

Development of WTX-921, A Conditionally Active IL-10 INDUKINE™ Molecule for the Treatment of Inflammatory Bowel Disease

Jenna M. Sullivan, Pamela Aderhold-Camiré, Heather R. Brodtkin, Celesztina Nagy-Domonkos, Connor J. Dwyer, Daniel J. Hicklin, Yuka Lewis, Leigh Magness, Christopher J. Nirschl, Zoe Steuert, Jessica Stieglitz, William M. Winston, and Andres Salmeron

Werewolf Therapeutics Inc., Watertown, MA

BACKGROUND

Development of WTX-921, an IL-10 INDUKINE Molecule for the Treatment of Inflammatory Bowel Disease (IBD)

Significant Unmet Medical Need for Patients with IBD

- o CDC estimated 7 million people worldwide had IBD in 2014
- o Significant unmet medical need for IBD despite progress achieved using biologics like anti-TNF, anti-IL-23, or $\alpha 4\beta 7$ blockers

Unrealized Potential for IL-10 Therapy

- o Clinical studies in IBD with IL-10 have shown promise but have been hampered by dose-dependent adverse events

Role of the Anti-inflammatory Cytokine IL-10 in IBD

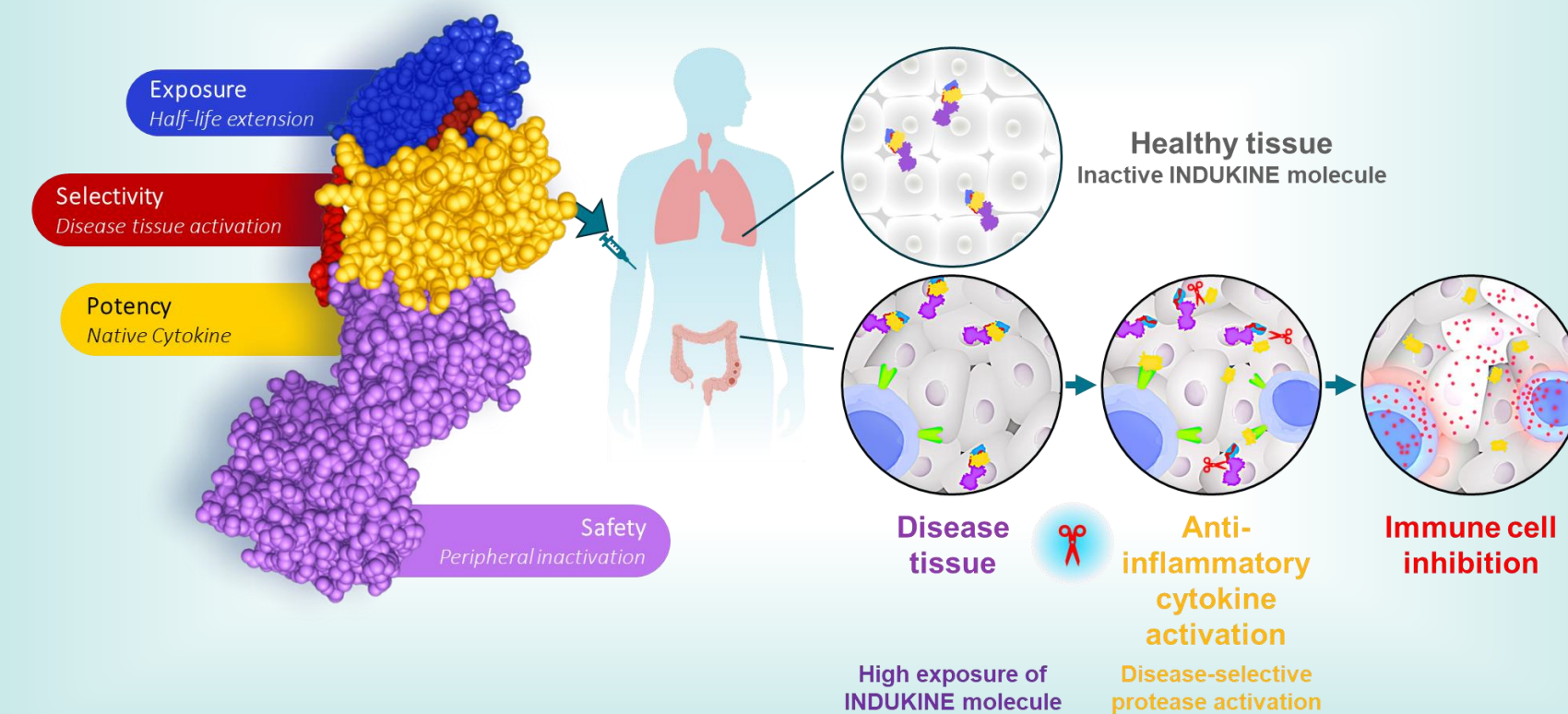
- o IL-10 maintains immune homeostasis in the intestinal mucosa
- o IL-10 SNP associated with susceptibility to IBD; individuals with missense mutations in IL-10 or IL-10 receptor develop IBD
- o Neutralizing IL-10 auto-antibodies can contribute to pediatric IBD

