



TNX-1700

Gastric and Colorectal Cancers

NASDAQ: TNXP

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¹human trefoil family factor 2 – human serum albumin fusion protein

²myeloid-derived suppressor cells

³azoxymethane/dextran sodium sulfate

⁴murine TFF-2 – murine serum albumin fusion protein

TNX-1700 (hTFF2-HSA) fusion protein)

Tumor Microenvironment, MDSCs



TNX-1700*: Gastric and Colorectal Cancers

Recombinant Trefoil Factor 2 (hTFF2) Fusion Protein

Potential New Cancer Treatment

- mTNX-1700 (mTFF2) has effects on cancer by altering the tumor micro-environment
- Mechanism of action: suppresses myeloid-derived suppressor cells and activates anti-cancer CD8+ T cells
- Potential synergy with anti-PD-1 or anti-PD-L1 monoclonal antibodies (mAbs)

Preclinical Evidence for Inhibiting Growth of Cancer Cells

- Data showed that mTFF2-CTP augmented the efficacy of mAb anti-PD-1 therapy. Anti-PD-1 in combination with mTFF2-CTP showed greater anti-tumor activity in PD-L1-overexpressing mice

Licensed from Columbia University

- Developing in partnership under sponsored research agreement

Market Entry: Immuno-oncology, combination therapy with PD1 blockers for gastric and colorectal cancer

Status: Preclinical

Next Steps: Animal studies ongoing

Differentiator: No product yet identified consistently augments PD1 effects on cold tumors

Patents Filed

*TNX-1700 is in the pre-IND stage of development and has not been approved for any indication.



Colorectal Cancer (CRC) is Common and Lethal

- ~150,000 new cases each year in the US
- ~53,000 expected to die
- 3rd most leading cause of cancer death in women
- 2nd most in men
- Steady increase in incidence in men/women under age 50 at a rate of 2.1%/yr since 1992
- 86% symptomatic at diagnosis, associated with more advanced disease and poorer outcomes
- Financial burden ~ \$17B (2018)

TNX-1700 (hTFF2-HSA): A Potential Treatment for Gastric and Colorectal Cancers



**Pre-IND
Candidate**

Targeted as a treatment for cancer

- Particularly for gastric and colorectal cancer
- Mechanism of Action (MOA) is different from checkpoint inhibitors
- Potential synergy with anti-PD-1 or anti-PD-L1 monoclonal antibodies

Patents and patent applications directed to recombinant TFF2 (rTFF2)

- Issued patent licensed from Columbia University

Inventor: Dr. Timothy Wang, MD

- Chief, Division of Digestive and Liver Diseases at Columbia University and Cancer Research Center and Silberberg Professor of Medicine
- Investigated the molecular mechanisms of gastrointestinal carcinogenesis for decades
- Leadership roles in gastroenterology and cancer biology fields

Pre-clinical evidence for inhibiting growth of cancer cells

- Several studies have shown rTFF2 to be active in the treatment of cancer¹⁻²

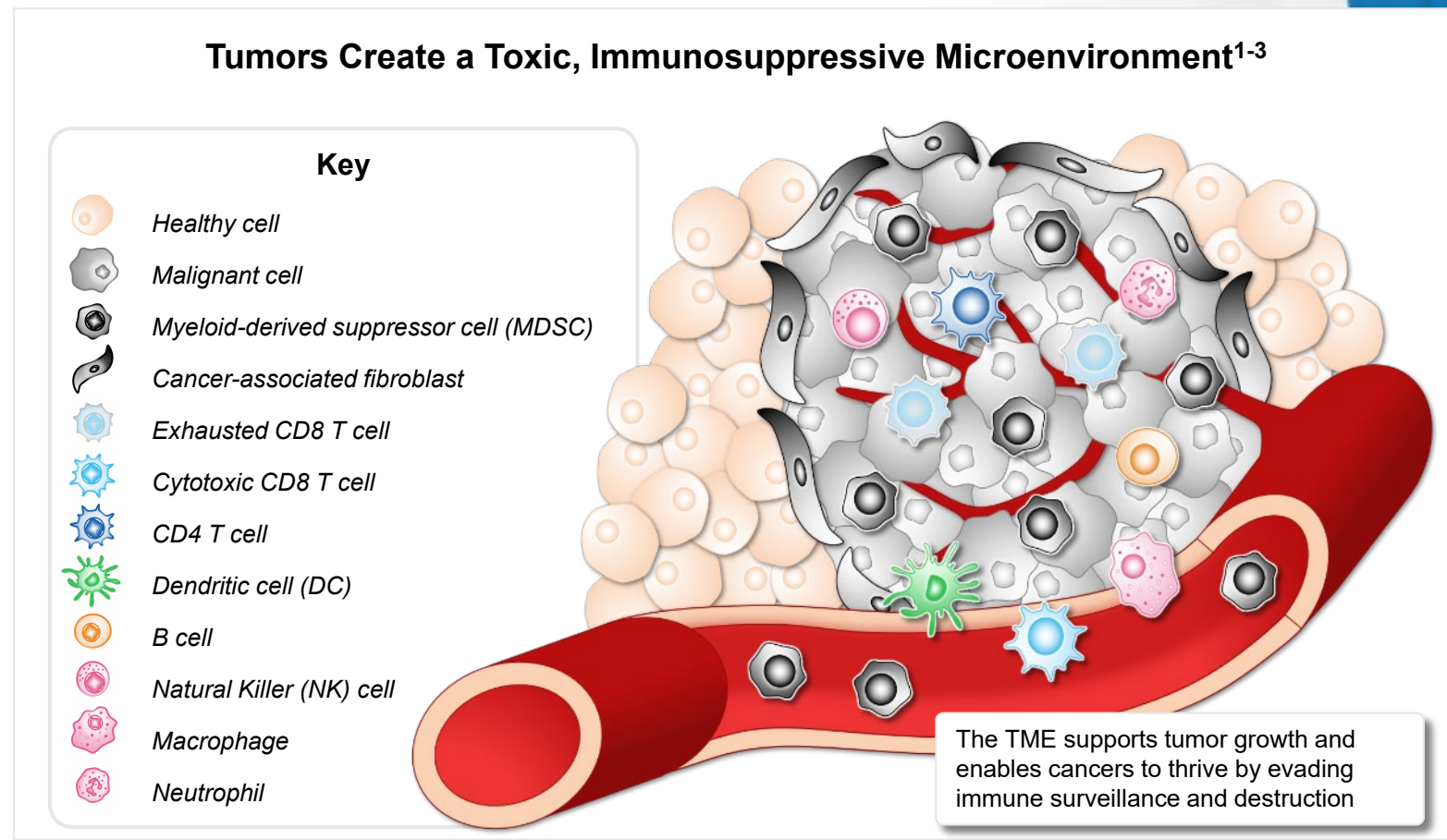
**Targeting a
Condition with
Significant Unmet
Need**

¹Dubeykovskaya Z, et al. Nat Commun. 2016 7:1-11

²Dubeykovskaya ZA, et al, Cancer Gene Ther. 2019 26(1-2):48-57

Cancers Create Toxic, Immunosuppressive Tumor Microenvironments (TME)

- Tumors are surrounded by endothelial and stroma cells, and invading immune cells, both innate and adaptive^{1,2}
- Complex regulatory network supports tumor growth, enabling cancers to thrive by evading immune surveillance and destruction²⁻³
- The TME sabotages tumor-killing cytotoxic CD8 T cells¹
- Myeloid-derived suppressor cells (MDSCs) interfere with anticancer immunity^{2,3}



¹Belli C, et al. *Cancer Treat Rev.* 2018;65:22-32.

²Roma-Rodriguez C, et al. *Int J Mol Sci.* 2019;20(4):840.

³Tsai M, et al. *ISRN Biochem.* 2014:351959.





MDSCs Are a Major Treatment Target

- Levels of MDSCs tend to correlate with tumor stage, patient survival, and metastatic burden and may predict poor response to certain cancer treatments¹
- MDSCs represent a central mechanism of immunosuppression in cancer; targeting these cells could significantly improve our ability to fight cancer^{2,3}
- Therapeutic strategies include³:
 - ▶ Promoting the differentiation of MDSCs to a non-immunosuppressive cell type
 - ▶ Blocking MDSC immunosuppressive functions
 - ▶ Inhibiting MDSC expansion
 - ▶ Eliminating MDSCs

¹Condamine T, et al. *Annu Rev Med*. 2015;66:97-110.

²Tuccito A, et al. *Virchows Arch*. 2019;474(4):407-420.

³Gabrilovitch DI, et al. *Nat Rev Immunol*. 2009;9(3):162-174.



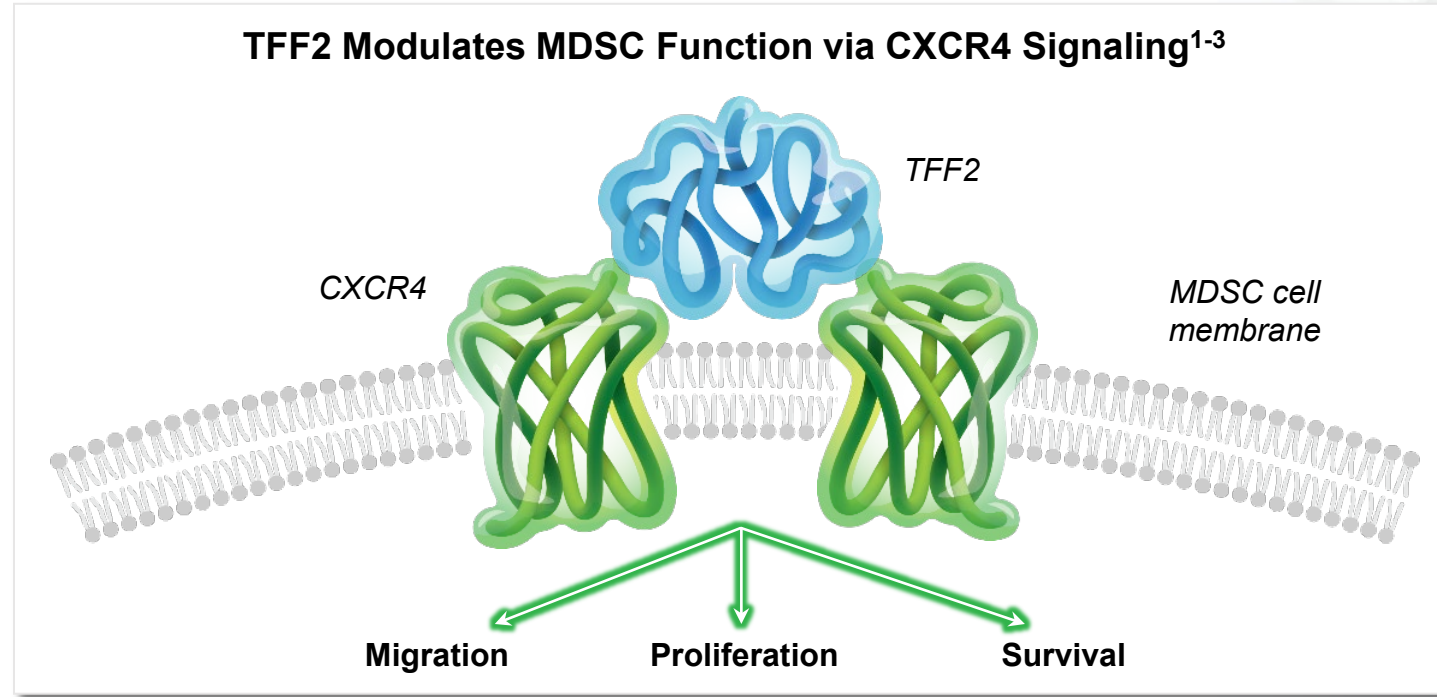
Trefoil Family Factor 2 (rTFF2) and Cancer Biology

- **TFF2 is a small secreted protein**
 - Encoded by the TFF2 gene in humans
 - Expressed in gastrointestinal mucosa where it functions to protect and repair mucosa
 - TFF2 is also expressed at low levels in splenic memory T cells
 - Upregulated in chronic inflammation
 - Activates the chemokine receptor CXCR4 in cancer cells
 - Blocked by AMD3100 (CXCR4 antagonist) or anti-CXCR4 mAb
- **TFF2 is epigenetically silenced in gastric cancer**
 - Postulated to protect against cancer development through multiple mechanisms
 - Has effects on cancer cells and tumor microenvironment, including marked suppression of MDSCs
 - Knockout of the TFF2 gene leads to faster tumor growth, while overexpression of TFF2 in T cells suppresses tumor growth in a manner dependent on CD8+ T cells.



