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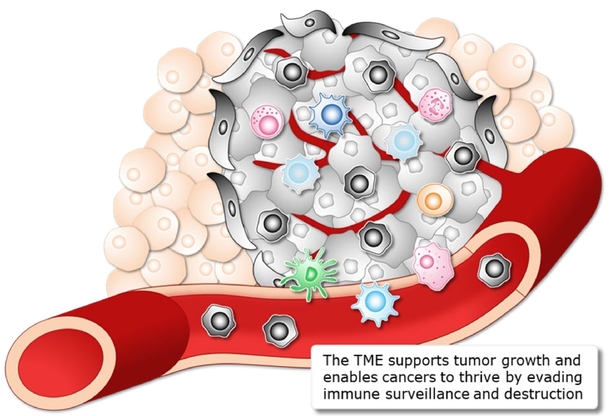
*TNX-1700 is an investigational new biologic and has not been approved for any indication

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)



- *Healthy cell*
- *Malignant cell*
- *Myeloid-derived suppressor cell (MDSC)*
- *Cancer-associated fibroblast*
- *Exhausted CD8 T cell*
- *Cytotoxic CD8 T cell*
- *CD4 T cell*
- *Dendritic cell (DC)*
- *B cell*
- *Natural Killer (NK) cell*
- *Macrophage*
- *Neutrophil*

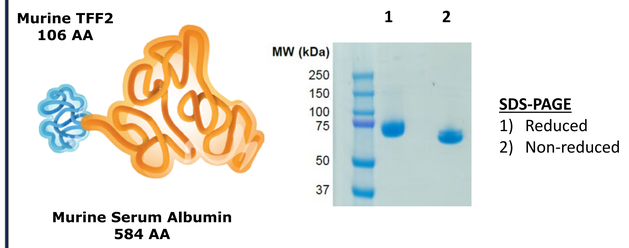
MDSCs are a Major Therapeutic Target

- 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



SDS-PAGE
1) Reduced
2) Non-reduced

Fig 2: Schematic of Syngeneic CRC Tumor Model

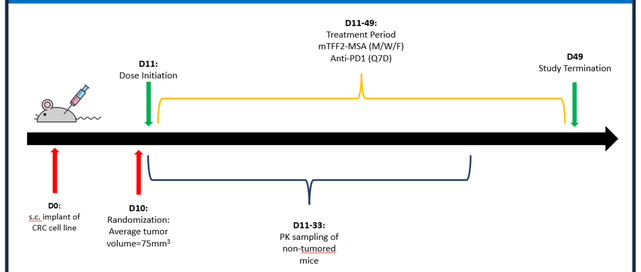


Fig 3: PK Analysis of mTFF2-MSA in Mice

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

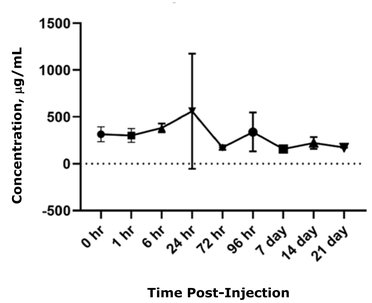


Fig 4: Inhibition of Tumor Growth in the MC38 CRC Model

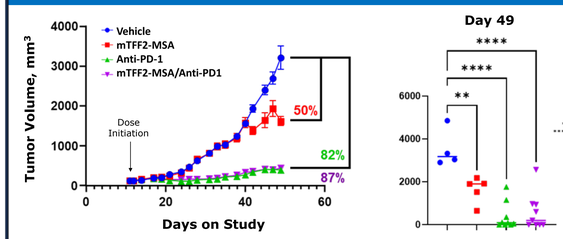
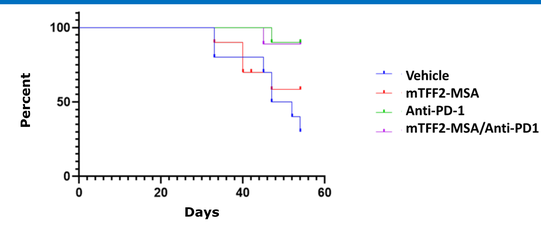


Fig 5: Probability of Survival in the MC38 CRC Model



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

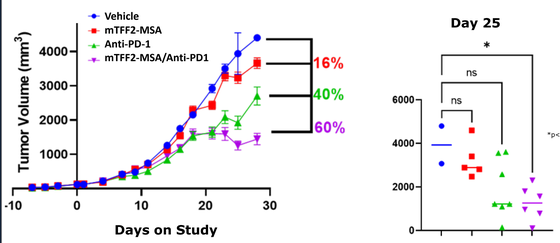
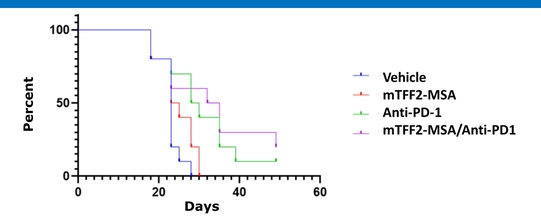
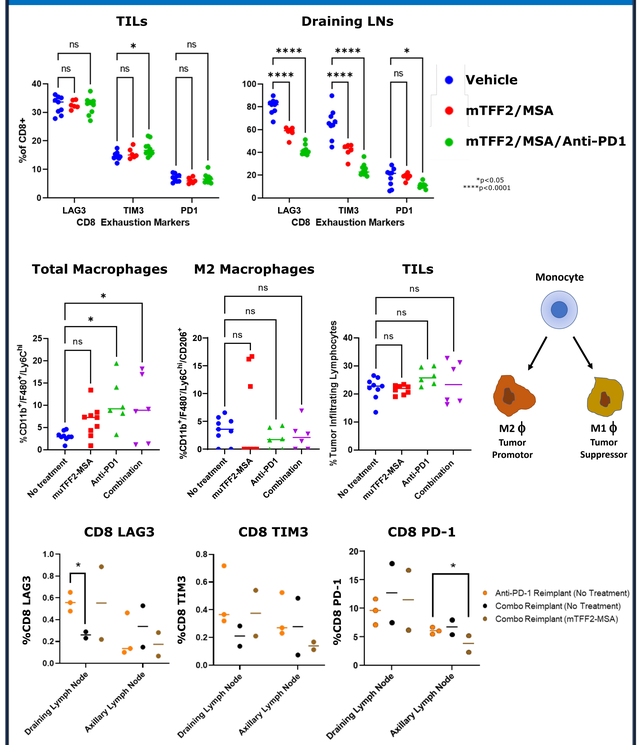


Fig 7: Probability of Survival in the CT26.wt CRC Model



Hazard Ratio (Mantel-Haenszel)	Vehicle/TFF2-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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