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A CXCR4 partial agonist TFF2-MSA improves anti-PD-1 immunotherapy in advanced gastric cancer by selectively targeting PMN-MDSC

Jin Qian[1], Chenkai Ma[2], Quin T. Waterbury[1], Christine S. Moon[1], Xiaofei Zhi[1], Hiroki Kobayashi[1], Feijing Wu[1], Ruhong Tu[1], Biyun Zheng[1], Leah B. Zamechek[1], Ryan H. Moy[1], Arnold Han[1], Bruce Daugherty[3], Seth Lederman[3], Timothy C. Wang[1]

[1]Division of Liver and Digestive Diseases, Irving Cancer Research Center, Columbia University Irving Medical Center, New York, NY,

[2]Integrated Diagnostic, Human Health, Health and Biosecurity, CSIRO, Westmead, Australia

[3]Tonix Pharmaceuticals, Inc., Chatham, NJ

Disclosure Information

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Jin Qian

I have no financial relationships to disclose.

Timothy C. Wang

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Polymorphonuclear Myeloid-Derived Suppressor Cell (PMN-MDSC) and HDC

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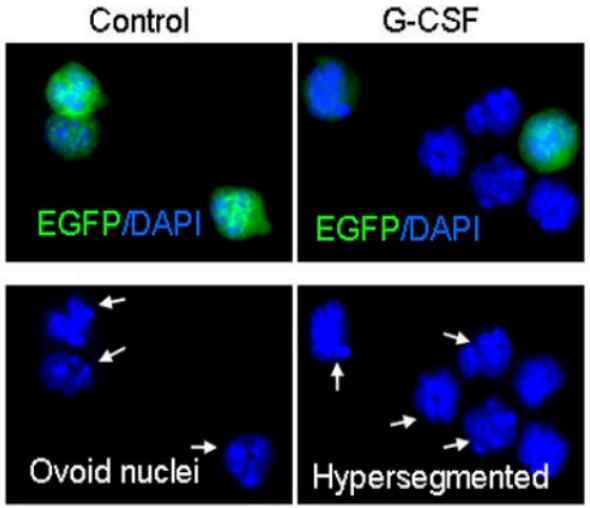
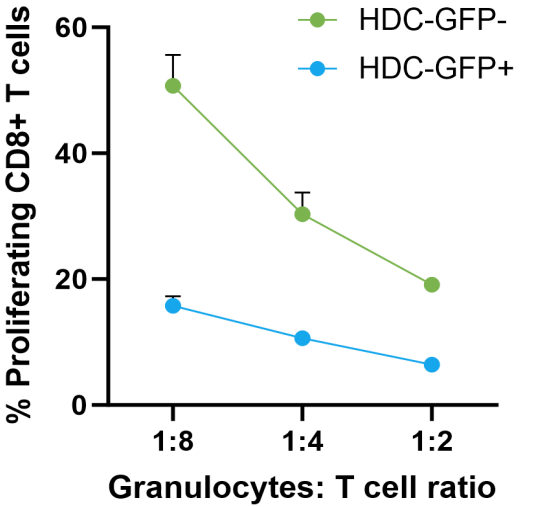
- Pathologically activated neutrophils with potent immunosuppression and abundant in tumors.
- Short half-life and continuously recruited from bone marrow
- Mostly immature neutrophils that share markers with polymorphonuclear neutrophil (PMN, both are LY6G$^+$ cells in mice), complicating its identification and targeting
- Therapeutic strategies targeting PMN-MDSCs in cancer: inhibition of recruitment (CXCR2 or CXCR4 blockade), depletion, reprogramming MDSCs or blocking their immunosuppressive functions.
- HDC (histidine decarboxylase) identifies immature immunosuppressive neutrophils.

HDC-GFP$^+$ and HDC-GFP$^-$ PMNs



T cell co-culture with LY6G$^+$ PMNs



Yang XD, *et al*. Nat Med. 2011

Stromal cell-derived factor-1(SDF1)-CXCR4 signaling as a master regulator of neutrophils in homeostasis and diseases

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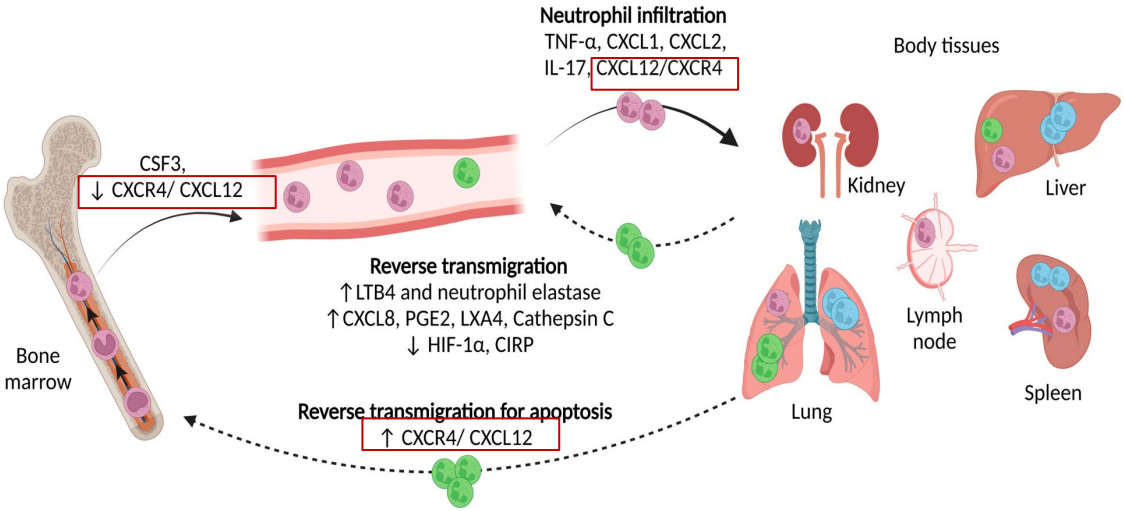
- Key retention signal for neutrophils and hematopoietic progenitors in bone marrow.
- Both agonism and antagonism of CXCR4 may induce bone marrow cell mobilization.
- Can be an inflammatory signal from tumor sites that mediates neutrophil recruitment.
- Both SDF1 and its antagonist have been reported to inhibit cancer development in preclinical models.
- SDF1-CXCR4 is important for development and functions of multiple immune cells (T, NK, B, macrophage and dendritic cells).
- Full antagonists of CXCR4 (Plerixafor, BL-8040) are being evaluated in clinical trials. <u>However, no partial agonists of CXCR4 have been tested in cancer treatments.</u>