TFF2 Signals Through CXCR4

- Importantly, TFF2 activates CXCR4 and may therefore modulate immune and tumorigenic responses, specifically by reducing the expansion or migration of immunosuppressive MDSCs¹⁻³
- TFF2 upregulates ApoE fifty-fold in myeloid progenitor cells; ApoE has been shown to suppress MDSCs⁴



¹Dubeykovskaya Z, et al. *J Biol Chem*. 2009;284(6):3650-3662.

²Balkwill F. *Semin Cancer Biol*. 2004;14(3):171-179.

³Teixidó J, et al. *Int J Biochem Cell Biol*. 2018;95:121-131.

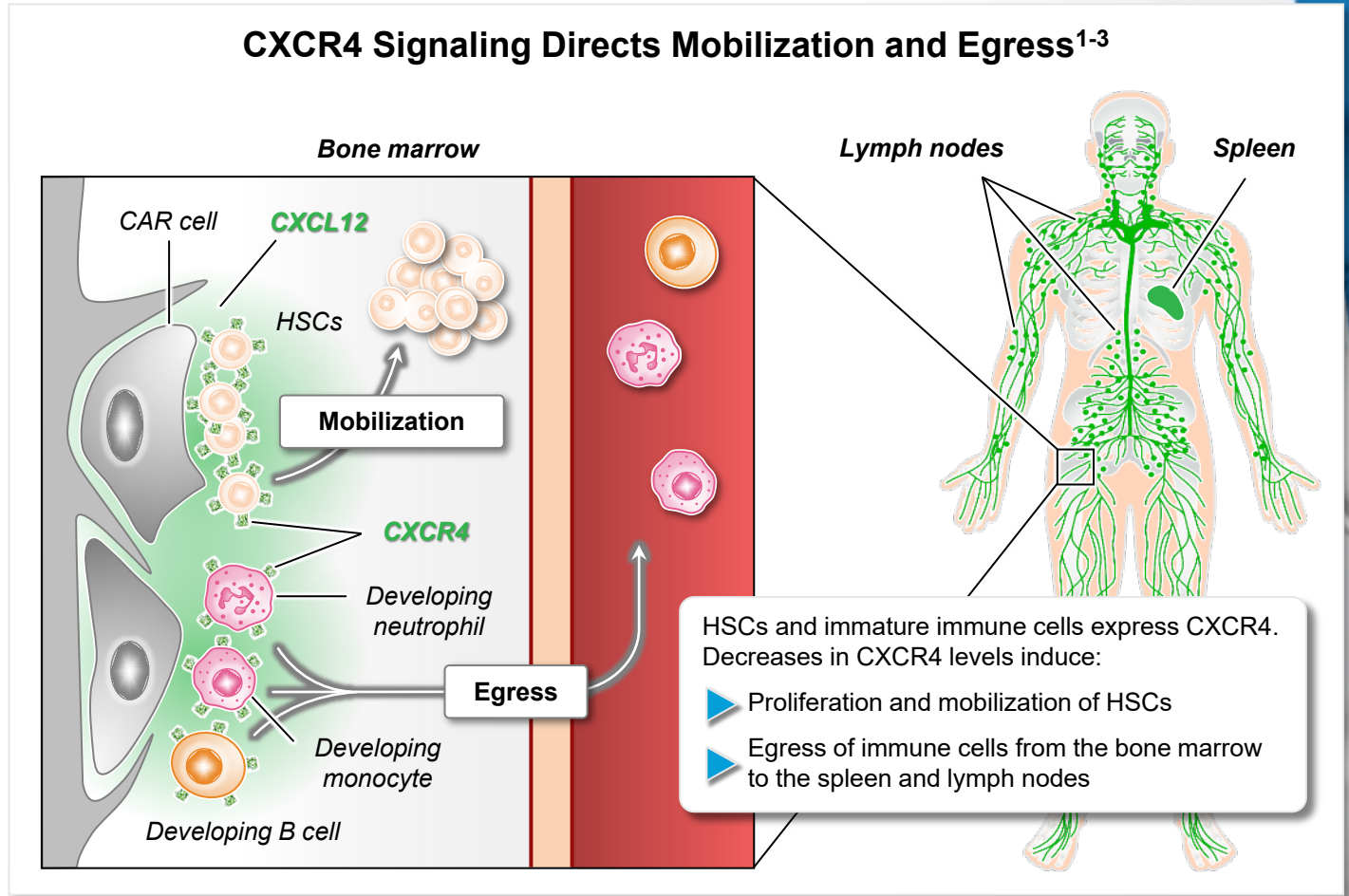
⁴Tavazoie MF et al, *Cell* 2018; 172:825-840.



Chemokines Direct Immune Cell Production and Migration

- Immune cells constantly migrate from the blood into and out of lymphoid organs, processes known as homing and egress^{1,2}
- Homing and egress are regulated by chemokines^{1,2}
- CXCL12-CXCR4 is a crucial chemokine signaling axis that regulates¹⁻³:

- ▶ Proliferation and mobilization of hemopoietic stem cells (HSCs)
- ▶ Retention of developing immune cells within the bone marrow



¹Griffith JW, et al. Annu Rev Immunol. 2014;32:659-702.
²Schultz O, et al. Annu Rev Immunol. 2014;34:203-242.
³Balkwill F. Semin Cancer Biol. 2004;14(3):171-179.

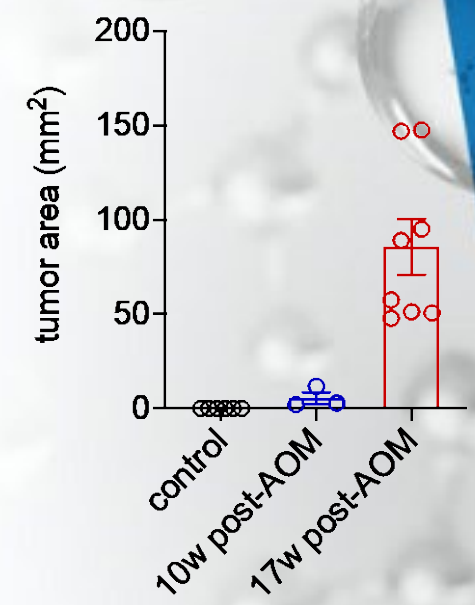
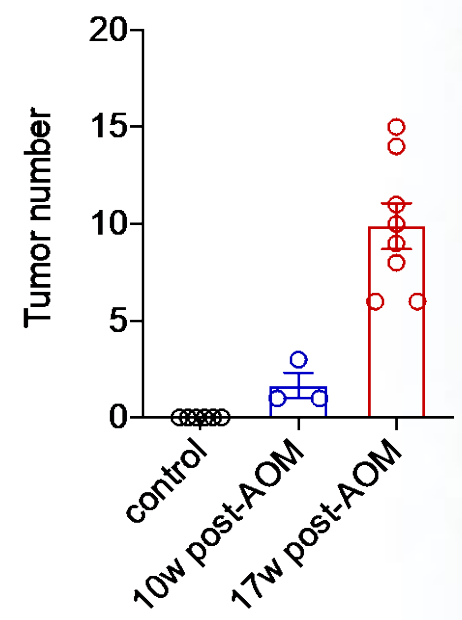
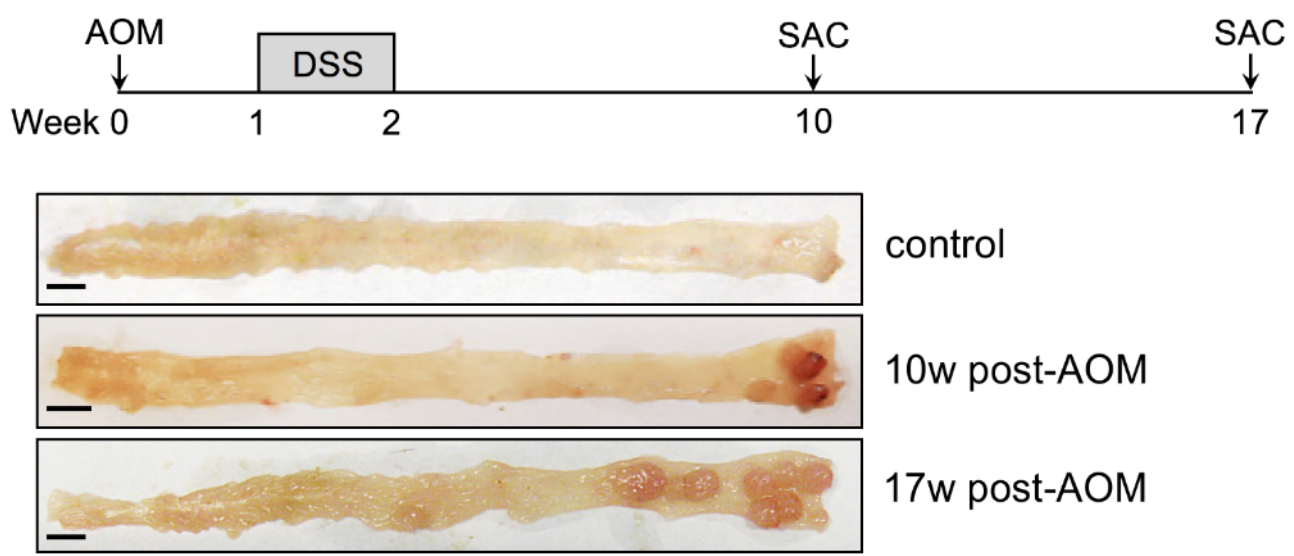
Chemoprevention Studies

Murine AOM/DSS Model, mTFF2-MSA

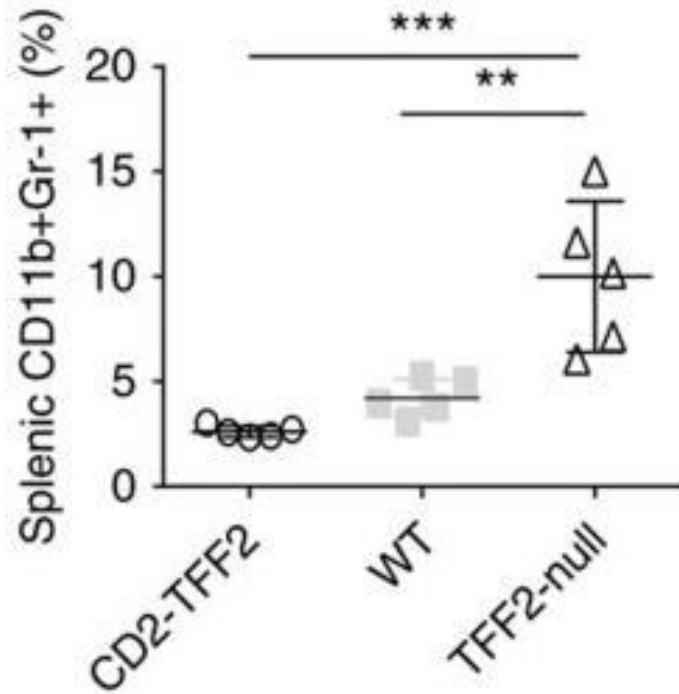


AOM/DSS induces Colorectal Cancer in a Mouse Model

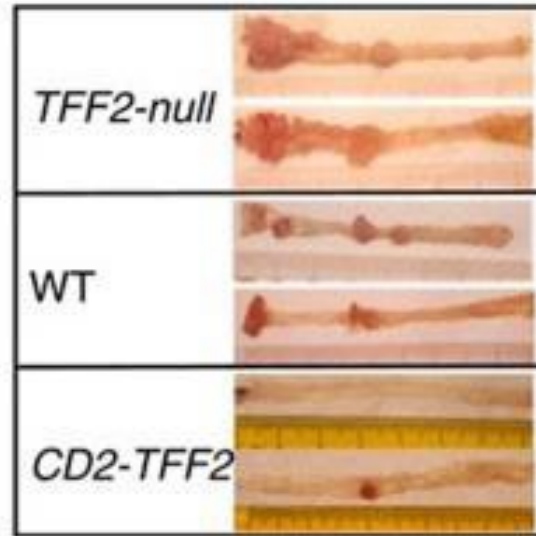
- The azoxymethane/dextran sodium sulfate (AOM/DSS) model is the most commonly used model of chemically-induced colon carcinogenesis
- Tumors display similar pathological and genetic features as human CRC
- AOM (carcinogen), DSS (inflammatory agent)



