UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
Washington, D.C. 20549

FORM 8-K

CURRENT REPORT

Pursuant to Section 13 or 15(d) of the Securities Exchange Act of 1934

Date of report (date of earliest event reported): April 19, 2023

TONIX PHARMACEUTICALS HOLDING CORP.

(Exact name of registrant as specified in its charter)

Nevada	**001-36019**	**26-1434750**
(State or Other Jurisdiction of Incorporation)	**(Commission File Number)**	**(IRS Employer Identification No.)**

26 Main Street, Chatham, New Jersey 07928
(Address of principal executive offices) (Zip Code)

Registrant's telephone number, including area code: (862) 904-8182

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions (see General Instruction A.2. below):

☐ Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)
☐ Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)
☐ Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))
☐ Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Securities registered pursuant to Section 12(b) of the Act:

Title of each class	Trading Symbol(s)	Name of each exchange on which registered
Common Stock	TNXP	The NASDAQ Capital Market

Indicate by check mark whether the registrant is an emerging growth company as defined in Rule 405 of the Securities Act of 1933 (§ 230.405 of this chapter) or Rule 12b-2 of the Securities Exchange Act of 1934 (§ 240.12b-2 of this chapter).

Emerging growth company ☐

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 13(a) of the Exchange Act. ☐

Item 7.01 Regulation FD Disclosure.

On April 19, 2023, Tonix Pharmaceuticals Holding Corp. (the "Company") announced the presentation of two posters with research results on the Company's TNX-1700 (recombinant TFF2 – albumin fusion peptide) product candidate at the American Association for Cancer Research ("AACR") Annual Meeting, held April 14, 2023 to April 19, 2023 (the "Posters"). A copy of the press release which discusses this matter is furnished hereto as Exhibit 99.01, and incorporated herein by reference. The Posters, which may contain nonpublic information, are filed as Exhibits 99.02 and 99.03 hereto and incorporated herein by reference.

The information in this Item 7.01 of this Current Report on Form 8-K, including Exhibits 99.01, 99.02 and 99.03 attached hereto, shall not be deemed "filed" for purposes of Section 18 of the United States Securities Exchange Act of 1934 (the "Exchange Act") or otherwise subject to the liabilities of that section, nor shall they be deemed incorporated by reference in any filing under the United States Securities Act of 1933 or the Exchange Act, except as shall be expressly set forth by specific reference in such a filing.

Item 8.01. Other Events.

On April 19, 2023, the Company announced the presentation of the Posters at the AACR. One poster presentation, entitled, "*MDSC-targeted TFF2-MSA suppresses tumor growth and increases survival in anti-PD-1 treated MC38 and CT26.wt murine colorectal cancer models,"* includes data demonstrating that targeting myeloid-derived suppressor cells ("MDSCs") using murine TNX-1700, or mTNX-1700 (TFF2-MSA fusion protein) synergizes with PD-1 blockade therapy in advanced syngeneic mouse models of colorectal cancer. The data show that mTNX-1700 and anti-PD-1 monotherapy each were able to evoke anti-tumor immunity in the MC38 and CT26.wt models of colorectal cancer, and that mTNX-1700 augmented the anti-tumor efficacy of anti-PD-1 therapy in both of these colorectal cancer models.

The Second poster presentation, entitled, "*MDSC-targeted TFF2-MSA synergizes with PD-1 blockade therapy in diffuse-type gastric cancer,"* includes data showing that targeting MDSCs using mTNX-1700 synergizes with PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of diffuse-type gastric cancer, suggesting combination therapy of mTNX-1700 and PD-1 blockade may also be applicable to gastric cancer. The Company believes these data demonstrate that targeting MDSCs using mTNX-1700 provides additive benefits to PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of colorectal and gastric cancer.

Forward- Looking Statements

This Current Report on Form 8-K contains certain forward-looking statements within the meaning of Section 27A of the Securities Act of 1933 and Section 21E of the

Securities Exchange Act of 1934 and Private Securities Litigation Reform Act, as amended, including those relating to the Company's product development, clinical trials, clinical and regulatory timelines, market opportunity, competitive position, possible or assumed future results of operations, business strategies, potential growth opportunities and other statement that are predictive in nature. These forward-looking statements are based on current expectations, estimates, forecasts and projections about the industry and markets in which we operate and management's current beliefs and assumptions.