Cambier S, *et al*. Cell Mol Immunol 2023
Tsioumpekou M, *et al*. Cells. 2023
Williams S.A , *et al*. Mol Cancer 2010

TFF2 is a partial agonist of CXCR4

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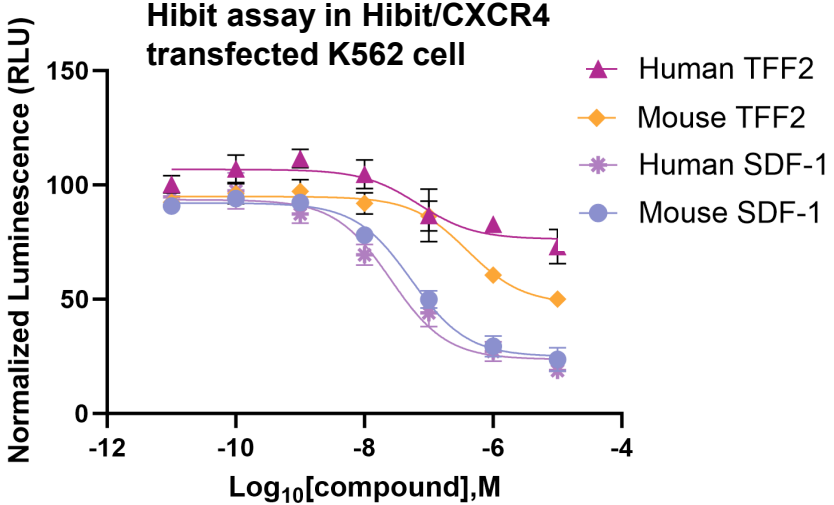
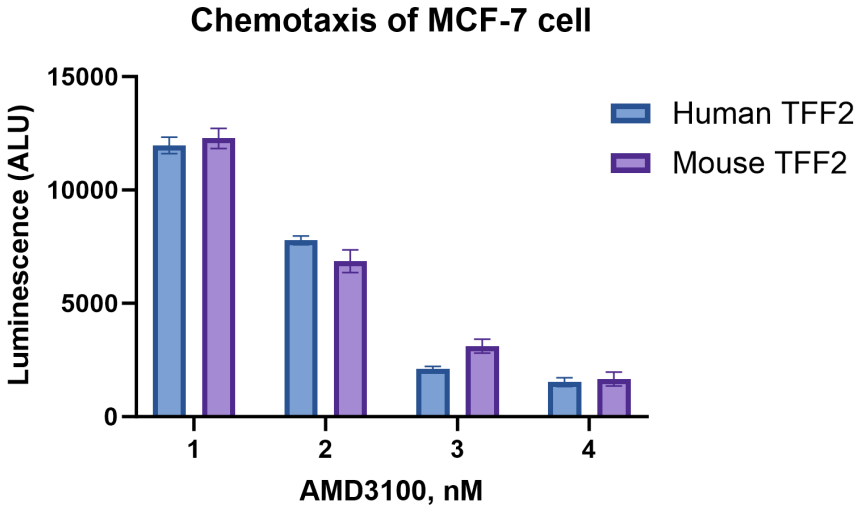
- A small secreted protein of the trefoil factor family.
- Partial agonist of CXCR4. Partially blocks SDF-1 mediated signaling and chemotaxis.
- TFF2 fused to mouse serum albumin (MSA) to generate a stabilized version TFF2-MSA peptide.





- TFF2 induced weaker CXCR4 receptor internalization than SDF-1.
- TFF2 function to induce chemotaxis (albeit weak) was dependent on CXCR4.

Dubeykovskaya Z, *et al*. *J Biol Chem*. 2009
Dubeykovskaya Z, *et al*. *Nat Commun*. 2016
White CW, *et al*. Cell Chem Biol. 2020

TFF2-MSA markedly improved aPD-1 efficacy in gastric cancer (ACKP cells) subcutaneous tumor model

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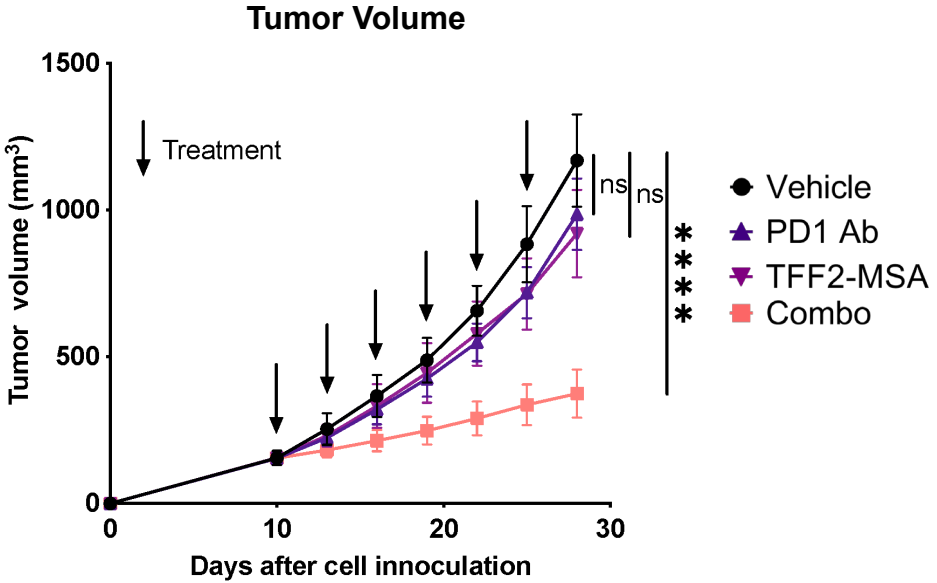

