The Biomarker Profile of PTG-200, an Oral Peptide Antagonist of IL-23 Receptor, Tracks with Efficacy in Preclinical Model of IBD

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INTRODUCTION

The recent regulatory approval of ustekinumab which targets IL-12/IL-23 and clinical data from several anti-IL-23 monoclonal antibodies, MEDI2070, BI655066 and LY3074828, support IL-23 as a therapeutic target for the treatment of inflammatory bowel disease (IBD).

To effectively treat IBD, Protagonist Therapeutics has generated a suite of oral peptides that would act locally in the gastrointestinal (GI) tissues and functionally block the IL-23 pathway by selectively antagonizing the IL-23 receptor (IL-23R). We have previously demonstrated that these peptides are: 1) Potential inhibitors of IL-23/IL-23R signaling in a human cell line and in human primary cells; 2) Selective for IL-23R, and may not inhibit binding to IL-6R or signaling through the IL-12 receptor; 3) Cross-reactive towards rat and cynomolgus homologs, enabling in vivo studies in these species; and 4) Resistant to the proteolytic and reducing environments of the GI tract, resulting in high drug levels in intestinal tissues while limiting drug exposure in the circulation, potentially addressing safety concerns associated with systemically delivered therapeutics.

In this study, we investigate the therapeutic potential of orally delivered PTG-200 in a 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced rat model of IBD. As there is interest in using pharmacodynamics (PD) biomarkers for early stage drug development, we sought to evaluate mechanism-specific and disease-related efficacy biomarkers of oral PTG-200 in the colons, feces and serum of colitic rats.

CONCLUSIONS

We demonstrate the *in vivo* activity of our lead candidate PTG-200, and show that PTG-200 primarily exerts its effects via the IL-23 pathway in an acute TNBS-induced rat colitis model. Specifically, blockade of IL-23R-mediated signaling by oral treatment with PTG-200 leads to: 1) Significant and dose-dependent attenuation of disease parameters, with activity comparable to that of a neutralizing anti-IL-23p19 antibody (mAb); 2) Significantly decreased colonic levels of myeloperoxidase (MPO; an indicator of neutrophil infiltration), of IL-17A and IL-22 (cytokines downstream of IL-23 signaling), and of phosphorylated Signal Transducer and Activator of Transcription 3 (pStat3; a transcription factor whose phosphorylation status is known to be regulated by IL-23). The dose-related responses in these markers track with PTG-200 treatment effects.

Moreover, we show that in the diseased animals, the levels of lipocalin 2 (LCN2; an anti-bacterial protein over-expressed in the inflamed colonic epithelium) are elevated in the serum, and MPO and LCN2 are increased in the feces. These inflammatory markers, MPO (in feces) and LCN2 (in serum and feces) are responsive to PTG-200 oral treatment, thus serving as non-invasive PD biomarkers for colitic activity.

Our results highlight the potential value of these biomarkers in translating preclinical efficacy to early clinical proof-of-concept for anti-IL-23R therapies.

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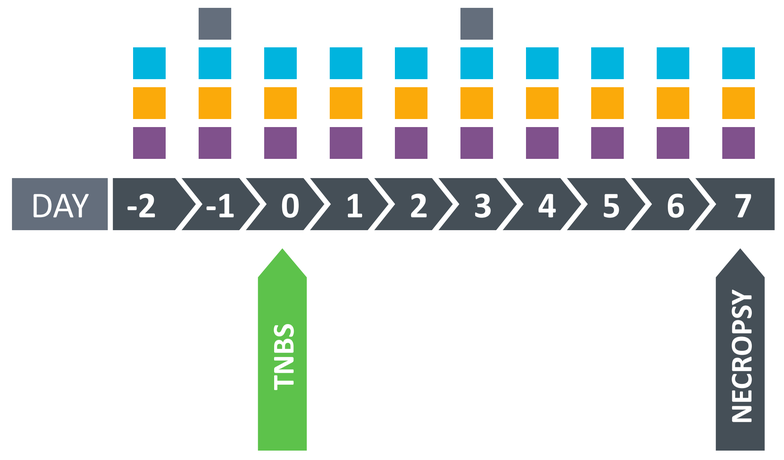
Protagonist Therapeutics