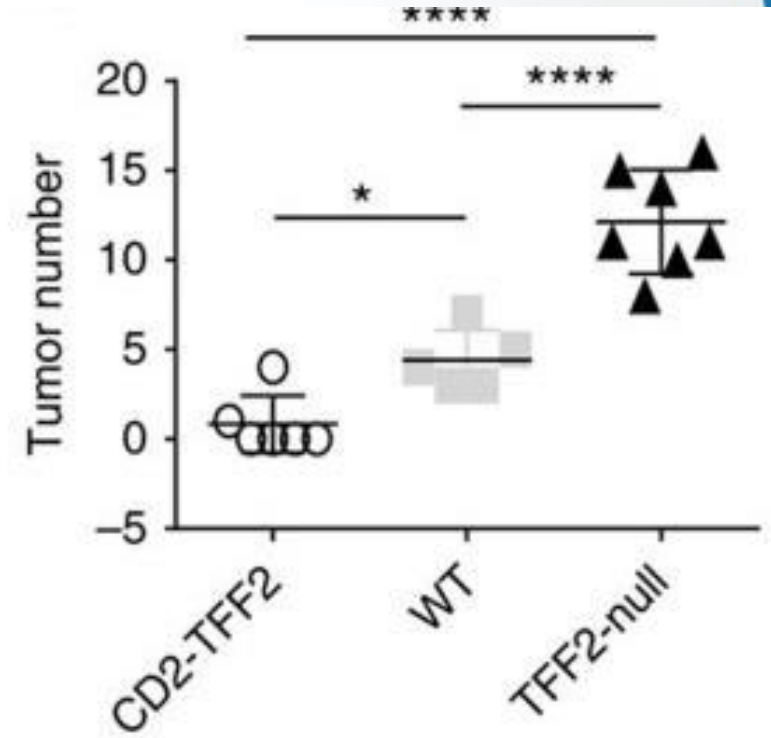
Transgenic Overexpression of mTFF2 Reduces Tumorigenesis *via* Suppression of MDSCs



MDSC number



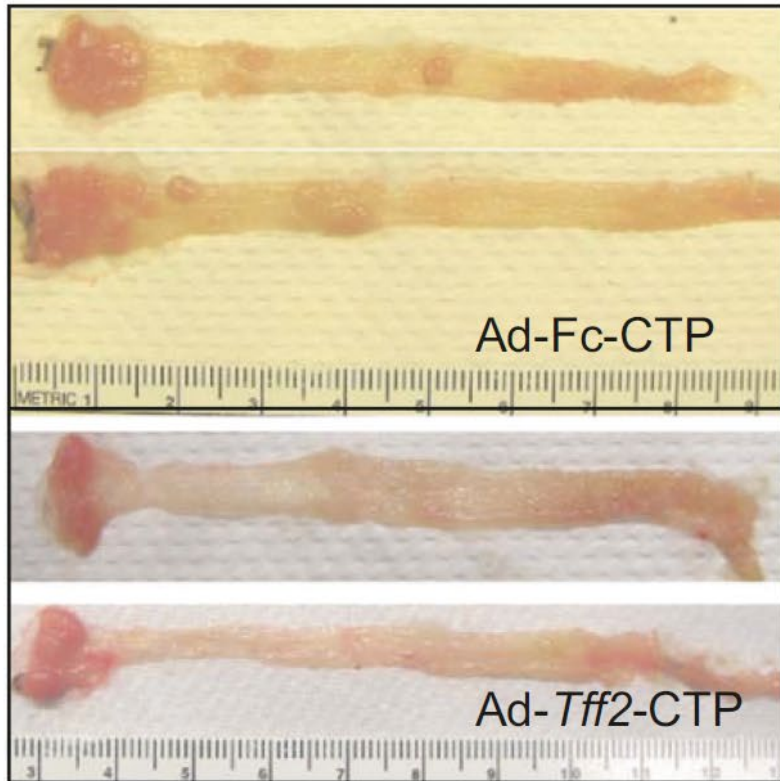
Colon appearance



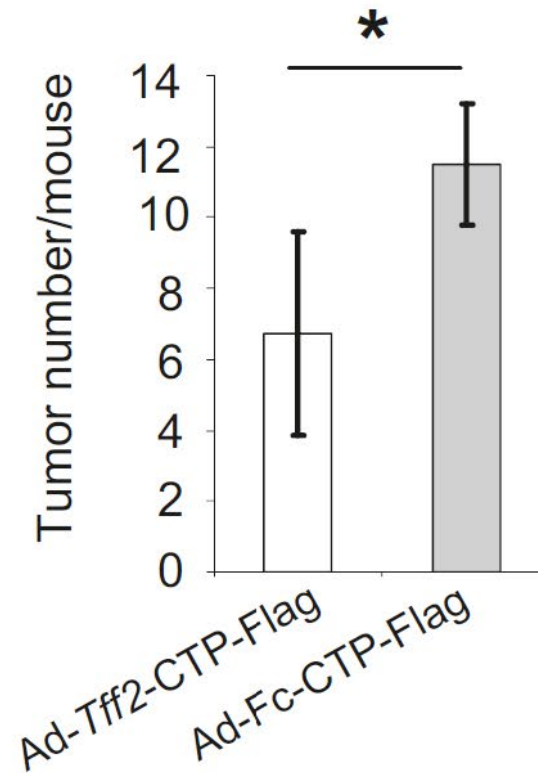
Tumor burden

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

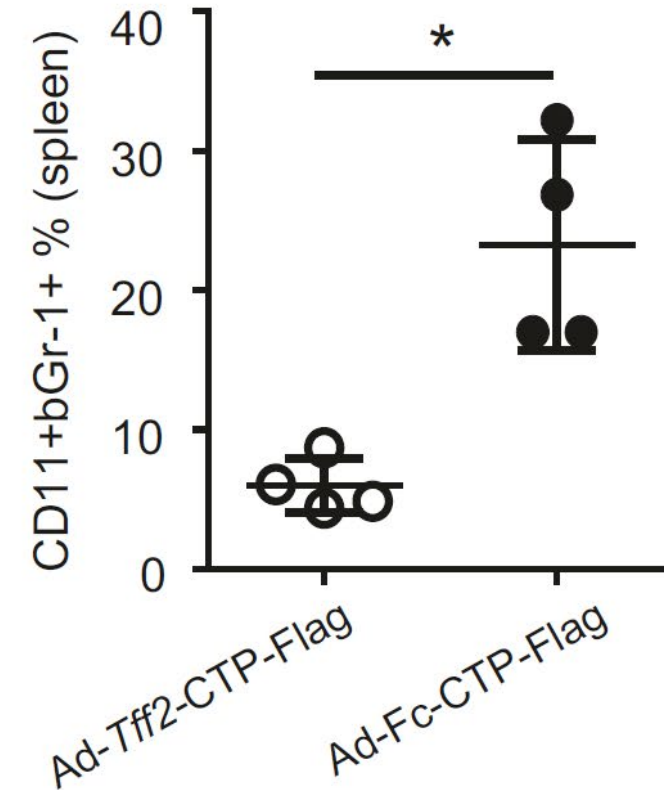
Adenoviral Delivery of mTFF2-CTP-Flag Reduces Tumorigenesis via Suppression of MDSCs



Colon appearance



Tumor burden

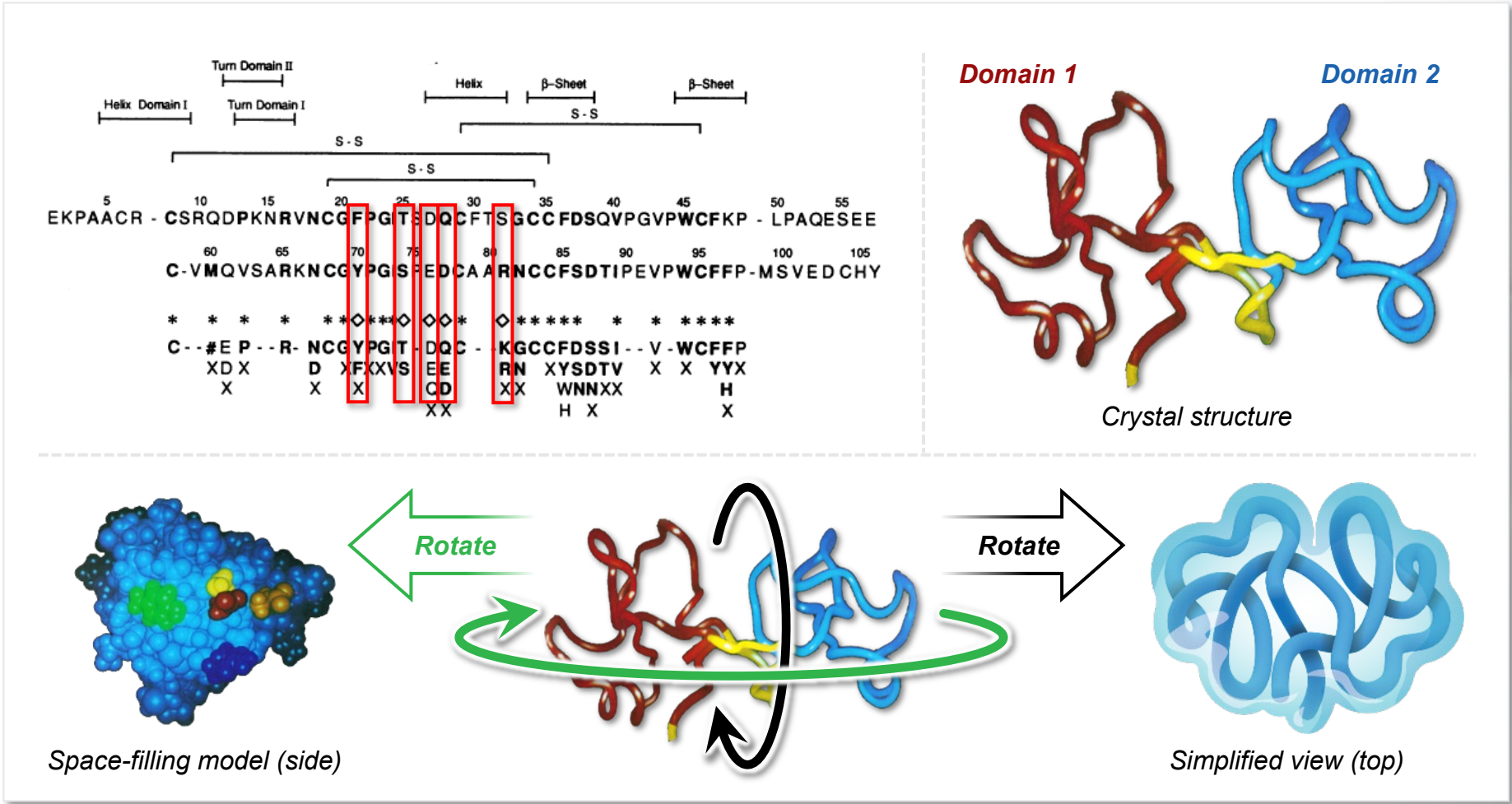


MDSC number

TNX-1700 Structure

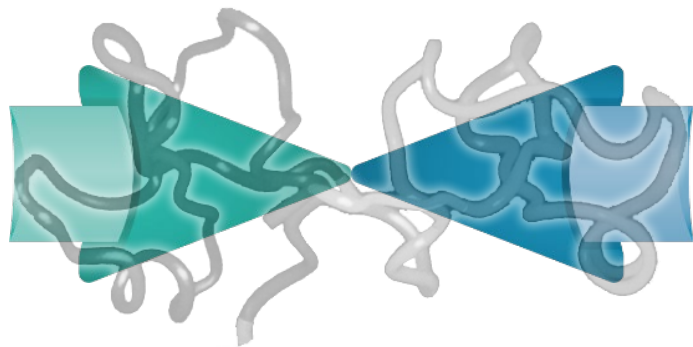
Domains, CXCR4 Interaction

TFF2 Contains 2 Trefoil Domains, Each Containing 5 Conserved Residues





Chimeric rTFF2 Domain (D) Swaps



Wild type = **DI/II**

Key

TFF2 Domains (D)

