MAYO CLINIC

Transforming Cancer Care and Research

Respectfully presented to Desert Mountain CARE | June 2025

MAYO CLINIC

Philanthropy accelerates hope and healing

Your remarkable partnership is helping drive Mayo Clinic in Arizona's bold vision to revolutionize cancer care. Together, with your support, we are pursuing innovative treatment options for some of the most difficult-to-treat and elusive cancers.

We are honored to share the enclosed updates on the projects that are advancing through your generous funding. In the following pages, Gloria Kim, Ph.D., and Mitesh Borad, M.D., outline their work in prostate and pancreatic cancer research.

RESEARCH UPDATES

Project 1: CAR-T Cell Therapy for Solid Tumors: A Potential Breakthrough for Prostate Cancer

Our research centers on developing a novel cancer immunotherapy using engineered immune cells known as chimeric antigen receptor (CAR) T cells. We've designed these CAR-T cells to target a protein called fibroblast growth factor-inducible 14 (Fn14), which appears at high levels in several aggressive cancers — most notably prostate cancer, as well as glioblastoma (a lethal brain tumor) and certain kidney cancers.

In our initial studies, we successfully created anti-Fn14 CAR-T cells and demonstrated their ability to recognize and eliminate Fn14-expressing cancer cells in laboratory models. This work lays the foundation for a targeted treatment strategy aimed at improving outcomes for patients with solid tumors, particularly those with advanced prostate cancer, where current therapies offer limited benefit.

To broaden the impact of our approach, we are now testing these CAR-T cells in patient-derived models of glioblastoma and Fn14-positive kidney cancer. At the same time, we are exploring combination therapies to enhance CAR-T cell performance. Specifically, we are evaluating whether pairing anti-Fn14 CAR-T cells with methylseleninic acid (MSA) — a selenium-based compound that blocks immune evasion pathways like TGF-β1 and PD-L1 — or with ascorbic acid (vitamin C), which boosts T-cell function and selectively targets cancer stem cells, can produce a more durable and comprehensive immune response.

By focusing on prostate cancer while exploring broader applications, this work has the potential to transform treatment for patients with some of the most challenging solid tumors.

Recent Accomplishments

Design anti-Fn14 CARS: We have successfully developed second-generation anti-Fn14 CAR-T cells to target Fn14-positive solid cancers. These CAR-T cells were designed using a humanized anti-Fn14 antibody. Because the antibody recognizes both human and mouse Fn14, it supports preclinical studies that can be translated to human applications.

The anti-Fn14 CAR constructs were engineered to include a single-chain variable fragment (scFv) that specifically recognizes Fn14. This fragment was linked to intracellular signaling domains that activate T-cell killing when the CAR-T cell encounters the Fn14 antigen. We generated three distinct CAR designs, bulleted below and illustrated in **Figure 1**.

- **CAR #1:** CD8S–Anti-Fn14 scFv–CD8H–CD8α transmembrane domain–4-1BB intracellular domain–CD3ζ signaling domain (P6)
- **CAR #2:** CD8S–Anti-Fn14 scFv–CD8H–CD28 transmembrane domain–CD28 intracellular domain–CD3ζ signaling domain (P7)
- **CAR #3:** CD8S–Anti-Fn14 scFv–CD8H–CD8α transmembrane domain–CD3ζ signaling domain (P9)

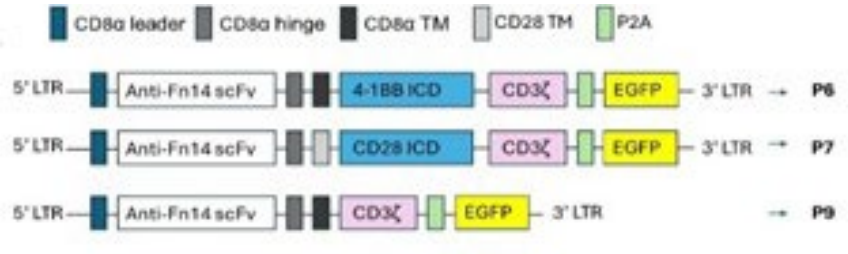
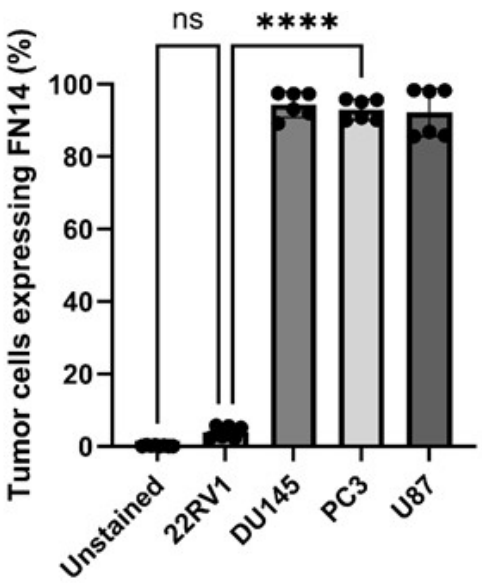


Figure 1: A schematic diagram of the anti-Fn14 CAR constructs.