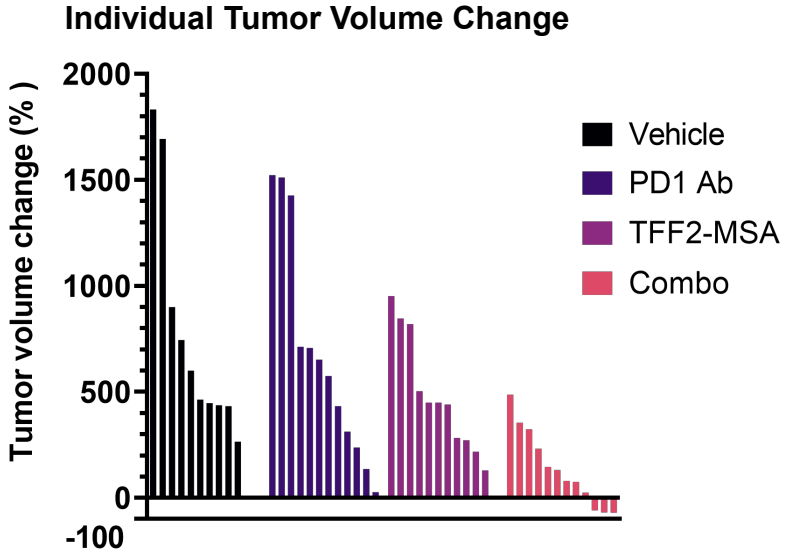

- ACKP syngeneic gastric cancer cells were developed from Atpb4-cre; LSL-KrasG12D, CDH1 fl/fl; p53 fl/fl, LSL-YFP mice induced by tamoxifen and developed highly metastatic gastric cancer.
- Although either TFF2-MSA or anti-PD1 monotherapy had limited effect, their combination resulted in tumor regression of s.c. implanted ACKP tumors.
- TFF2-MSA 22.5mg/kg, aPD-1 10mg/kg, i.p. every 3 days

Till JE, *et al*. Cancer Res. 2017

TFF2-MSA markedly improved aPD-1 efficacy in ACKP gastric orthotopic and lung metastatic models

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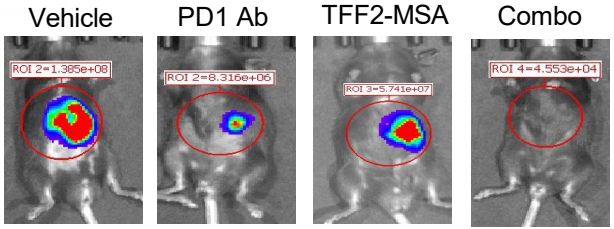
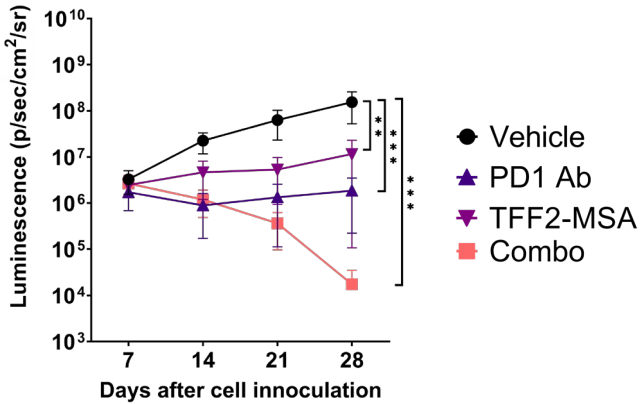
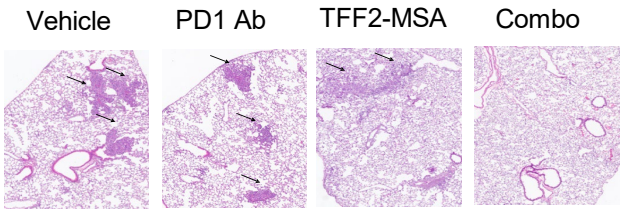
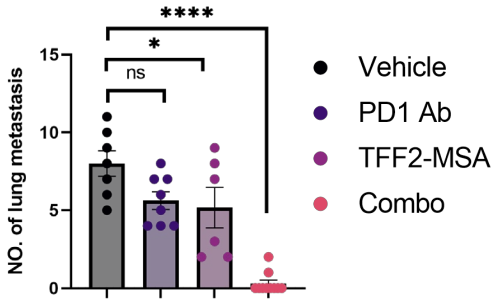
Orthotopic tumor imaging



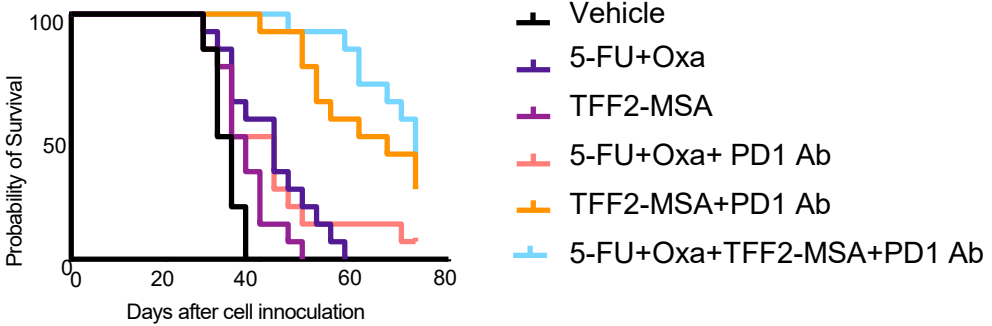


Lung metastasis after tumor resection





Survival curve



- TFF2-MSA and anti-PD1 combination robustly inhibited tumor growth of orthotopic ACKP tumors, and spontaneous lung metastasis after tumor resection, and extended mouse survival.