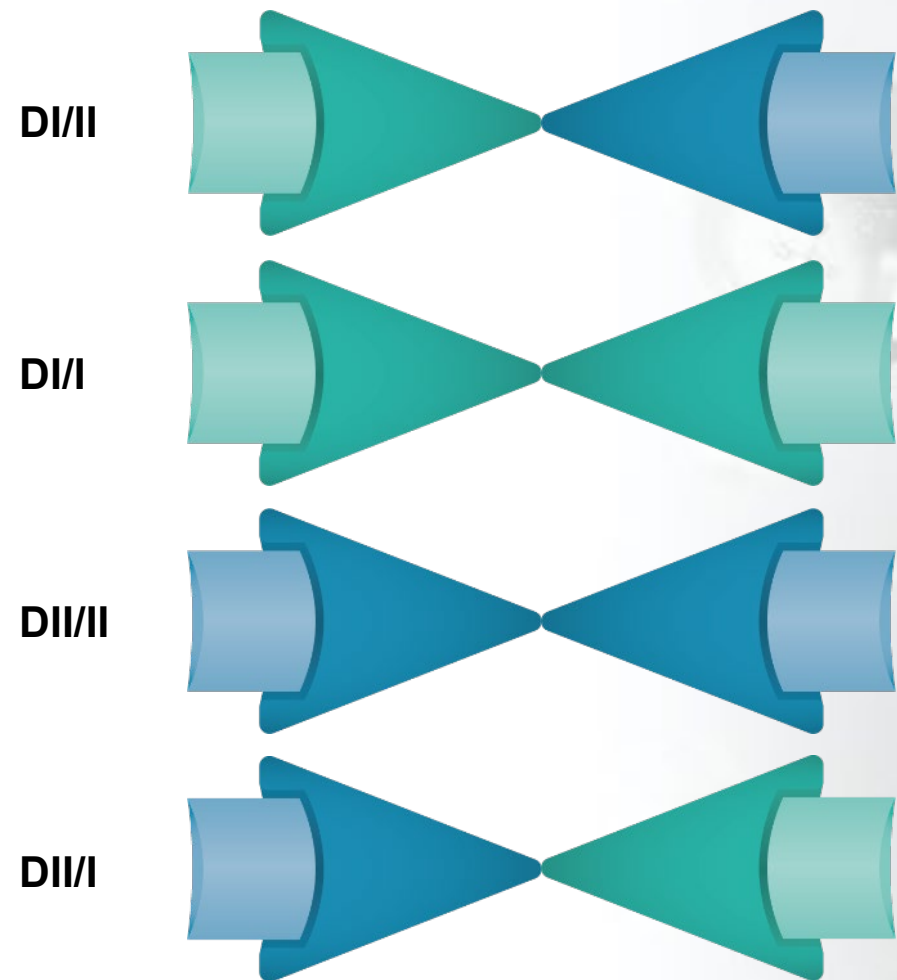
DI

DII

TFF2 Ligand Binding Domains (LBD)

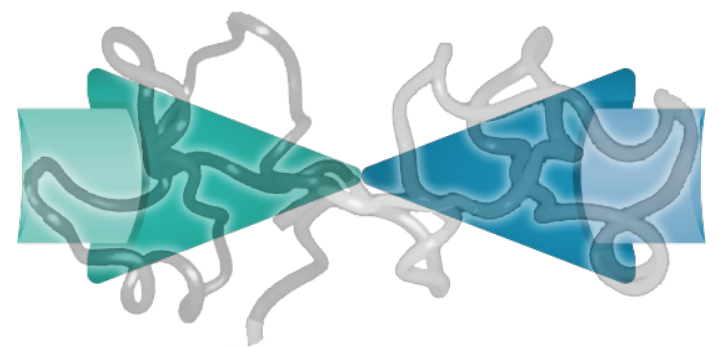
LBDI

LBDII





Chimeric rTFF2 Ligand Binding Domain (LBD) Swaps



Wild type = **LBDI/II**

Key

TFF2 Domains (D)

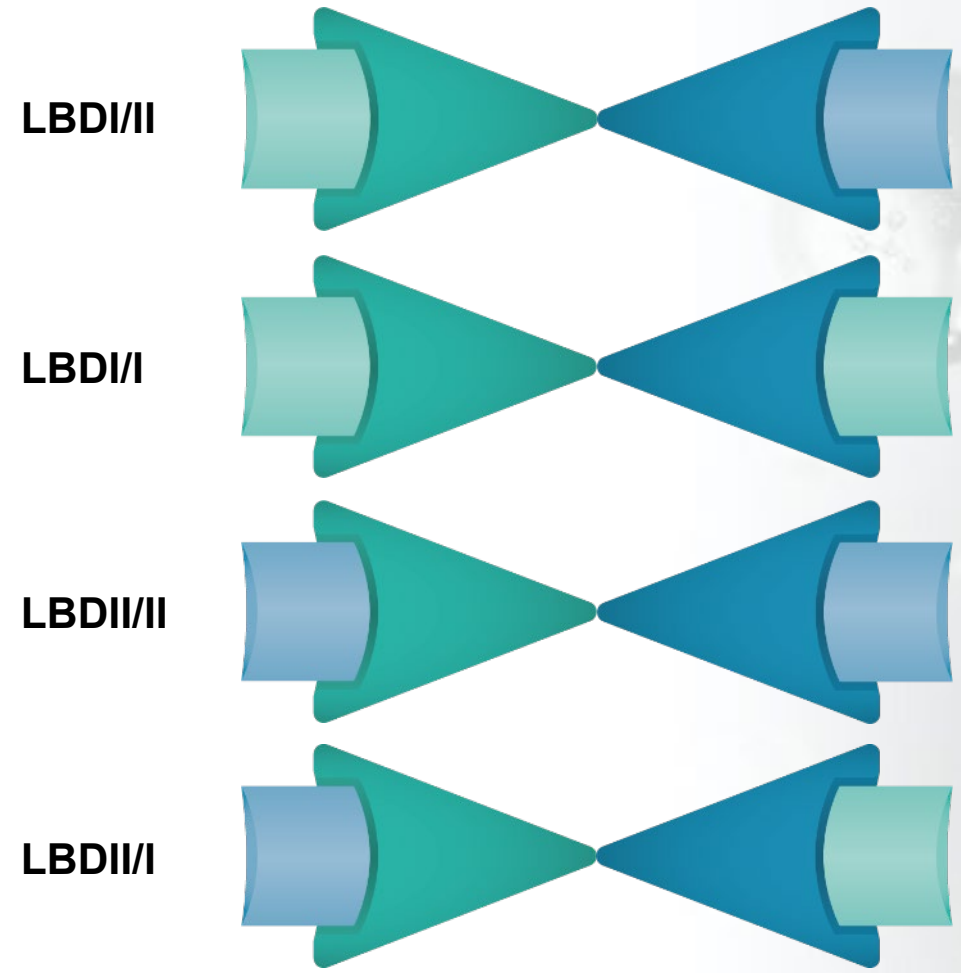
DI

DII

TFF2 Ligand Binding Domains (LBD)

LBDI

LBDII



TNX-1700 Protein Design

Albumin Fusion Proteins, Pharmacokinetics



Strategies for Half-Life Extension: Albumin Fusion

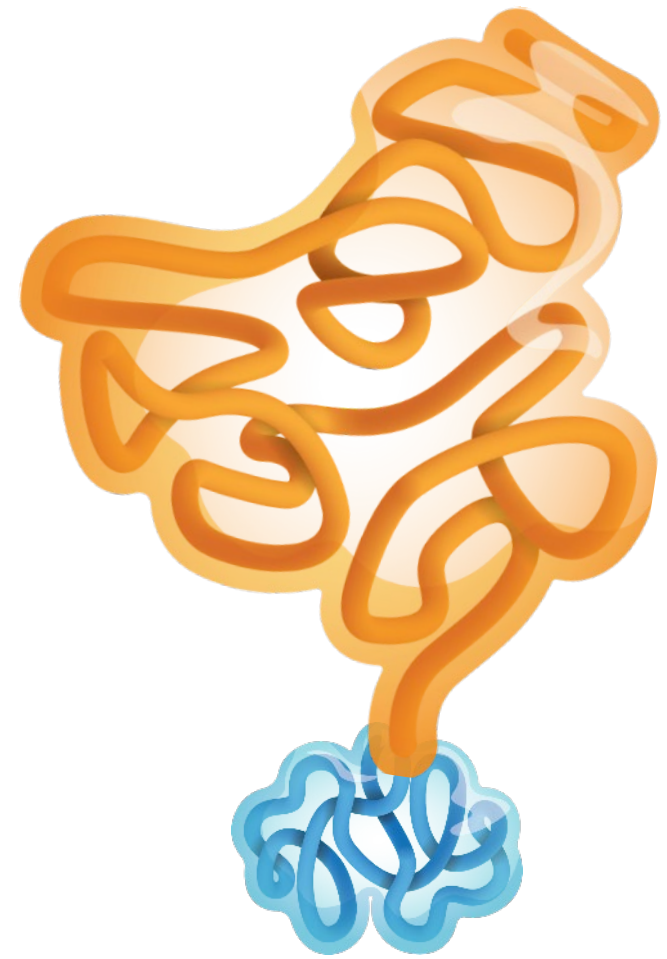
- **Albumin**
 - Most abundant plasma protein
 - Involved in transport of nutrients in the body
 - Interaction with cellular receptors Gp18, Gp30, and Gp60, which regulate transcytosis/endocytosis of albumin across the endothelial cell surface
 - High circulatory half-life of ~ 19 days mediated mainly due to neonatal Fc (FcRn)-mediated recycling
- **Marketed albumin fusions and conjugates**
 - Levemir
 - Eperzan/Tanzeum
 - Victoza
 - Abraxane



hTFF2-HSA Fusion Protein

**Human
Serum
Albumin**

hTFF2

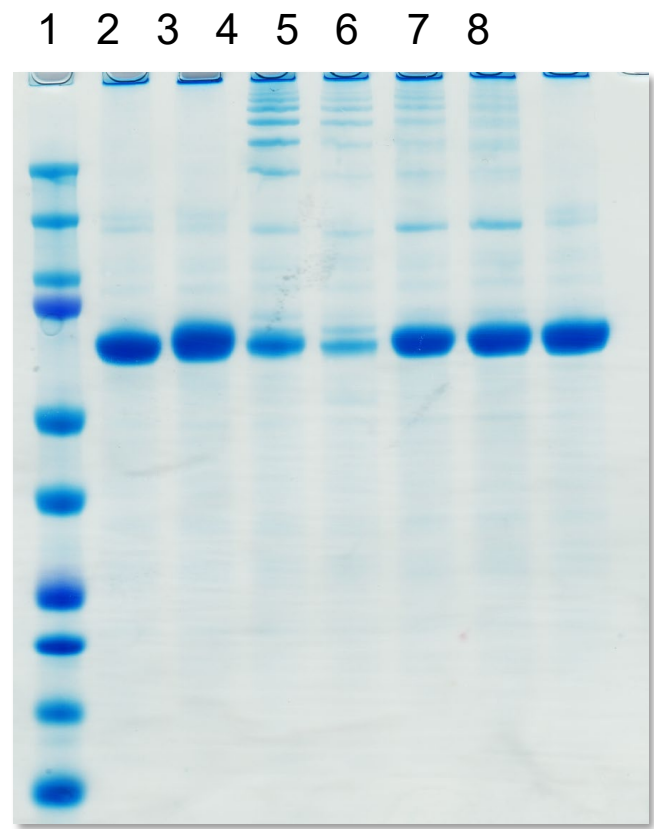


Predicted Mw ~ 78,000 daltons

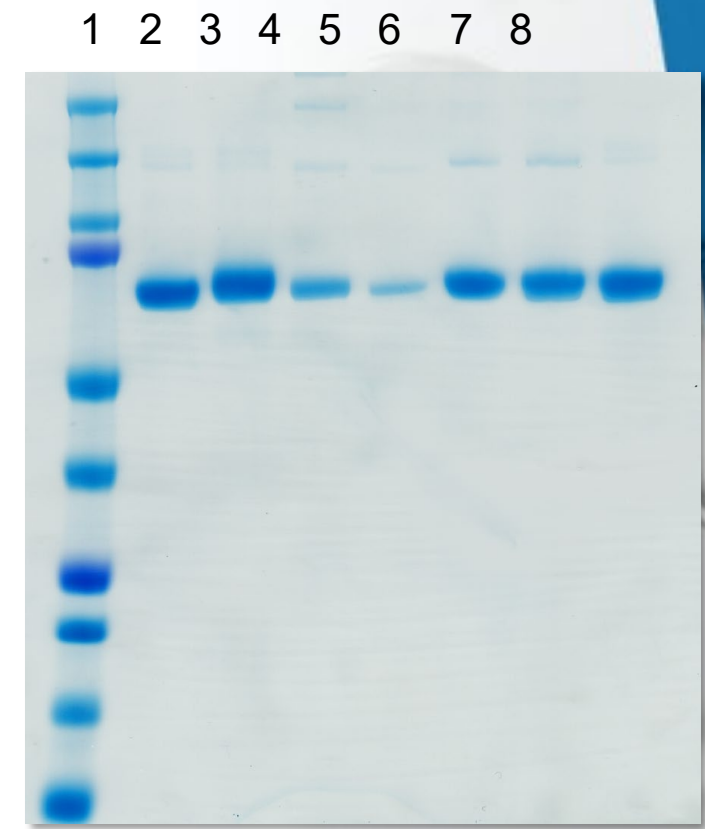


SDS-PAGE of hTF2-HSA Fusion Proteins

- Lane 1: Marker
- Lane 2: TFF2-HSA [WT]
- Lane 3: TFF2-HSA [DI/I]
- Lane 4: TFF2-HSA [DII/I]
- Lane 5: TFF2-HSA [DII/II]
- Lane 6: TFF2-HSA [LBDI/I]
- Lane 7: TFF2-HSA [LBDII/I]
- Lane 8: TFF2-HSA [LBDII/II]



