Digestive Disease Week 2026
May 2nd-5th, 2026
Chicago, Illinois

Small-Molecule TNF Inhibition Blocks Pro-inflammatory Soluble TNF Signaling While Maintaining Potentially Beneficial TNFR2 Signaling in Regulatory T Cells and Epithelial Cells

Archana S. Nagaraja, Rameshwari G. Rayaji, Bindi K. Patel, Rajesh K. Singh, Saranya Chandrasekar, Yihong Guan, Ritu Kushwaha, Yue Tong Lee, Cesar A. Meleza, Matthew R. Epplin, Brandon R. Rosen, Manmohan R. Leleti, Matthew J. Walters, Daniel DiRenzo
Arcus Biosciences, Hayward, CA, USA

Abstract Sa1453

Background

- Anti-TNF biologics are effective in the treatment of both Crohn's disease (CD) and ulcerative colitis (UC). However, nearly half of patients treated with anti-TNF therapies discontinue within 2 years due to a loss of therapeutic response or intolerable adverse events.
- While blocking soluble TNF (sTNF)/TNFR1 signaling reduces pro-inflammatory signaling, blocking membrane TNF (mTNF)/TNFR2 signaling may compromise T cell homeostasis and intestinal tissue repair.
- TNFR2 has been shown to be upregulated in intestinal epithelial cells in both CD and UC. Anti-TNF biologics block membrane TNF signaling and can potentially compromise barrier healing.
- In an intestinal epithelial cell line, we show TNFR2 is increased in response to proinflammatory cytokines and membrane TNF signaling
- Herein, we describe a small-molecule TNF inhibitor (SMI) that blocks sTNF/TNFR1 comparably to an anti-TNF biologic while preserving potentially beneficial mTNF/TNFR2 signaling

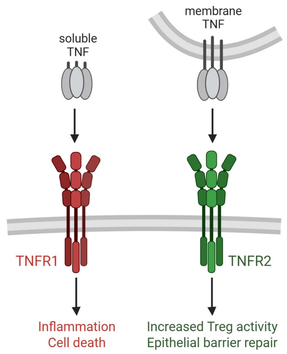

Figure 1. Soluble TNF (sTNF) activates TNFR1 which mediates inflammation and cell death. In contrast, membrane-bound TNF (mTNF) activates TNFR2 to promote Treg activity and intestinal epithelial repair

Methods

- TNF SMI refers to a compound synthesized from PCT publication WO 2020/084008 A1
- HEK293 expressing an NF-κB-SEAP reporter and K562 cells expressing an NF-κB luciferase reporter were used; membrane TNF expressing HeLa cells (mTNF HeLa) cells were generated using CRISPR. Reporter cells were cultured with mTNF HeLa for 4 hours.
- Primary HUVEC cells were cultured in presence of small molecule inhibitors of TNF or adalimumab in presence of 1 ng/ml TNF for 24h. Cell supernatant was quantified for IL-8 using Meso Scale Discovery (MSD) assays
- HIEC6 intestinal epithelial cells were cultured with cytokines or mTNF-expressing cells for 24h. Cells and supernatant were collected to quantify TNFR2 levels by MSD or flow cytometry
- Tregs were isolated using the $CD4^+$ $CD127^{low}$ $CD25^+$ Treg isolation kit from Stem Cell Technologies and cultured with mTNF-expressing cells for 36 hours. Cells were collected for flow cytometry and cytokines were measured in cell supernatants by cytokine bead array.
- DSS was purchased from MP Biochemicals for murine colitis model. C57bl6 female mice were given 3% DSS in drinking water for 7 days. 1 cm of Distal colon was incised on day 8 and incubated in RPMI media. Supernatants were assessed for TNFR2 levels using MSD.