Generation of human anti-Fn14 CAR-T cells: We isolated primary human CD4⁺ and CD8⁺ T cells from de-identified blood samples donated by healthy individuals through Mayo Clinic, following an Institutional Review Board–approved protocol. We cultured the T cells in CTS OpTmizer™ T Cell Expansion medium, supplemented with GlutaMAX™, penicillin/streptomycin, and HEPES to support optimal growth and function.

On Day 0, we combined CD4⁺ and CD8⁺ T cells in a 1:1 ratio and activated them using anti-CD3/CD28 Dynabeads, which mimic signals that naturally stimulate T cells in the body. The next day, we introduced a lentiviral vector carrying our gene of interest at a multiplicity of infection (MOI) of 5 to genetically modify the activated T cells. We removed the activation beads on Day 3, performed cell phenotyping on Day 4 to assess their characteristics, and began functional testing on Day 5.

Figure 2



To evaluate how well our engineered T cells could recognize their target, we conducted in vitro functional assays. We tested a panel of tumor cell lines and used flow cytometry — a technique that measures protein levels on the surface of cells — to quantify their expression of fibroblast growth factor-inducible 14 (Fn14), the protein our CAR-T cells are designed to target. A graphical summary of Fn14 surface expression across tumor cell lines is shown in **Figure 2.**

After characterization, we evaluated the ability of our transduced anti-Fn14 CAR-T cells to selectively kill Fn14-expressing prostate cancer and glioblastoma cells. A lactate dehydrogenase (LDH) release assay demonstrated that the CAR-T cells effectively induced cytotoxicity against these tumor cells in vitro, confirming the successful generation of functional, antigen-specific CAR-T cells suitable for preclinical testing.

Figure 3, below, shows the antitumor activity of our Fn14-targeting CAR-T cells against prostate carcinoma (DU145, RRID:CVCL_0105; and PC3, RRID:CVCL_0035) and glioblastoma (U87, RRID:CVCL_GP63) cell lines, all of which express Fn14 on their surface.

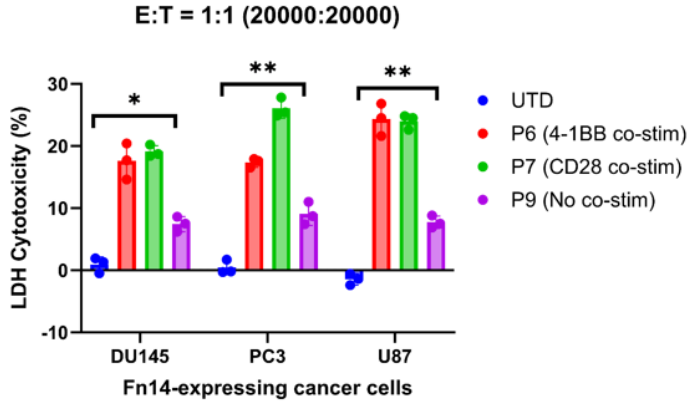


Figure 3: Bar graphs showing cytotoxicity assays (e.g., % tumor cell killing) measured by LDH assay. P6, P7, P9 are different anti-Fn14 CAR constructs.