WTX-921 Advantages

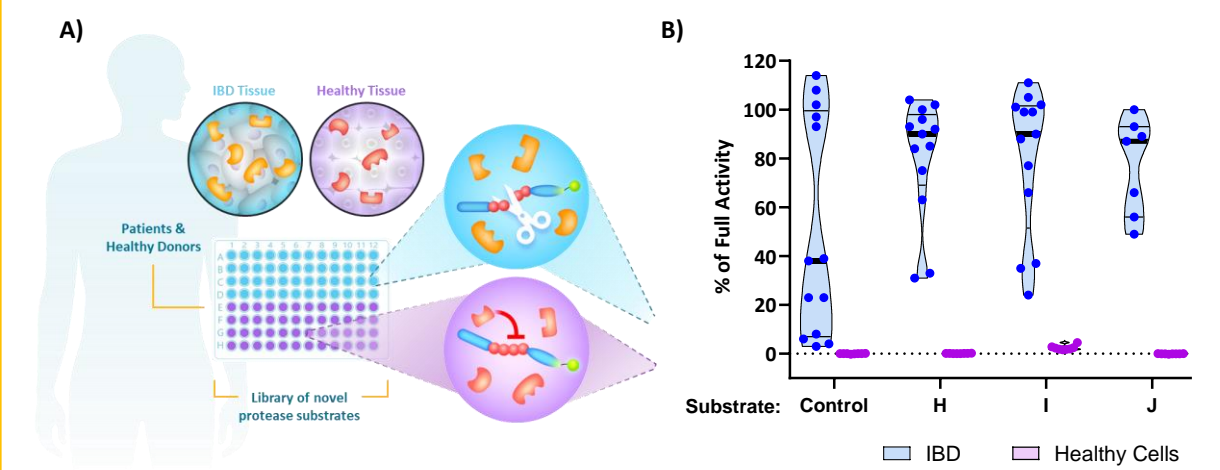
- o Selective delivery of native IL-10 to inflamed tissues in preclinical models, providing therapeutically-relevant exposure while minimizing systemic toxicity
- o Potential to block several disease driving effector molecules and cytokines, eliciting a multipronged effect by inhibiting disease driving innate and adaptive immune cell populations

INDUKINE Molecules:

Targeting Cytokine Activity to Disease Tissue

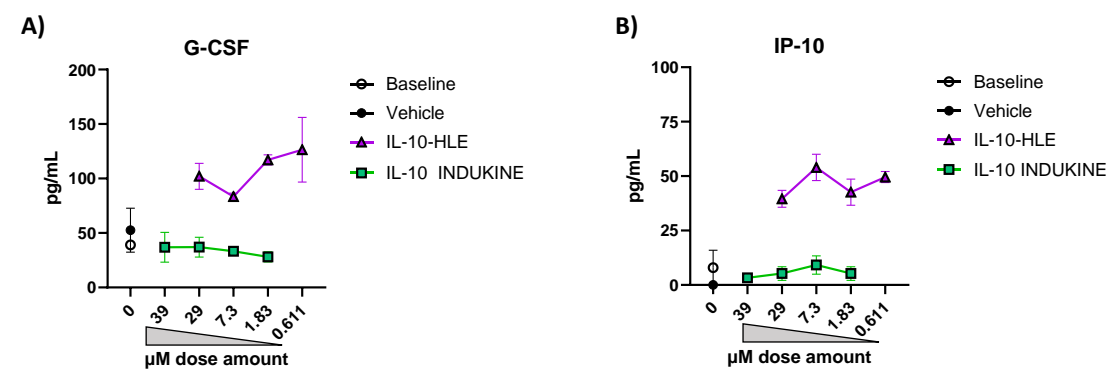


Novel IBD Specific Linkers Preferentially Cleaved by Human IBD Colon Samples



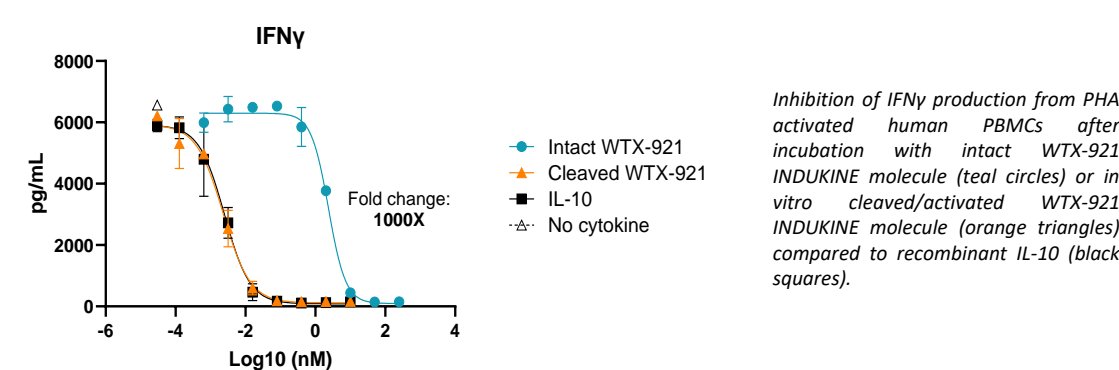
(A) Schematic depicting library screening of novel protease substrates. (B) Dissociated colon samples from patients with Ulcerative Colitis or Crohn's Disease (IBD) or healthy human primary cells were cultured with IL-10 INDUKINE molecules containing different linker substrates. Cleavage was measured by activity of free IL-10 in HEK-Blue IL-10 reporter assay. Data was normalized to pre-cut control. Healthy human primary cells included various cells cell types and multiple donors.

