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mTFF2-MSA (mTNX-1700) Suppresses Tumor Growth and Increases Survival in Anti-PD-1 Treated CT26.WT Subcutaneous and CT26-Luciferase Orthotopic Syngeneic Colorectal Cancer Models by Targeting MDSCs in BALB/C Mice

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

Abstract

Introduction: Myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment are potential therapeutic targets in immune checkpoint cancer therapy, particularly for cancers that are unresponsive to anti-PD-1 therapy. It has previously been demonstrated that trefoil factor family 2 (TFF2), a secreted anti-inflammatory peptide, can partially suppress MDSC expansion and partially activate tumor immunity through agonism of the CXCR4 receptor[1-3]. We investigated whether a novel fusion protein, murine TFF2-murine serum albumin (mTFF2-MSA), has single agent activity and can improve on the therapeutic effects of anti-PD-1 in CT26.WT subcutaneous and CT26-Luciferase (CT26-Luc) orthotopic syngeneic mouse models of advanced colorectal cancer (CRC).

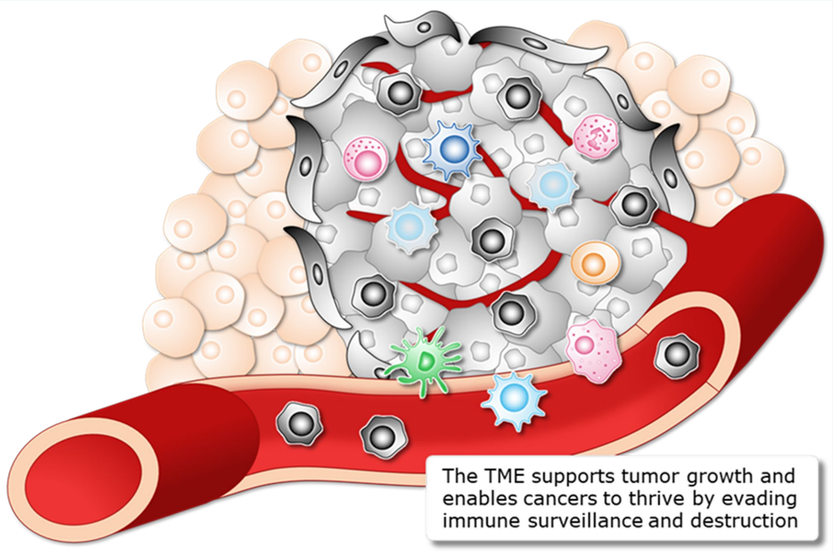
Materials and Methods: Two syngeneic colon carcinoma mouse models were developed using the CT26.WT and CT26-Luc CRC cell lines grafted subcutaneously and orthotopically, respectively, into BALB/C mice. We generated a recombinant fusion protein, designated mTFF2-MSA, which contains murine TFF2 fused to murine serum albumin (MSA), for the purpose of increasing half-life and reducing the frequency of dosing. Mice subsequently received mTFF2-MSA, anti-PD-1 antibody (clone 29F.1A12 for subcutaneous study; clone RMP-1-14 for orthotopic study) or combination of mTFF2-MSA and anti-PD-1. Tumor volume, and survival were measured. At the endpoint, flow cytometry was performed on the blood, bone marrow, tumor, and lymph nodes, to examine treatment-induced effects on cellular immune profiles.

Results: In the CT26.WT model, tumor growth was suppressed by mTFF2-MSA, anti-PD-1 and by the combination of mTFF2-MSA and anti-PD-1 by 16%, 40% and 60%, respectively. Survival in the CT26.WT model on Day 30 treated with vehicle, mTFF2-MSA, anti-PD1 and the combination of mTFF2-MSA and anti-PD-1 was 0%, 40%, 60% and 60%, respectively. In the CT26-Luc model, mTFF2-MSA, anti-PD-1, and the combination of mTFF2-MSA and anti-PD-1 suppressed tumor growth by 42%, 94%, and 94%, respectively. In the CT26-Luc model, neutrophils were significantly reduced in the blood in all treatment groups by flow cytometry. In the bone marrow, a significant reduction in total macrophages, M2 macrophages, and neutrophils was also observed but only in the group treated with anti-PD-1 and mTFF2-MSA. In the axillary lymph node, there was a significant reduction in TOX+ cells in both CD4+ and CD8+ T-cells in all treatment groups. In the tumor, there was a significant reduction in total macrophages and M2 macrophages in all treatment groups, while NK cells were also increased, but only in the combination anti-PD-1/mTFF2-MSA treated group.