Enhancing Precision and Power in CAR-T Cell Therapy

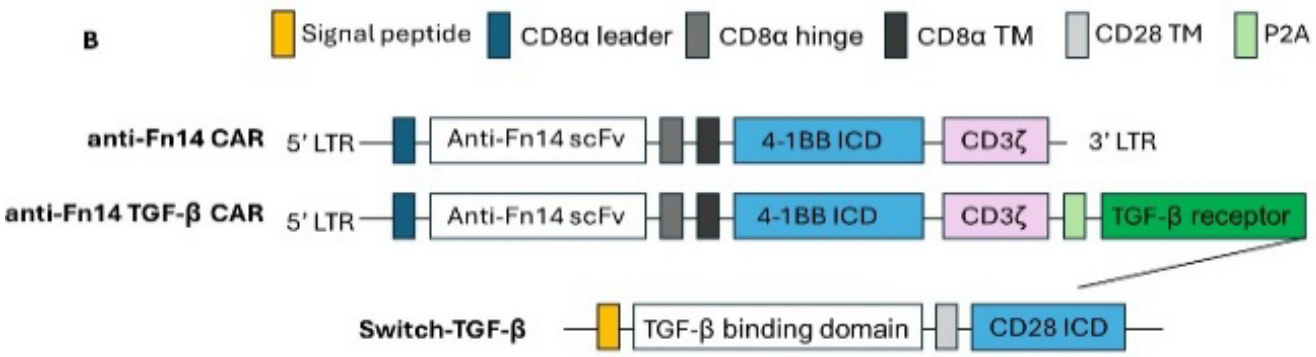
To advance our anti-Fn14 CAR-T cell platform, we are expanding efficacy testing using additional patient-derived xenograft (PDX) glioblastoma models. These models include both high-Fn14-expressing lines (GBM8, GBM39, GBM44) and low-Fn14-expressing lines (GBM6, GBM12, GBM5), allowing us to rigorously evaluate the specificity and potency of our CAR-T cells across a clinically relevant spectrum of tumors.

We are complementing standard LDH cytotoxicity assays with more detailed in vitro analyses. These include intracellular cytokine staining to profile T-cell activation and xCELLigence real-time killing assays to dynamically track tumor cell destruction. Together, these methods will provide a deeper understanding of how our CAR-T cells function and how quickly they eliminate cancer cells.

One of the major hurdles in treating solid tumors is the immunosuppressive tumor microenvironment (TME), which is heavily shaped by transforming growth factor-β (TGF-β). TGF-β suppresses effector T-cell activity, promotes regulatory T cell (Treg) development, drives T-cell exhaustion, and accelerates tumor progression, especially in advanced cancers.

To overcome this, we engineered TGF-β chimeric switch receptors (CSRs) (**Figure 4**) that convert TGF-β's suppressive signals into activating ones. When integrated into CAR-T cells, these CSRs enhance T-cell persistence, activation and infiltration.

Figure 4:



This strategy offers three key advantages: resistance to TGF-β–mediated suppression, improved co-stimulation for stronger antitumor responses, and better trafficking to TGF-β–rich tumor sites. This approach is particularly promising for clear cell renal cell carcinoma (ccRCC), where TGF-β levels are especially high.

We are also exploring combination strategies to further boost CAR-T cell performance. Two promising small molecules include:

1. **Methylseleninic acid (MSA):** A selenium-based compound that directly inhibits TGF-β1, PD-L1 and VEGF expression, sensitizes tumors to immune attack, and shows no toxicity to natural killer (NK) or T cells.
2. **Ascorbic acid (AA):** A well-tolerated antioxidant that selectively kills cancer stem cells, enhances T-cell cytokine production and infiltration, and disrupts TGF-β signaling by remodeling the tumor's extracellular matrix.

By integrating these combination therapies, we aim to mount a more comprehensive and durable immune response — one that could significantly improve tumor control and long-term outcomes for patients with Fn14-positive cancers.

Expanding the Horizon and Impact of Prostate Cancer Immunotherapy

This work opens the door to a new generation of precision immunotherapies for patients facing some of the most aggressive solid tumors. By targeting Fn14, we aim to deliver a treatment that not only improves survival but also reduces the burden of side effects, offering patients a better quality of life.

Looking ahead, this platform holds promise beyond a single cancer type. With further development, it could serve as a foundation for multitargeted CAR-T therapies that adapt to the complexity of solid tumors. This approach has the potential to reshape how we treat prostate cancer and extend hope to patients with other Fn14-positive cancers, including glioblastoma and kidney cancer.

A Note of Gratitude

“We are profoundly grateful to Desert Mountain CARE whose generosity fuels this work. Your support empowers us to push the boundaries of cancer immunotherapy and pursue bold ideas that may one day save lives. Because of you, we are not only advancing science — we are offering hope. Thank you for standing with us in this mission.”

— Gloria B. Kim, Ph.D.
Assistant Professor of Biomedical Engineering

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Project 2: Targeting GATA6 in Pancreatic Cancer: Enhancing Treatment Efficacy

Pancreatic cancer remains one of the deadliest malignancies in the United States, with limited treatment options and a high mortality rate. Over 70% of patients are diagnosed at an advanced stage, when surgery — the only potential cure — is no longer viable. Standard chemotherapy offers only modest survival benefits, underscoring the urgent need for more effective, less toxic therapies. Advancing our mechanistic understanding of this disease is essential to drive the development of transformative treatments.

GATA6, a regulatory protein in pancreatic cancer, plays a critical role in controlling the expression of other proteins. In patients with pancreatic cancer, GATA6 can be either missing or not working correctly. When GATA6 isn't doing its job, it disrupts normal cell growth and contributes to the development and progression of cancer.

