TFF2-mediated CXCR4 partial agonism outperforms CXCR4 antagonism in reducing murine gastric cancer by suppressing PMN-MDSC generation

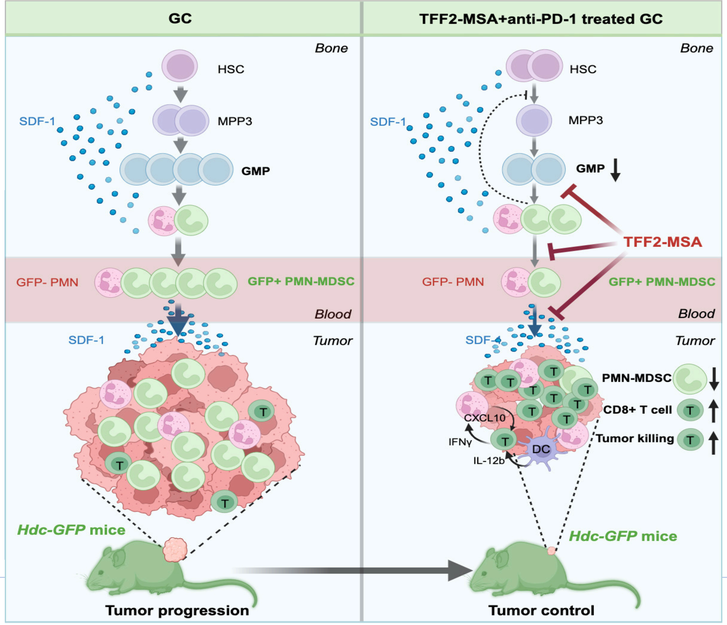
Jin Qian[1], Chenkai Ma[2], Quin T. Waterbury[1], Xiaofei Zhi[1], Christine S. Moon[1], Ruhong Tu[1], Hiroki Kobayashi[1], Feijing Wu[1], Biyun Zheng[1], Yi Zeng[1], Hualong Zheng[1], Yosuke Ochiai[1], Ruth A. White[1], David W. Harle[1], Jonathan S. LaBella[1], Leah B. Zamechek[1], Lucas ZhongMing Hu[1], Ryan H. Moy[1], Arnold S. Han[1], Bruce L. Daugherty[3], Seth Lederman[3], Timothy C. Wang[1]*

1. Irving Cancer Research Center, Columbia University Medical Center, New York, NY 10032, USA.
2. Integrated Diagnostic, Human Health, Health and Biosecurity, CSIRO, Westmead, New South Wales 2070, Australia
3. Tonix Pharmaceuticals, Inc., 26 Main Street, Suite 101, Chatham, NJ 07928

Background

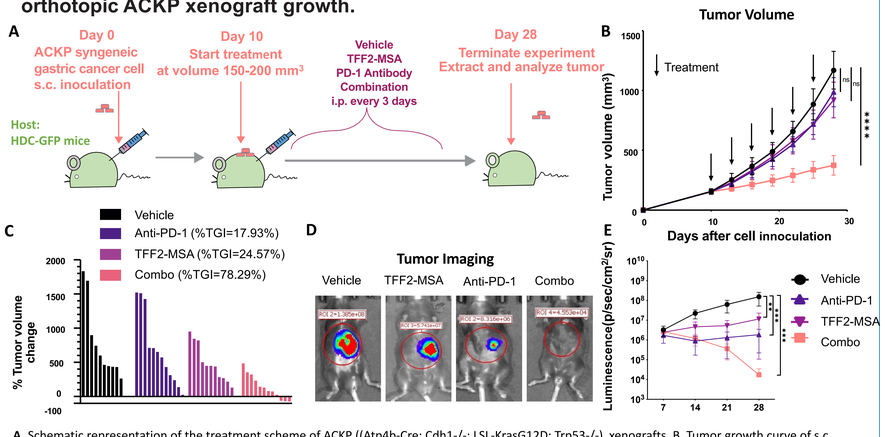
- Immunosuppressive neutrophils, also known as polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), are a major component in solid tumors that significantly hinder anti-tumor immunity. These cells comprise a heterogeneous group of neutrophils pathologically programmed by cancer-derived signals[1,2].
- Despite being short-lived, their continuous replenishment from aberrant myelopoiesis in the bone marrow (BM) sustains their potent immunosuppression in the tumor microenvironment (TME)[3].
- CXCR4 is a key bone marrow retention signal for hematopoietic stem and progenitor cells (HSPCs) and neutrophils. In many cancers, stromal cells in the TME highly secreted CXCL12, promoting immunosuppression by recruiting MDSCs. Tumor-derived signals also dampen the CXCL12-CXCR4 bone marrow retention signal, leading to accelerated neutrophil mobilization and increased granulopoiesis.
- Trefoil Factor 2 (TFF2), a secreted peptide of the trefoil factor family, acts as a partial agonist of CXCR4, dampening CXCL12-mediated intense signaling while eliciting weak CXCR4 signaling[4,5]. The *Tff2* gene is epigenetically silenced during gastric preneoplasia.
- Histidine decarboxylase (Hdc), an enzyme that catalyzes histamine synthesis, is expressed in immature granulocytes that predominate in cancer, suggesting its potential utility in distinguishing tumor PMN-MDSC[6].