Conclusions: mTFF2-MSA has single agent activity and is additive to anti-PD-1 antibody checkpoint inhibition in treating two syngeneic (subcutaneous and orthotopic) mouse models of advanced CRC.

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]

- **<u>Therapeutic Strategies Include[9]:</u>**
  - 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

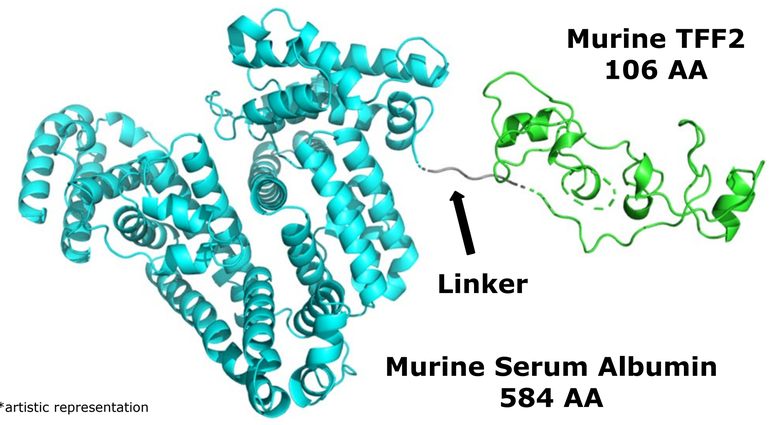


Fig 2: Schematic of Subcutaneous Tumor Model

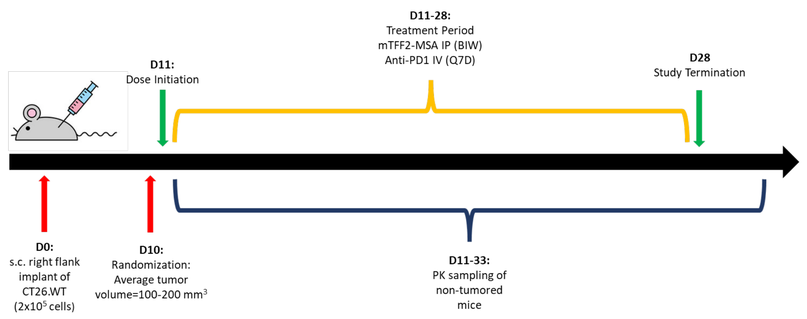


Fig 3: PK Analysis of mTFF2-MSA in Mice

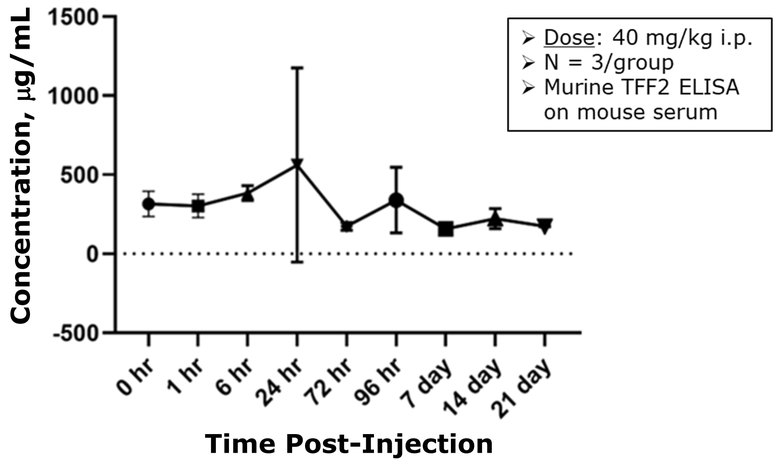


Fig 4: Inhibition of Tumor Growth in the CT26.WT Subcutaneous Model

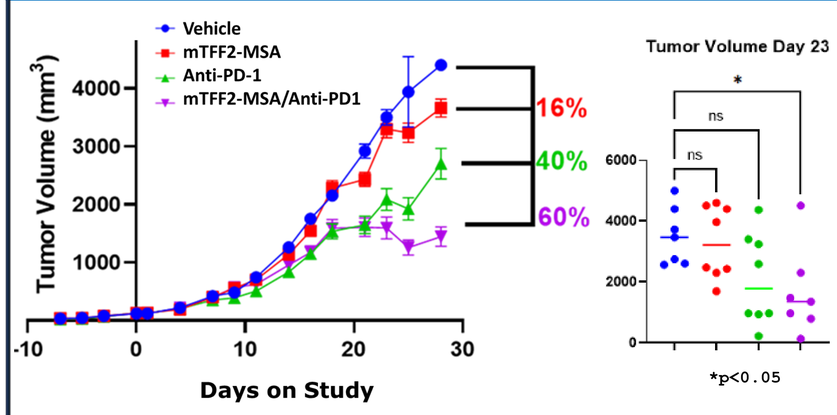
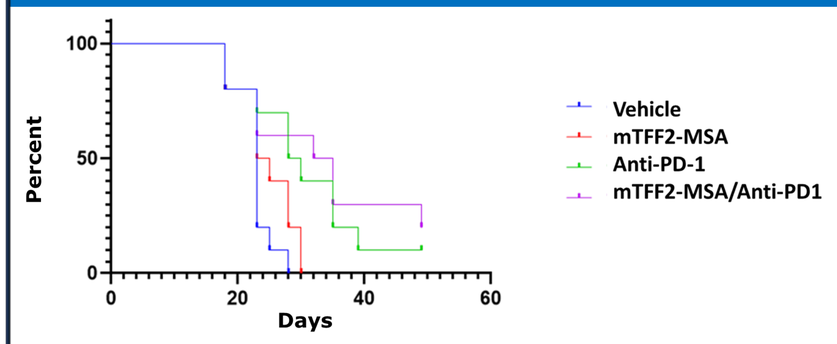


Fig 5: Probability of Survival in the CT26.WT Subcutaneous Model



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 6: Immunophenotyping of the TME in the CT26.WT Subcutaneous Model

