PRECLINICAL STUDIES OF TAC01-CLDN18.2, AN AUTOLOGOUS CLAUDIN 18.2-DIRECTED TAC T CELL THERAPY, FOR THE TREATMENT OF GASTRIC CANCER

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ABSTRACT

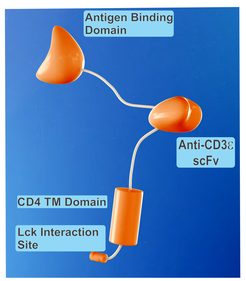
Background
The T cell antigen coupler (TAC) is a novel, proprietary chimeric receptor that facilitates the redirection of T cells to tumor cells and activates T cells by co-opting the endogenous T cell receptor complex with the goal to elicit safe and durable anti-tumor responses. TAC01-HER2, a first-in-class TAC T product targeting HER2 (ERBB2), has entered a phase I/II clinical trial in patients with HER2-positive solid tumors. The subject of this presentation is a new TAC T product, TAC01-CLDN18.2, targeting claudin 18.2 (CLDN18.2) to treat gastric cancer. CLDN18.2 belongs to a family of claudin tight junction proteins and is naturally restricted to epithelia of normal stomach. In gastric cancer cells, CLDN18.2 expression can go awry, is no longer confined to tight junctions and, thus, targetable by CLDN18.2-TAC T cells.

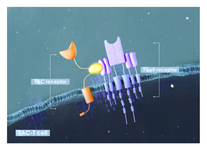
Materials and Methods
CLDN18.2-TAC T cells were evaluated using a variety of in vitro and in vivo assays. In vitro assays were based on flow cytometric analysis of T cell proliferation and surface activation marker expression. Cytotoxicity was assessed via real-time microscopy-based co-culture assays. In vivo studies examined the anti-tumor effect of CLDN18.2-TAC T cells against established solid CLDN18.2-expressing tumors.

Results
CLDN18.2-TAC T cells showed specific anti-tumor cytotoxicity in CLDN18.2-expressing gastric spheroid models as well as 2D-co-cultures with tumor cells expressing endogenous CLDN18.2. In contrast, CLDN18.2-TAC T cells lacked activity when cultured with CLDN18.2-negative cells derived from normal human tissues . While CLDN18.2-TAC T cells also cross-reacted with murine CLDN18.2, mice showed no signs of toxicity, suggesting that CLDN18.2-TAC T cells do not induce off-tumor effects. The in vitro repeat killing assay demonstrated strong and persistent anti-tumor activity of CLDN18.2-TAC T cells against CLDN18.2-expressing target cells. Lastly, treatment with CLDN18.2-TAC T cells in MHC DKO mice bearing CLDN18.2-positive tumors led to complete and sustained tumor clearance, even after a secondary tumor re-challenge, indicating long-term persistence of TAC cells up to 56 days after initial dosing.

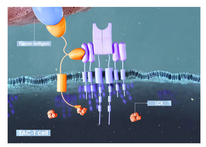
Conclusion
The in vitro and in vivo data confirm strong and specific activity of CLDN18.2-targeted TAC T cells against CLDN18.2-expressing solid tumor models and highlight the versatility of the TAC platform for therapeutic applications in solid tumors.

TAC SCIENCE

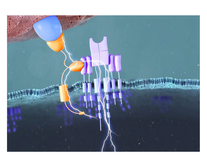




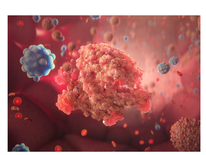
The membrane-bound TAC receptor interacts directly with the TCR-CD3 epsilon domain and...



...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.

