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 ligand IL-23 is also a heterodimer of the unique p19 subunit coupled with the common p40 subunit shared with IL-12. Binding of IL-23 ligand to the 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 briakinumab (which target the common p40 subunit) and tildrakizumab, guselkumab, MEDI2070, 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-23R antagonist peptides that act locally in the intestinal tissue for treatment of IBD.

Methods

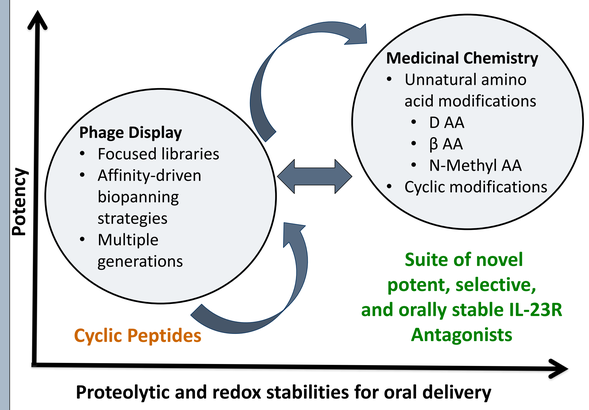
Potent, selective and orally stable peptide antagonists of IL-23R were identified through a combination of phage display technology and medicinal chemistry. To evaluate oral stability, the peptides were incubated in a variety of exvivo intestinal/colonic washes or simulated gastric/intestinal fluids, 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-23R 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 lymphoblast DB cell line, and block IL-23 stimulated IFNγ release from human primary NK cells. They are also active against the rat and cynomolgus monkey IL-23R. 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-trinitrobenzenesulfonic 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.

PEPTIDE DISCOVEY AND OPTIMIZATION

Figure 1: Optimization using phage display technology and medicinal chemistry.

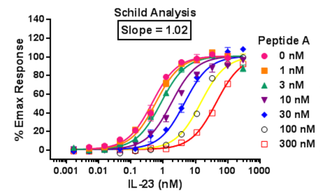


IN VITRO CHARACTARIZATION

Table 1: Peptide X is potent and competitive inhibitor of IL-23R.

	IC_{50}		KD
	pSTAT3/DB Cell Assay	IFNγ/PB NK Primary Cell Assay	IL-23R Surface
Peptide X	4 nM	27 nM	2.4 nM

Figure 2: Schild analysis.



- IL-23-dependent upregulation of phospho-STAT3 (pSTAT3) in human DB cell line and IFNγ production in human peripheral blood natural killer (PB NK) cells
- Binding kinetics to the immobilized human IL-23R by surface SPR
- Schild analysis from pSTAT3/DB cell assay shows Peptide X is a competitive inhibitor

Table 2: Peptide X is a selective inhibitor

	IC_{50}		KD
	IL-6/IL-6R ELISA	IFNγ/IL-12 PBMC Cell Assay	IL-12Rβ1 Surface
Peptide X	> 100 uM	> 100 uM	None

Cell-free ELISA for human IL-6R; IL-12-dependent production of IFNγ in human peripheral blood mononuclear cells (PBMC); binding kinetics to the immobilized human IL-12Rβ1 by SPR.

Table 3: Peptide X cross-reacts with cynomolgus and rat IL-23R.

	IC_{50}		
	Cyno IL-23R ELISA	Rat IL-23R ELISA	IL-17A/Rat Splenocyte Assay
Peptide X	7 nM	17 nM	130 nM

Cell-free ELISA and IL-23-dependent IL-17A production in splenocytes for the indicated IL-23 receptor.

Table 4: Peptide X is stable in a variety of gastrointestinal fluids and reducing environment

	Half Life			
	Simulated Intestinal Fluid	Simulated Gastric Fluid	Human Intestinal Fluid	Reducing Environment
Peptide X	> 24 hr.	> 24 hr.	> 24 hr.	> 2 hr.

Half lives determined by mass spectrometry.

IN VIVO EFFICACY IN A TNBS-INDUCED RAT MODEL OF IBD

Table 5: Study design

SD Rats Groups (N = 8-10) – Dosing starts Day -1
Sham
Vehicle
Anti-IL-23p19 monoclonal antibody◊, 4 mg/kg (IP, Day -1 and Day 3) (Anti-IL-23p19 PK is studied to optimize its use for positive control)
Peptide X, 160 mg/kg/d (PO TID + in drinking water)
Peptide X, 55 mg/kg/d (PO TID + in drinking water)

◊: Anti-IL-23p19 antibody neutralizes IL-23-dependent IL-17A production in the rat splenocyte assay with an IC_{50} value of 1 nM.