Clarified Harvest

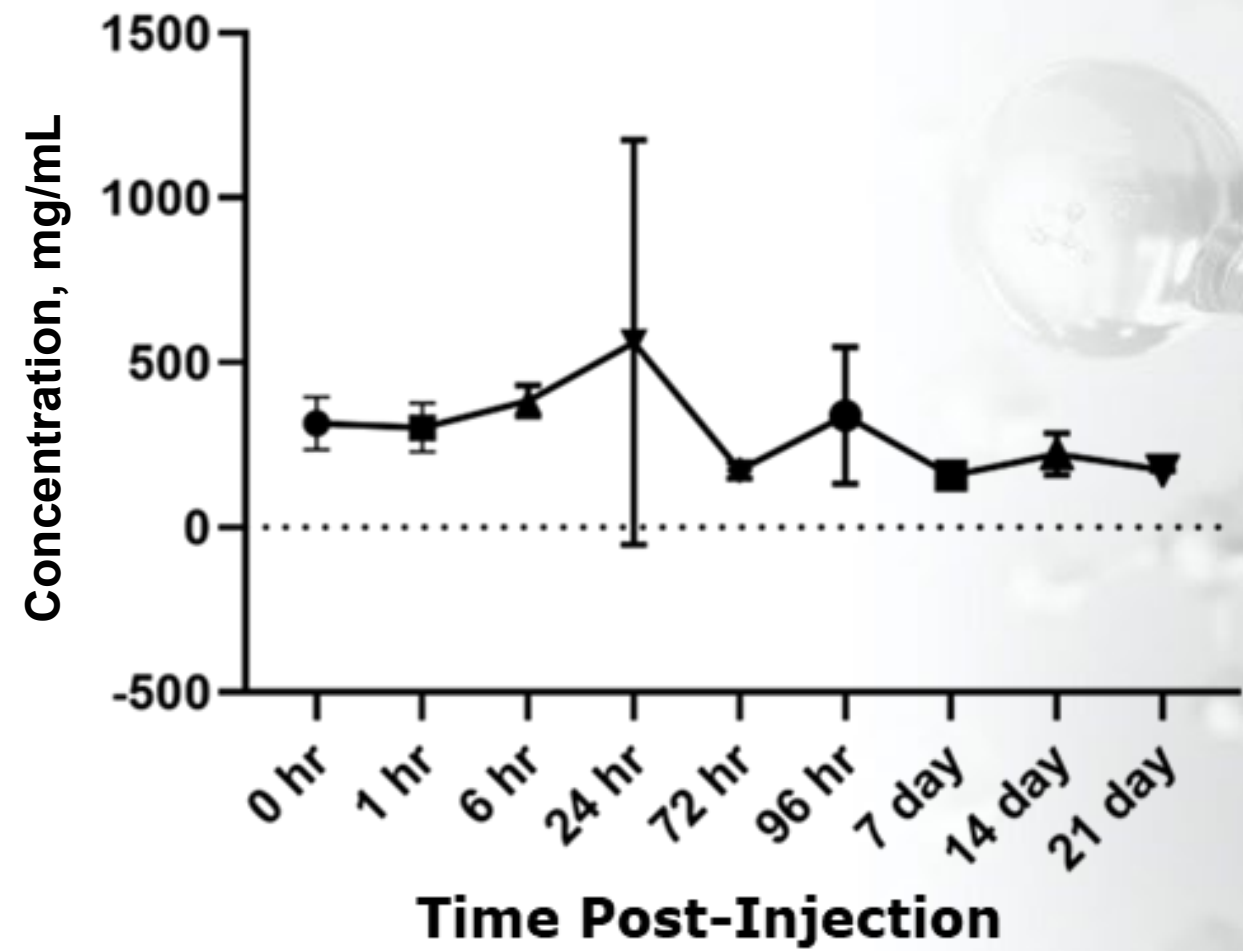


AlbuPure Elution



Pharmacokinetic Analysis of mTNX-1700 (mTTF2-MSA) in Mice

- Dose: 40 mg/kg i.p.
- N = 3/group
- Murine TFF2 ELISA on mouse serum



Therapeutic Studies

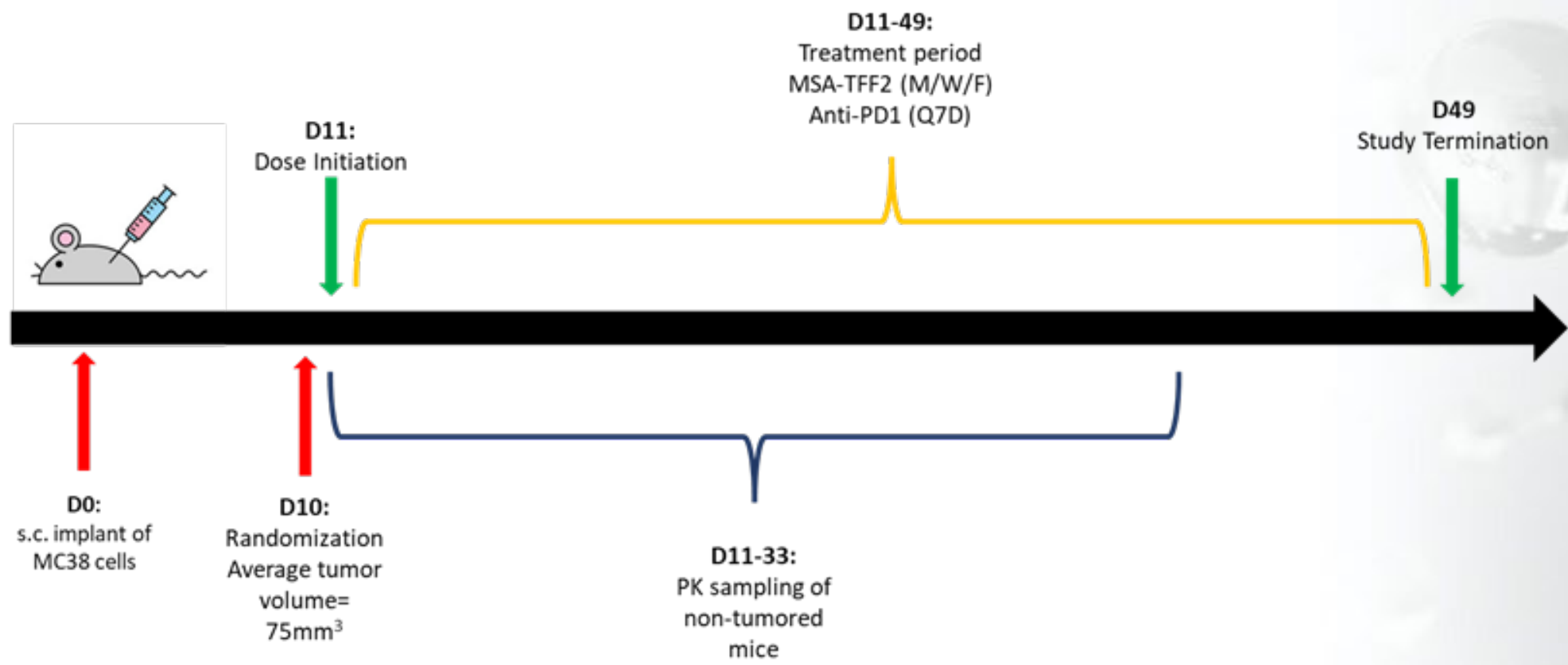
Synergy with PD-1 Blockade

Colorectal Cancer (CRC)

MC38 and CT26.wt Murine Models



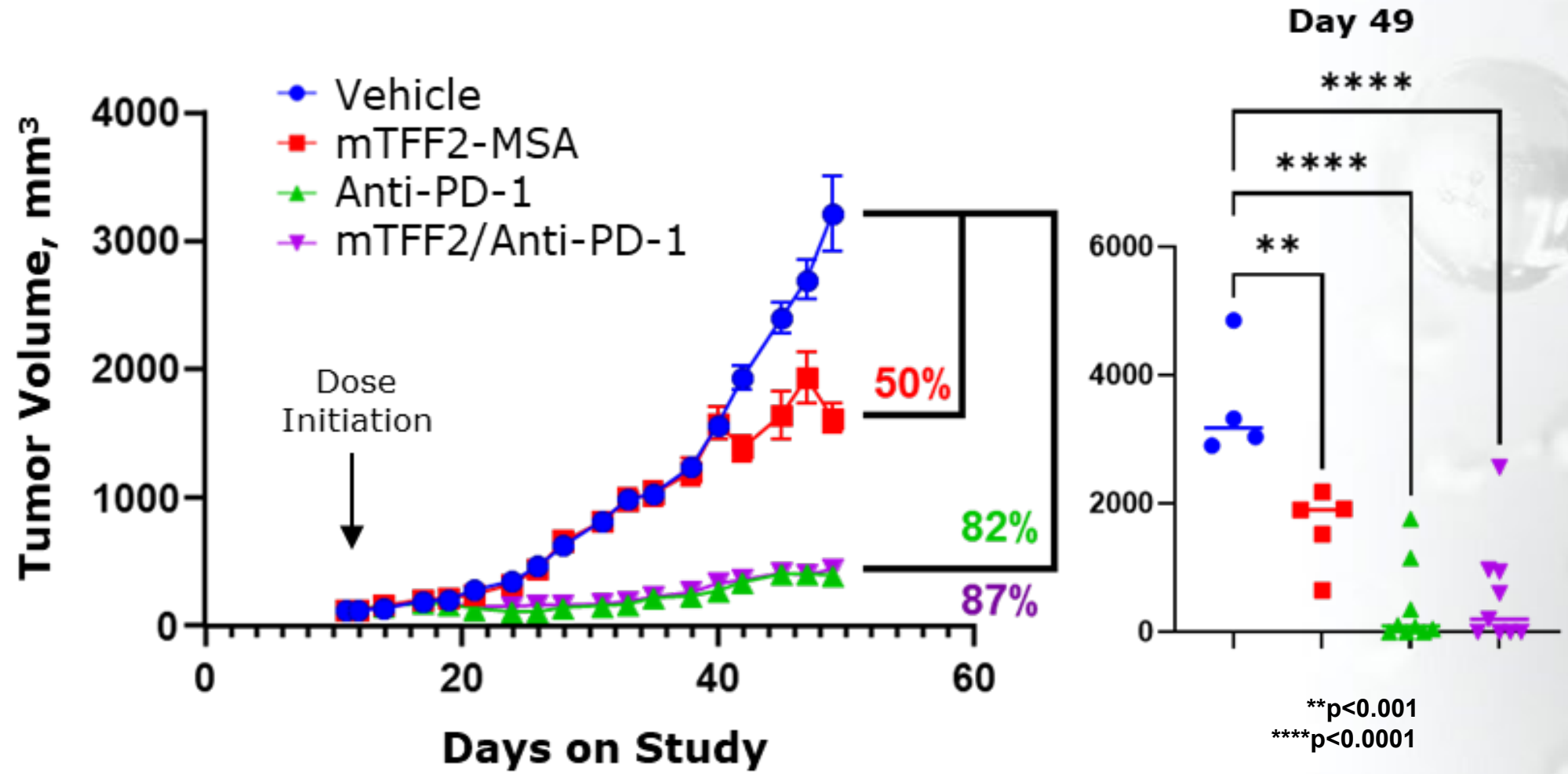
Schematic of Syngeneic MC38 CRC Tumor Model