TFF2-MSA reduced HDC-GFP$^+$ PMN-MDSC abundance in the TME and blood, and aberrant myelopoiesis in the bone marrow

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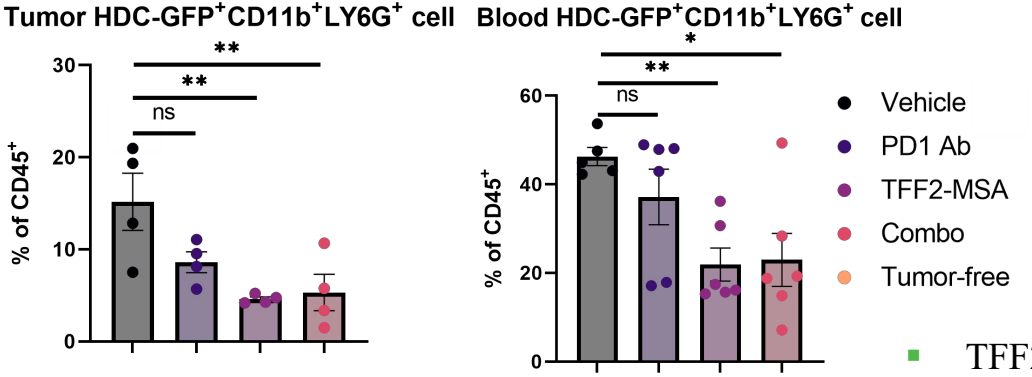

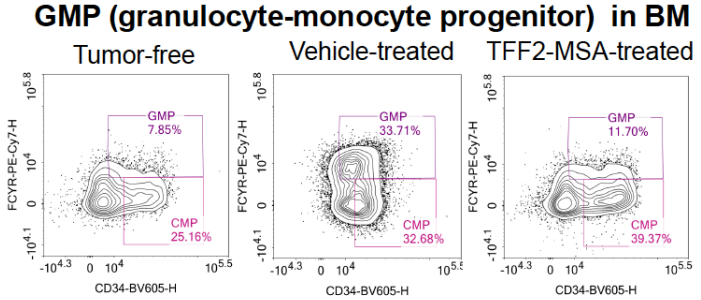

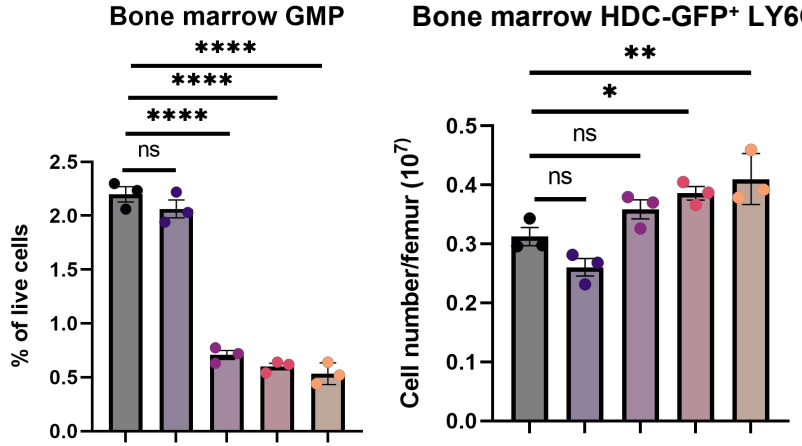

- TFF2-MSA reduced accumulation of HDC-GFP$^+$ PMN-MDSCs in the TME and blood of ACKP s.c. tumor-bearing HDC-GFP mice.
- TFF2-MSA decreased granulocyte-monocyte progenitor (GMP) while increasing long-term repopulating hematopoietic stem cells (LT-HSC). The reduction in myelopoiesis achieved a level that was similar to that of tumor-free mice.
- TFF2-MSA reduced aberrant myelopoiesis in the bone marrow, possibly via increasing retention of HDC-GFP$^+$ neutrophils.

scRNA-seq of gastric cancer TME revealed PMN reduction and T cell expansion with TFF2-MSA treatments

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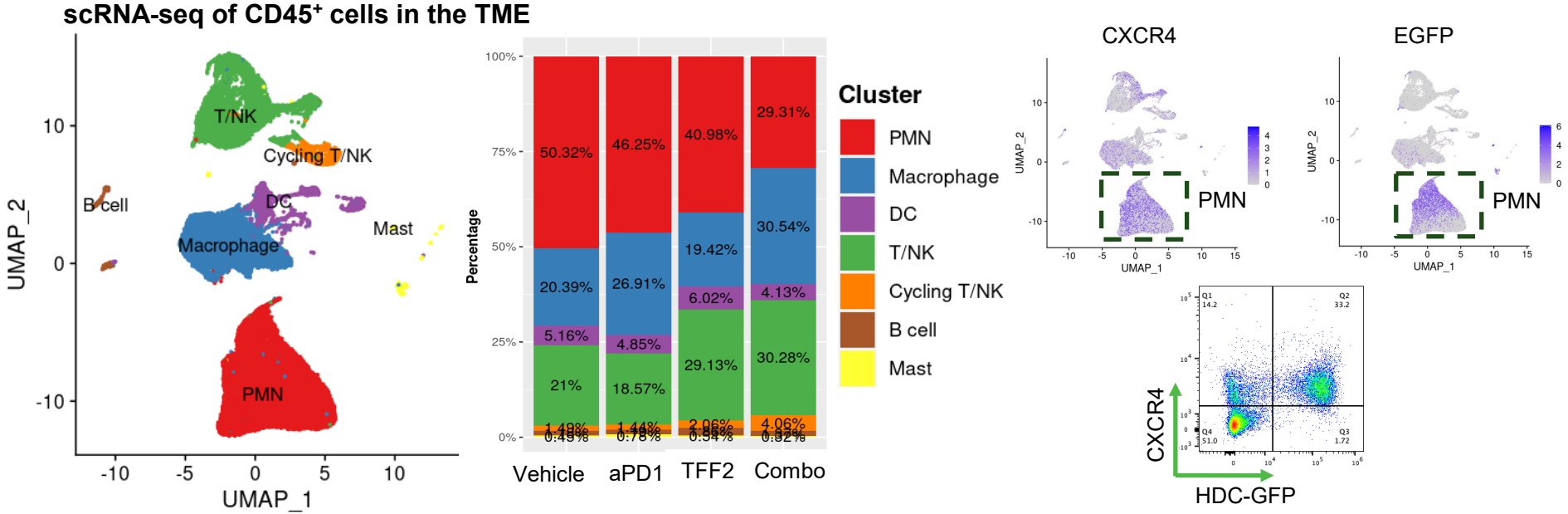