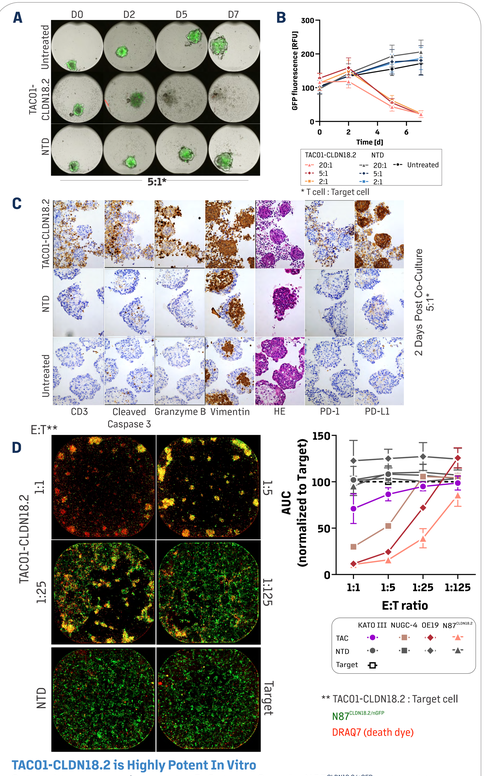


This results in effective cell lysis of multiple tumor cells during multiple killing events.

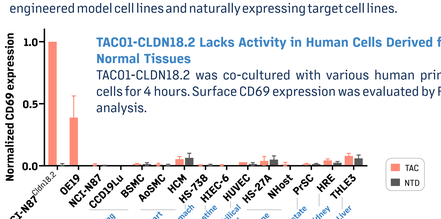
Key features of TAC technology:

- 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

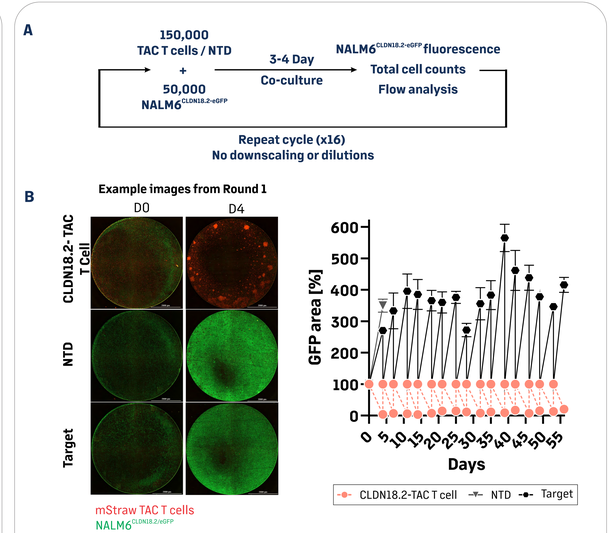
Watch a short animation to understand the TAC mechanism



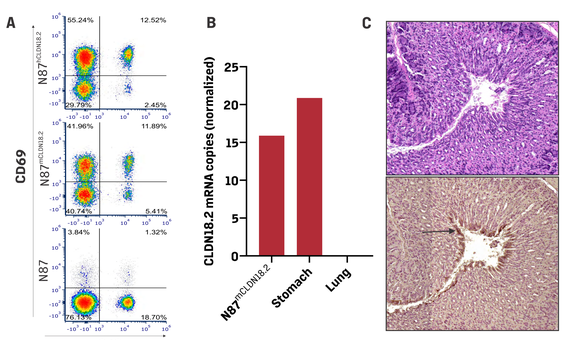
TAC01-CLDN18.2 is Highly Potent In Vitro
Spheroid assay performed by InSphero. Gastric N87$^{CLDN18.2/nGFP}$ spheroids were generated with human cancer associated fibroblast cells resulting in dense spheroids. (**A** and **B**) In co-cultures, TAC01-CLDN18.2 rapidly engage and eradicate spheroids at all E:T ratios tested. (**C**) Histology and immuno-histochemistry analysis reveals the infiltration of TAC01-CLDN18.2, as well as their ability to efficiently engage and eliminate target cells. (**D**) In a 2D cytotoxicity assay, TAC01-CLDN18.2 was co-cultured with N87$^{CLDN18.2/nGFP}$ at low E:T ratios. Cytotoxicity was observed across multiple cell lines, including CLDN18.2 engineered model cell lines and naturally expressing target cell lines.