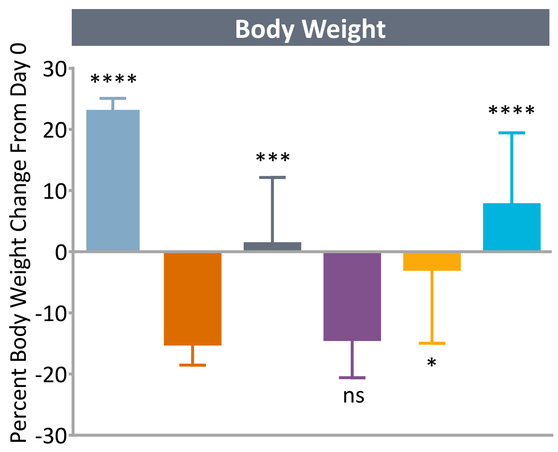
ECCO European Crohn's and Colitis Organisation

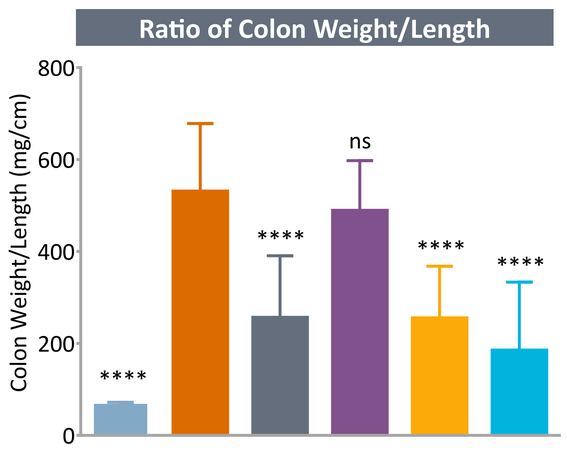
RESULTS: *IN VIVO* EFFICACY AND EFFICACY BIOMARKER RESPONSES IN AN ACUTE TNBS-INDUCED RAT MODEL OF IBD

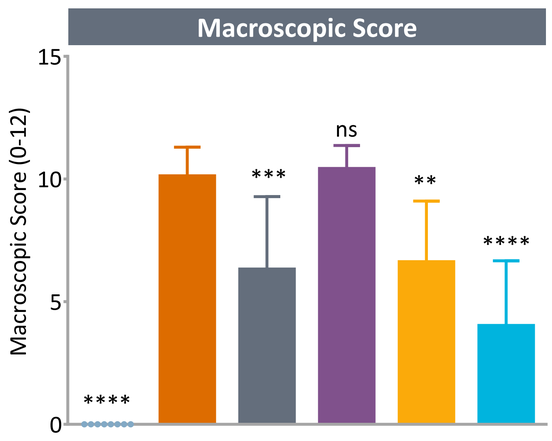
Figure 1. Study outline: Acute colitis was induced in Sprague-Dawley rats by a single intra-rectal instillation of TNBS (48mpk) followed by efficacy analysis at Day 7.

Sprague-Dawley Rats	N = 8-10 per group
Sham + Vehicle	PO TID
TNBS + Vehicle	PO TID
TNBS + Anti-IL-23p19 mAb at 4 mpk	IP, Day -1 and Day 3
TNBS + PTG-200 at 9 mpk/d	PO TID + in drinking water
TNBS + PTG-200 at 28 mpk/d	PO TID + in drinking water
TNBS + PTG-200 at 61 mpk/d	PO TID + in drinking water









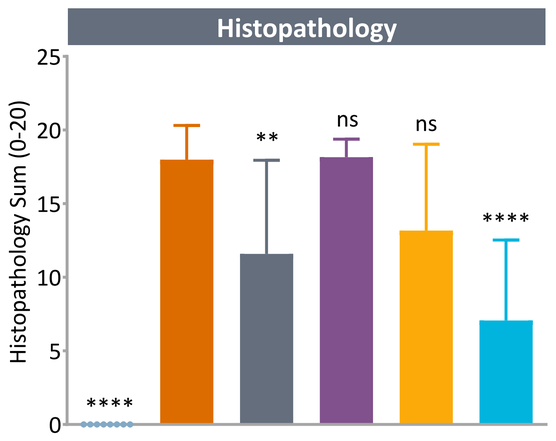


Figure 2. Oral treatment with PTG-200 resulted in significant and dose–dependent improvement in animal body weight, reduction in colon weight-to-length ratio, and normalization in macroscopic and histopathological changes in the colon: Body weight was from each animal; weight and length were from entire colon; colonic score was evaluated as sum of adhesion (0-2), stricture (0-3), ulcer (0-5), and colon wall thickness (0-2); and histology was evaluated as sum of mucosal inflammation (0-5), transmural inflammation (0-5), erosion (0-5), and gland loss (0-5). Values shown as mean + SD. Statistical significance was assessed by One-way ANOVA with post-hoc Dunnett's vs. Vehicle control: *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001; ns, not significant.