Daugherty et al., AACR Poster. 2023. MDSC-targeted TFF2-MSA suppresses tumor growth and increases survival in anti-PD-1 treated MC38 and CT26.wt murine colorectal cancer models. <https://bit.ly/45XbGK9>

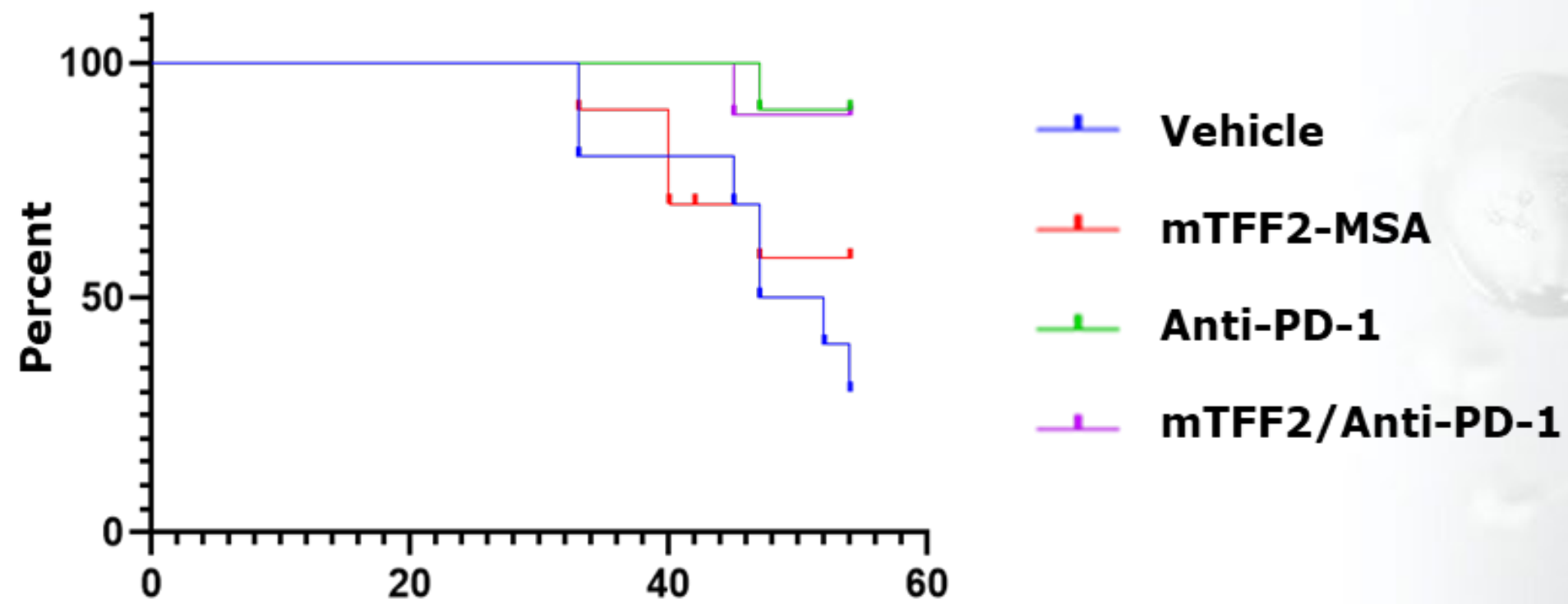


Inhibition of Tumor Growth in the MC38 CRC Model





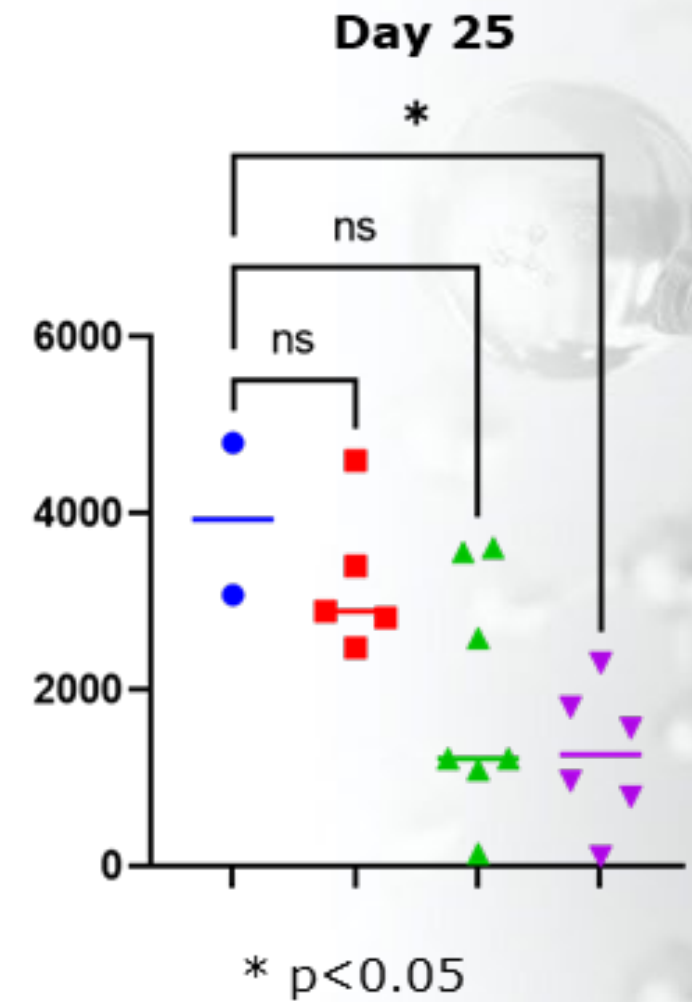
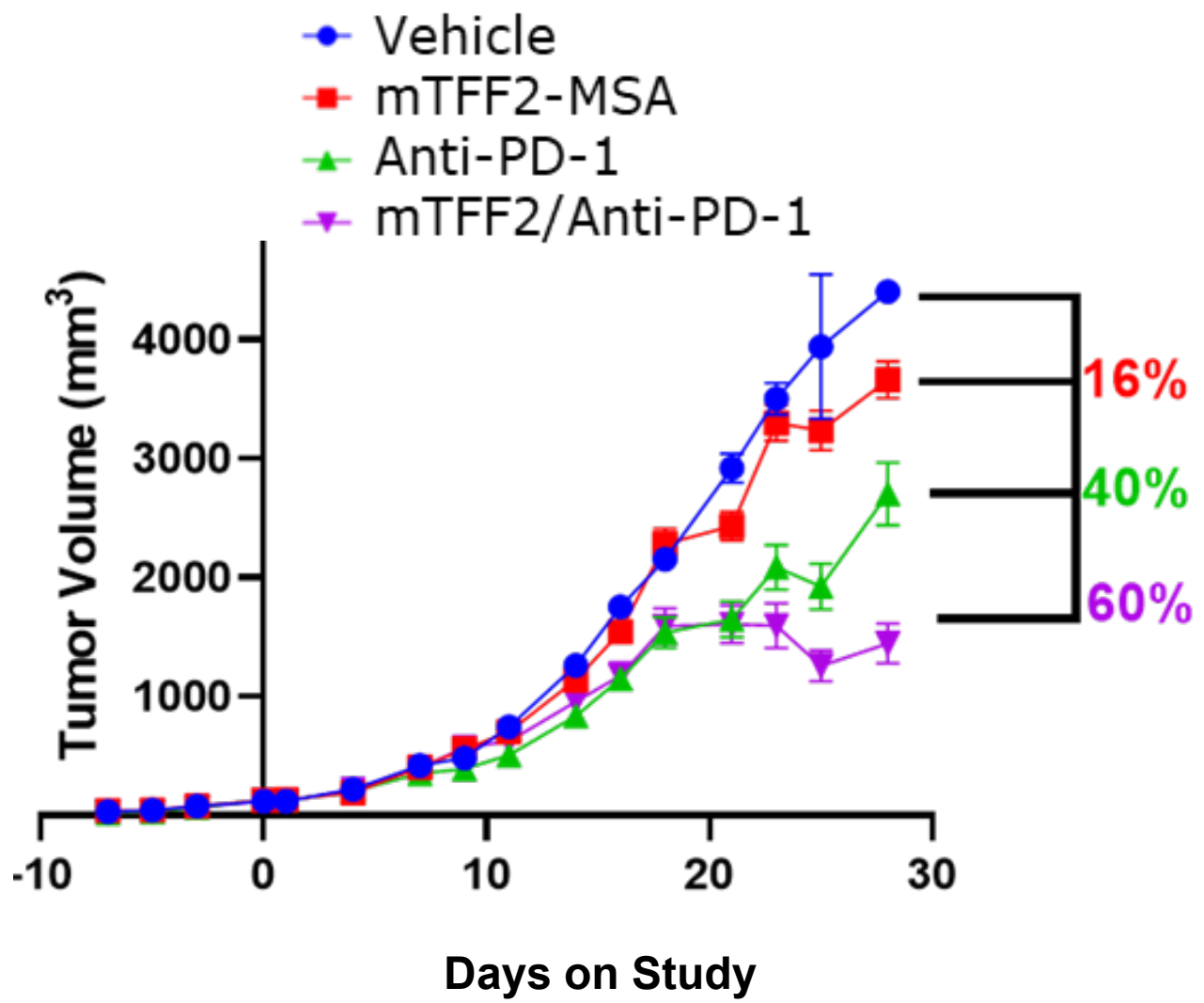
Probability of Survival in the MC38 CRC Model



Hazard Ratio (Mantel-Haenszel)	Vehicle/mTFF2-MSA	Vehicle/Anti-PD-1	Vehicle/Combo
Ratio	2.57	5.46	5.08
95% CI	0.74 – 8.92	1.50 – 19.88	1.36 – 18.95

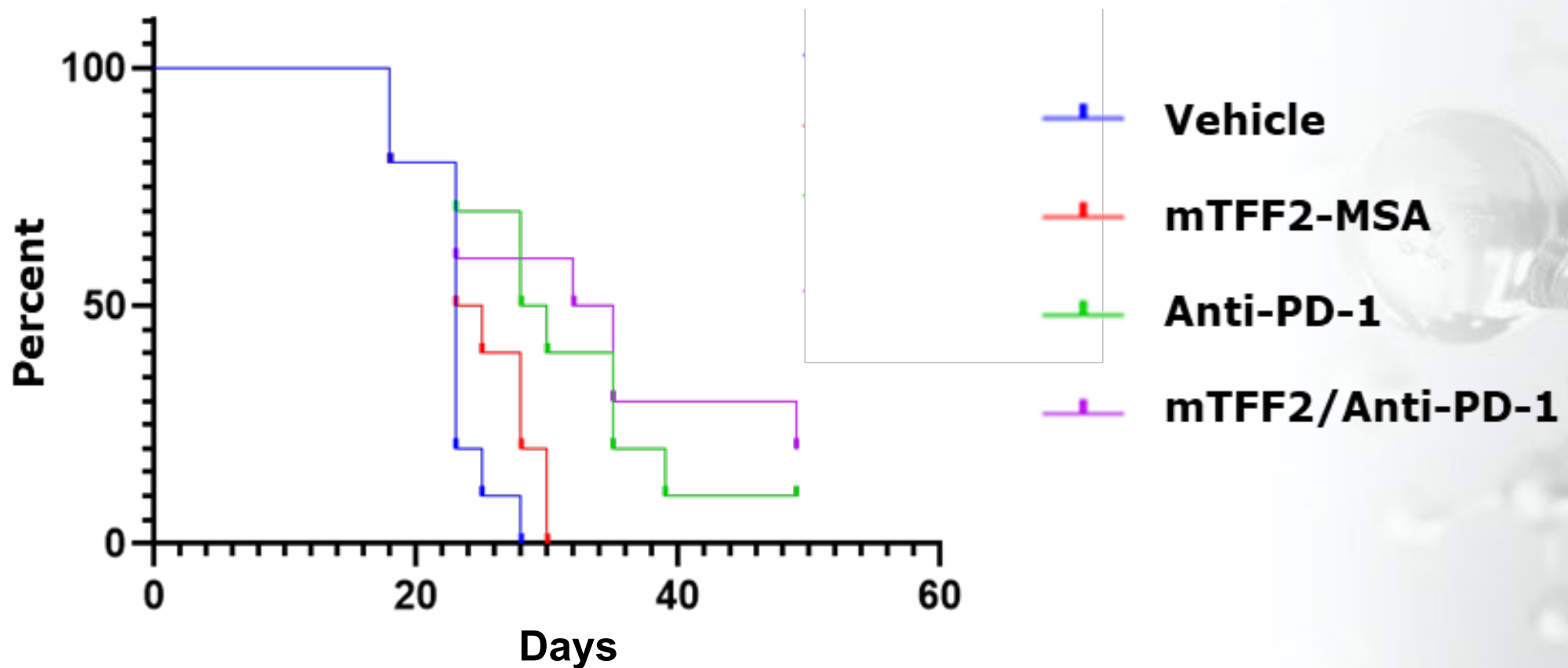


Inhibition of Tumor Growth in the CT26.wt CRC Model





Probability of Survival in the CT26.wt CRC Model



Hazard Ratio (Mantel-Haenszel)	Vehicle/mTFF2-MSA	Vehicle/Anti-PD-1	Vehicle/Combo
Ratio	2.57	5.46	5.08
95% CI	0.74 – 8.92	1.50 – 19.88	1.36 – 18.95