Results

Small-Molecule TNF Inhibition Fully Blocks Soluble TNF-Driven Activity

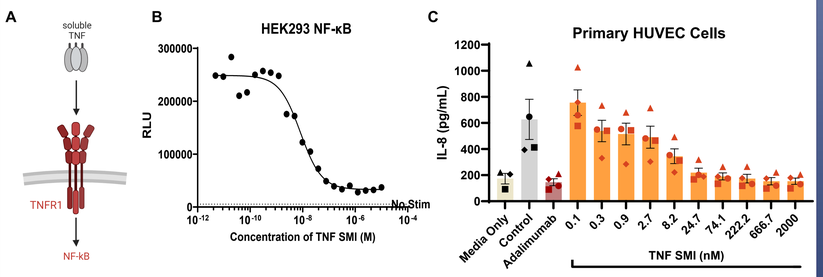

Figure 2 (A) Model of TNFR1 activation by soluble TNF. **(B)** NF-κB reporter HEK293 cells were incubated with 0.125 pg/ml of TNF and increasing concentrations of TNF SMI, reporter activity was measured by luminescence. **(C)** Secreted IL-8 levels were measured in HUVEC media at 24 hours post incubation with 1 ng/ml of soluble TNF and increasing concentrations of TNF SMI or anti-TNF biologic, adalimumab, added as a control. N=4 donors

Results

Small-Molecule TNF Inhibition Does Not Block mTNF Signaling in TNFR1/TNFR2 Dual-Expressing Cells

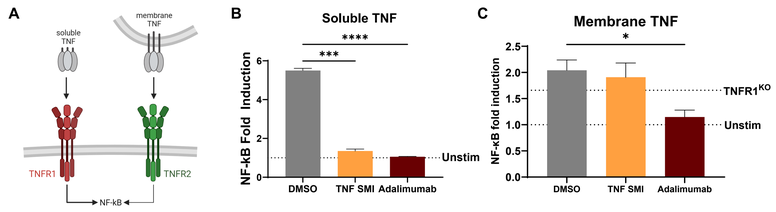

Figure 4 (A) Model of NF-kB activation by soluble or membrane TNF. **(B)** Both a small-molecule TNF inhibitor and adalimumab inhibit soluble TNF activation of NF-κB in HEK293 cells. **(C)** Adalimumab, but not the small-molecule TNF inhibitor, suppressed membrane TNF-stimulated NF-κB activation through TNFR2 in K562 cells. TNFR1 was blocked using an anti-TNFR1 antibody. *p<0.05, ****p<0.0001.

T Cells Can Express High Levels of TNFR2 and mTNF Signaling Enhances Features of Highly Suppressive Regulatory T Cells

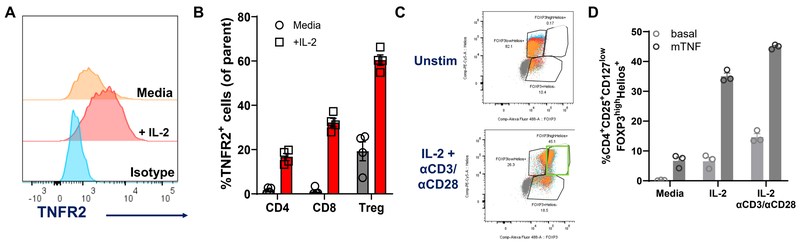

Figure 5. (A) Total T cells were isolated from PBMC and cultured for 48 hours with or without IL-2 prior to surface TNFR2 analysis by flow cytometry. **(B)** A greater percentage of T regulatory cells express surface TNFR2 compared to $CD4^+$ and $CD8^+$ T cells. **(C)** Helios+ FoxP3+ Treg populations at basal conditions and with IL-2 and CD3/CD28 activation in mTNF Hela coculture. **(D)** Helios+ FoxP3+ Tregs increase when cultured in the presence of mTNF Hela cells

Small-Molecule Inhibition of TNF Does Not Block mTNF-Driven Enhancement of Treg Activity

