Stabilized recombinant trefoil factor 2 (TFF2-CTP) enhances anti-tumor activity of PD-1 blockade in mouse models of colorectal cancer

Woosook Kim[1], Na Fu[1], Phaneendra Duddempudi[2], Zinaida Dubeykovskaya[1], Steven Almo[2], Chandan Guha[2], Seth Lederman[3], Timothy Wang[1]

[1]Columbia University, New York, NY, [2]Albert Einstein College of Medicine, Bronx, NY, [3]Tonix Pharmaceuticals, Inc., New York, NY

Woosook Kim (wk2277@cumc.columbia.edu)

Abstract

Despite remarkable responses to immune checkpoint blockade across multiple tumor types, the clinical benefit in colorectal cancer (CRC) is limited to microsatellite unstable tumors. PD-L1 expression is a negative prognostic marker in CRC but correlates with a better response to PD-1 blockade. Here, we 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 CRC. We generated knockin mice that conditionally express the murine Pdl1 gene (*R26*-LSL-*Pdl1*-EGFP) and crossed them with *LysM*-Cre mice to overexpress PD-L1 specifically in the myeloid lineage. AOM/DSS-treated mice formed tumors at 10 weeks and developed adenocarcinoma at 17 weeks post-AOM. AOM/DSS treatment led to a significant expansion of myeloid cells, particularly CD11b$^+$Gr-1$^+$ MDSCs, compared to untreated mice. Furthermore, there was a significant decrease in intratumoral CD8$^+$ T cells, indicating attenuated anti-tumor immunity. AOM/DSS-treated PD-L1-overexpressing *LysM*-Cre; *R26*-PD-L1 mice showed markedly enhanced early colorectal tumorigenesis, with a significant increase in tumor number and size. Trefoil factor 2 (TFF2), a secreted anti-inflammatory peptide, inhibits colon tumor growth by suppressing the expansion of CD11b$^+$Gr-1$^+$ MDSCs. TFF2 fused with two carboxyl-terminal peptide and three Flag motifs (TFF2-CTP-Flag) prolonged the circulation time in blood but retained bioactivity. We induced tumors in *R26*-PD-L1 and *LysM*-Cre; *R26*-PD-L1 mice with AOM/DSS, administered fusion recombinant TFF2-CTP-Flag and/or anti-PD-1. Anti-PD-1 in combination with TFF2-CTP showed a marked reduction in tumor growth while anti-PD-1 monotherapy failed to suppress growth. Interestingly, combined treatment showed greater anti-tumor activity in PD-L1-overexpressing mice than control animals. Treatment responders showed significantly increased tumor-infiltrating CD8$^+$ T cells and concomitantly decreased CD11b$^+$Gr-1$^+$ myeloid cells. These early findings suggest that TFF2 augments the response rate of CRC to PD-1 blockade, possibly through suppressing MDSC expansion, supporting the potential of TFF2-CTP in combination I-O treatment for CRC.

AOM/DSS-induced CRC in the murine model

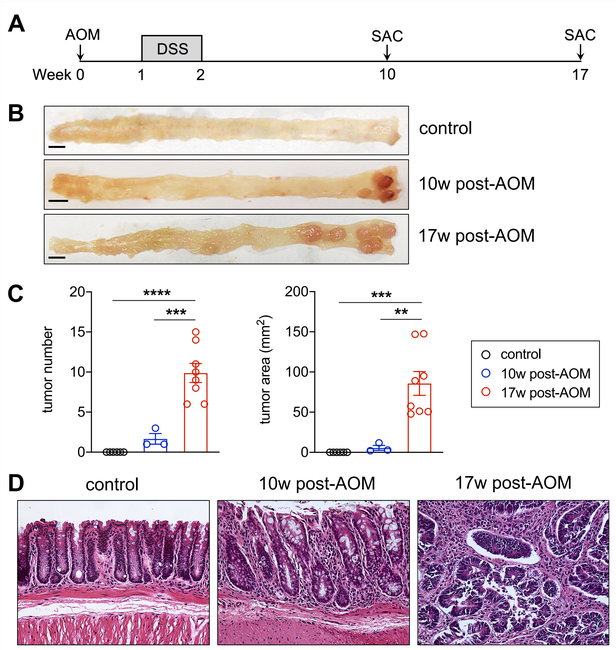

Figure 1. (A) Mice (C57BL/6 WT) received azoxymethane (AOM; 10 mg/kg i.p.) followed one week later with 2.5% dextran sodium sulfate (DSS) in the drinking water for 7 days. (B-D) AOM/DSS-treated mice formed tumors at 10 weeks and developed adenocarcinoma at 17 weeks post-AOM. (B) Gross images. Scale bars, 5mm. Tumors were more frequently observed in the distal colon. (C) Macroscopically visible tumors were counted and tumor area was measured using ImageJ Fiji. (D) H&E stains. Increased intramucosal immune cell infiltrates were detected at 10 weeks post-AOM.

