Striking a Balance in Anti-tumoral Immunity: A CXCR4 Partial Agonist Overcomes Immune Suppression in Gastric Cancer

Solid tumors such as gastric cancer exploit the CXCL12–CXCR4 axis to recruit large numbers of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs), orchestrating an immunosuppressive microenvironment and leading to resistance to PD-1/PD-L1 antibodies. Previous attempts to block this pathway using CXCR4 full antagonists have limited treatment efficacy, due to compensatory granulopoiesis triggered in the bone marrow. Further, neutrophils in tumors are highly heterogeneous—some are pro-tumorigenic, while others have anti-tumor functions. Is it possible to eliminate the "bad" ones while preserving the "good"?

On June 26, ***Cancer Cell*** (IF 44.5) published a study entitled ″A CXCR4 partial agonist improves immunotherapy by targeting immunosuppressive neutrophils and cancer-driven granulopoiesis″, conducted by Columbia University in collaboration with Tonix Pharmaceuticals. The study proposes a novel strategy: TFF2-MSA, a CXCR4 partial agonist, which precisely modulates CXCR4 signaling by selectively reducing immunosuppressive neutrophils.

Trefoil Factor 2 (TFF2) is a secreted partial agonist of CXCR4 that can diminish activation induced by the full agonist CXCL12, while preserving milder and more selective chemotaxis. To effectively target PMN-MDSCs, the researchers fused TFF2 with mouse serum albumin (MSA), creating a more stable peptide, TFF2-MSA. TFF2-MSA plus anti-PD-1 significantly inhibits tumor growth and metastases while extending survival across various gastric cancer models. The authors also explored the mechanism of action via multiple approaches and assessed clinical relevance in patient samples.

Columbia University has licensed the therapeutic application of TFF2 technology to Tonix Pharmaceuticals. Tonix Pharmaceuticals' TNX-1700 is the human version of mouse TFF2-MSA, a fusion protein of human TFF2 (hTFF2) and human serum albumin (HSA). And it is under preclinical development for the treatment of gastric and colorectal cancers.

Bio X Cell *in vivo* Antibodies Empower the Study

This study made extensive use of mouse models—Figure 1 alone involved nearly 250 mice, and with relevant Supplementary Figures 1–3, nearly 400 mice were used to evaluate the combination treatment's efficacy. Twelve high-quality *in vivo* antibodies from Bio X Cell were utilized throughout efficacy and mechanistic studies, making a significant contribution to the research.

- **Robust baseline for combination therapy evaluation**

Bio X Cell's anti-PD-1, #BE0273 (clone 29F.1A12) and #BE0146 (clone RMP1-14), were used as monotherapy baseline of immune checkpoint inhibitor. When combining TFF2-MSA, they synergistically inhibit tumor growth and metastases and prolong survival in mouse models of gastric cancer. Anti-LAG-3 antibody (clone C9B7W) was also deployed, demonstrating TFF2-MSA's broad applicability.

Mouse models used to evaluate monotherapy vs combination therapy:

- ACKP (*Atp4b*-Cre;*Cdh1*$^{-/-}$;LSL-*Kras*$^{G12D/+}$;*Trp53*$^{-/-}$) cell line-based subcutaneous, orthotopic, liver metastases models via portal vein, and spontaneous lung metastases following surgical resection, representing mixed-type gastric cancer; standard chemotherapy was also included as a first-line treatment in the subcutaneous model.
- PC (*Trp53*$^{-/-}$;*CCNE1*-overexpressed) organoid-transplant model representing intestinal-type gastric cancer.
- Autochthonous diffuse-type gastric cancer model with an inducible *RHOA*$^{Y42C/Y42C}$ mutation and *Cdh1* loss (*Mist1*-CreERT;*Cdh1*$^{flox/flox}$;LSL- *RHOA*$^{Y42C/Y42C}$;*Hdc*-GFP).
- Other gastrointestinal cancer models include subcutaneous and orthotopic CT26 colon cancer, and Panc02 pancreatic cancer.
- Anti-LAG-3 combined with TFF2-MSA in the ACKP model to evaluate generalizability.