IL-10 Blocking Domain Prevents Peripheral Pharmacodynamic Effects of IL-10



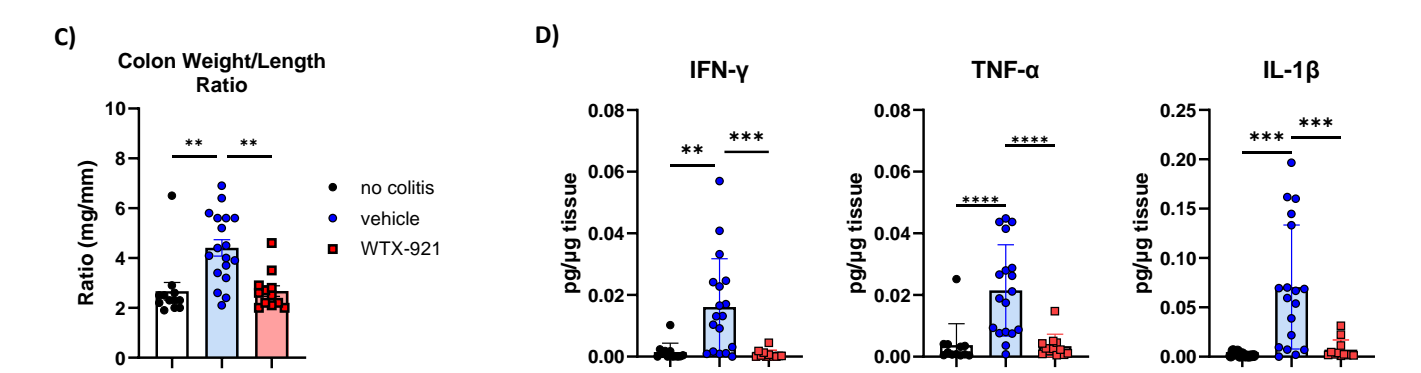
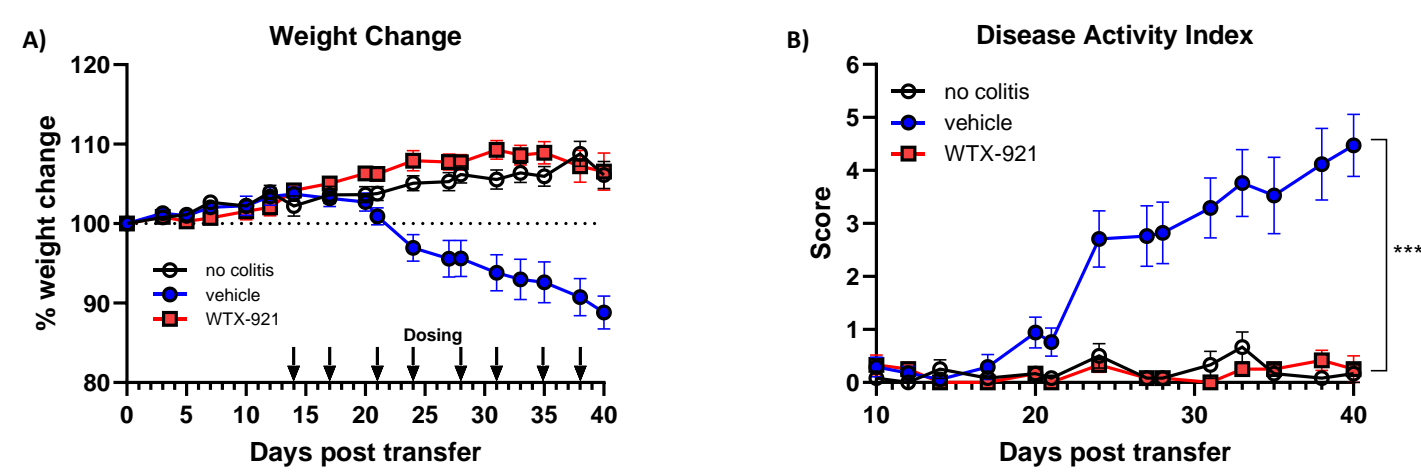
Naive Balb/c mice were dosed IP with equimolar amounts of half-life extended IL-10 or intact IL-10 INDUKINE molecule. Serum was collected after 24 hours and cytokines measured by Luminex assay. (A) In vivo production of G-CSF in response to IL-10 and (B) in vivo production of IP-10 in response to IL-10 comparing IL-10 INDUKINE molecule (green squares), IL-10-HLE (purple triangles), vehicle (black circles) and baseline (open circles).

