ARMORING IPSC-DERIVED ALLOGENEIC THERAPIES WITH A TGF-B NEUTRALIZING SYNTHETIC RECEPTOR THAT ENHANCES SOLID TUMOR ELIMINATION IN TME-MIMICKING CONDITIONS IN VITRO

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INTRODUCTION

- Allogeneic immunotherapy holds immense promise for solid tumor treatment with significant challenges remaining in overcoming the barriers imposed by the solid tumor microenvironment (TME).
- Transforming Growth Factor Beta (TGF-β) pathway plays a key role in regulating the TME, driving immunosuppression, stromal remodelling, and angiogenesis to support tumor progression.
- The TME can disarm CAR engineered immune effector cells intended to target and drive anti-tumoral activity at the tumor site, inhibiting the ability to irradicate the disease.
- TGF-β dominant-negative receptor (DNR) is a genetically engineered receptor designed to block TGF-β signaling.

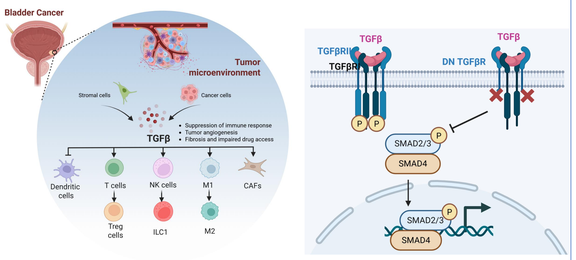


Figure 1. TGF-ß role in TME (left) and TGF-ß pathway (right). *TGF-ß, secreted by tumor and stromal cells, suppresses immune responses by converting cytotoxic T cells into Tregs, downregulating NK cell receptors (NKG2D, NKp30), and reducing MHCII expression in dendritic cells. It also promotes pro-tumor M2 macrophages and N2 neutrophils through Snail and arginase upregulation (Left).TGF-ß receptors are dual-specificity kinases. Upon ligand binding, TGFBR2 activates TGFBR1, which phosphorylates SMAD2/3. These associate with SMAD4 to form a complex that enters the nucleus and regulates gene expression in a context-dependent manner. (Right)*

- We have developed a solid tumor-targeted, induced pluripotent stem cell (CAR-iPSC) armored with a synthetic TGF-β DNR that neutralizes TGF-β in the TME, thus inhibiting tumor growth and enhancing immune responses against cancer.

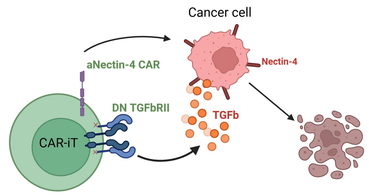


Figure 2. TGF-ß DNR armored anti-Nectin-4 CAR-iT design

RESULTS

TGF-β DNR design and neutralizing activity

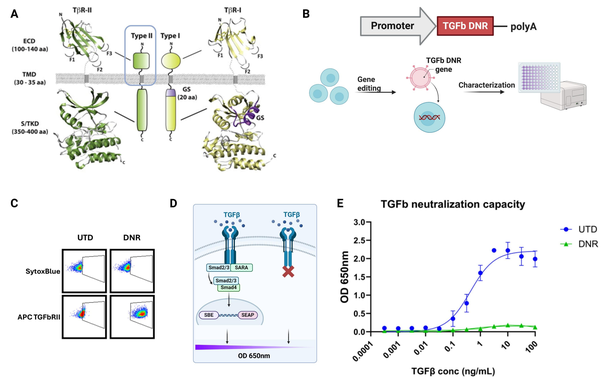
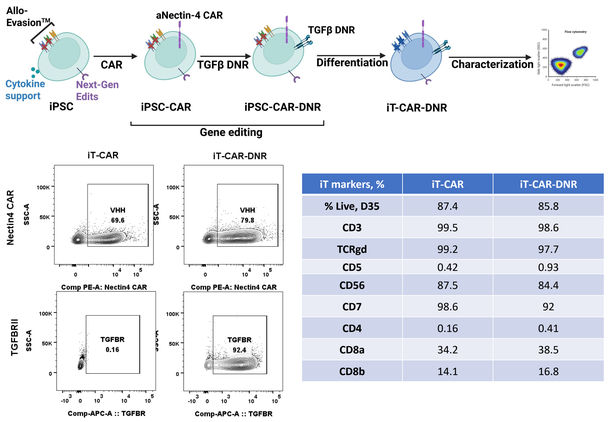