- scRNA-seq of CD45$^+$ TME cells revealed a decrease of PMNs with TFF2-MSA and combo, and a simultaneous increase in both T cells and NK cells with TFF2-MSA and combo.
- As expected, HDC-GFP mainly marks PMNs. CXCR4 is highest expressed in HDC-GFP$^+$ PMN-MDSCs in the TME.

2 Clusters of PMN were differentially modulated by TFF2-MSA treatments

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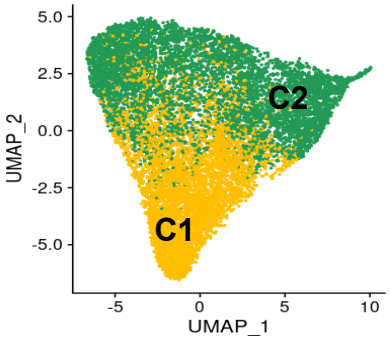
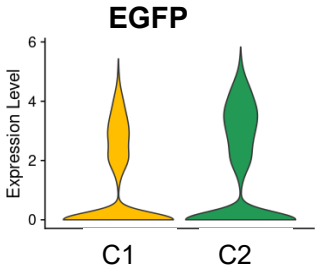
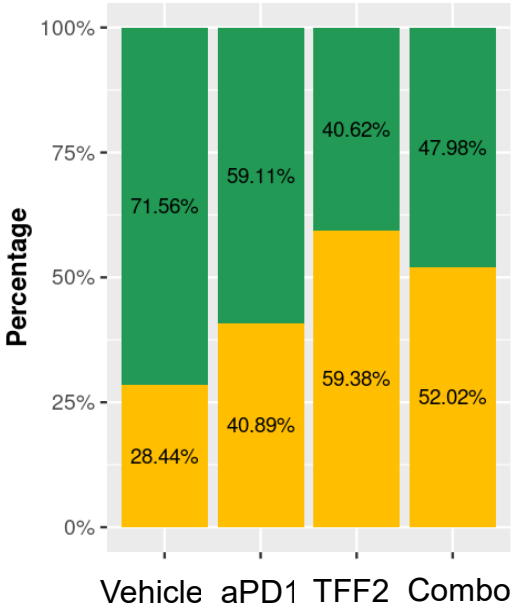
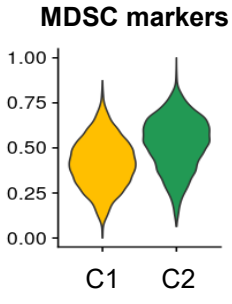
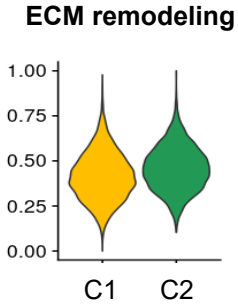
Subclustering of tumor PMNs

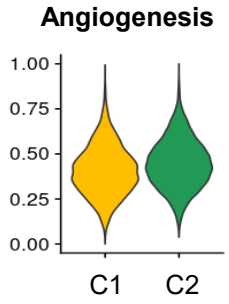


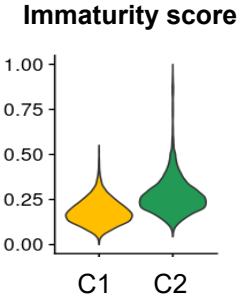


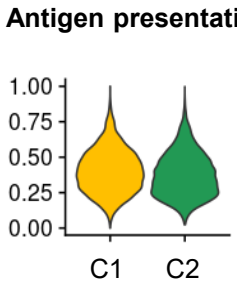


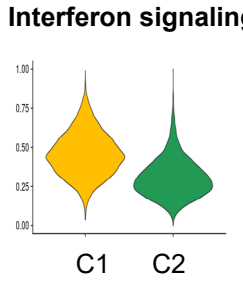












- Cluster 2 reduced by TFF2-MSA enriched for MDSC markers, ECM remodeling and angiogenesis genes.
- Cluster 1 increased by TFF2-MSA was characterized by antigen presentation genes and interferon signaling.

