GUCY2C-TAC T cells effectively eradicate both solid and liquid tumor xenografts expressing GUCY2C