CD11b$^+$Gr-1$^+$ MDSCs markedly increase as tumors progress

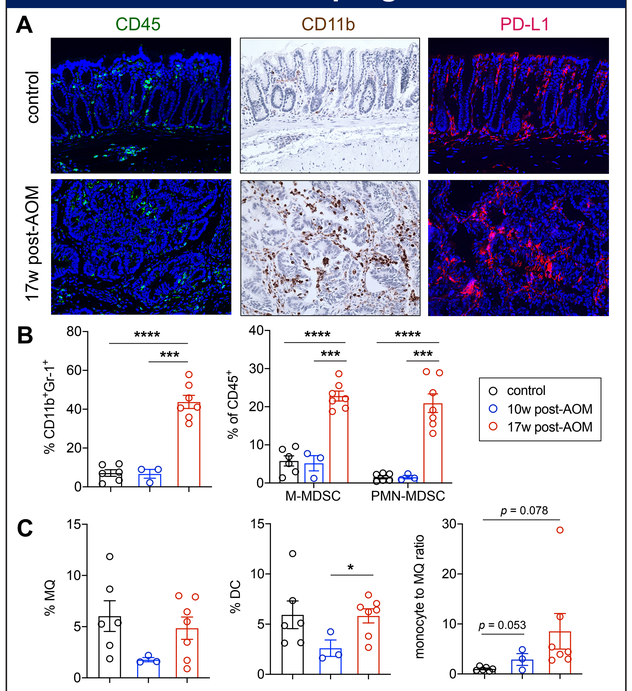

Figure 2. (A) Immunostaining for CD45, CD11b and PD-L1 on colon tissues from AOM/DSS-treated C57BL/6 WT mice. CD11b$^+$ myeloid cells and PD-L1 expression were increased as tumors progressed. (B and C) Immunophenotyping of intratumoral myeloid cells by flow cytometry (% of CD45$^+$). CD11b$^+$Gr-1$^+$ MDSCs and both granolulocytic (CD11b$^+$Ly6G$^+$) and monocytic (CD11b$^+$Ly6G$^-$Ly6C$^+$) MDSC subsets were markedly increased in tumors (B). Macrophages (MQ; CD11b$^+$Ly6C$^-$F4/80$^+$) and dendritic cells (DC; CD11c$^+$F4/80$^-$) (C).

CD8$^+$ T cells : Treg ratio is decreased as tumors develop

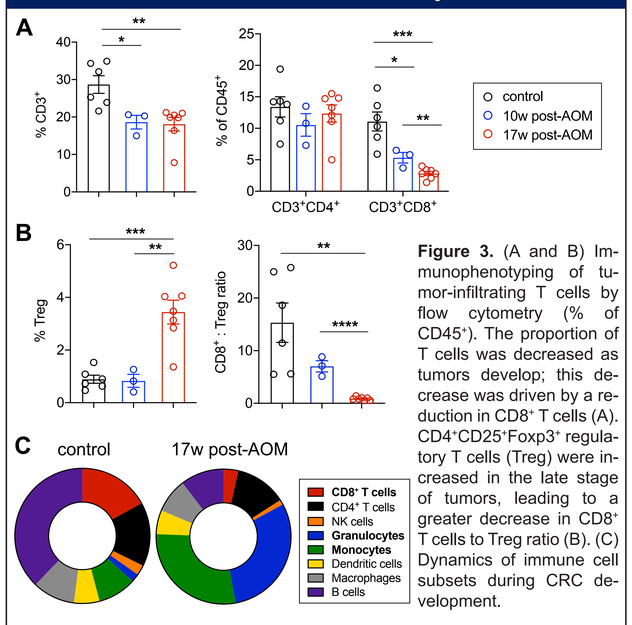

Figure 3. (A and B) Immunophenotyping of tumor-infiltrating T cells by flow cytometry (% of CD45$^+$). The proportion of T cells was decreased as tumors develop; this decrease was driven by a reduction in CD8$^+$ T cells (A). CD4$^+$CD25$^+$Foxp3$^+$ regulatory T cells (Treg) were increased in the late stage of tumors, leading to a greater decrease in CD8$^+$ T cells to Treg ratio (B). (C) Dynamics of immune cell subsets during CRC development.

