DISCOVERY AND CHARACTERIZATION OF NOVEL ORAL PEPTIDE ANTAGONISTS OF HUMAN IL-23 RECEPTOR THAT ARE EFFICACIOUS IN A RAT MODEL OF IBD

Xiaoli Cheng, Ashok Bhandari, Greg Bourne, Brian Frederick, Jenny Zhang, Jaimee McMahon, Sonya Scott, Herodion Celino, Genet Zemede, Natalie Spencer, Vinh Tran, Mridula Dogra, Jennifer Dias, Lu Bai, Thamil Annamalai, Mark Smythe, Larry Mattheakis, Dinesh Patel, David Liu

Protagonist Therapeutics, Milpitas CA USA

ABSTRACT

Genome-wide association studies (GWAS) have demonstrated significant association of the IL-23 receptor (IL-23R) gene with inflammatory bowel disease (IBD), suggesting that perturbation of IL-23 signaling could be relevant to the pathogenesis of the disease. Here, we propose to modulate the IL-23 pathway through selective antagonism of IL-23R by oral treatment with peptides that are stable and restricted to the gastrointestinal (GI) tissue. Using a combination of phage display technology and medicinal chemistry, we identified inhibitory peptides that are uniquely resistant to oxidative/reductive conditions and proteolytic degradation in a variety of assays that mimic the various compartments of the GI environment. Functionally, these peptides potently neutralize IL-23-mediated signaling in a transformed human cell line and in human primary cells. The binding of IL-23R is selective since the peptides do not block the interaction between IL-6 to IL-6R or antagonize the IL-12 signaling pathway. Furthermore, we show that these orally delivered peptides are efficacious in attenuating colitis in a 2,4,6-trinitrobenzenesulfonic acid (TNBS) -induced acute rat model of IBD, as shown by a significant reduction in the ratio of colon weight to length, colon macroscopic score, neutrophil infiltration, and histopathology comparable to that of the control anti-IL-23p19 mAb. Overall, these data support the therapeutic potential of GI-restricted IL-23R antagonists for the treatment of IBD.

BACKGROUND

Increasing understanding of the mechanisms underpinning immune-mediated inflammatory diseases supports a pivotal role for the IL-23/IL-23R signaling axis in the pathogenesis of these diseases. IL-23 is composed of a unique p19 subunit coupled with the common p40 subunit shared with IL-12, and signals through the heterodimeric IL-23R complex. This complex consists of the IL-23R subunit paired with the IL-12Rβ1 subunit shared with the IL-12R complex. Binding of IL-23 to the IL-23R complex leads to phosphorylation of STAT3, and IL-23-dependent expression of proinflammatory 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. Protagonist Therapeutics has developed oral peptide inhibitors that selectively target IL-23R for the treatment of IBD. GWAS in IBD patients indicated a prominent role of IL-23R variants in defining disease susceptibility. Further studies in acute and chronic mouse models of IBD revealed a primary role of IL-23R and downstream effector cytokines in disease pathogenesis. IL-23R is expressed on various adaptive and innate immune cells including Th17 cells, γδ T cells, natural killer (NK) cells, dendritic cells, macrophages, and innate lymphoid cells, which are found abundantly in the intestine. At the intestine mucosal surface, the gene expression and protein levels of IL-23R are found to be elevated in IBD patients. Thus, specific targeting of IL-23R from the luminal side of the gut may provide therapeutic benefit to IBD patients suffering from local inflammation of the intestinal tissue. Here, we present exciting proof of concept data on oral GI-restricted peptide inhibitors of IL-23R that are efficacious in a rat model of IBD.

RESULTS

DISCOVERY AND OPTIMIZATION

Figure 1: Discovery using phage display technology.