- ***In vivo* depletion to identify key anti-tumor cell type**

The results of flow cytometry and scRNA-seq showed that TFF2-MSA and the combination therapy increased intra-tumoral CD8$^+$ T cells, especially cytotoxic and IFNγ$^+$TNFα$^+$ multifunctional CD8$^+$ cells. To identify key players in combination treatment synergy, *in vivo* lymphocyte depletion was performed using #BE0003-3 (anti-CD4, clone YTS177), #BE0004-1 (anti-CD8α, clone 53-6.7) and #BE0036 (anti-NK1.1, clone PK136), and confirmed by flow cytometry analysis. Only CD8$^+$ T cell depletion completely abolished combination therapy-mediated tumor control, indicating that CD8$^+$ T cells are essential for TFF2-MSA's synergy with PD-1 therapy.

- **Cytokine/chemokine neutralization reveals immune cooperation**

scRNA-seq also revealed that dendritic cells expressing high levels of *IL12b* expanded by combination treatment. The authors reasoned that the synergistic efficacy requires the cooperation between IL-12$^+$ DCs, IFNγ$^+$ CD8$^+$ T cells, and remaining immunostimulatory PMNs. Neutralizing IFNγ (by #BE0054, anti-IFNγ, clone R4-6A2), IL-12 (by #BE0051, anti-IL-12p40, clone C17.8), or CXCL10 (by #BE0440, anti-

CXCL10, clone 1F11) expressed by *Hdc*-GFP^{-} PMNs reversed the tumor response to combination therapy, indicating that the regimen activates both innate and adaptive immunity to maximize CD8^{+} T cell anti-tumor activity.

- **Comparison with existing PMN-Targeted strategies**

The study compared TFF2-MSA to existing PMN-targeted strategies, such as anti-LY6G (#BE0075-1, clone 1A8). Despite adopting an optimized protocol (anti-LY6G combined with anti-rat Kappa Immunoglobulin Light Chain, #BE0122, clone MAR 18.5) enhancing peripheral PMN depletion, anti-LY6G did not significantly enhance ACKP tumor responsiveness to anti-PD-1, suggesting compensatory PMN production. Interestingly, adding anti-LY6G to the combo of TFF2-MSA plus PD-1 diminished therapeutic efficacy, revealing that combo efficacy is reliant in part on peripheral PMNs, consistent with the role of CXCL10^{+} PMNs.

From immune checkpoint blockade to lymphocyte depletion, cytokine and chemokine neutralization, and reliable isotype controls, twelve *InVivo*MAb antibodies with high purity, low endotoxin, and azide-free formulations ensure the safety of *in vivo* applications up to several weeks and the reproducibility of the results.

By proving the feasibility of "partial activation" via TFF2-MSA, this study offers new insights into overcoming anti-PD-1 resistance. TNX-1700 is advancing toward clinical development—and Bio X Cell antibodies will continue to empower oncology research and therapeutic innovation.

FEATURED PRODUCTS:

The following Bio X Cell antibodies are featured in the study:

- *InVivo*MAb anti-mouse PD-1 (CD279) (BE0273)
- *InVivo*MAb anti-mouse PD-1 (CD279) (BE0146)
- *InVivo*MAb anti-mouse LAG-3 (BE0174)
- *InVivo*MAb anti-mouse CD4 (BE0003-3)
- *InVivo*MAb anti-mouse CD8α (BE0004-1)
- *InVivo*MAb anti-mouse NK1.1 (BE0036)
- *InVivo*MAb anti-mouse IFNγ (BE0054)
- *InVivo*MAb anti-mouse IL-12 p40 (BE0051)
- *InVivo*MAb anti-mouse CXCL10 (IP-10) (BE0440)
- *InVivo*MAb anti-mouse Ly6G (BE0075-1)
- *InVivo*MAb anti-rat Kappa Immunoglobulin Light Chain (BE0122)
- *InVivo*Mab rat IgG2a isotype control, anti-trinitrophenol (BE0089)

Reference

- Qian J, et al. A CXCR4 partial agonist improves immunotherapy by targeting immunosuppressive neutrophils and cancer-driven granulopoiesis. *Cancer Cell*. 2025 Online ahead of print. https://doi.org/10.1016/j.ccell.2025.06.006
- Tonix Pharmaceuticals Announces Peer-Reviewed Publication in Cancer Cell Journal Highlighting Positive Preclinical Data of mTNX-1700 in Gastric Cancer Animal Models. Tonix Pharmaceuticals Press Release.