Figure 3. TGF-ß DNR design and POC study in a reporter line. *TGF-ß receptor I and II structures. The extracellular domain used in design of the DNR consists of the EC and TM domains indicated by the blue box (Figure 3A). The vector and initial in vitro study design, involving lentiviral vector packaging and transduction of HEK-Blue TGFb line (Invivogen) is shown in Figure 3B. TGFBRII staining confirmed >95% positive population in the transduced cells (Figure 3C). HEK-Blue TGF-ß cells were stimulated with recombinant human TGF-ß (rhTGF-ß) to assess signaling. In untransduced (UTD) cells, TGF-ß stimulation induced formation of Smad3/Smad4 complexes, resulting in activation of the SEAP reporter and detection via Quanti-Blue assay. In contrast, cells expressing TGF-ß DNR showed minimal reporter activity, indicating impaired downstream SMAD signaling. These data support the functional blockade of TGF-ß signaling by DNR expression (Figures 3D and E).*

TGF-β DNR engineering is compatible with iPSC differentiation



iT markers, %	iT-CAR	iT-CAR-DNR
% Live, D35	87.4	85.8
CD3	99.5	98.6
TCRgd	99.2	97.7
CD5	0.42	0.93
CD56	87.5	84.4
CD7	98.6	92
CD4	0.16	0.41
CD8a	34.2	38.5
CD8b	14.1	16.8

Figure 4. TGF-ß DNR expression is compatible with iPSC cell differentiation to effector T-cells. *iPSC lines expressing the DNR were differentiated using a standard iT protocol. Both DNR-expressing and control iPSC lines generated CD4⁺ and CD8⁺ iT cells with comparable efficiencies, as measured by flow cytometry at the final differentiation stage. No impairment in lineage commitment or T cell marker expression was observed, indicating that DNR integration does not disrupt iT cell development.*

RESULTS

TGF-β DNR-engineered CAR-iPSCs have reduced SMAD signaling

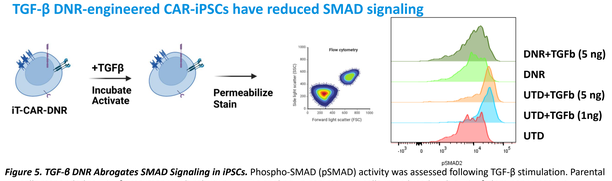


Figure 5. TGF-ß DNR Abrogates SMAD Signaling in iPSCs. *Phospho-SMAD (pSMAD) activity was assessed following TGF-ß stimulation. Parental iPS cells responded to TGF-ß treatment with robust pSMAD signaling. In contrast, iPS cells transduced with a TGF-ß dominant-negative receptor (DNR) failed to induce pSMAD, indicating successful blockade of TGF-ß signaling. These results suggest that the TGF-ß DNR functions as a ligand sink, effectively neutralizing extracellular TGF-ß.*

Nectin-4 expressing tumor cell lines growth in the presence of TGFb

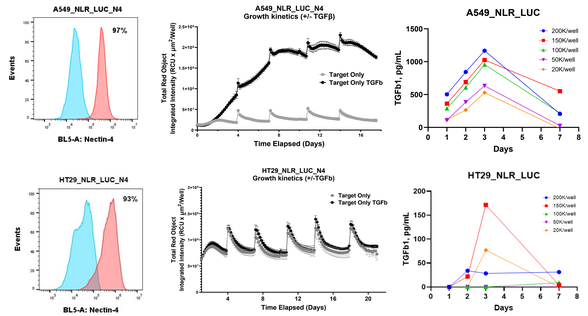


Figure 6. Characterization of Nectin-4 overexpressing lines. *A549 (epithelial, lung adenocarcinoma) and HT-29 (epithelial, colorectal adenocarcinoma) cell lines (ATCC) were engineered to stably overexpress huNectin-4. Cells were characterized for the surface expression of Nectin-4. Cells were cultured with and without rhTGFb and growth kinetics curves were obtained. Both cell lines naturally express TGFb, as measured by ELISA.*

DNR boosts CAR-iT activity in the presence of recombinant human TGF-β

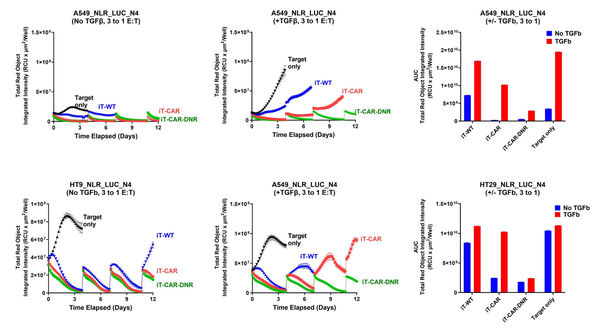
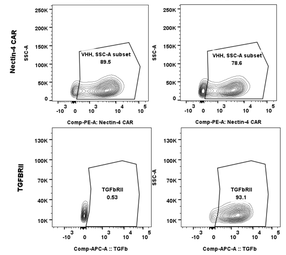