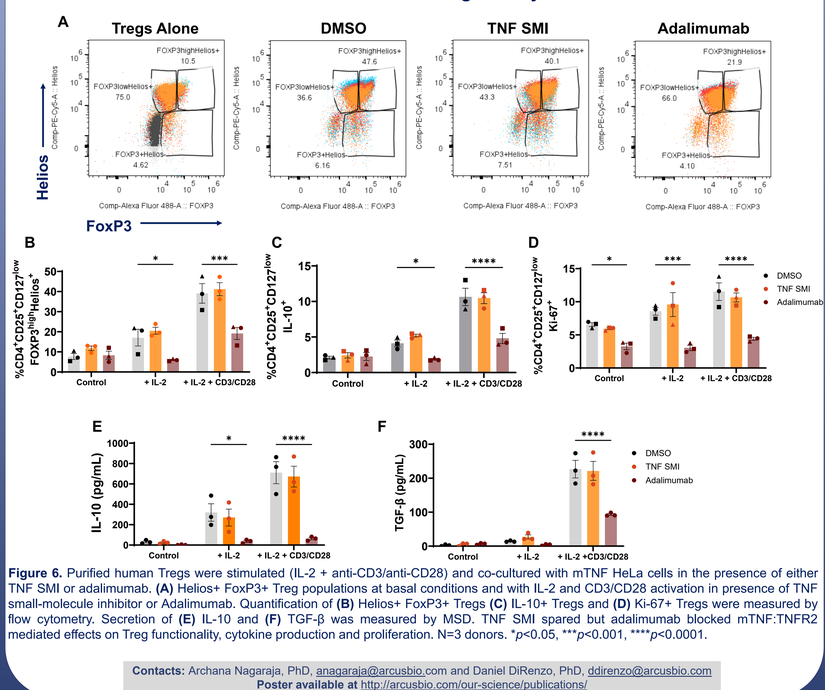

Figure 6. Purified human Tregs were stimulated (IL-2 + anti-CD3/anti-CD28) and co-cultured with mTNF HeLa cells in the presence of either TNF SMI or adalimumab. **(A)** Helios+ FoxP3+ Treg populations at basal conditions and with IL-2 and CD3/CD28 activation in presence of TNF small-molecule inhibitor or Adalimumab. Quantification of **(B)** Helios+ FoxP3+ Tregs **(C)** IL-10+ Tregs and **(D)** Ki-67+ Tregs were measured by flow cytometry. Secretion of **(E)** IL-10 and **(F)** TGF-β was measured by MSD. TNF SMI spared but adalimumab blocked mTNF:TNFR2 mediated effects on Treg functionality, cytokine production and proliferation. N=3 donors. *p<0.05, ***p<0.001, ****p<0.0001.

Contacts: Archana Nagaraja, PhD, anagaraja@arcusbio.com and Daniel DiRenzo, PhD, ddirenzo@arcusbio.com
Poster available at http://arcusbio.com/our-science/publications/

Results

TNFR2 Levels Are Increased in a DSS Model of Murine Colitis and Administration of an Anti-TNF Biologic Exacerbates Weight Loss

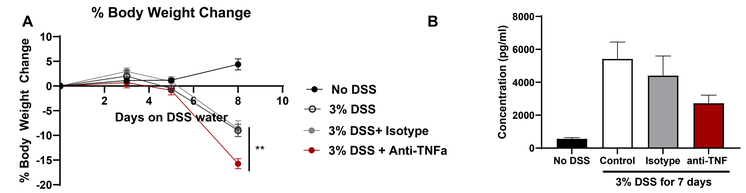

Figure 7. (A) C57BL/6 females were given 3% DSS in their drinking water for 7 days and their bodyweights were evaluated at regular intervals. An anti-TNF biologic appeared to exacerbate the colitis model which is driven by epithelial barrier loss. **(B)** Analysis of distal colon explants at the end of study (Day 8) showed an increase in TNFR2 levels. The Anti-TNF biologic decreased TNFR2 levels, suggesting pro-inflammatory signaling and membrane TNF signaling may drive TNFR2 upregulation. **p<0.01