Gungabeesoon J, *et al*. Cell. 2023
Vafadarnejad E *et al*. Circ Res. 2020

TFF2-MSA treatment inhibited immunosuppression of PMNs

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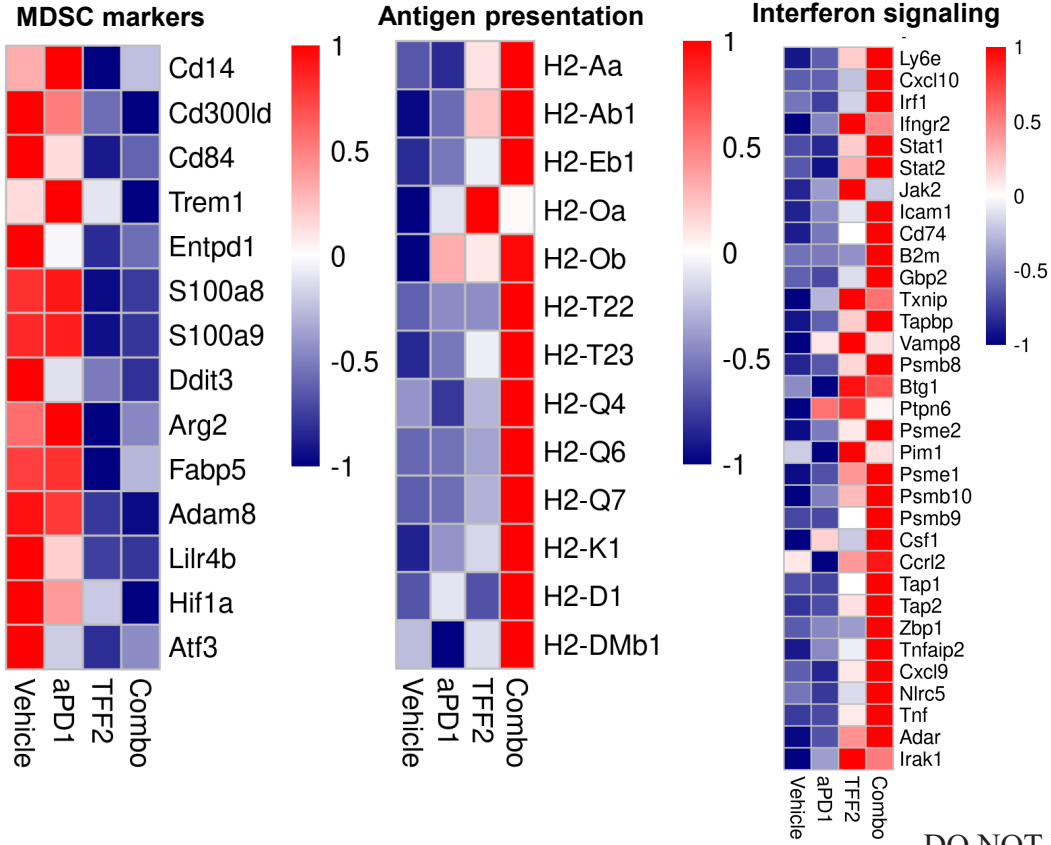

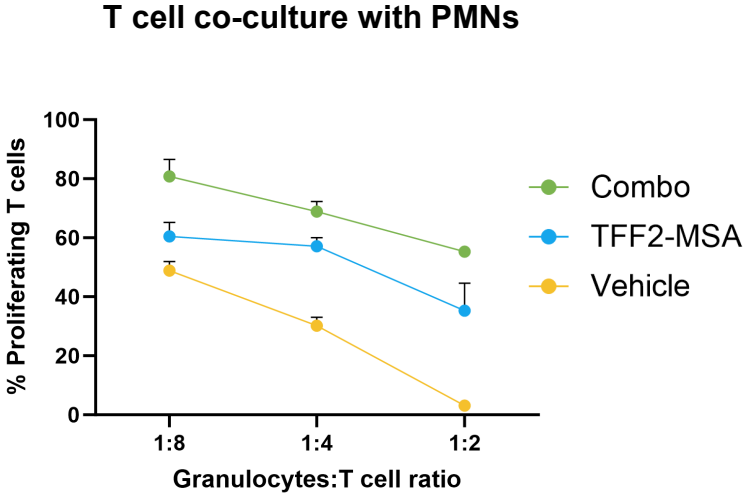

- Tumor PMNs from TFF2-MSA and combo treated mice exhibited less immunosuppression against T cell proliferation.

Combination therapy of TFF2-MSA and aPD-1 induced robust anti-tumor cytotoxic CD8 T cell response