These statements may be identified by the use of forward-looking expressions, including, but not limited to, "expect," "anticipate," "intend," "plan," "believe," "estimate," "potential," "predict," "project," "should," "would" and similar expressions and the negatives of those terms. These statements relate to future events or our financial performance and involve known and unknown risks, uncertainties, and other factors which may cause actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. Such factors include those set forth in the Company's filings with the SEC. Prospective investors are cautioned not to place undue reliance on such forward-looking statements, which speak only as of the date of this press release. The Company undertakes no obligation to publicly update any forward-looking statement, whether as a result of new information, future events or otherwise.

Item 9.01 Financial Statements and Exhibits.

(d)

Exhibit No.	Description.
99.01	Press Release of the Company, dated April 19, 2023
99.02	MDSC-targeted TFF2-MSA suppresses tumor growth and increases survival in anti-PD-1 treated MC38 and CT26.wt murine colorectal cancer models
99.03	MDSC-targeted TFF2-MSA synergizes with PD-1 blockade therapy in diffuse-type gastric cancer
104	Cover Page Interactive Data File (embedded within the Inline XBRL document)

SIGNATURE

Pursuant to the requirement of the Securities Exchange Act of 1934, the registrant has duly caused this report to be signed on its behalf by the undersigned thereunto duly authorized.

TONIX PHARMACEUTICALS HOLDING CORP.

Date: April 19, 2023

By: /s/ Bradley Saenger
Bradley Saenger
Chief Financial Officer

Exhibit 99.01

Tonix Pharmaceuticals Announces Presentations of Pre-Clinical Data on TNX-1700 in Syngeneic Models of Colorectal and Gastric Cancer at the American Association for Cancer Research Annual Meeting 2023

CHATHAM, N.J., April 19, 2023 – Tonix Pharmaceuticals Holding Corp. (Nasdaq: TNXP), a clinical-stage biopharmaceutical company, today announced the presentation of two posters with research results on TNX-1700 (recombinant TFF2 – albumin fusion peptide) at the American Association for Cancer Research (AACR) Annual Meeting, held April 14-19, 2023, in Orlando, Fla. Copies of the Company's posters are available under the Scientific Presentations tab of the Tonix website at www.tonixpharma.com.

The poster presentation, titled, *"MDSC-targeted TFF2-MSA suppresses tumor growth and increases survival in anti-PD-1 treated MC38 and CT26.wt murine colorectal cancer models,"* includes data demonstrating that targeting myeloid-derived suppressor cells (MDSCs) using murine TNX-1700, or mTNX-1700 (TFF2-MSA fusion protein) synergizes with PD-1 blockade therapy in advanced syngeneic mouse models of colorectal cancer. The data show that mTNX-1700 and anti-PD-1 monotherapy each were able to evoke anti-tumor immunity in the MC38 and CT26.wt models of colorectal cancer, and that mTNX-1700 augmented the anti-tumor efficacy of anti-PD-1 therapy in both of these colorectal cancer models.

The poster presentation, titled, "*MDSC-targeted TFF2-MSA synergizes with PD-1 blockade therapy in diffuse-type gastric cancer,*" includes data showing that targeting MDSCs using mTNX-1700 synergizes with PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of diffuse-type gastric cancer, suggesting combination therapy of mTNX-1700 and PD-1 blockade may also be applicable to gastric cancer.

"We believe these data demonstrate that targeting MDSCs using mTNX-1700 provides additive benefits to PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of colorectal and gastric cancer," said Seth Lederman, M.D., Chief Executive Officer of Tonix Pharmaceuticals.

About Trefoil Factor Family Member 2 (TFF2)