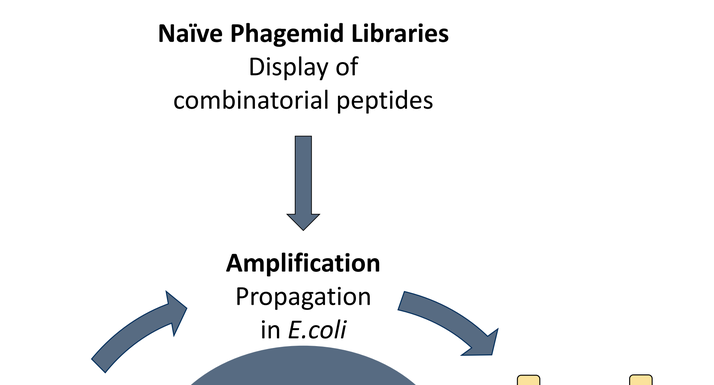
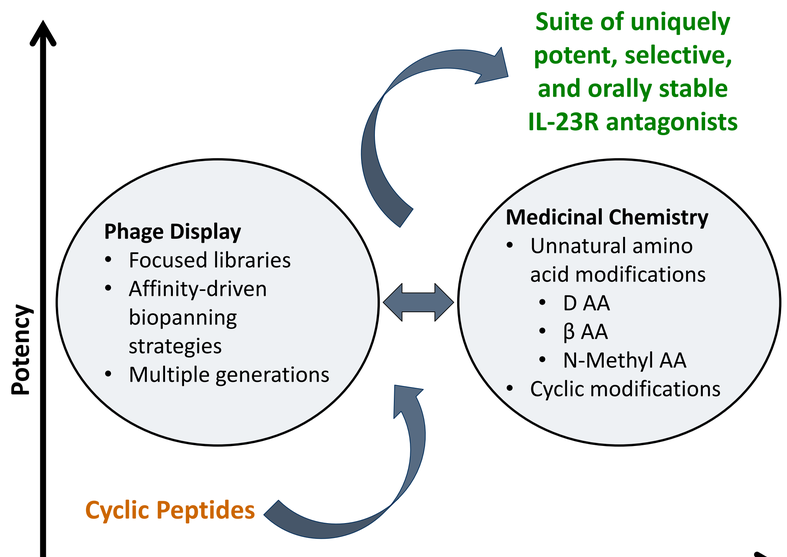


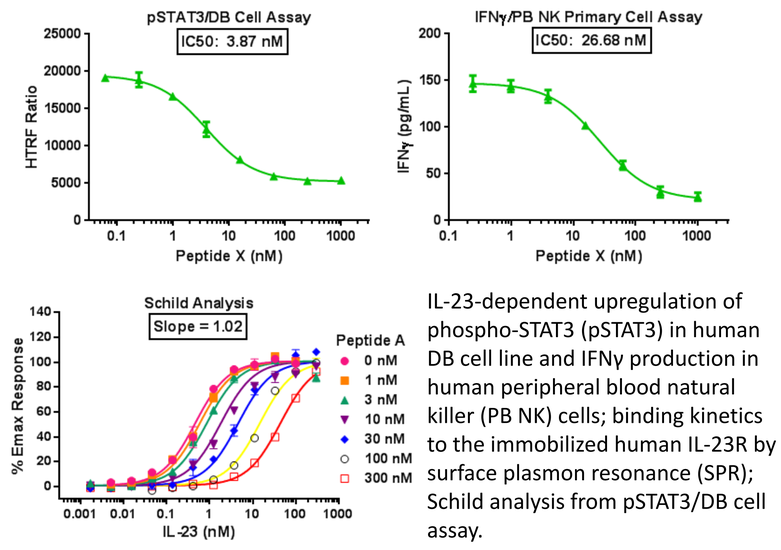
Figure 2: Optimization using phage display technology and medicinal chemistry.



IN VITRO CHARACTERIZATION

Figure 3: Peptide X is a 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



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 plasmon resonance (SPR); Schild analysis from pSTAT3/DB cell assay.

Figure 4: 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.

Figure 5: 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.

Figure 6: 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.

Figure 7: Exposure of Peptide X is restricted to the GI.

	Area Under Curve			Recovery
	Small Intestine Mucosa	Colon Mucosa	Plasma	Feces
Peptide X	355 ug.h/g	77 ug.h/g	0.3 ug.h/mL	40%

Sprague Dawley (SD) rats PO dosed at 20 mg/kg

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

Figure 8: 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)
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.