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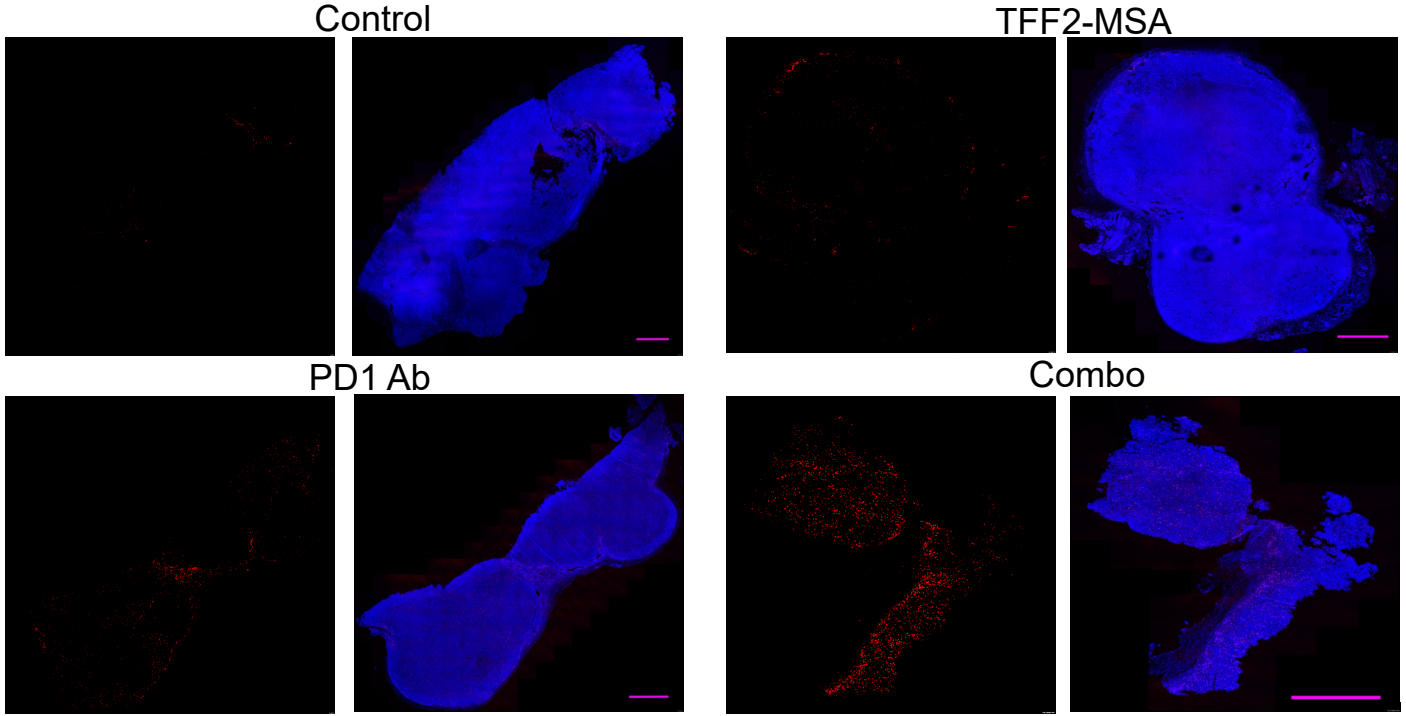
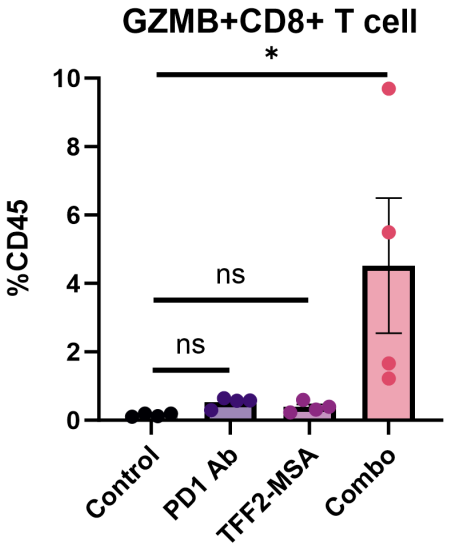
CD8 staining on whole tumor sections





Combination therapy of TFF2-MSA and aPD-1 induced robust anti-tumor cytotoxic CD8 T cell response

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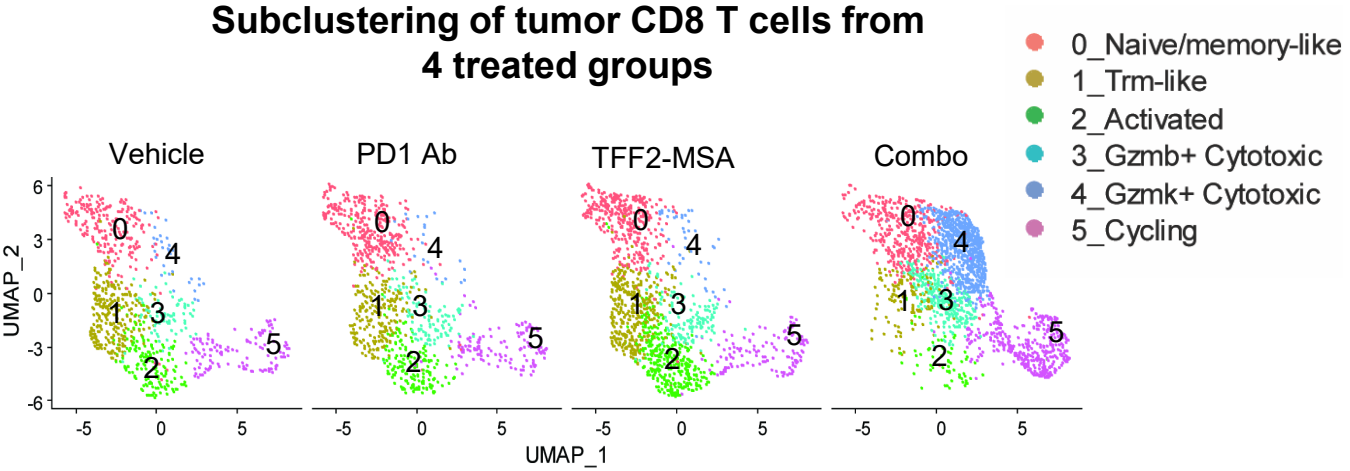

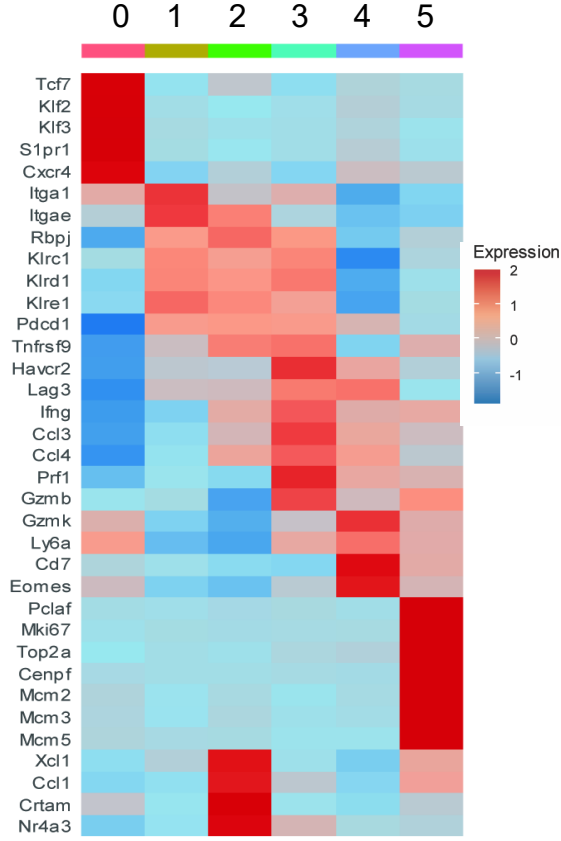

- Cytotoxic CD8 T cells enriched in only combination-treated tumor:
  - Cluster 3 - Gzmb+ Prf1+IFNg+ cells
  - Cluster 4 - Gzmk+ Eomes+ cells
  - Cluster 5 – proliferating/cycling Gzmk+ cells