PD-L1 overexpression in myeloid cells promotes early colorectal tumorigenesis

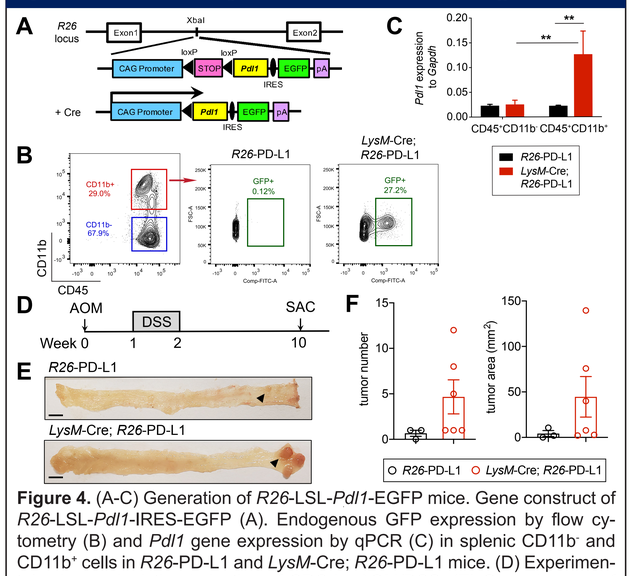

Figure 4. (A-C) Generation of *R26*-LSL-*Pdl1*-EGFP mice. Gene construct of *R26*-LSL-*Pdl1*-IRES-EGFP (A). Endogenous GFP expression by flow cytometry (B) and *Pdl1* gene expression by qPCR (C) in splenic CD11b$^-$ and CD11b$^+$ cells in *R26*-PD-L1 and *LysM*-Cre; *R26*-PD-L1 mice. (D) Experimental scheme depicting induction of CRC by AOM/DSS. (E) Gross images of colorectal tumors at 10 weeks post-AOM. Scale bars, 5 mm. (F) The tumor numbers counted and tumor area measured. Note that *LysM*-Cre; *R26*-PD-L1 mice treated with AOM/DSS showed markedly enhanced early colorectal tumorigenesis.

TFF2 inhibits colorectal tumor growth by suppresing the expansion of MDSCs

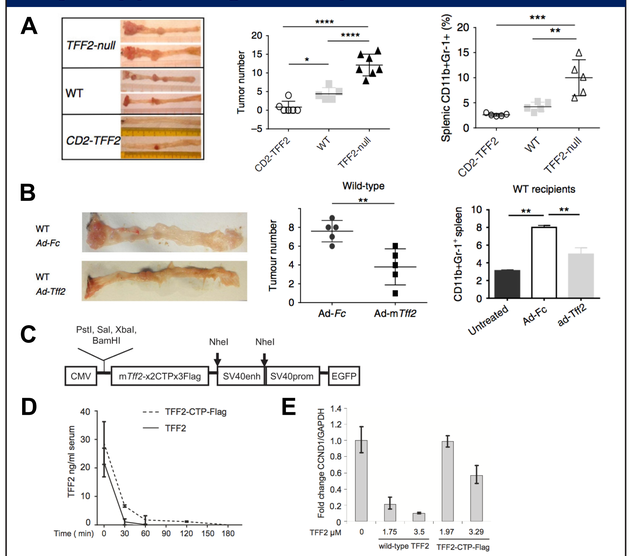

Figure 5. (A and B) TFF2 overexpression (CD2-*Tff2* mice) (A) and treatment with adenovirus Ad-*Tff2* (B) conferred resistance to colon carcinogenesis through suppression of MDSCs. (C) Fusion construct *Tff2*-2CTP-3Flag. (D and E) TFF2-CTP-Flag prolonged the circulation time in blood (D) but retained bioactivity (E). Dubeykovskaya *et al*. 2016 *Nat Commun*. (A-B); 2019 *Cancer Gene Ther*. (C-E).