Human TFF2 is a secreted protein, encoded by the TFF2 gene in humans, that is expressed in gastrointestinal mucosa where it functions to protect and repair mucosa. TFF2 is also expressed at low levels in splenic immune cells and is now appreciated to have intravascular roles in the spleen and in the tumor microenvironment. In gastric cancer, TFF2 is epigenetically silenced, and TFF2 is suggested to be protective against cancer development through several mechanisms. Tonix is developing TNX-1700 (rTFF2-HSA) for the treatment of gastric and colon cancers under a license from Columbia University. The inventor at Columbia is Dr. Timothy Wang, who is an expert in the molecular mechanisms of carcinogenesis whose research has focused on the carcinogenic role of inflammation in modulating stem cell functions. Dr. Wang demonstrated that knocking out the mTFF2 gene in mice leads to faster tumor growth and that overexpression of TFF2 markedly suppresses tumor growth by curtailing the homing, differentiation, and expansion of MDSCs to allow activation of cancer-killing $CD8^+$ T cells.[1] He went on to show that a novel engineered form of recombinant murine TFF2 (mTFF2-CTP) had an extended half-life *in vivo* and was able to suppress MDSCs and tumor growth in an animal model of colorectal cancer. Later, he showed in gastric cancer models that suppressing MDSCs using chemotherapy enhances the effectiveness of anti-PD1 therapy and significantly reduces tumor growth.[2] Dr. Wang proposed the concept of employing rTFF2 in combination with other therapies in cancer prevention and early treatment. Dr. Wang presented data at the American Association for Cancer Research (AACR) conference as a collaboration between Tonix and Columbia University in 2020 that includes data from a preclinical study which investigated the role of PD-L1 in colorectal tumorigenesis and evaluated the utility of targeting myeloid-derived suppressor cells (MDSCs) in combination with PD-1 blockade in mouse models of colorectal cancer. The data show that anti-PD-1 monotherapy was unable to evoke anti-tumor immunity in this model of colorectal cancer, but mTFF2-CTP augmented the efficacy of anti-PD-1 therapy. Anti-PD-1 in combination with TFF2-CTP showed greater anti-tumor activity in PD-L1-overexpressing mice.

Tonix Pharmaceuticals Holding Corp.[*]

Tonix is a clinical-stage biopharmaceutical company focused on discovering, licensing, acquiring and developing therapeutics to treat and prevent human disease and alleviate suffering. Tonix's portfolio is composed of central nervous system (CNS), rare disease, immunology and infectious disease product candidates. Tonix's CNS portfolio includes both small molecules and biologics to treat pain, neurologic, psychiatric and addiction conditions. Tonix's lead CNS candidate, TNX-102 SL (cyclobenzaprine HCl sublingual tablet), is in mid-Phase 3 development for the management of fibromyalgia with topline data expected in the fourth quarter of 2023. TNX-102 SL is also being developed to treat Long COVID, a chronic post-acute COVID-19 condition. Enrollment in a Phase 2 study has been completed, and topline results are expected in the third quarter of 2023. TNX-1900 (intranasal potentiated oxytocin), in development for chronic migraine, is currently enrolling with topline data expected in the fourth quarter of 2023. TNX-601 ER (tianeptine hemioxalate extended-release tablets), a once-daily formulation being developed as a treatment for major depressive disorder (MDD), is also currently enrolling with interim data expected in the fourth quarter of 2023. TNX-1300 (cocaine esterase) is a biologic designed to treat cocaine intoxication and has been granted Breakthrough Therapy designation by the FDA. A Phase 2 study of TNX-1300 is expected to be initiated in the second quarter of 2023. Tonix's rare disease portfolio includes TNX-2900 (intranasal potentiated oxytocin) for the treatment of Prader-Willi syndrome. TNX-2900 has been granted Orphan Drug designation by the FDA. Tonix's immunology portfolio includes biologics to address organ transplant rejection, autoimmunity and cancer, including TNX-1500, which is a humanized monoclonal antibody targeting CD40-ligand (CD40L or CD154) being developed for the prevention of allograft and xenograft rejection and for the treatment of autoimmune diseases. A Phase 1 study of TNX-1500 is expected to be initiated in the second quarter of 2023. Tonix's infectious disease pipeline includes TNX-801, a vaccine in development to prevent smallpox and mpox, for which a Phase 1 study is expected to be initiated in the second half of 2023. TNX-801 also serves as the live virus vaccine platform or recombinant pox vaccine platform for other infectious diseases. The infectious disease portfolio also includes TNX-3900 and TNX-4000, classes of broad-spectrum small molecule oral antivirals.

[*] *All of Tonix's product candidates are investigational new drugs or biologics and have not been approved for any indication.*

[1] *Dubeykovskaya ZA et al, Nat Commun 2016*

[2] *Kim W et al, Gastroenterology 2021*

This press release and further information about Tonix can be found at www.tonixpharma.com.