The superior efficacy of combo therapy (TFF2-MSA+aPD-1) was due to selective targeting of PMN-MDSCs

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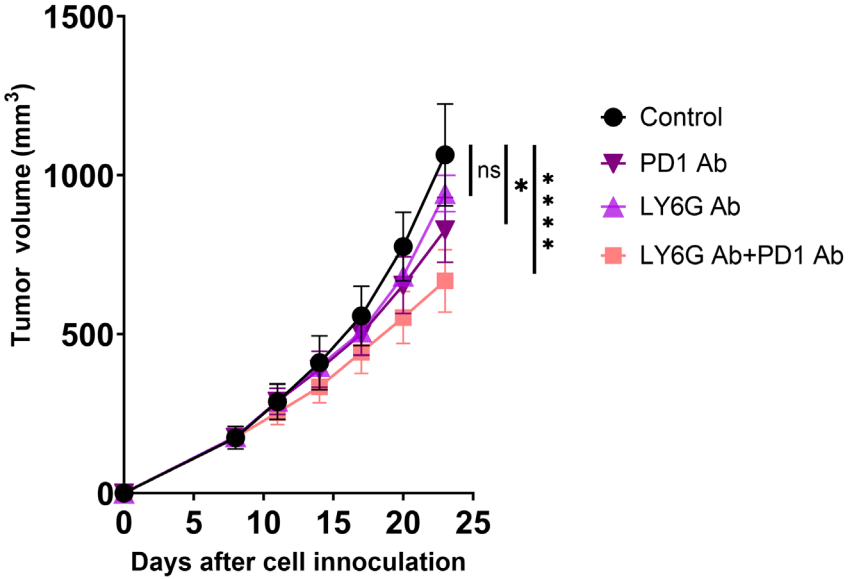

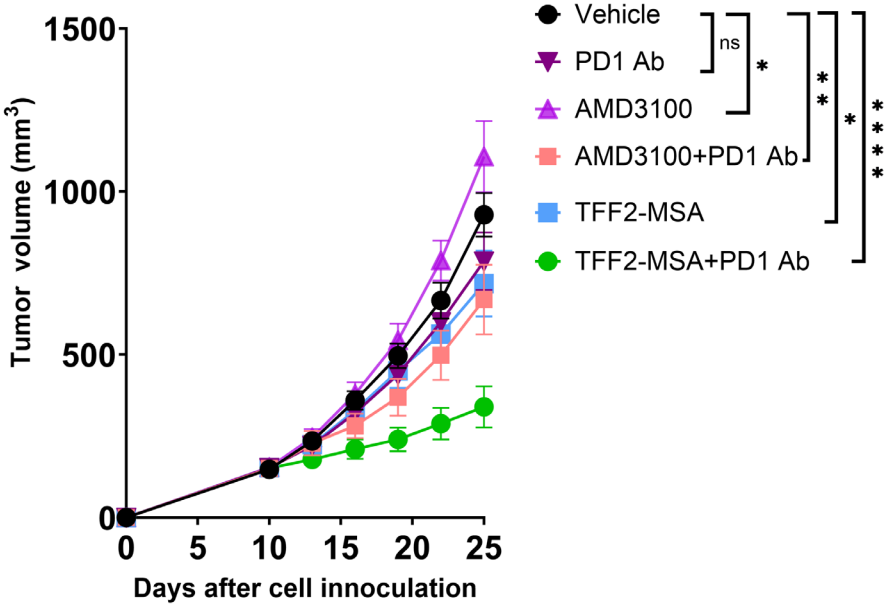

- Total neutrophil depletion by anti-LY6G antibody only slightly improved aPD-1 therapy efficacy.
- AMD3100 as a CXCR4 full antagonist alone accelerated tumor growth, while its combination with aPD-1 antibody did not effectively inhibit tumor growth as TFF2-MSA.

For neutrophil depletion, mice received 75 μg Ly6G-specific antibody (clone 1A8) i.p. every second day, and 50 μg anti-rat immunoglobulin (clone MAR 18.5) i.p. every 24 hours. AMD3100 was given at 5mg/kg i.p. every day).
Boivin G, *et al*. Nat Commun. 2020

Summary

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- TFF2-MSA enhanced treatment efficacy of anti-PD-1 immunotherapy in advanced mouse models of syngeneic gastric cancer.
- TFF2-MSA reduced HDC-GFP$^+$ PMN-MDSC accumulation in the periphery and myelopoiesis in the bone marrow.
- TFF2-MSA inhibited PMN-MDSC immunosuppression, thereby inducing robust anti-tumor cytotoxic T cell response when combined with anti-PD-1 immunotherapy.
- TFF2-MSA functioned as a CXCR4 partial agonist that preferentially targets HDC-GFP$^+$ PMN-MDSCs.
- CXCR4 partial agonism may be a more efficacious strategy than its antagonism to improve responsiveness of anti-PD-1 immunotherapy.

Acknowledgement

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Current Wang lab

Xiaofei Zhi
Quin T. Waterbury
Hiroki Kobayashi
Feijing Wu
Ermanno Malagola
Ruhong Tu
Biyun Zheng
Ruth A. White
Yosuke Ochiai
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Bioinformatics

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