Natasha Kodgi
BIOL7015
Fall 2025

Armored CAR T Cells Resistant to TGF-β Mediated Immunosuppression in Triple-Negative Breast Cancer (TNBC)

Abstract

Triple-negative breast cancer (TNBC) remains one of the most aggressive breast cancer subtypes. Although chimeric antigen receptor (CAR) T cell therapy has transformed blood cancer treatment, its efficacy in solid tumors like TNBC is hindered by TGF-β-mediated immunosuppression and poor immune infiltration. Over three years, I will engineer MUC1-targeted CAR T cells that co-express a dominant-negative TGF-β receptor II (dnTGFBR2) to block inhibitory signaling. I will assess their cytotoxicity, cytokine secretion, and infiltration in 3D TNBC spheroid models, followed by a pilot xenograft study in immunodeficient mice. I hypothesize that these "armored" CAR T cells will resist TGF-β-driven exhaustion and maintain antitumor activity within the suppressive TNBC microenvironment, providing a foundation for microenvironment-resilient CAR T therapies for solid tumors.

Project Aims

The primary goal is to develop and evaluate a TGF-β-resistant CAR T-cell therapy for triple-negative breast cancer (TNBC) that can maintain strong antitumor activity within the suppressive tumor microenvironment. The three main aims of this study are as follows.

Aim 1: Engineer and validate MUC1-targeted CAR T cells co-expressing a dominant-negative TGF-β receptor II (dnTGFBR2). I will construct a lentiviral MUC1-CAR-dnTGFBR2 vector and test expression, viability, and cytokine secretion under TGF-β exposure.

Aim 2: Assess cytotoxicity and persistence of dnTGFBR2-CAR T cells in 2D co-cultures and 3D TNBC spheroids. I will quantify cytotoxicity, cytokine release, and CAR T infiltration using confocal imaging and viability assays.

Aim 3: Evaluate antitumor potential in a small pilot TNBC xenograft model. Tumor burden and CAR T persistence will be tracked using bioluminescence and flow cytometry.

Significance

Triple-negative breast cancer (TNBC) accounts for 10-20% of all breast cancers and remains one of the most aggressive subtypes with limited treatment options (Bianchini et al., 2022; Lehmann et al., 2011). Because TNBC lacks estrogen, progesterone, and HER2 receptors, chemotherapy remains the standard of care, but recurrence and poor survival are common (Lehmann et al., 2011; Nasiri et al., 2022). These challenges highlight the need for new strategies to overcome TNBC's resistance to conventional treatment.

CAR T-cell therapy has transformed outcomes in blood cancers, but its efficacy in solid tumors like TNBC remains limited due to the tumor microenvironment (TME), which restricts immune infiltration and suppresses T-cell activity through inhibitory cytokines such as TGF-β (Bianchini et al., 2022). TGF-β is a major driver of immunosuppression in the TME, promoting T-cell exhaustion and reducing proliferation and persistence (Stüber et al., 2020).

Prior work has explored strategies to enhance CAR T-cell efficacy in solid tumors. Zhou et al. (2019) developed MUC1-targeted CAR T cells that effectively killed TNBC cells in vitro and reduced tumor growth in mice, but their responses were short-lived due to suppression by the tumor microenvironment (TME). Tang et al. (2020) showed that disrupting TGF-β receptor signaling via CRISPR deletion of *TGFBR2* enhanced CAR T-cell function and cytokine secretion despite TGF-β exposure, resulting in improved tumor control in vivo. Similarly, Li et al. (2024) engineered a bicistronic CAR T construct targeting EGFR and IL13Rα2 with a co-expressed dominant-negative TGF-β receptor II (dnTGFβRII), which significantly improved T-cell proliferation, functional fitness, and tumor clearance in TGF-β-rich glioblastoma models. However, no study has optimized TGF-β-resistant CAR T cells for TNBC specifically, and most prior work uses 2D cultures rather than 3D models that better capture tumor architecture and immune interactions (Bittman-Soto et al., 2024).

This project builds on these advances by engineering MUC1-targeted CAR T cells co-expressing a dominant-negative TGF-β receptor II (dnTGFBR2) and evaluating them in 3D TNBC spheroids and a pilot xenograft model. This approach both targets the main immunosuppressive pathway limiting CAR T efficacy and uses physiologically relevant models to test function and infiltration.

Innovation

This project is innovative because it strengthens the CAR T cell itself to withstand the suppressive TNBC microenvironment rather than relying on external checkpoint inhibitors or new antigen targets. It builds on prior MUC1-CAR studies (Zhou et al., 2019) by introducing a dnTGFBR2-based "armoring" strategy that blocks inhibitory signaling intracellularly, creating a self-shielded immune effector that maintains cytotoxicity and cytokine secretion in TGF-β-rich conditions.

The integration of 3D TNBC spheroid assays adds a second layer of novelty by modeling CAR T infiltration and persistence in an environment closer to human tumors. Together with a small proof-of-concept xenograft study, these experiments bridge mechanistic insight and translational relevance, advancing the design of microenvironment-resilient CAR T therapies for solid cancers.

Research Plan and Key Methods

Aim 1: Engineer and validate MUC1-targeted CAR T cells co-expressing a dominant-negative TGF-β receptor II (dnTGFBR2).

