Development of GUCY2C-TAC T Cells for the Treatment of Colorectal Cancer

Tania Benatar, Ling Wang, Thanyashanthi Nitya-Nootan, Heather L. MacGregor, Suzanna L. Prosser, Philbert Ip, Prabha Lal, Gargi U. Thakor, Stacey X. Xu, Laura M. Shaver, 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

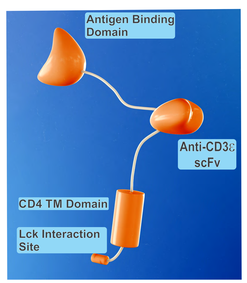
Background
The T cell antigen coupler (TAC) is a novel, proprietary chimeric receptor that facilitates the redirection of T cells to tumor cells and activates T cells by co-opting the endogenous T cell receptor complex with the goal to elicit safe and durable anti-tumor responses. TAC01-HER2, a first-in-class TAC T product targeting HER2 (ERBB2), has entered a phase I/II clinical trial in patients with HER2-positive solid tumors. Here, we present the development of a new TAC T product targeting guanylyl cyclase 2C (GUCY2C) to treat colorectal cancer. GUCY2C belongs to a family of membrane-bound mucosal guanylate cyclase receptors and is selectively 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 and, thus, a preferred antigen for the specific targeting of tumor cells via TAC T cells.

Materials and Methods
GUCY2C-TAC receptor functionality was characterized using a variety of in vitro and in vivo assays. In vitro assays were based on flow cytometric analysis of TAC surface staining, CD69 upregulation, and T cell proliferation. Cytotoxicity was assessed via real-time microscopy co-culture assays. In vivo studies examined the anti-tumor effect of TAC-engineered T-cells against established GUCY2C-expressing tumor xenografts.

Results
The GUCY2C-TAC receptor showed strong surface expression and specific activation when co-cultured with a variety of cancer cells expressing GUCY2C in vitro. Upregulation of the activation-induced CD69 marker was comparable with levels induced by activated control TAC T cells. Proliferation of GUCY2C-TAC T cells was induced by 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. No increases in T cell activation, proliferation, and no cytotoxicity were observed in non-transduced T cells and GUCY2C-TAC T cells co-cultured with GUCY2C-negative target cells, indicating that the T cell response is specific to the GUCY2C antigen. Intravenous administration of GUCY2C-TAC T cells in mice carrying GUCY2C-positive tumor xenografts led to a sustained anti-tumor response.

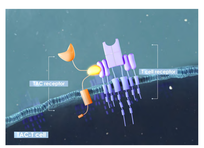
Conclusion
The in vitro and in vivo data confirm strong and specific activity of GUCY2C-targeted TAC T cells against GUCY2C-expressing tumor models and highlight the versatility of the TAC platform for therapeutic applications in solid tumors.

TAC SCIENCE

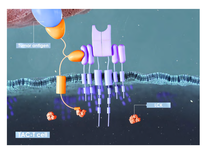


Key features of TAC technology:

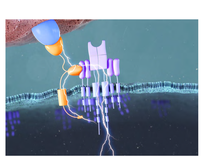
- TAC functions independently of MHC
- TAC activates T cells via the endogenous TCR
- TAC incorporates the co-receptor and recruits the TCR complex, mimicking natural TCR activation



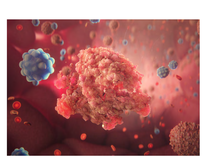
The membrane-bound TAC receptor interacts directly with the TCR-CD3 epsilon domain and...



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

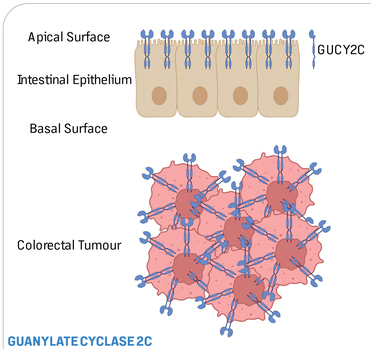


...initiates T cell activation via the endogenous CD3-TCR complex.



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

Watch a short animation to understand the TAC mechanism



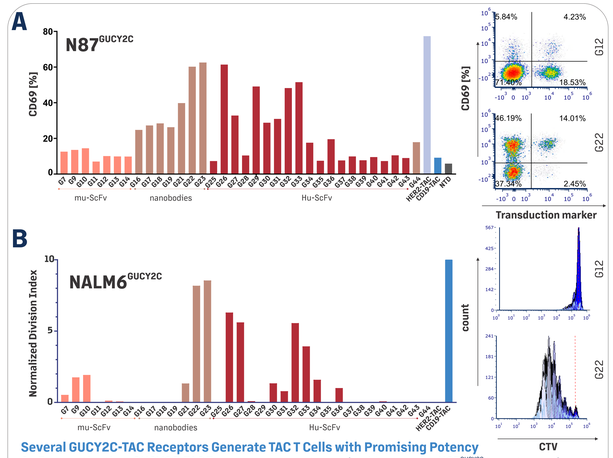
GUANYLATE CYCLASE 2C

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.