Forward Looking Statements

Certain statements in this press release are forward-looking within the meaning of the Private Securities Litigation Reform Act of 1995. These statements may be identified by the use of forward-looking words such as “anticipate,” “believe,” “forecast,” “estimate,” “expect,” and “intend,” among others. These forward-looking statements are based on Tonix's current expectations and actual results could differ materially. There are a number of factors that could cause actual events to differ materially from those indicated by such forward-looking statements. These factors include, but are not limited to, risks related to the failure to obtain FDA clearances or approvals and noncompliance with FDA regulations; delays and uncertainties caused by the global COVID-19 pandemic; risks related to the timing and progress of clinical development of our product candidates; our need for additional financing; uncertainties of patent protection and litigation; uncertainties of government or third party payor reimbursement; limited research and development efforts and dependence upon third parties; and substantial competition. As with any pharmaceutical under development, there are significant risks in the development, regulatory approval and commercialization of new products. Tonix does not undertake an obligation to update or revise any forward-looking statement. Investors should read the risk factors set forth in the Annual Report on Form 10-K for the year ended December 31, 2022, as filed with the Securities and Exchange Commission (the “SEC”) on March 13, 2023, and periodic reports filed with the SEC on or after the date thereof. All of Tonix's forward-looking statements are expressly qualified by all such risk factors and other cautionary statements. The information set forth herein speaks only as of the date thereof.

Contacts

Jessica Morris (corporate)
Tonix Pharmaceuticals
investor.relations@tonixpharma.com
(862) 904-8182

Maddie Stabinski (media)
Russo Partners
Madeline.Stabinski@russopartnersllc.com
(212) 845-4273

Peter Vozzo (investors)
ICR Westwicke
peter.vozzo@westwicke.com
(443) 213-0505

Exhibit 99.02

MDSC-Targeted mTFF2-MSA (mTNX-1700*) Suppresses Tumor Growth and Increases Survival in Anti-PD-1 Treated MC38 and CT26.wt Murine Colorectal Cancer Models

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Bruce L. Daugherty[1], Rebecca J. Boohaker[2], Rebecca Johnstone[2], Karr Stinson[2], Jin Qian[3], Timothy C. Wang[3], Seth Lederman[1]

[1]Tonix Pharmaceuticals, Inc., Chatham, NJ, [2]Southern Research, Birmingham, AL, [3]Division of Digestive and Liver Diseases, Irving Cancer Research Center, Columbia University Medical Center, New York, NY

*TNX-1700 is an investigational new biologic and has not been approved for any indication

Abstract

Aims: Myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment are a potential therapeutic target in immune checkpoint cancer therapy, but MDSC-targeted therapies have yet been shown to improve survival. Trefoil factor family 2 (TFF2), a secreted anti-inflammatory peptide, can suppress MDSC expansion and activate tumor immunity in part through agonism of the CXCR4 receptor[1-3]. The aim of this study is to investigate whether a novel TFF2 – albumin fusion peptide (TFF2-MSA) can improve survival in anti-PD-1 treated syngeneic colorectal cancer (CRC) mouse models.

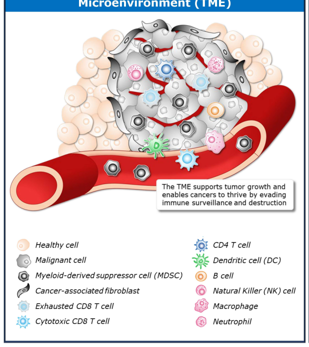
Methods: Two syngeneic colon carcinoma mouse models were developed using cell lines grafted subcutaneously into mice. MC38 CRC cells were engrafted into C57BL/6 mice while CT26.wt CRC cells were implanted into BALB/C mice We generated a recombinant fusion protein, designated mTFF2-MSA, which contains murine TFF2 fused to murine serum albumin (MSA), for the purpose of increasing half-life and reducing dose frequency. Mice subsequently received either mTFF2-MSA or anti-PD-1 antibody (clone 29F.1A12) or both, and tumor volume, and survival were measured. At the endpoint, flow cytometry was performed to examine treatment-induced effects on immune profiles.

Results: In the MC38 model, administration of mTFF2-MSA suppressed tumor growth (TGI 50%), the combination of mTFF2-MSA and anti-PD-1 had an additive effect and suppressed tumor growth dramatically (TGI 87%). The combination also exhibited a survival rate of 90% after 50 days, while vehicle and single mTFF2-MSA therapy were 20% and 50%, respectively. The percentage of exhausted CD8+ T cells was markedly reduced in the draining lymph node by the combination treatment, as measured by flow cytometry using antibodies against LAG3, TIM3 and PD-1. In the CT26.wt model, administration of mTFF2-MSA alone exhibited little effect, but the combination of anti-PD-1 and mTFF2-MSA showed a profound effect. In the CT26.wt model, administration of mTFF2-MSA suppressed tumor growth (TGI 16%), anti-PD-1 alone (TGI 40%) and the combination of mTFF2-MSA and anti-PD-1 (TGI 60%).