Inducibility of WTX-921 INDUKINE Molecule



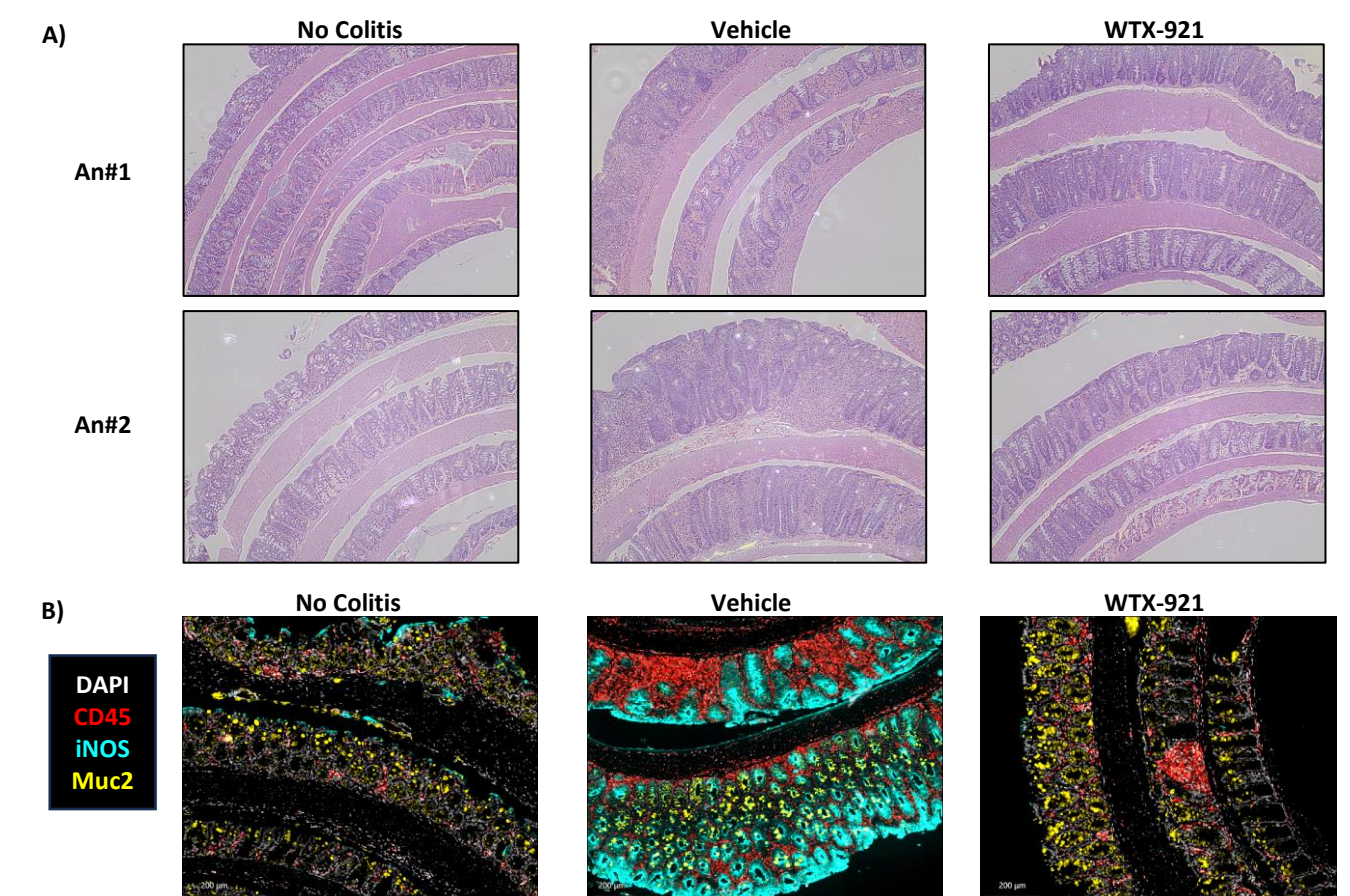
Inhibition of IFN γ production from PHA activated human PBMCs after incubation with intact WTX-921 INDUKINE molecule (real circles) or in vitro cleaved/activated WTX-921 INDUKINE molecule (orange triangles) compared to recombinant IL-10 (black squares).

