**Abstract**

Myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment are a potential therapeutic target in immune checkpoint therapy, but improved survival has yet to be shown targeting MDSCs. 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.

We investigated whether a novel recombinant fusion protein, designated murine TNX-1700, which contains murine TFF2 fused to murine serum albumin (mTFF2-MSA), can improve survival in an anti-PD-1 treated syngeneic mouse model of colorectal cancer (CRC). The fusion protein was designed with the goal of increasing half-life and reducing dose frequency. We developed a model using MC38 CRC cells grafted subcutaneously into C57BL/6 mice. Mice subsequently received either mTFF2-MSA, anti-PD-1 antibody (clone 29F.1A12), or both, and tumor volume, and survival were measured. Flow cytometry was performed to examine treatment-induced effects on immune profiles. Administration of mTFF2-MSA suppressed tumor growth (TGI 50%), while the combination of mTFF2-MSA and anti-PD-1 antibody had an additive effect and suppressed tumor growth dramatically (TGI 87%). Mice receiving both mTFF2-MSA, and anti-PD-1 exhibited a survival rate of 90% after 50 days, while vehicle and single mTFF2-MSA therapy were 30% and 60%, 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. mTFF2-MSA in combination with checkpoint inhibition via anti-PD-1 antibody is additive in an advanced syngeneic mouse model of colorectal cancer.

**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 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.

**References**