**ABSTRACT**

Background

The αβ T cell antigen receptor (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. TAC1-H3-HER2, a first-in-class, autologous TAC T cell product targeting HER2 (BBR2), has entered a phase 1/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 accessible to T cells. In cancer, however, GUCY2C is frequently overexpressed in primary and metastatic colorectal cancer tissues, 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 T cells against GUCY2C-expressing tumor models.

Materials and Methods

The top 2 GUCY2C-TAC constructs were modified to improve efficiency by mutation of the CD3 binding domain and humanization of the nanobody, antigen binding domain. These new GUCY2C-TACs were functionally evaluated using various in vitro and in vivo assays. In vitro assays included proliferation, cytotoxicity, and recruitment of kinases (Lck) via TAC-TCR complexes, leading to effective cell killing. In vivo studies examined the anti-tumor effect of these GUCY2C-TACs in both liquid and solid tumor models.

Results

The GUCY2C-TAC constructs 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-targeting cell lines. Immunohistochemical analysis 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**

The membrane-bound TAC receptor interacts directly with the TCR-COD3 epitope 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...

**CONCLUSIONS**

GUCY2C-TAC T cells lack signs of terminal exhaustion. GUCY2C-TAC T cells from cytotoxicity assay were phenotyped by flow cytometry at day 6 and found to maintain high levels of CD69 and GUCY2C-TAC expression, similar to CD19-TAC T cell positive controls. Percentage of CD8+ and CD4+ T cells were determined by flow cytometry and calculated as a percentage of total CD8+ and CD4+ cells. GUCY2C-TAC T cells from cytotoxicity assay were phenotyped by flow cytometry and calculated as a percentage of total CD8+ and CD4+ cells.

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


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