WTX-921 Inhibits Colitis in Adoptive Cell Transfer Mouse Model of Colitis



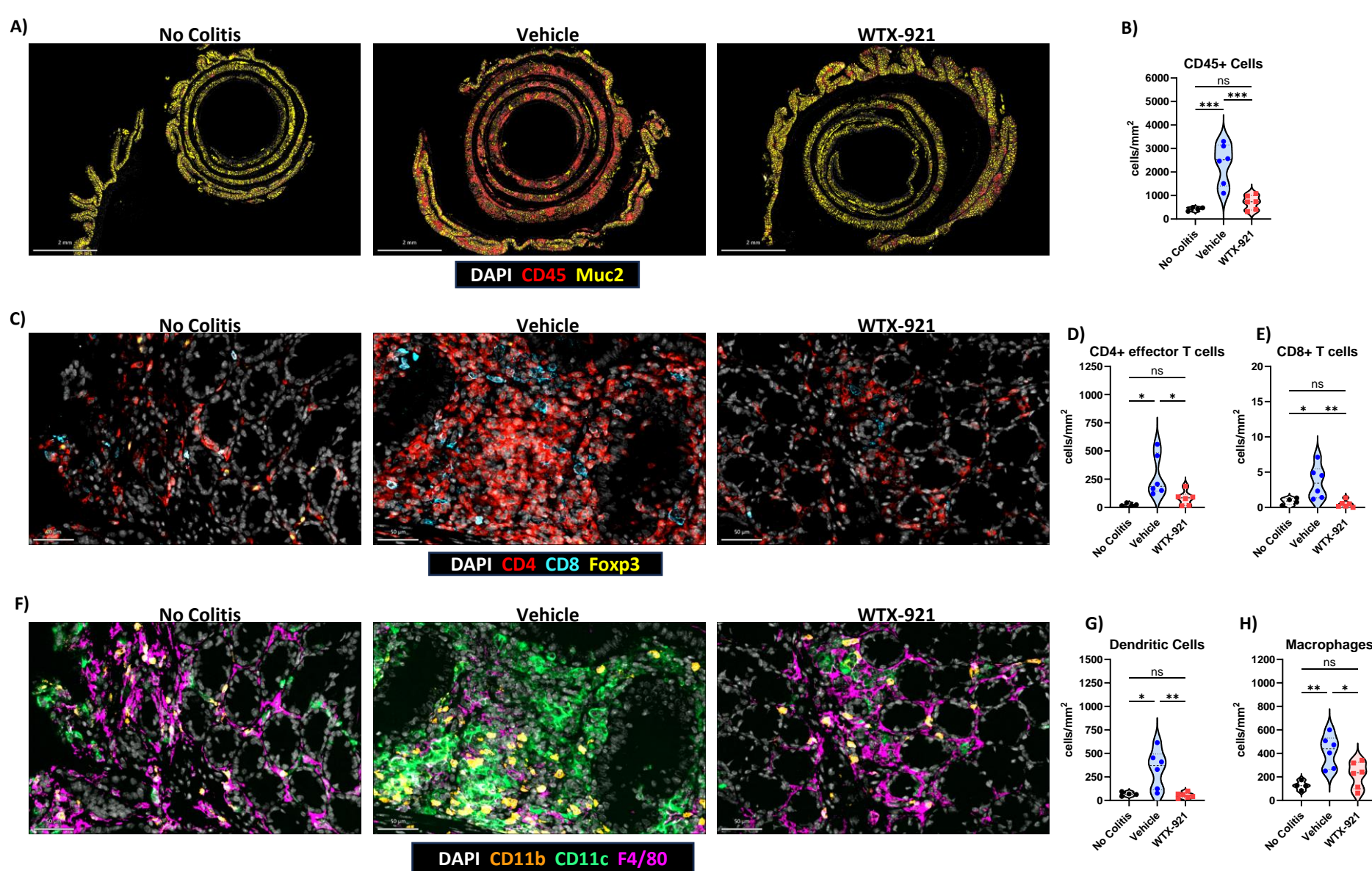
ACT colitis was induced by transferring naive T cells (CD4⁺ CD25⁻ CD45RBhi) into Balb/c SCID mice. Mice were dosed with WTX-921 or vehicle twice weekly starting on day 14 for 4 weeks. (A) Percent weight change over time. (B) Disease Activity Index (DAI) Score over time. DAI score is a combination of weight loss and stool score at indicated timepoint. (C) Colon weight/length ratio at day 40. (D) Levels of inflammatory cytokines in colon tissue lysate at day 40. p values represent the results of a one-way ANOVA: *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001. Studies performed at Hooke Laboratories, Lawrence, MA.