Conclusion: Targeting MDSCs using mTFF2-MSA fusion protein synergizes well with PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of colorectal cancer. In a separate abstract, additive effects between mTFF2-MSA and anti-PD-1 antibody were also demonstrated in a separate ACKP (Atp4b-Cre; Cdh1-/-; LSL-KrasG12D; Trp53-/-) gastric cancer model, suggesting combination therapy may also be applicable to gastric cancer.

Introduction

Tumors Create a Toxic, Immunosuppressive Microenvironment (TME)



MDSCs are a Major Therapeutic Target

- Tumors are surrounded by endothelial and stroma cells, and invading immune cells, both innate and adaptive[4,5]
- Complex regulatory network supports tumor growth, enabling cancers to thrive by evading immune surveillance and destruction[5,6]
- The TME sabotages tumor-killing cytotoxic CD8 T cells[1]
- Myeloid-derived suppressor cells (MDSCs) interfere with anticancer immunity[5,6]
- Levels of MDSCs tend to correlate with tumor stage, patient survival, and metastatic burden and may predict poor response to certain cancer treatments[7]
- MDSCs represent a central mechanism of immunosuppression in cancer; targeting these cells could significantly improve our ability to fight cancer[8,9]

Therapeutic Strategies Include[9]:

- Promoting differentiation of MDSCs to a non-immunosuppressive cell type
- Blocking MDSC immunosuppressive functions
- Inhibiting MDSC expansion
- Eliminating MDSCs

Results

Fig 1: mTFF2-MSA is a Novel Fusion Protein

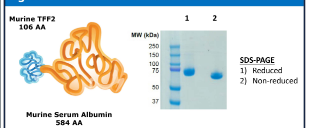


Fig 2: Schematic of Syngeneic CRC Tumor Model

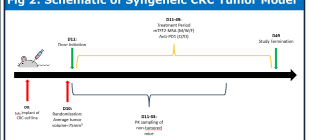


Fig 3: PK Analysis of mTFF2-MSA in Mice

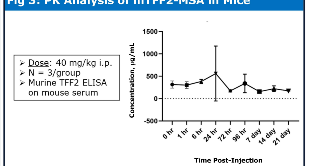


Fig 4: Inhibition of Tumor Growth in the MC38 CRC Model

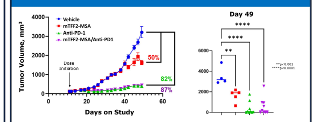
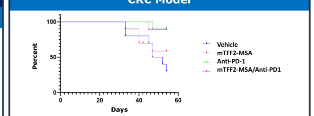


Fig 5: Probability of Survival in the MC38 CRC Model



Hazard Ratio (Mantel-Haenszel)	Vehicle/TFF2-MSA	Vehicle/Anti-PD-1	Vehicle/Combo
Ratio	1.77	7.63	6.08
95% CI	0.51 – 6.11	1.77 – 32.89	1.44 – 25.58

Fig 6: Inhibition of Tumor Growth in the CT26.wt CRC Model

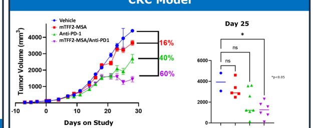
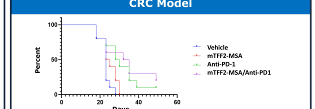
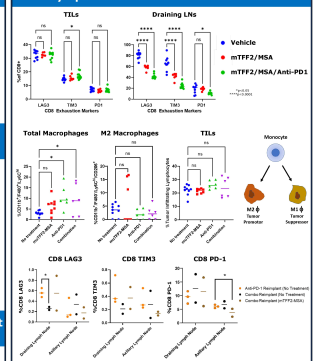