Graphic Abstract



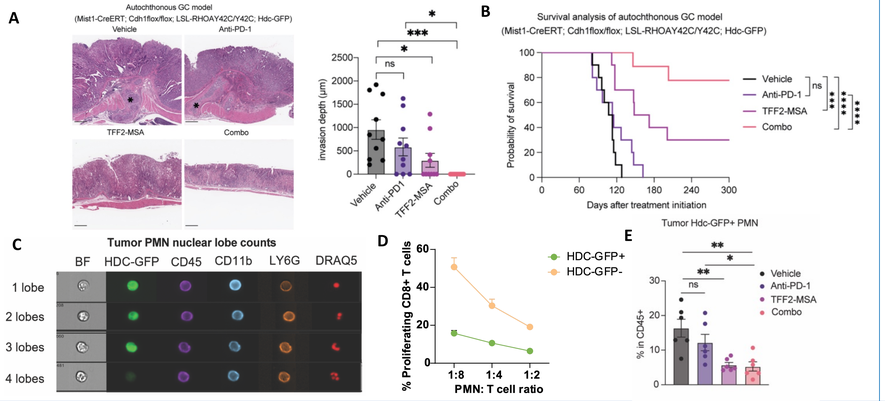
Result

Figure 1. TFF2-MSA showed synergy with anti-PD-1 antibody in inhibition of s.c. and orthotopic ACKP xenograft growth.



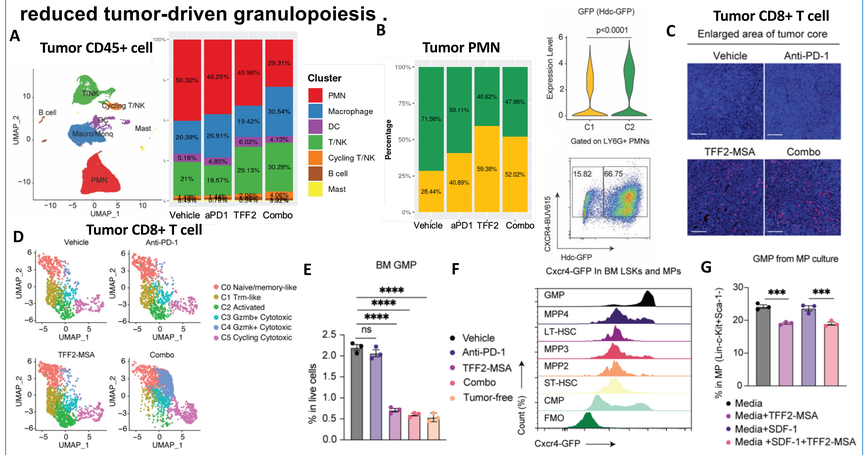
A. Schematic representation of the treatment scheme of ACKP ((Atp4b-Cre; Cdh1-/-; LSL-KrasG12D; Trp53-/-) xenografts. B. Tumor growth curve of s.c. implanted ACKP tumors in response to anti-PD1 antibody, TFF2-MSA or their combination. **C.** Tumor volume change relative to the initial volume of each tumor. Each bar represents one tumor. TGI: Tumor Growth Inhibition. Tumor Positive or negative value represents volume increase or decrease respectively. **D.** Representative bioluminescence images showing orthotopically injected ACKP tumors with different treatments. **E.** Bioluminescent intensity curves showing changes of orthotopic tumors. **P < 0.01, ***P < 0.001, ****P < 0.0001.

Figure 2. TFF2-MSA inhibited tumor invasion, and reduced Hdc-GFP+ PMN-MDSCs in the tumor.