Daugherty, *op. cit*

Therapeutic Studies

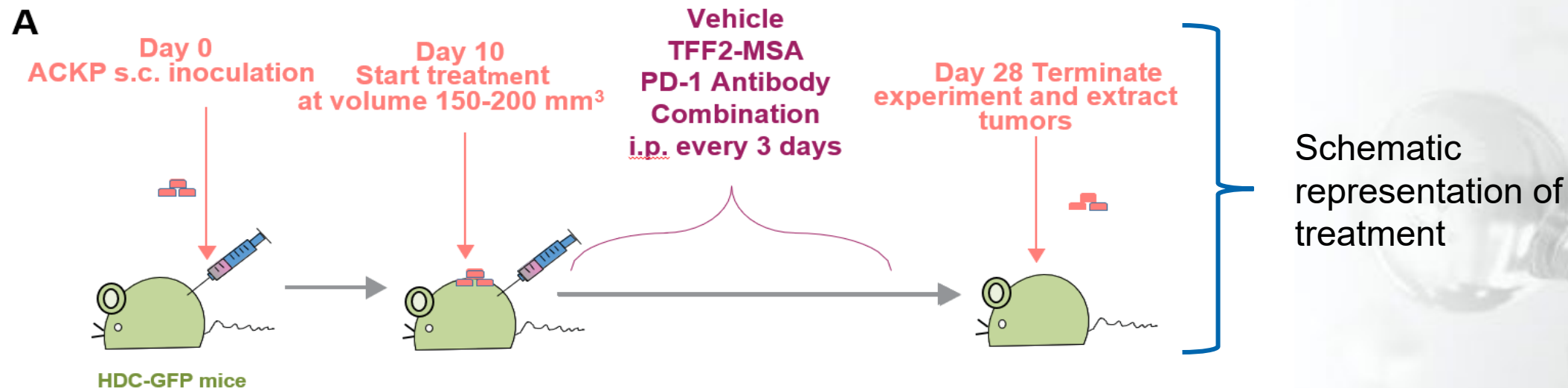
Synergy with PD-1 Blockade

Gastric Cancer

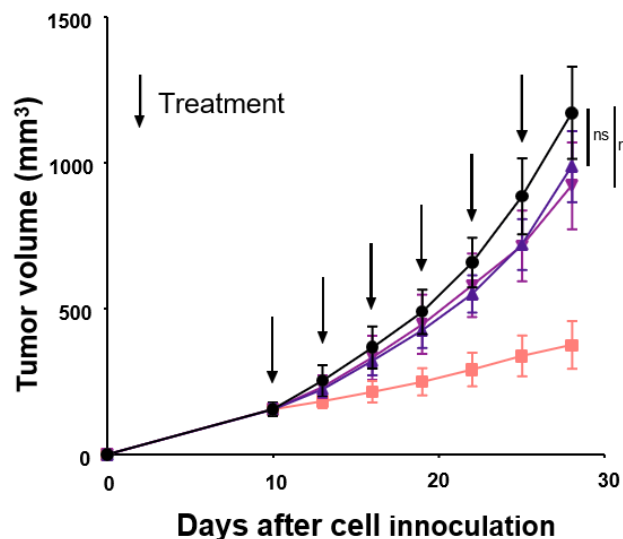
ACKP Murine Model



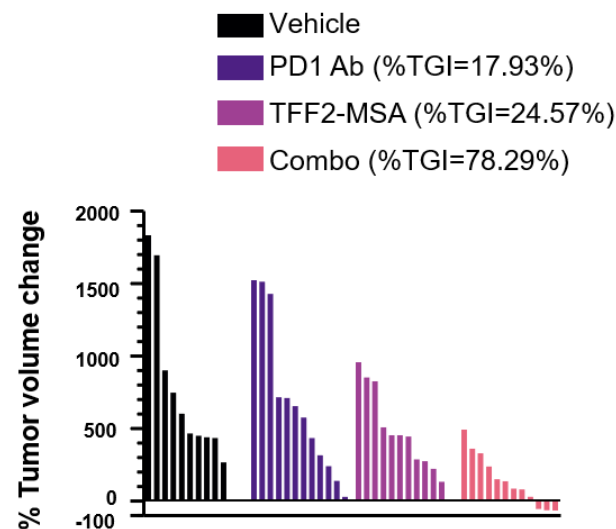
mTNX-1700 (mTFF2-MSA) Showed Synergy with anti-PD1 Antibody in Inhibition of s.c. ACKP Xenograft Growth



B Tumor Volume



C Individual Tumor Volume Change



B. Tumor growth curve of s.c. implanted ACKP tumors in response to anti-PD1 antibody, mTFF2-MSA or their combination.

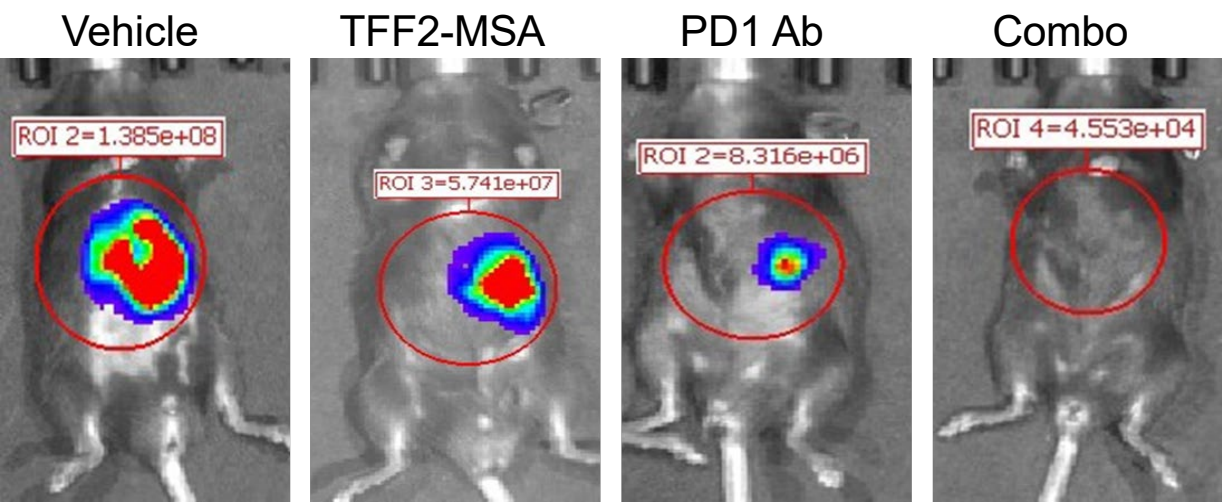
C. Tumor volume change relative to the initial volume of each tumor. Each bar represents one tumor. Positive or negative value represents volume increase or decrease respectively. $P < 0.0001$.

Qian et al., AACR Poster. 2023. MDSC-targeted TFF2-MSA synergizes with PD-1 blockade therapy in diffuse-type gastric cancer. <https://bit.ly/3qCQsku>

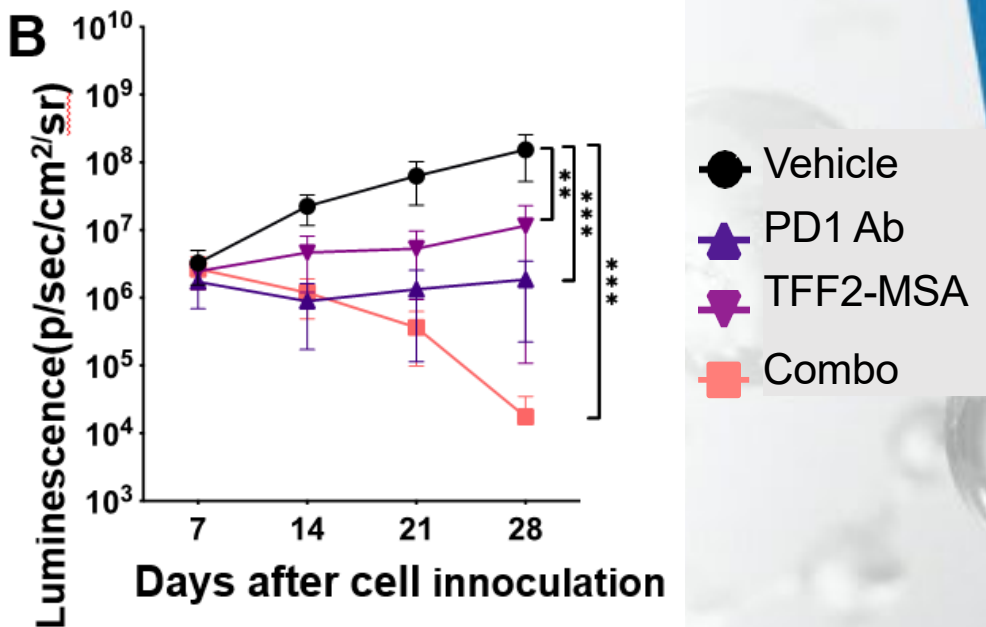


mTNX-1700 Showed Synergy w/ anti-PD1 Antibody in Inhibition of Orthotopic ACKP Xenograft Growth & Spontaneous Lung Metastasis

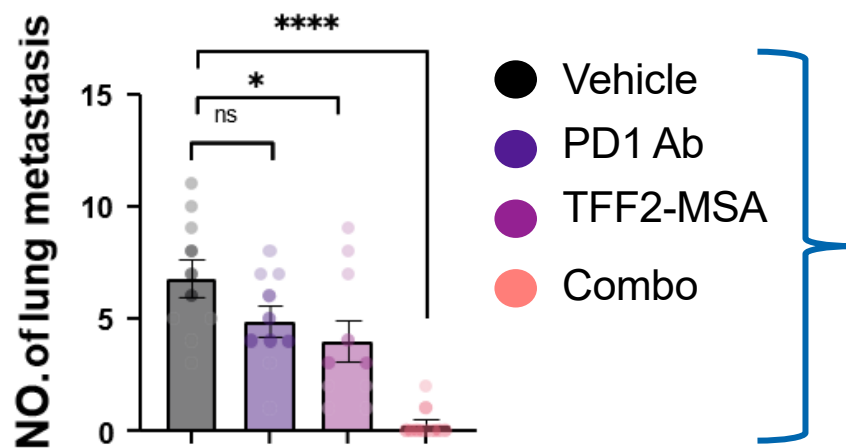
Tumor Imaging



Representative bioluminescence images showing orthotopically injected ACKP tumors in response to different treatments



Bioluminescent intensity curves showing changes of orthotopic tumors



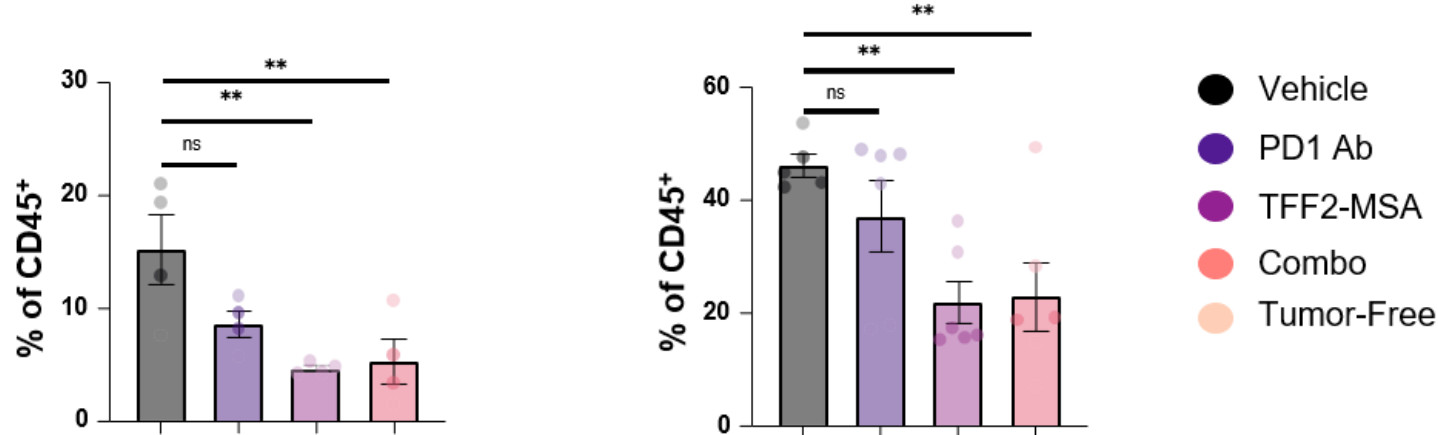
Number of lung micrometastasis in mice from different treatment groups