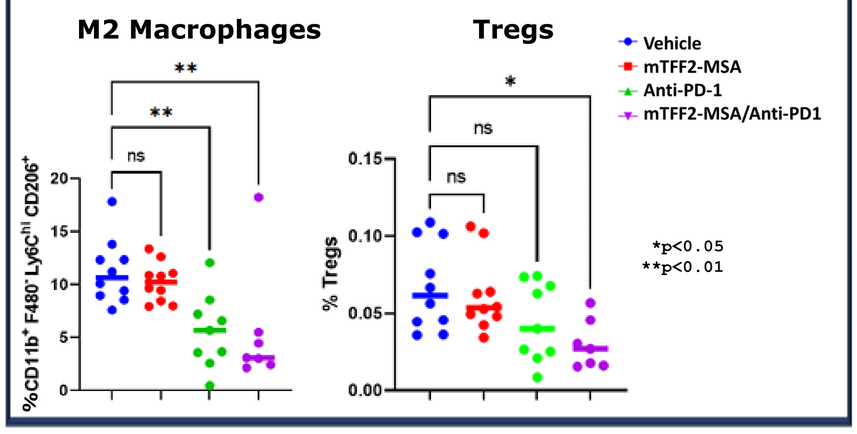


Fig 7: Schematic of Orthotopic Tumor Model

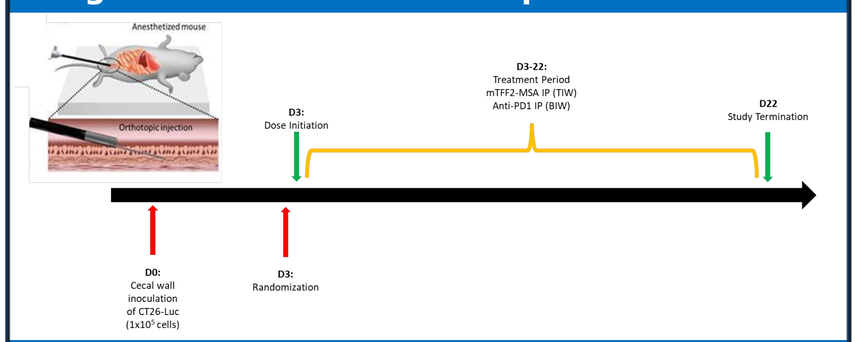


Fig 8: Inhibition of Tumor Growth in the CT26-Luc Orthotopic Model

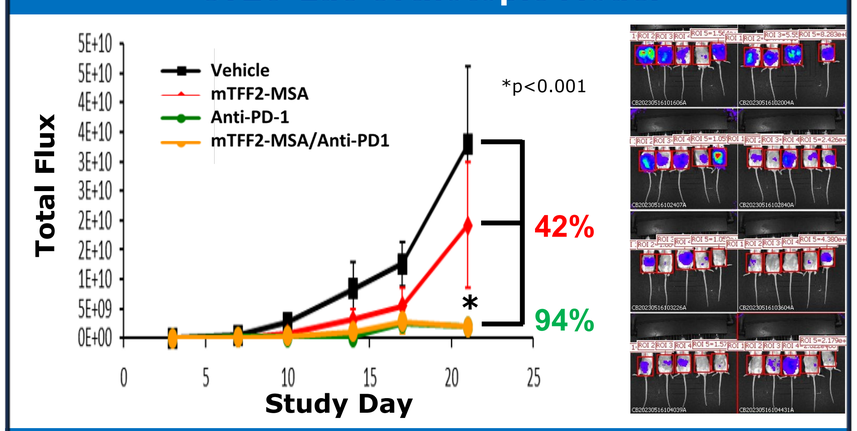


Fig 9: Tumor Weight on Day 22

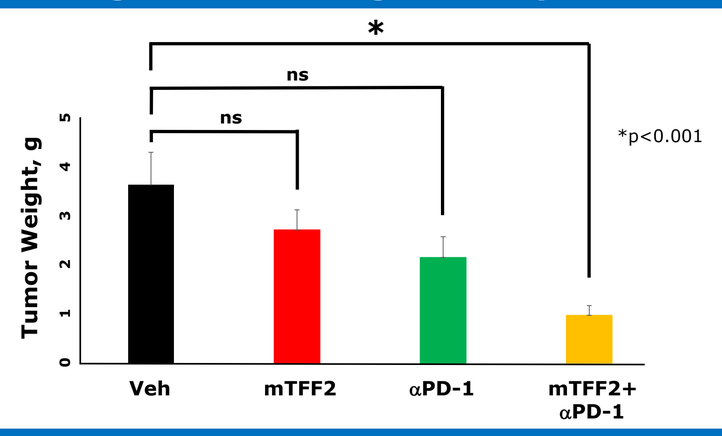
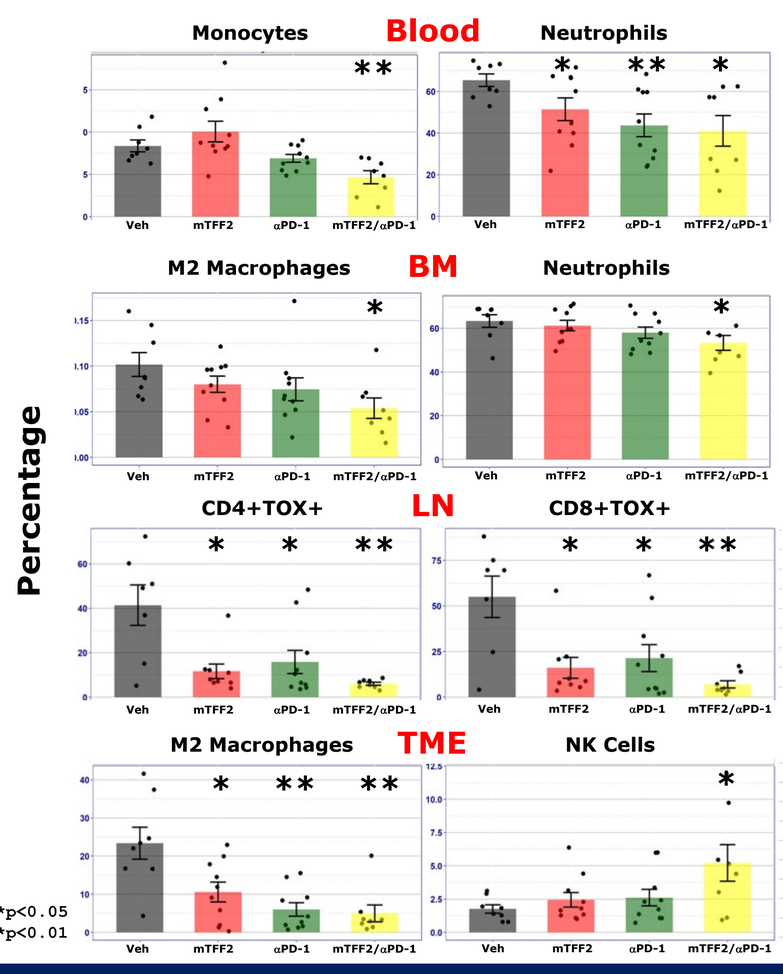


Fig 10: Immunophenotyping of Various Tissues in the CT26-Luc Orthotopic Model



Conclusions

- mTFF2-MSA (mTNX-1700) is a novel fusion protein and exhibits an extended half-life *in vivo* in mice.
- In the CT26.WT subcutaneous 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 subcutaneous model, survival was 60% in the combination treated group after 30 days, while 0% survived in the untreated group.
- In the CT26-Luc model, mTFF2-MSA alone inhibited tumor growth by 42%, while anti PD-1 and the combination treated groups inhibiting tumor growth by 94%.
- Immunophenotyping in both models revealed a decrease in tumor promoting M2 macrophages in the tumor.
- 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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