GUCY2C construct	Binder type	Transduction %	GUCY2C construct	Binder type	Transduction %
G7	Mu-scFv	44.4	G28	Hu-scFv	49.5
G9	Mu-scFv	28.3	G29	Hu-scFv	52.8
G10	Mu-scFv	26.3	G30	Hu-scFv	54.3
G11	Mu-scFv	41.4	G31	Hu-scFv	42.8
G12	Mu-scFv	42.3	G32	Hu-scFv	34.4
G13	Mu-scFv	53.3	G33	Hu-scFv	36.0
G14	Mu-scFv	54.7	G34	Hu-scFv	37.8
G16	Nanobody	49.9	G35	Hu-scFv	48.7
G17	Nanobody	57.4	G36	Hu-scFv	47.8
G18	Nanobody	49.6	G37	Hu-scFv	56.6
G19	Nanobody	51.8	G38	Hu-scFv	43.3
G21	Nanobody	43.8	G39	Hu-scFv	40.6
G22	Nanobody	42.3	G40	Hu-scFv	51.6
G23	Nanobody	46.5	G41	Hu-scFv	56.8
G25	Hu-scFv	48.0	G42	Hu-scFv	48.8
G26	Hu-scFv	41.0	G43	Hu-scFv	39.4
G27	Hu-scFv	45.1	G44	Nanobody	52.3

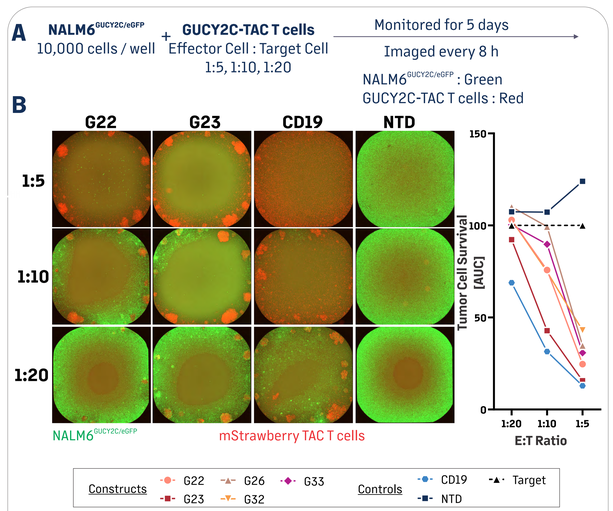
Successful Generation of GUCY2C-TAC T Cells

A total of 34 GUCY2C-TAC receptor candidates employing both nanobody and single chain variable fragment binding domains were screened. **A.** Comparison between scFv and nanobody structure. **B.** Representative flow plots of the transduction marker, mStrawberry, vs TAC expression from a variety of candidates including G12 (Mu-scFv), G22 (nanobody), and G26 and G36 (Hu-scFv). **C.** Summary chart of the 34 GUCY2C-TAC binders, their structures and transduction status.



Several GUCY2C-TAC Receptors Generate TAC T Cells with Promising Potency

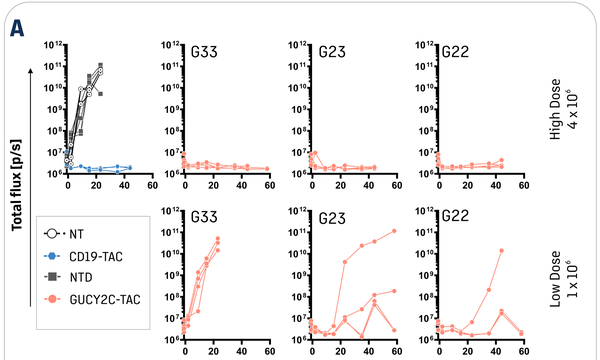
A. Early activation: GUCY2C-TAC T cells were co-cultured for 4 hours with N87^{GUCY2C} target cells, followed by staining for T cell activation via CD69 upregulation. **B.** Proliferation: The GUCY2C-TAC T cells were co-cultured for 4 days with NALM6^{GUCY2C} target cells. Proliferation was measured by the proportion of CTV dye remaining in the T cells. Results show that several of the GUCY2C-TAC T cell products are activated and proliferate in response to co-culture with the GUCY2C target.



GUCY2C-TAC T Cells Demonstrate Highly Potent Cytotoxicity

A. GUCY2C-TAC T cells, CD19-TAC T cells (positive control), 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 h. Tumor cell growth was monitored by GFP fluorescence. **B.** Example final images after 5 days co-culture. GUCY2C-TAC T cells co-cultured with NALM6$^{GUCY2C/eGFP}$ cells controlled growth of the cancer cells at E:Ts of 1:5 and 1:10. GUCY2C-TAC T cells were able to control tumor cells at a ratio as low as 1:20. The total GFP area of each well was plotted against time. The area under the curve (AUC) was calculated for each plot. The AUC is a cumulative measure of target cell survival, normalized to target alone, then plotted per E:T ratio. The G23 construct displayed the greatest control of target cell growth across each E:T.

triumvira



GUCY2C-TAC T Cells Control Tumor at Low Dose Levels

A. Mice were inoculated with NALM6$^{GUCY2C/eLUC}$ tumors via tail vein injection. Treatment with three different GUCY2C-TAC constructs at either a high dose or a low dose occurred on Day 0 (12 days following tumor seeding). Control animals had either no treatment (NT), or were administered non-transduced (NTD) T cells or CD19-TAC T cells. Mice were monitored for tumor burden by bioluminescent imaging weekly. **B.** IVIS images of tumor burden in individual mice at different times post-ACT.

Summary

- **Identified multiple functional GUCY2C-TAC candidates**
- **GUCY2C-TAC candidates display high in vitro and in vivo potency**
- **GUCY2C-TAC T cells effectively control aggressive NALM6^{GUCY2C} tumor xenografts in vivo at low T cell doses**