Fig 7: Probability of Survival in the CT26.wt CRC Model



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

Fig 8: Immunophenotyping of the TME and the Lymph Nodes in the MC38 CRC Model



Conclusions

- mTFF2-MSA (mTNX-1700) is a novel fusion protein and exhibits an extended half-life *in vivo* in mice.
- In the MC38 mouse model of colorectal cancer, mTFF2-MSA alone inhibited tumor growth by 50%, and is additive with anti-PD-1 by inhibiting tumor growth by 87%.
- In the MC38 model, survival was 90% in the combination treated group after 50 days, with 40% exhibiting a complete response, while 20% survived in the untreated group.
- In the MC38 model, the percentage of exhausted CD8+ T cells was markedly reduced in the draining lymph node by treatment with TFF2-MSA alone, and the combination treated group, as measured by flow cytometry using antibodies against LAG3, TIM3 and PD-1.
- In the MC38 model, the percentage of total macrophages in the tumor microenvironment were markedly increased in the anti-PD-1 and the combination treated groups.
- In the MC38 model, in animals with complete remission, comparison of exhaustion markers on CD8+ T cells, LAG3+ T cells are reduced in the draining lymph node in the combination treated group, while suppression of PD-1+ T cells are observed in the axillary lymph node.
- In the CT26.wt mouse model of colorectal cancer, mTFF2-MSA alone inhibited tumor growth by 16%, and is additive with anti-PD-1 by inhibiting tumor growth by 60%.
- In the CT26.wt model, survival was 60% in the combination treated group after 30 days, while 0% survived in the untreated group.
- TNX-1700 is a novel mechanism for suppressing MDSCs and has the potential to synergize with other immuno-oncology drugs.

References

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AACR Annual Meeting, Orlando, FL, April 16, 2023

COLUMBIA UNIVERSITY

Abstract number 5088
MDSC-targeted TFF2-MSA synergizes with PD-1 blockade therapy in advanced gastric cancer models

Jin Qian[1], Sandra Ryeom[1], Bruce Daugherty[2], Seth Lederman[2], Timothy C. Wang[1]*

1. Irving Cancer Research Center, Columbia University Medical Center, New York, NY 10032, USA
2. Tonix Pharmaceuticals, Inc., 26 Main Street, Suite 101, Chatham, NJ 07928

Abstract

Recent studies revealed chemotherapy increases anti-PD1 response of gastric cancer (GC) by reducing tumor myeloid-derived cell (MDSC). However, a more potent MDSC-targeted treatment is needed to further improve anti-PD1 efficacy in advanced GC. Trefoil factor family 2 (TFF2), a partial agonist of CXCR4 and a secreted anti- inflammatory peptide, can decrease MDSCs. Here, we developed a novel peptide TFF2-MSA (mTNX-1700) with an extended serum half-life by fusing murine TFF2 to murine serum albumin. Using a syngeneic mouse model of transplanted ACKP (Atp4b-Cre; Cdh1-/-; LSL-KrasG12D; Trp53-/-) GC cells, we investigated whether TFF2-MSA can synergize with anti-PD1 therapy by reducing MDSC accumulation and biogenesis. When the subcutaneously implanted ACKP tumors reached 150-200 mm^3, TFF2-MSA or anti-PD-1 antibody or both was given to tumor-bearing mice. Intriguingly, while either TFF2-MSA or PD-1 antibody showed little benefit as a single agent (TGI 18% and 25% respectively, p>0.05), their combination dramatically suppressed ACKP tumor growth (TGI 78%, p<0.0001) and prolonged mouse median survival (64 days vs. 32.5 days in control) in a synergistic manner. Mechanistically, the combination therapy efficiently reduced intratumoral MDSCs, and profoundly increased tumor-infiltrating CD8$^+$ T cells accompanied by a better effector phenotype. In the bone marrow, biogenesis of MDSC from its progenitors was markedly decreased by TFF2-MSA to the level of tumor-free mice. In an orthotopic model, with implantation of ACKP-luc cells into the stomach submucosa, the TFF2-MSA/PD-1 antibody combo regimen eradicated GC in 80% mice compared to 0% in either monotherapy treatment. Finally, the combination significantly reduced spontaneous lung metastasis in s.c. xenograft resected mice (vs. control, p<0.0001), compared to minimal inhibition with either monotherapy (p>0.05). Overall, our data indicate that targeting MDSCs using TFF2-MSA synergizes with PD-1 blockade therapy in advanced and metastatic syngeneic mouse models of GC.