Reduced Colon Damage and Immune Infiltrates After WTX-921 INDUKINE Molecule Treatment in ACT Colitis



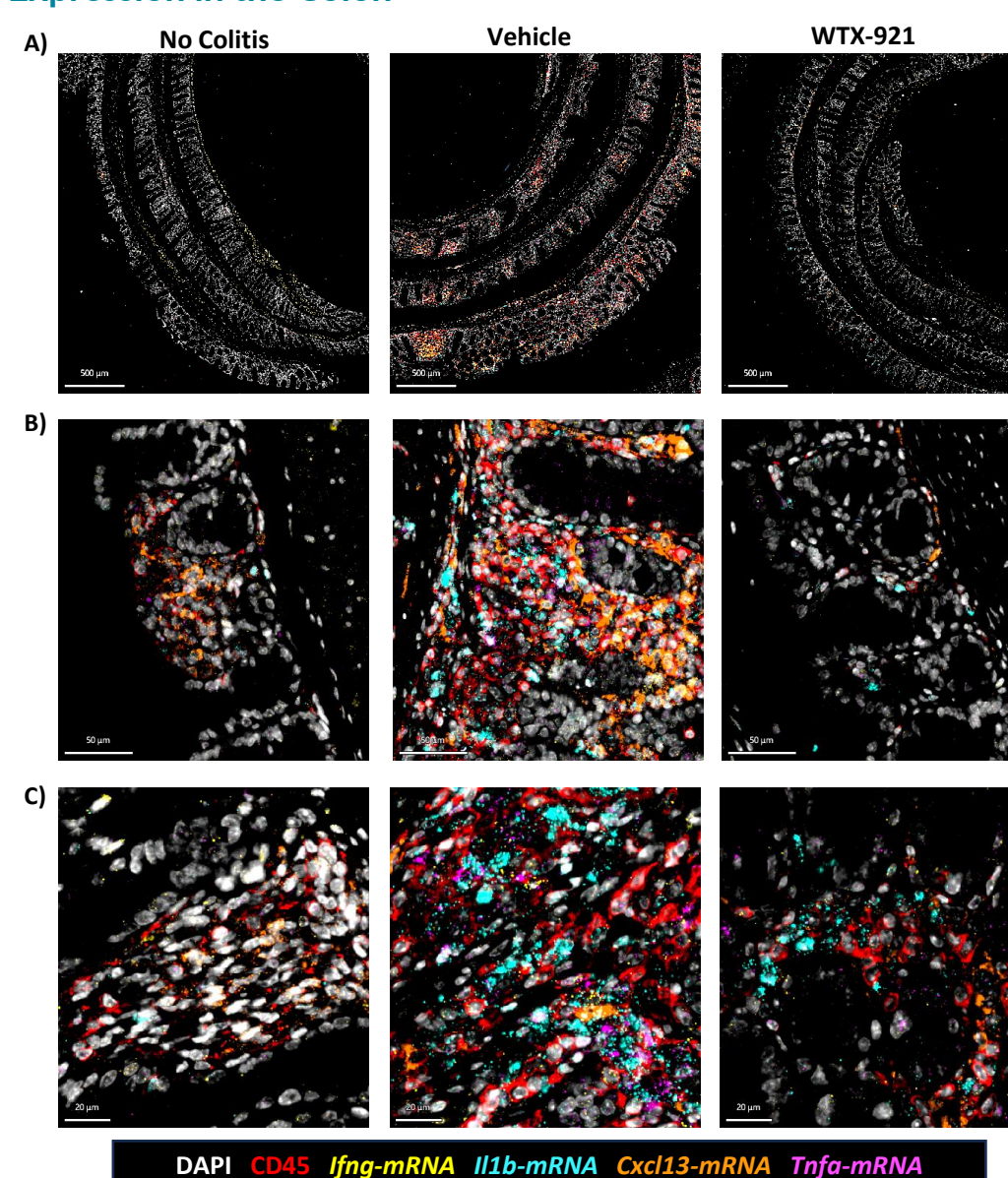
ACT colitis was induced by transferring naive T cells (CD4⁺ CD25⁻ CD45RBhi) into Balb/c SCID mice at Hooke Laboratories, Lawrence, MA. Mice were dosed with WTX-921 or vehicle twice weekly starting on day 14 for 4 weeks. Colons were collected on day 40 (endpoint) and fixed in formalin, embedded in paraffin and sectioned. Colons were either stained for H&E or stained and imaged using a Lunaphore COMET multiplex immunofluorescence platform. (A) Representative images of H&E stained colons at 10x magnification. (B) Representative images of CD45, iNOS and Muc2 staining.