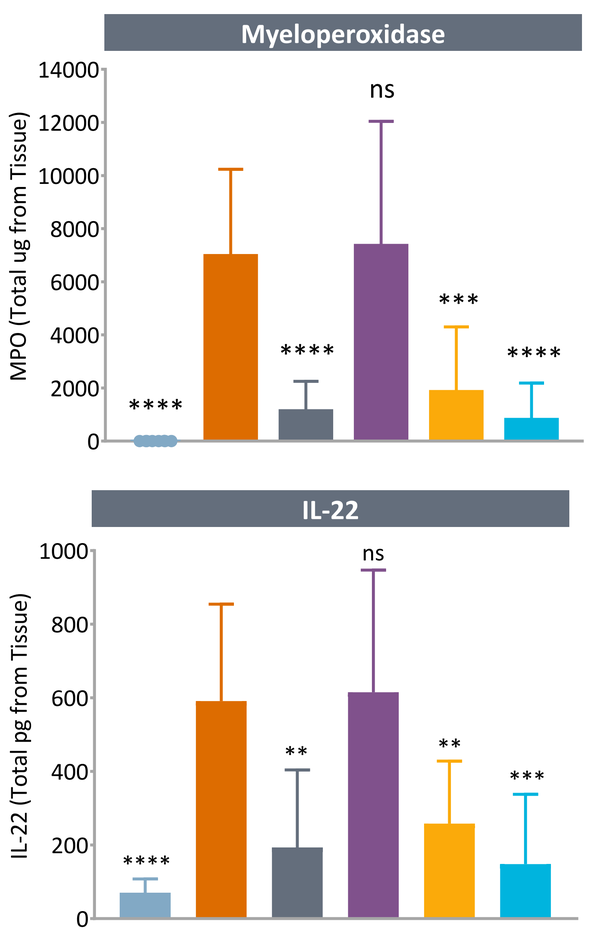


Figure 3. Oral treatment with PTG-200 resulted in significant and dose-dependent reduction of disease-related and IL-23-directed markers in the distal colons of TNBS-treated rats: MPO, IL-17A, and IL-22 in sampled tissues were quantified by enzyme-linked immunosorbent assay (ELISA); percentage of pStat3 was normalized to the area of the distal colon quantified by immunohistochemistry (IHC). Values shown as mean + SD. Statistical significance was assessed by One-way ANOVA with post-hoc Dunnett's vs. Vehicle control: *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001; ns, not significant.

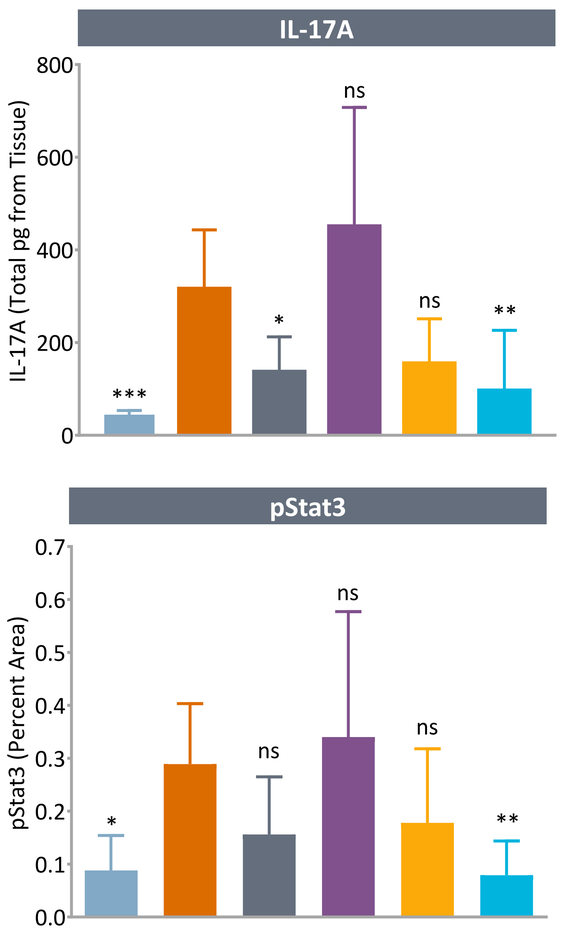


Figure 4. Oral treatment with PTG-200 resulted in significant down-regulation of LCN2 in the serum, and attenuation of LCN2 and MPO in the feces collected from the distal colons of TNBS-induced colitis rats. Disease induction and oral treatments with PTG-200 (31 mpk/d) were performed as outlined in the study design in Figure 1. LCN2 and MPO detected from sampled tissues were quantified by ELISA. Statistical significance was assessed by One-way ANOVA with post-hoc Dunnett's vs. Vehicle control: *p≤0.05; **p≤0.01; ***p≤0.001.