Hypothesis

Stabilized recombinant trefoil factor 2 (TFF2-CTP) enhances anti-tumor activity of PD-1 blockade in colorectal cancer by suppressing MDSCs

TFF2-CTP + anti-PD-1 showed greater anti-tumor activity in PD-L1-overexpressing mice

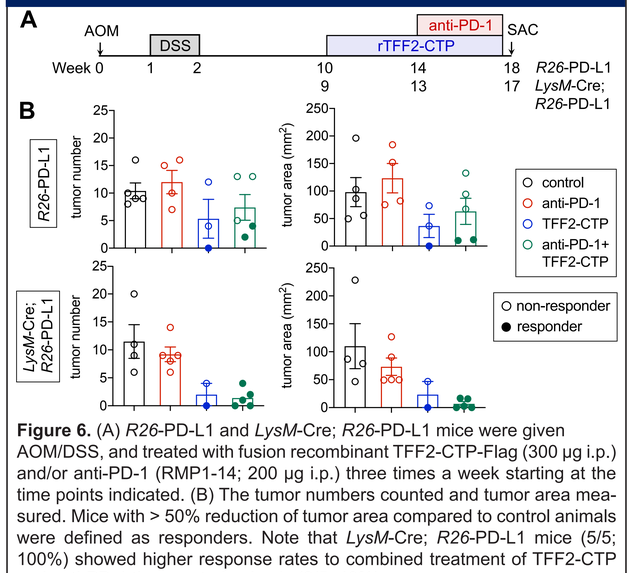

Figure 6. (A) *R26*-PD-L1 and *LysM*-Cre; *R26*-PD-L1 mice were given AOM/DSS, and treated with fusion recombinant TFF2-CTP-Flag (300 μg i.p.) and/or anti-PD-1 (RMP1-14; 200 μg i.p.) three times a week starting at the time points indicated. (B) The tumor numbers counted and tumor area measured. Mice with > 50% reduction of tumor area compared to control animals were defined as responders. Note that *LysM*-Cre; *R26*-PD-L1 mice (5/5; 100%) showed higher response rates to combined treatment of TFF2-CTP and anti-PD-1 than control animals (2/5; 40%).

Responders showed increased CD8$^+$ T cells and decreased MDSCs in tumors

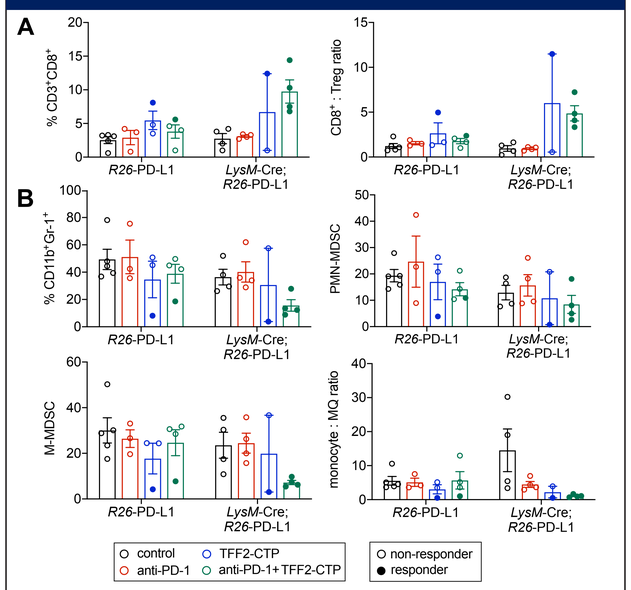

Figure 7. (A) The proportion of CD3$^+$CD8$^+$ T cells in CD45$^+$ cells and a ratio of CD8$^+$ T cells to Treg in tumors. Note that responders had more abundant tumor-infiltrating CD8$^+$ T cells and a higher ratio of CD8$^+$ T cells to Treg. (B) Immunophenotyping of intratumoral myeloid cells following different treatments. A marked reduction in MDSCs, in particular M-MDSC, was observed in responders. Responders also showed a lower ratio of monocyte to MQ.

Conclusions

- ❑ Anti-PD-1 monotherapy was unable to evoke anti-tumor immunity in CRC but TFF2-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.
- ❑ Responders to TFF2-CTP alone or in combination with PD-1 blockade had increased tumor-infiltrating CD8$^+$ T cells, along with decreased MDSCs.