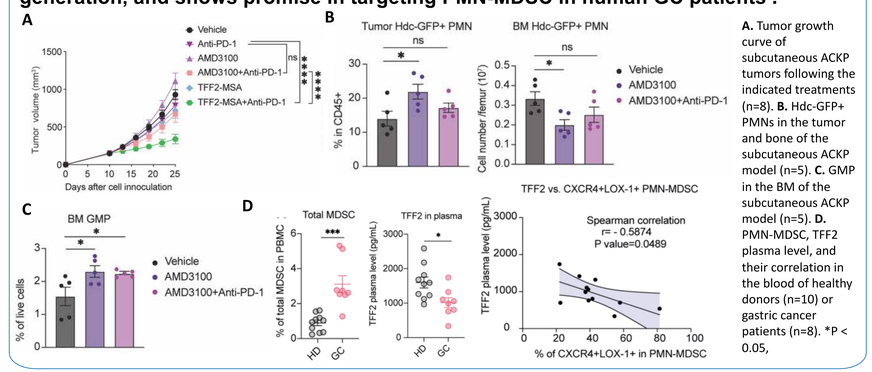
A. Representative H&E staining images of autochthonous GC in Mist1-CreERT; Cdh1flox/flox; LSLRHOAY42C/Y42C; Hdc-GFP mice and quantification of tumor invasion depths following treatments at 10 months post-tamoxifen induction (n=10). **B.** Survival curve of the mice in **A** (n=10). **C.** Imaging flow cytometry showing the nuclear segmentation in Hdc-GFP- and Hdc-GFP+ subsets of neutrophils (CD45+CD11b+LY6G+LY6C-/low). **D.** T cell proliferation co-cultured with HDC-GFP+ or HDC-GFP- LY6G+ PMNs. **E.** Flow cytometry showing the Hdc-GFP+ PMN in subcutaneous ACKP tumors following the treatments (n=6). *P < 0.05, ***P < 0.001, ****P < 0.0001.

Figure 3. TFF2-MSA in combination with anti-PD-1 activated anti-tumor immunity and it reduced tumor-driven granulopoiesis .



A. scRNA-seq of tumor CD45+ cells following the treatments.. **B.** Tumor PMN changes in the scRNA-seq Representative flow cytometry plots of CXCR4 in tumor PMNs.**C.** Immunostaining of CD8+ T cells in the treated tumors.. **D.** CD8+ T cell changes in the scRNA-seq. **E.** Granulocyte-monocyte progenitor (GMP) percentage in the bone marrow (BM) by flow cytometry. **F.** Cxcr4-GFP expression in LSK (Lin-C-kit+Sca-1+) and MP (myeloid progenitor) cells in the BM of Cxcr4-GFP mice with subcutaneous ACKP tumors (n=3). **G.** Frequency of GMP after culture of sorted MP (myeloid progenitor, Lin-C-kit+Sca-1-) cells in myeloid differentiation media for 5 days (n=3). ***P < 0.001, **** P < 0.0001.

Figure 4. TFF2-MSA outperforms CXCR4 antagonist AMD3100 by reducing PMN-MDSC generation, and shows promise in targeting PMN-MDSC in human GC patients .



A. Tumor growth curve of subcutaneous ACKP tumors following the indicated treatments (n=8). **B.** Hdc-GFP+ PMNs in the tumor and bone of the subcutaneous ACKP model (n=5). **C.** GMP in the BM of the subcutaneous ACKP model (n=5). **D.** PMN-MDSC, TFF2 plasma level, and their correlation in the blood of healthy donors (n=10) or gastric cancer patients (n=8). *P < 0.05,

Conclusion

- TFF2-MSA, a CXCR4 partial agonist, sensitizes mouse gastric cancer to anti-PD-1.
- TFF2-MSA selectively reduces immunosuppressive neutrophils and cancer-driven granulopoiesis.
- TFF2-MSA plus anti-PD-1 induces robust anti-tumoral CD8⁺ T cell responses
- TFF2 reduction correlates with elevated PMN-MDSCs in gastric cancer patients.

- **Lead contact: Timothy C. Wang M.D.**

Email: tcw21@cumc.columbia.edu
Chief, Division of Digestive and Liver Diseases Silberberg Professor of Medicine
Department of Medicine
Irving Cancer Research Center Columbia University Medical Center

References

1. Kim W, *et al.* PD-1 Signaling Promotes Tumor-Infiltrating Myeloid-Derived Suppressor Cells and Gastric Tumorigenesis in Mice. Gastroenterology. 2021 Feb;160(3):781-796. 2.Veglia F, *et al.* Analysis of classical neutrophils and polymorphonuclear myeloid-derived suppressor cells in cancer patients and tumor-bearing mice. J Exp Med. 2021 Apr 5;218(4):e20201803. 3. Colligan SH, *et al.* Inhibiting the biogenesis of myeloid-derived suppressor cells enhances immunotherapy efficacy against mammary tumor progression. J Clin Invest. 2022 Dec 1;132(23):e158661.4.Dubeykovskaya Z, *et al.* Secreted trefoil factor 2 activates the CXCR4 receptor in epithelial and lymphocytic cancer cell lines. J Biol Chem. 2009 Feb 6;284(6):3650-62. 5.Dubeykovskaya Z, *et al.* Neural innervation stimulates splenic TFF2 to arrest myeloid cell expansion and cancer. Nat Commun. 2016 Feb 4;7:10517.6.Chen X, *et al.* Histidine decarboxylase (HDC)-expressing granulocytic myeloid cells induce and recruit Foxp3+ regulatory T cells in murine colon cancer. Oncoimmunology. 2017 Feb 10;6(3):e1290034.