This aim establishes the core armoring strategy by creating MUC1-directed CAR T cells co-expressing dnTGFBR2 to test whether blocking TGF-β signaling preserves CAR T-cell function under immunosuppressive conditions. Primary human T cells will be isolated, activated, and transduced using a lentiviral vector, and expression will be verified by flow cytometry. Functional assays will test cytokine release (IL-2, IFN-γ), viability, and cytotoxicity against MUC1-positive TNBC cells (e.g., MDA-MB-231, a widely used human TNBC line) (Lehmann et al., 2011) under TGF-β exposure. By the end of the first year, the engineered CAR T cells should maintain their ability to kill tumor cells and secrete cytokines even when exposed to TGF-β, confirming successful resistance.

Aim 2: Evaluate cytotoxicity and persistence in 2D and 3D TNBC models.
This aim evaluates how TGF-β-resistant CAR T cells behave in increasingly realistic tumor environments. To better mimic the TNBC tumor environment, I will develop 3D spheroids from MDA-MB-231 cells embedded in a matrix and supplemented with recombinant TGF-β to model an immunosuppressive microenvironment. Engineered and control CAR T cells will be compared for tumor killing, cytokine secretion, and infiltration using imaging and viability assays. By the second year, dnTGFBR2-CAR T cells are expected to show stronger cytotoxicity, better infiltration, and longer persistence in 3D models compared to controls.

Aim 3: Test antitumor activity in vivo using a pilot xenograft study.
This aim provides proof-of-concept evidence for in vivo efficacy and durability. Immunodeficient NSG mice will be implanted with human MUC1-positive TNBC cells and treated with either control or dnTGFBR2-CAR T cells. Tumor growth will be tracked by imaging, and CAR T persistence will be measured at the study endpoint. By the third year, dnTGFBR2-CAR T cells should demonstrate better tumor control and persistence in vivo, supporting their potential for future translational studies.

These experiments require access to a cell therapy lab equipped for primary T-cell culture, flow cytometry, and small animal studies. All proposed techniques are well-established and feasible within three years, providing a systematic test of whether blocking TGF-β signaling improves CAR T durability against TNBC.

Alternative Approach

While established immunoengineering methods are the basis of this work, I still anticipate potential challenges and have contingency plans to maintain testing of the central hypothesis. If dnTGFBR2 expression is inefficient, I will compare partial versus complete TGF-β blockade to define the level of inhibition needed for functional improvement. If 3D spheroid models prove inconsistent, I will use simpler co-culture or migration assays that still capture key features of immune suppression. If the in vivo studies face delays or show limited CAR T persistence, I will expand in vitro analyses of cytokine production, exhaustion, and memory markers to assess functional durability. These adjustments will keep the project feasible within the three-year timeline while ensuring that the hypothesis remains testable.

Long-Term Impact and Goals

This project will lay the groundwork for developing microenvironment-resilient CAR T therapies capable of overcoming the immunosuppressive barriers that limit efficacy in solid tumors. By demonstrating that TGF-β-resistant CAR T cells maintain cytotoxicity and persistence in TNBC, this work will provide preclinical evidence to advance armored CAR designs toward clinical translation. Beyond TNBC, the framework developed here, combining genetic engineering with 3D modeling of tumor-immune interactions, can be applied to other TGF-β-rich cancers, advancing the broader goal of making cellular immunotherapy effective across solid malignancies.

References

1. Bianchini, Giampaolo et al. “Treatment landscape of triple-negative breast cancer - expanded options, evolving needs.” *Nature reviews. Clinical oncology* vol. 19,2 (2022): 91-113. doi:10.1038/s41571-021-00565-2

2. Lehmann, Brian D et al. “Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies.” *The Journal of clinical investigation* vol. 121,7 (2011): 2750-67. doi:10.1172/JCI45014

3. Nasiri, Fatemeh et al. “CAR-T cell therapy in triple-negative breast cancer: Hunting the invisible devil.” *Frontiers in immunology* vol. 13 1018786. 22 Nov. 2022, doi:10.3389/fimmu.2022.1018786

4. Stüber, Tanja et al. “Inhibition of TGF-β-receptor signaling augments the antitumor function of ROR1-specific CAR T-cells against triple-negative breast cancer.” *Journal for immunotherapy of cancer* vol. 8,1 (2020): e000676. doi:10.1136/jitc-2020-000676

5. Zhou, Ru et al. “CAR T Cells Targeting the Tumor MUC1 Glycoprotein Reduce Triple-Negative Breast Cancer Growth.” *Frontiers in immunology* vol. 10 1149. 24 May. 2019, doi:10.3389/fimmu.2019.01149

6. Tang, Ning, et al. “TGF-β Signaling Blockade Augments the Efficacy of CAR T Cells against Solid Tumors.” *JCI Insight*, vol. 5, no. 4, 2020, article e133977, https://doi.org/10.1172/jci.insight.133977

7. Li, Nannan et al. “Armored bicistronic CAR T cells with dominant-negative TGF-β receptor II to overcome resistance in glioblastoma.” *Molecular therapy : the journal of the American Society of Gene Therapy* vol. 32,10 (2024): 3522-3538. doi:10.1016/j.ymthe.2024.07.020

8. Bittman-Soto, María, et al. “The Transformative Role of 3D Culture Models in Triple-Negative Breast Cancer Research.” *Cancers*, vol. 16, no. 10, 2024, article 1859. MDPI, https://doi.org/10.3390/cancers16101859.