Patients with high GATA6 expression experience significantly longer survival, prompting us to explore the biological and clinical differences between high and low GATA6 tumors. We aim to identify drugs that replicate the favorable characteristics of high GATA6 expression in patients with low levels. To achieve this, we are analyzing patient samples and tumor models to uncover actionable insights that could guide future therapies.

We launched an international, multicenter collaboration with Mayo Clinic, Princess Margaret Cancer Center in Toronto, and CNIO in Madrid to access clinically annotated cohorts of pancreatic cancer samples. Our goal was to compare patients who survived more than five years with those who did not and determine whether high GATA6 expression correlated with long-term survival. To investigate this, we conducted gene copy analysis using fluorescent in situ hybridization (FISH) and assessed protein expression through immunohistochemistry, as demonstrated in **Figure 1** below.

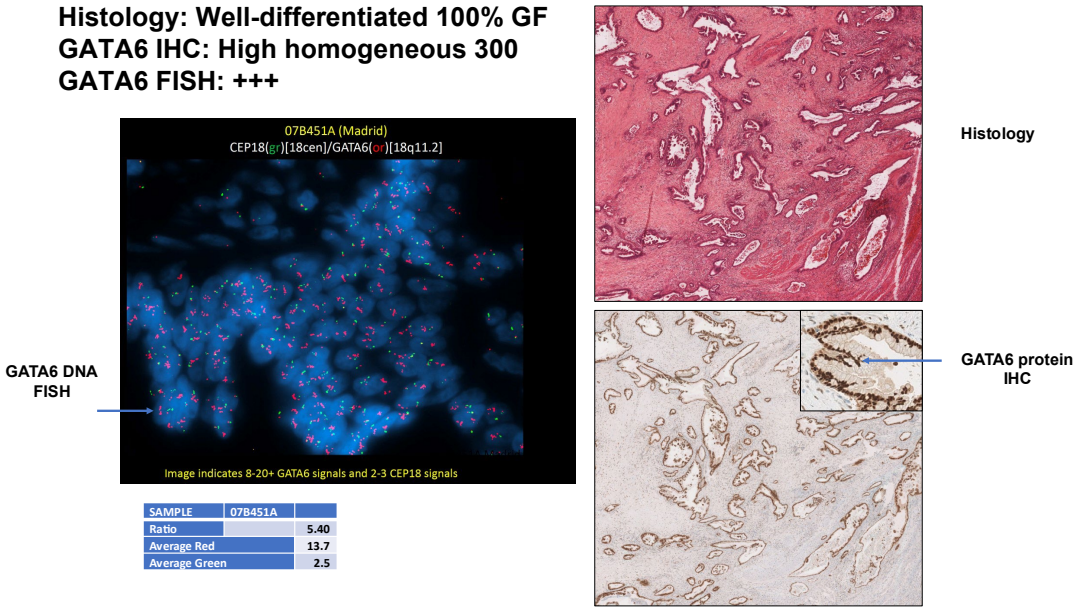

Figure 1. GATA6 expression in pancreatic cancer. *Left*: GATA6 gene copy number assessed by FISH. *Upper right*: Representative pancreatic tumor histology. *Lower right*: GATA6 protein expression detected by immunohistochemistry.

This study included 229 patients, with 169 long-term survivors and 60 short-term survivors. Immunohistochemistry revealed a strong correlation between GATA6 protein expression and long-term survival, while FISH testing showed no such correlation. We are also using exome sequencing, RNA-Seq and ATAC-Seq to characterize organoids with high and low GATA6 expression derived from patients with pancreatic cancer. These ongoing studies, conducted in partnership with Martin E. Fernandez-Zapico, M.D., aim to elucidate the biological basis for these GATA6-related differences.

If our hypotheses are accurate, this work will reveal why patients with high GATA6 expression live longer and develop less aggressive pancreatic cancer than those with low expression. These insights will lay the groundwork for therapies that shift GATA6-low tumors toward a GATA6-high profile, potentially improving outcomes for a broader group of patients. Building on these findings, we will identify a panel of drugs that act differently on GATA6-high and GATA6-low tumors and test whether any can induce a GATA6-high-like effect in low-expressing tumors.

“We are deeply grateful for Desert Mountain CARE’s extraordinary generosity in advancing our research. This support not only fuels our current efforts but also strengthens our ability to secure additional funding from peer-reviewed organizations and biopharmaceutical partners — accelerating the translation of these discoveries into meaningful clinical applications.”

— Mitesh Borad, M.D.
Getz Family Research Professor of Mayo Clinic Arizona
Professor of Medicine