WTX-921 INDUKINE Molecule Inhibits Inflammation Driven by Influx/Expansion of Immune Cells in the Colon



ACT colitis was induced by transferring naive T cells (CD4⁺ CD25⁻ CD45RBhi) into Balb/c SCID mice. Mice were dosed with WTX-921 twice weekly starting on day 14 for 4 weeks. Colons collected at day 40, study endpoint and fixed in formalin. Colons were embedded in paraffin, sectioned, dewaxed and antigen retrieved. Colons were stained and imaged using a Lunaphore COMET multiplex immunofluorescence platform. Four to six individual colons were imaged and analyzed per group. Images were analyzed using HALO Software from Indica Labs, and indicated cell populations were quantified and normalized to tissue area. Representative staining of (A) DAPI, CD45 and Muc2, (C) DAPI, CD4, CD8 and Foxp3, (E) DAPI, CD11b, CD11c and F4/80. Quantification of (B) CD4⁺ cells (D) CD4⁺ effector T cells (E) CD8⁺ T cells, (G) Dendritic cells, and (H) Macrophages. p values represent the results of a one-way ANOVA: *p<0.05, **p<0.005.

WTX-921 Treatment Inhibits Inflammatory Cytokine Expression in the Colon



ACT colitis was induced by transferring naive T cells (CD4⁺ CD25⁻ CD45RBhi) into Balb/c SCID mice. Mice were dosed with WTX-921 twice weekly starting on day 14 for 4 weeks. Colons collected at day 40, study endpoint and fixed in formalin. Colons were embedded in paraffin, sectioned, dewaxed and antigen retrieved. Colons were stained and imaged using a Lunaphore COMET multiplex immunofluorescence platform utilizing RNA probes from ACD. Representative staining of DAPI, CD45 protein, Ifng RNA, Il1b RNA, Tnfa RNA and Cxcl13 RNA at (A) 500μm (B) 50μm and (C) 20μm magnifications.

SUMMARY and CONCLUSIONS

- WTX-921 is a novel IL-10 INDUKINE molecule engineered to overcome toxicities associated with systemic IL-10 delivery for IBD patients
- Novel linker substrates were discovered which are selectively cleaved by human colon samples from patients with IBD
- In vivo stability of IL-10 INDUKINE molecule demonstrated with optimized blocking domain to prevent off tissue activity
- WTX-921 demonstrated *in vitro* inducibility and activity
- WTX-921 is efficacious in a mouse model of colitis resulting in reduced tissue damage and inflammatory cytokine production
- Multi-IF imaging demonstrated reduced cell infiltration/expansion of CD4⁺ effector T cells and myeloid cells in colons of WTX-921 treated animals
- RNAScope images demonstrated reduced inflammatory cytokine RNA in the colons of WTX-921 treated animals

Werewolf Therapeutics
200 Talcott Avenue
Watertown, MA 02472

media@werewolf.com
info@werewolf.com
https://werewolf.com/