·CONTACT:

Timothy C. Wang M.D.
tcw21@cumc.columbia.edu
Chief, Division of Digestive and Liver Diseases
Silberberg Professor of Medicine
Department of Medicine
and Irving Cancer Research Center
Columbia University Medical Center

AACR Annual Meeting, Orlando, FL, April 18, 2023
Poster #22

Introduction

- Immune suppression within the tumor microenvironment (TME) has been demonstrated as an integral barrier to the efficacy of immune checkpoint blockade therapy. A major tumor- driven mechanism of immune suppression is the generation of myeloid-derived suppressor cells (MDSCs), which impede antitumor T cell activity within the TME[1].
- Granulocytic MDSCs (PMN-MDSCs) are a heterogeneous group of immature myeloid cells that greatly expand in malignancies. They are functionally and transcriptionally distinct from mature neutrophils[2]. PMN-MDSCs are short-lived and constantly replenished by the bone marrow progenitors[3].
- Trefoil factor family 2 (TFF2) is a partial agonist for CXCR4, able to activate Ca$^+$ signaling but in the presence of SDF-1, TFF2 partial inhibits SDF-1-dependent signaling and chemotaxis[4].
- TFF2 has been shown to inhibit tumor formation by reducing MDSC expansion and proliferation in a colorectal cancer model[5].
- HDC$^+$ MDSCs expressed higher levels of CXCR4 and are more immunosuppressive than their HDC$^-$ counterparts. HDC$^+$ MDSCs profoundly expand in colorectal cancer and its reduction leads to tumor control[6].

References

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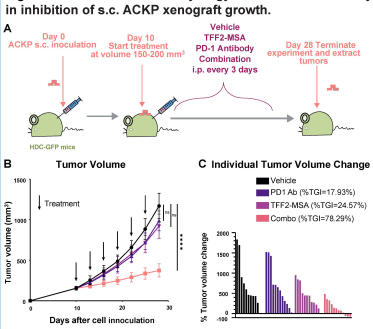
6. Chen X, *et al.* Histidine decarboxylase (HDC)-expressing granulocytic myeloid cells induce and recruit Foxp3+ regulatory T cells in murine colon cancer. Oncoimmunology. 2017 Feb 10;6(3):e1290034.

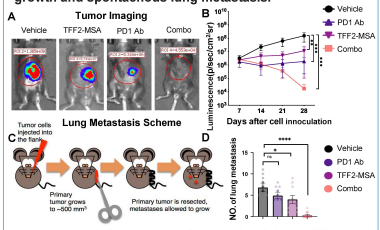
Results

Figure 1. TFF2-MSA showed synergy with anti-PD1 antibody in inhibition of s.c. ACKP xenograft growth.



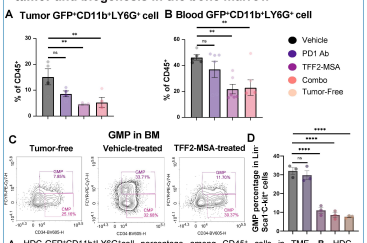
A. Schematic representation of the treatment scheme. **B.** Tumor growth curve of s.c. implanted ACKP tumors in response to anti-PD1 antibody, TFF2-MSA or their combination. **C.** Tumor volume change relative to the initial volume of each tumor. Each bar represents one tumor. Positive or negative value represents volume increase or decrease respectively. **** P < 0.0001.

Figure 2. TFF2-MSA showed synergy with anti-PD1 antibody in inhibition of orthotopic ACKP xenograft growth and spontaneous lung metastasis.



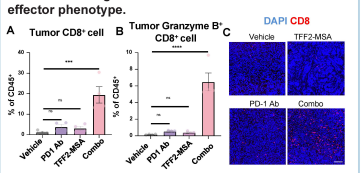
A. Representative bioluminescence images showing orthotopically injected ACKP tumors in response to different treatments. **B.** Bioluminescent intensity curves showing changes of orthotopic tumors. **C.** Schematic representation of the s.c. tumor resection scheme. **D.** Number of lung micrometastasis in mice from different treatment groups. * P < 0.05, **** P < 0.0001.

Figure 3. TFF2-MSA reduced MDSC accumulation in the tumor and biogenesis in the bone marrow



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.

Figure 4. TFF2-MSA/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.

Conclusion

TFF2-MSA (mTNX-1700) peptide synergizes with PD1 blockade therapy in advanced and metastatic GC syngeneic mouse models by reducing MDSC biogenesis and promoting a T cell-infiltrated tumor microenvironment.