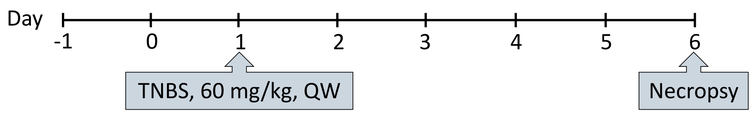
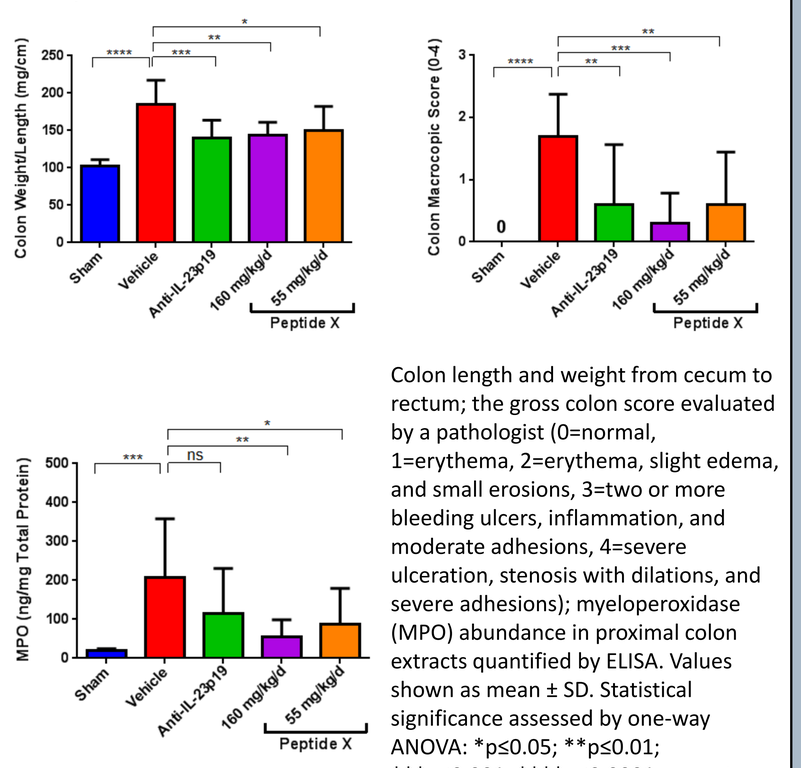
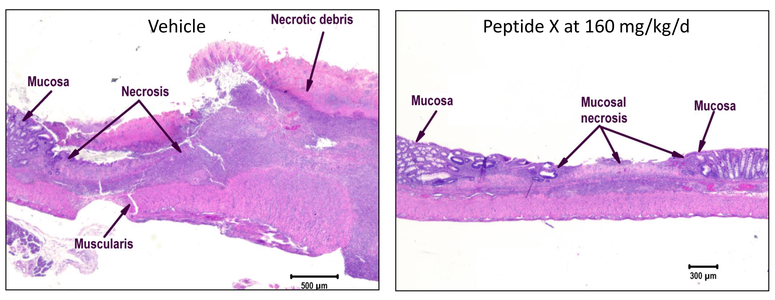


Figure 9: Oral treatment reduces colonic inflammation and neutrophil infiltration.



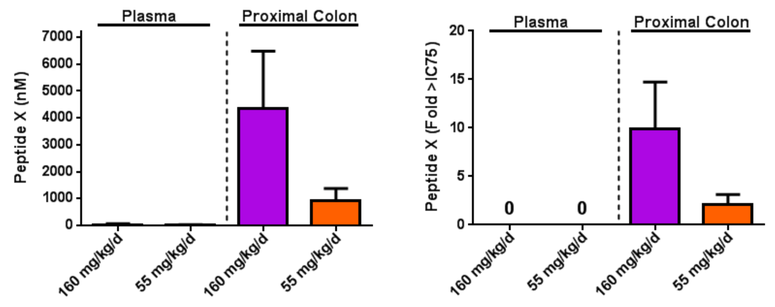
Colon length and weight from cecum to rectum; the gross colon score evaluated by a pathologist (0=normal, 1=erythema, 2=erythema, slight edema, and small erosions, 3=two or more bleeding ulcers, inflammation, and moderate adhesions, 4=severe ulceration, stenosis with dilations, and severe adhesions); 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 10: Oral treatment improves histopathology.



Histological scores evaluated by a pathologist (inflammation, mucosal necrosis, gland loss, colon wall thickness, 0-5); photomicrograph of lesions from the vehicle group represented by transmural inflammation, presence of necrotic tissue, and mucosa devoid of crypts; photomicrograph of inflammation from the Peptide X-treated group, characterized by restriction of lesions to the mucosa. Values and statistical significance as shown in Figure 9.

Figure 11: Oral efficacy observed is most likely due to local activity in the colon.



Peptide X concentration and fold above IC_{75} in the IL-17A/rat splenocyte assay, determined from plasma and proximal colon extracts 1 hour post-terminal PO dose.

CONCLUSIONS AND DISCUSSION

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 environment 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;
- 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.

CONTACT INFORMATION

Lili Cheng, Ph.D.
Protagonist Therapeutics
521 Cottonwood Drive
Milpitas, CA 95035 USA
l.cheng@progtagonist-inc.com
www.progtagonist-inc.com

Protagonist Therapeutics