Discovery of Novel Oral Peptide Antagonists of IL23-Receptor That are Efficacious in Rat Model of IBD

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ABSTRACT
Background
The heterodimeric IL-23 receptor is comprised of the IL-12Rβ1 subunit in complex with IL-23R subunit. The IL-23R is also a heterodimer of the unique p19 subunit coupled with the common p40 subunit shared with IL-12. Binding of IL-23 to IL-23R complex leads to phosphorylation of STAT3, and IL-23-dependent expression of pro-inflammatory cytokines. Clinical trials in Crohn’s Disease or psoriasis with ustekinumab and broilimumab (which target the common p40 subunit) and tildrakizumab, guselkumab, MED1001, and BI-655066 (which target the unique p19 subunit of IL-23) highlight the potential of IL-23 signaling blockade in treatment of human inflammatory diseases. The aim of this study is to develop orally stable IL-23 antagonist peptides that act locally in the intestinal tissue for treatment of IBD.

Methods
Potent, selective and orally stable peptide antagonists of IL-23 were identified through a combination of phage display technology and medicinal chemistry. To evaluate oral stability, the peptides were incubated in a variety of ex vivo intestinal/coliconic washes or simulated gastric/intestinal fluids, and half-lives determined by mass spectrometry. Pharmacokinetic (PK), pharmacodynamic (PD) and colitis studies were conducted in rats.

Results
Using a combination of phage display technology and medicinal chemistry, we identified functional inhibitory peptides of IL-23 that are stable in assays that mimic the harsh redox and proteolytic conditions of the GI environment. These peptides potently neutralize IL-23-mediated STAT3 signaling in the transformed human B lymphoblastoid DB cell line, and block IL-23-induced IL-6 production from human primary NK cells. They are also active against the rat and cynomolgus monkey IL-23. The peptides do not block the interaction between IL-6 and IL-6R or antagonize the IL-12 signaling pathway. In PK studies, oral dosing of the peptides results in high exposure in GI tissues, but very low exposure in the blood. In a rat TNBS (2,4,6-trinitrobenzene-sulfonic acid)-induced acute colitis model, oral dosing of the peptides caused a significant reduction in neutrophil infiltration as measured by myeloperoxidase (MPO) activity, and a reduction in inflammation and other disease parameters as assessed by histopathology. These in vivo activities of the peptides were comparable to that of an anti-IL-23p19 mAb.

CONCLUSIONS
Protagonist Therapeutics has generated a suite of potent, selective, orally efficacious IL-23R peptide antagonists that are promising therapeutics for the treatment of IBD. We have demonstrated that these peptides are:

- Potent blockers of IL-23p19 interacting with a human primary cell line and in human primary cells
- Selective for IL-23R, and do not inhibit binding to IL-6R or signaling through IL-12R
- Cross-reactive towards rat and cynomolgus but not mouse homologs, enabling in vivo studies in these species
- Resistant to proteolytic and reducing environments of the GI, resulting in high drug levels in the intestinal tissues and limited drug concentrations in the circulation, potentially improving safety concerns associated with systemically delivered therapeutics
- Reduces IL-23 pathway directed biomarkers in both TNBS induced rat colitis and S. typhimurium induced inflammation models
- Effective and comparable to an anti-IL-23p19 monoclonal antibody in attenuating colitis in a TNBS-induced rat colitis model, most likely through GI restricted activities

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