* P < 0.05, **** P < 0.0001

mTNX-1700 Reduced MDSC Accumulation in the Tumor and Biogenesis in the Bone Marrow

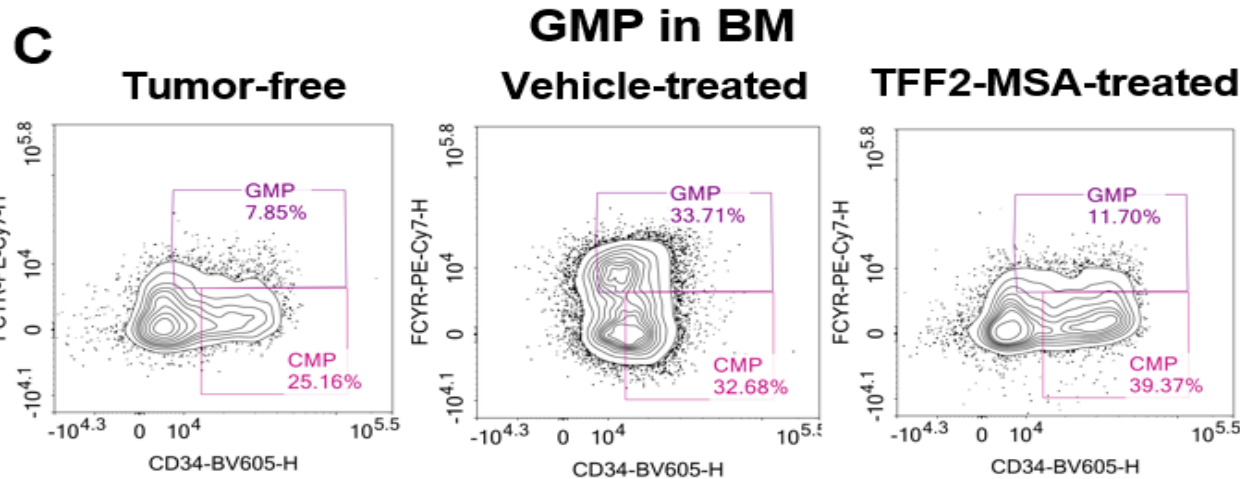


A Tumor GFP⁺CD11b⁺LY6G⁺ cell B Blood GFP⁺CD11b⁺LY6G⁺ cell

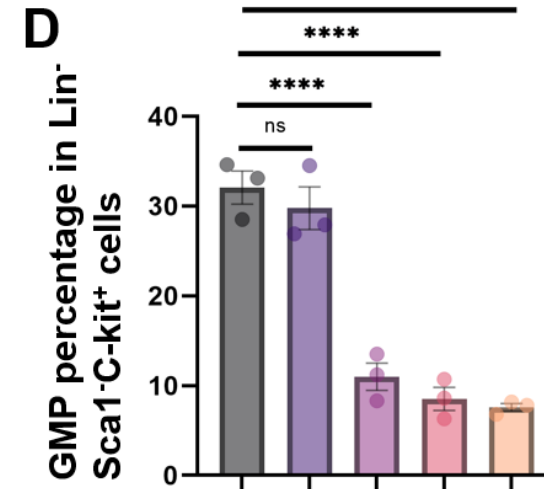


A. HDC-GFP⁺CD11b⁺LY6G⁺cell percentage among CD45⁺ cells in TME.

B. HDC-GFP⁺CD11b⁺LY6G⁺cell percentage among CD45⁺ cells in blood.



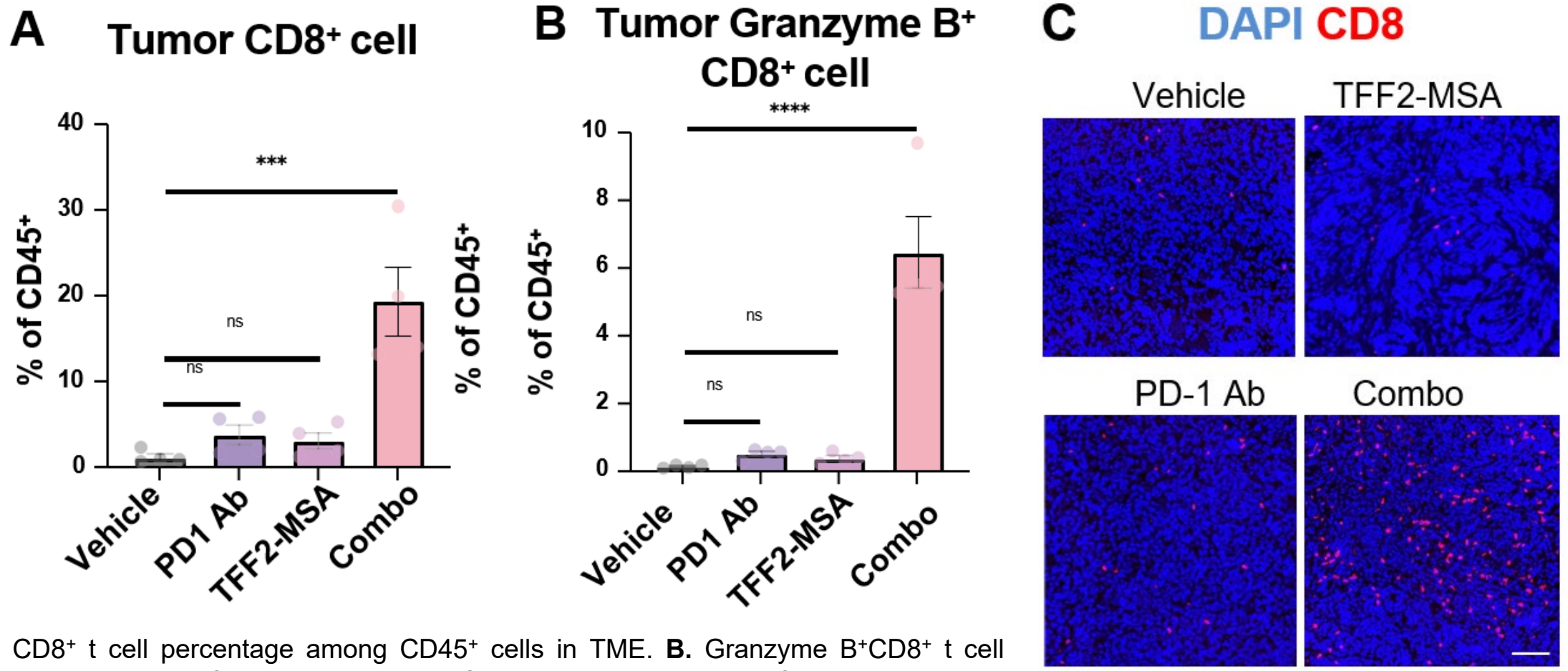
C. Representative flow cytometry plots showing ACKP tumor-bearing mice has increased granulocyte-monocyte progenitor (GMP) percentage in the bone marrow (BM) than tumor-free mice, while TFF2-MSA reduces GMP to a level similar to tumor-free mice.



D. GMP percentage in Lin⁻Sca1⁺C-kit⁺ cells within the BM. Data are presented as means ± SEM. One-way ANOVA. ** P < 0.01 **** P < 0.0001.



mTNX-1700/Anti-PD1 Ab Combination Increased Tumor-Infiltrating CD8⁺ T cell Associated with a Better Effector Phenotype



A. CD8⁺ t cell percentage among CD45⁺ cells in TME. **B.** Granzyme B⁺CD8⁺ t cell percentage among CD45⁺ cells in TME. **C.** Representative immunofluorescent images showing CD8⁺ T cell infiltration into the TME. Scale bars: 100μm. Data are presented as means ± SEM. One-way ANOVA. *** P < 0.001, **** P < 0.0001.

Summary



Conclusions

- TFF2 is a naturally occurring anti-inflammatory peptide that is a key part of the inflammatory reflex.
- TFF2 is a partial agonist for CXCR4, upregulates ApoE and suppresses the proliferation and expansion of myeloid progenitors, thus reducing MDSCs.
- Overexpression of TFF2, either through transgenic or adenoviral expression, reduces the development of colorectal cancer (CRC) following AOM/DSS treatment.
- mTFF2-MSA (mTNX-1700) peptide synergizes with PD1 blockade therapy to reduce tumor size and increase survival in two CRC syngeneic mouse models.
- mTNX-1700 synergizes with anti-PD1 blockade to increase survival and eradicate gastric cancer (GC) in advanced orthotopic and metastatic models.
- mTNX-1700 reduces the production of MDSC and promotes a T-cell rich microenvironment, inducing a 50-fold increase in intratumor CD8+ T cells.



Product Development

- Progress
 - Expression of TNX-1700 (hTFF2-HSA fusion protein)
- Next Steps
 - File the Investigational New Drug (IND) application in the US
 - Scale-up and produce GMP TNX-1700 for human clinical trials
 - Phase 1 – Evaluate safety and pharmacokinetics
 - Phase 2 – Study effects on tumors in anti-PD1 treated patients



TNX-1700*: Gastric and Colorectal Cancers

Recombinant Trefoil Factor 2 (rTFF2) Fusion Protein

Potential New Cancer Treatment

- TNX-1700 (rTFF2) has effects on cancer by altering the tumor micro-environment
- Mechanism of action: suppresses myeloid-derived suppressor cells and activates anti-cancer CD8+ T cells
- Potential synergy with anti-PD-1 or anti-PD-L1 monoclonal antibodies (mAbs)

Preclinical Evidence for Inhibiting Growth of Cancer Cells

- Data showed that TFF2-CTP augmented the efficacy of mAb anti-PD-1 therapy. Anti-PD-1 in combination with TFF2-CTP showed greater anti-tumor activity in PD-L1-overexpressing mice

Licensed from Columbia University

- Developing in partnership under sponsored research agreement

Market Entry: Immuno-oncology, combination therapy with PD1 blockers for gastric and colorectal cancer

Status: Preclinical

Next Steps: Animal studies ongoing

Differentiator: No product yet identified consistently augments PD1 effects on cold tumors

Patents Filed

*TNX-1700 is in the pre-IND stage of development and has not been approved for any indication.

THANK YOU

APPENDIX





AACR Presentations

Presentation #1

Title: MDSC-targeted TFF2-MSA suppresses tumor growth and increases survival in anti-PD-1 treated MC38 and CT26.wt murine colorectal cancer models
Authors: Bruce L. Daugherty¹, Rebecca J. Boohaker², Rebecca Johnstone², Karr Stinson², Jin Qian³, Timothy C. Wang³, Seth Lederman¹
Tonix Pharmaceuticals, Inc., 26 Main Street, Suite 101, Chatham, NJ 07928
Southern Research, 2000 9th Ave S, Birmingham, AL 35205
Division of Digestive and Liver Diseases, Irving Cancer Research Center, Columbia University Medical Center, New York, NY 10032, USA

Topic: Oncolytic Viruses, Anticancer Vaccines, and Other Immunomodulatory Therapies
Location: Orange County Convention Center, Orlando, Fla.
Section: 24, #704
Date: Sunday, April 16, 2023
Time: 1:30 p.m. – 5:00 p.m. ET
Abstract: [Click here](#)

Presentation #2

Title: MDSC-targeted TFF2-MSA synergizes with PD-1 blockade therapy in diffuse-type gastric cancer
Authors: Jin Qian¹, Sandra Ryeom¹, Bruce Daugherty², Seth Lederman², Timothy C. Wang¹²
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Location: Orange County Convention Center, Orlando, Fla.
Section: 21, #5088
Date: Tuesday, April 18, 2023
Time: 1:30 p.m. – 5:00 p.m. ET
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