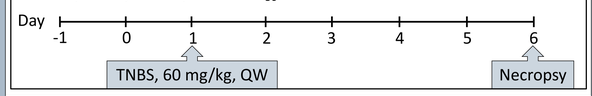
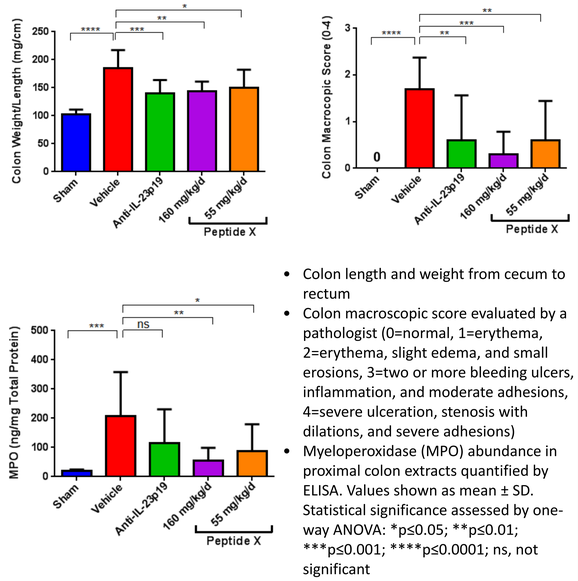
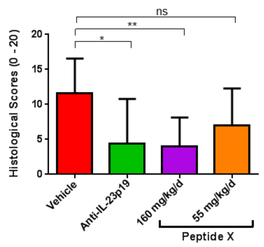


Figure 3: Oral treatment reduces colonic inflammation and neutrophil infiltration



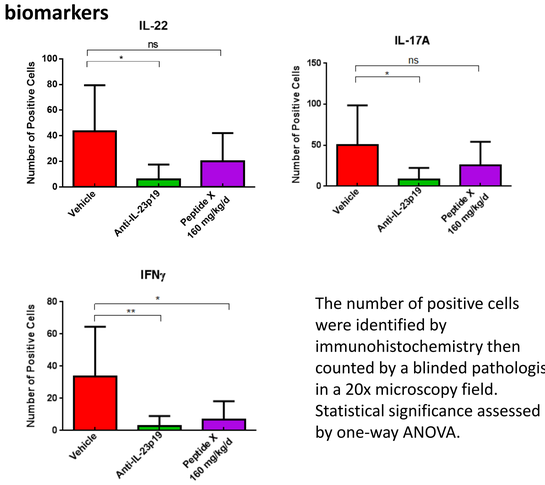
- Colon length and weight from cecum to rectum
- Colon macroscopic score evaluated by a pathologist (0=normal, 1=erythema, 2=erythema, slight edema, and small erosions, 3=two or more bleeding ulcers, inflammation, and moderate adhesions, 4=severe ulceration, stenosis with dilations, and severe adhesions)
- Myeloperoxidase (MPO) abundance in proximal colon extracts quantified by ELISA. Values shown as mean ± SD. Statistical significance assessed by one-way ANOVA: *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001; ns, not significant

Figure 4: Oral treatment improves histopathology



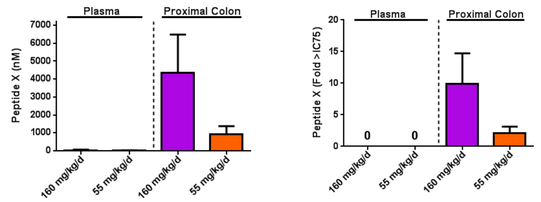
Histological scores evaluated by a blinded pathologist (inflammation, mucosal necrosis, gland loss, colon wall thickness, 0-5)

Figure 5: Oral treatment reduces IL-23 pathway directed biomarkers



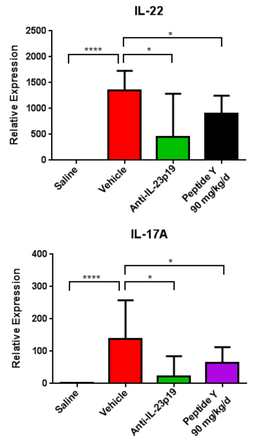
The number of positive cells were identified by immunohistochemistry then counted by a blinded pathologist in a 20x microscopy field. Statistical significance assessed by one-way ANOVA.

Figure 6: Oral efficacy observed is most likely due to local activity in the colon



IN VIVO ACTIVITY IN A RAT FLAGELLIN MEDIATED INFLAMATION MODEL

Figure 7: Oral treatment reduced the expression of IL-23 pathway directed signature genes in *S. typhimurium* flagellin induced inflammation model



- SD rats received saline or *S. typhimurium* derived flagellin with vehicle, an anti-IL-23p19 antibody or Peptide Y
- Two hours after iv injection, mRNA extracted from the small intestine
- Cytokine expression was assessed by quantitative PCR. Values normalized to HPRT1 expression were used to calculate relative expression with ddCt analysis.
- Statistical significance assessed by T-test

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-23/IL-23R signaling in a human 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* flagellin 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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