Soluble TNF and IFN-γ Robustly Induce TNFR2 Expression in Intestinal Epithelial Cell Lines

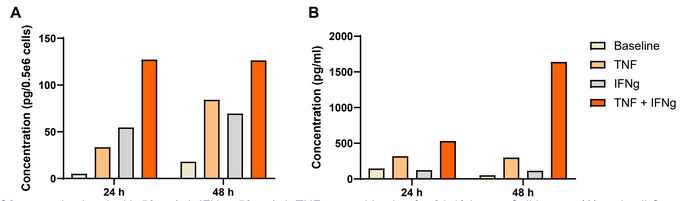

Figure 8. HIEC6 were stimulated with 50 ng/mL IFN-γ, 50 ng/mL TNF or combination for 24-48 hours. Cell lysates **(A)** and cell Supernatants **(B)** were assessed for TNFR2 protein. Both TNF and IFN-γ are elevated in IBD and they robustly increase TNFR2 in combination compared to single cytokine alone in a time-dependent manner

mTNF Signaling Induces TNFR2 Expression in Intestinal Epithelial Cells

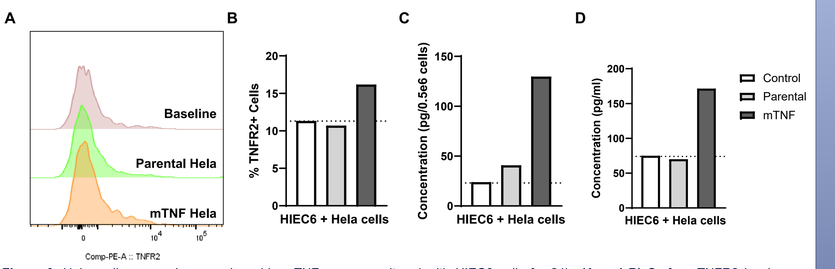

Figure 9. HeLa cells expressing non-cleavable mTNF were co-cultured with HIEC6 cells for 24h. **(A and B)** Surface TNFR2 levels were assessed by flow cytometry. Cell lysates **(C)** and cell supernatants **(D)** were assessed for TNFR2 protein levels. Membrane TNF signaling increased TNFR2 expression in HIEC6 cells at both cell surface and in secretion.

Conclusions

- Small-molecule inhibitors of TNF show equivalent blockade of pro-inflammatory sTNF/TNFR1 signaling as anti-TNF biologics but do not block membrane TNF signaling through TNFR2.
- Membrane TNF signaling enhances the suppressive capacity of regulatory T cells which is not blocked with a small-molecule TNF inhibitor but is abrogated by an anti-TNF biologic.
- TNFR2 is upregulated in an acute DSS model of murine colitis and an anti-TNF antibody exacerbates DSS-mediated weight loss. Analysis of in colon explants show increased TNFR2 during DSS treatment and this is decreased by anti-TNF which blocks mTNF/TNFR2 signaling.
- Intestinal epithelial cell lines express low levels of TNFR2 at baseline but these are increased robustly in response to proinflammatory cytokines and membrane TNF signaling.

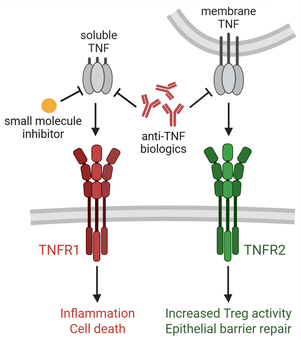

Figure 11. Anti-TNF biologics block both soluble and membrane TNF signaling whereas small molecule inhibitors block only soluble TNF leaving TNFR2 signaling available to promote Treg activity and epithelial repair.

Created with BioRender.com