Figure 7. TGF-ß DNR enhances repeat-killing capacity of CAR-iT cells under suppressive conditions. *CAR-iT cells with or without TGF-ß DNR were evaluated in a repeat-killing assay against Nectin-4⁺ target cells in the presence of inhibitory TGF-ß (5 ng/mL, added with every refeed). In conditions mimicking the TME, TGF-ß DNR expression enhanced cytotoxic function, maintaining prolonged target cell clearance as measured by Incucyte imaging. TGF-ß DNR CAR-iT cells demonstrated ~4-fold greater cumulative killing compared to controls (2.5×10^9 vs. 1.05×10^{10} total activity).*

RESULTS

TGF-β DNR in iNK: Differentiation and expansion compatibility and function



iNK markers, %	iNK-CAR	iNK-CAR-DNR
CD3+/CD56+	0.44	0.29
CD45+/CD56+	99.4	99.6
CD56+/PANKIR2D+	20.3	20.6
CD16+/CD56+	99.4	99.5
CD56+/DNAM+	95.3	88.6
CD56+/NKP46+	14	7.3
CD56+/NKG2C+	98.1	89.9

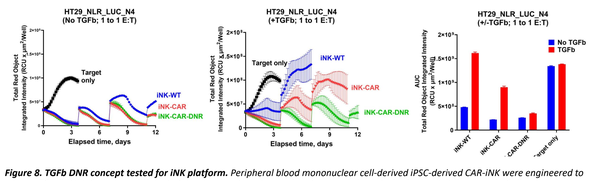


Figure 8. TGFb DNR concept tested for iNK platform. *Peripheral blood mononuclear cell-derived iPSC-derived CAR-iNK were engineered to express TGF-ß DNR. Cells were differentiated according to a standard protocol and characterized using iNK flow panels. Cells were challenged in a repeat-killing assay against Nectin-4 expressing lines. Targeted CAR-iNK engineered with TGF-ß DNR demonstrated enhanced cytotoxicity activity against solid tumor cell lines in the presence of recombinant human TGF-ß as compared to CAR-iNK (no DNR) and iNK-WT cells.*

Enhanced Cytotoxicity of iT-CAR DNR Cells Against Bladder Cancer Cells

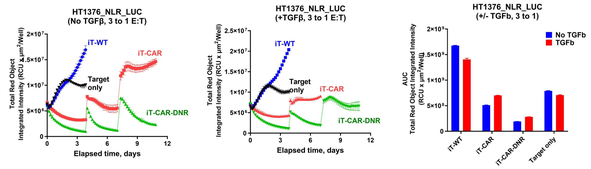


Figure 9. TGFb DNR protective effect in bladder cancer line. *Repeat-killing assays were performed using HT1365 bladder cancer cells with or without recombinant human TGF-ß. CAR-iT cells expressing TGF-ß DNR exhibited enhanced cytotoxicity compared to controls, regardless of TGF-ß presence, indicating a protective effect of DNR against immunosuppressive signaling.*

TGF-β DNR Restores CAR-iT Cell Function in a 3D Tumor Microenvironment Model

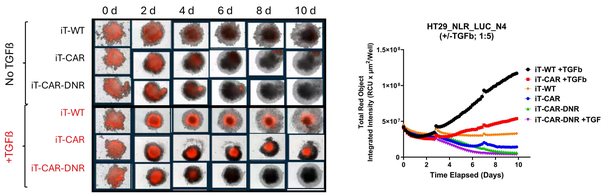


Figure 10. TGFb DNR restores activity of CAR-iT in TME. *To evaluate the functional advantage of TGF-ß DNR in TME-like conditions, CAR-iT cells were tested in a 3D spheroid model in the presence of rhTGF-ß. DNR-expressing CAR-iT cells demonstrated superior tumoroid clearance compared to iT-CAR or iT-WT cells, indicating robust resistance to TGF-ß–mediated suppression.*

References
Kuburich, N. A., Sabapathy, T., Demestichas, B. R., Maddela, J. J., Den Hollander, P., & Mani, S. A. (2023). Proactive and reactive roles of TGF-β in cancer. Seminars in Cancer Biology, 95, 120-139.

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