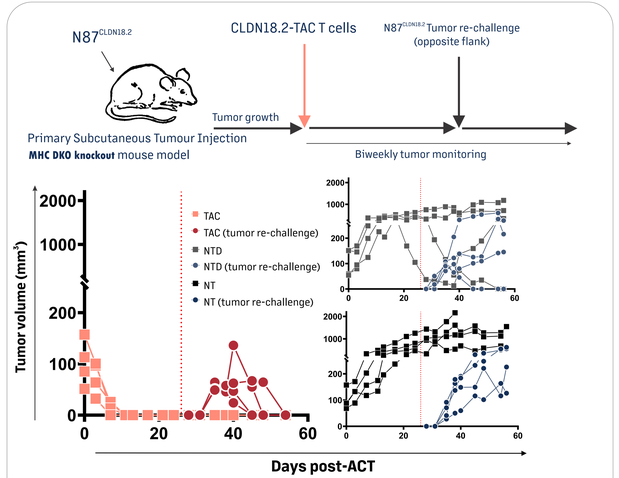
TAC01-CLDN18.2 Lacks Activity in Human Cells Derived from Normal Tissues
TAC01-CLDN18.2 was co-cultured with various human primary cells for 4 hours. Surface CD69 expression was evaluated by FACS analysis.



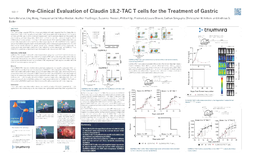
CLDN18.2-TAC T Cells Demonstrate Persistent Cytotoxicity Against Tumor Cells
CLDN18.2-TAC T cells were co-cultured with NALM6$^{CLDN18.2/nGFP}$ target cells at a 3:1 E:T ratio (**A**) Alternating rounds of 3 and 4 days of co-culture were used. At the end of each round, cytotoxicity was evaluated by GFP fluorescence. (**B**) The T cells were then carried forward into a new round with fresh target cells. The assay was performed in a 96-well plate with 8-wells setup per condition.



TAC01-CLDN18.2 Cross-Reacts with Murine CLDN18.2 While Showing no Signs of Toxicity In Vivo
(**A**) TAC01-CLDN18.2 was co-cultured with N87 cells engineered with human or murine CLDN18.2 for 4 hours. CD69 expression was evaluated by FACS analysis. (**B**) CLDN18.2 mRNA copies from N87$^{mCLDN18.2}$, mouse stomach, and lung tissue were quantified by ddPCR. (**C**) Stomach tissue from CLDN18.2-TAC treated mice was stained with H&E (top) or anti-CLDN18.2 (bottom, as indicated by arrow).



CLDN18.2-TAC T Cells Demonstrate Long-Term Persistence
MHC DKO knockout mice were inoculated subcutaneously with N87$^{CLDN18.2}$ tumor cells. Once tumors grew to an average size of 100 mm^3, mice were treated with TAC01-CLDN18.2 T cells. Two weeks after the primary tumors regressed, mice were re-challenged with tumor cells on the opposing hind flank in both TAC T cell and non-transduced (NTD)-treated groups. Mice receiving no treatment (NT) were separated into two cohorts, with each cohort receiving a single tumor inoculation at the same time when the other treatment groups received either their initial or re-challenge tumor dose. Individual tumor measurements are shown, as measured twice a week.



Please see AACR Poster 2022 for specificity of CLDN18.2-TAC in broad protein array screen

Previously Presented
- CLDN18.2 - TAC T cells are highly specific for CLDN18.2 showing no cross-reactivity in a membrane proteomic screen
- CLDN18.2 TAC T cells do not cross-react with CLDN18.1
- CLDN18.2 TAC T cells are efficacious in various tumor models

Summary

- **CLDN18.2-TAC T cells effectively eradicate Claudin 18.2-expressing tumor cells in vitro and in vivo**
- **CLDN18.2-TAC T cells demonstrate persistent cytotoxicity against Claudin 18.2-expressing tumor cells in vitro and in vivo**
- **CLDN18.2-TAC T cells demonstrate cross-reactivity with murine Claudin 18.2 while preserving normal stomach histology in mice**
- **CLDN18.2-TAC T cells lack off-target effects in human cells derived from normal tissues**