PTG-100, an oral peptide antagonist of integrin α4β7 that alters trafficking of gut homing T cells in preclinical animal models

Larry Mattheakis, Ashok Bhandari, Lu Bai, Genet Zemede, Vinh Tran, Herodion Celino, Brian Frederick, Li Zhao, Mridula Dogra, Harriet Lister, James Tovera, Shairaz Shah, Thamil Annamalai, Dinesh Patel , David Liu

Protagonist Therapeutics, Milpitas, CA

Protagonist Therapeutics

ABSTRACT

Background

The α4β7 integrin is a clinically validated target in inflammatory bowel disease (IBD). Vedolizumab (Entyvio®), a humanized mononclonal antibody that specifically binds to the α4β7 integrin, is FDA-approved for the treatment of moderate-to-severe ulcerative colitis and Crohn's disease. Vedolizumab binds to α4β7 on circulating memory/effector T cells in the blood and blocks their homing to intestinal tissues expressing the ligand MAdCAM-1. The aim of this study is to characterize PTG-100, a novel oral α4β7 antagonist peptide that is largely restricted to the gut tissues, and is pharmacologically active in murine colitis models and in normal cynomolgus monkeys.

Methods

Pharmacokinetic (PK) studies of PTG-100 were conducted in mice, rats, and cynomolgus monkeys, with peptide concentrations measured by mass spectrometry. Pharmacodynamic (PD) studies were conducted in murine colitis models and in healthy cynomolgus monkeys. Cell trafficking in blood and gut lymphoid tissues was measured by FACS or immunohistochemistry (IHC).

Results

PTG-100 is a potent antagonist of α4β7 (IC50 = 1 nM), but inactive against α4β1, αLβ2 or αEβ7 as measured in a variety of biochemical and cellular assays. Oral dosing of PTG-100 in normal or dextran sodium sulfate (DSS)-treated mice and rats showed dose-dependent exposure in the small intestine, colon, mesenteric lymph nodes (MLN) and Peyer's Patches (PP), but much lower exposure if any in blood and urine. Oral dosing of a fluorescent dye conjugate of PTG-100 and imaging by fluorescence microscopy or IHC showed the peptide accumulates in the lamina propria of tissues from the small intestine. Daily dosing with PTG-100 in murine DSS colitis models showed a dose-dependent reduction of CD4+ CD44high CD45RBlow β7+ T cells in the MLN and PP, and a concomitant increase in the spleen and blood as measured by FACS. There was also a strong reduction of β7+ cell infiltration into lamina propria lesions of the distal colon as measured by IHC. PTG-100 also caused a dose-dependent reduction in body weight loss and mucosal injury as assessed by endoscopy. Daily oral dosing of PTG-100 in normal cynomolgus monkeys resulted in high blood receptor occupancy of memory T cells, and a dose-dependent increase in the percentage of α4β7 memory CD4+ T cells in the blood. There were no adverse clinical or microscopic changes with PTG-100 administration in six week GLP toxicology studies in rat and monkey up to 90 and 75 mg/kg/day, respectively. Safety pharmacology and mutagenesis studies demonstrated no adverse findings.

Conclusions

PTG-100 is a first-in-class oral α4β7-selective antagonist being developed for the treatment of patients with IBD. PTG-100 reaches high concentrations in gut tissues and alters the trafficking of gut-homing T cells in mice and cynomolgus monkeys. The lack of toxicity in the full battery of safety and toxicology studies to date coupled with low exposure in blood suggest that PTG-100 will be suitable for human trials.

BACKGROUND

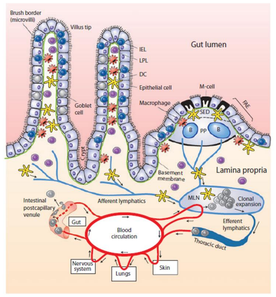


Figure 1. Lymphocyte trafficking. Oral PTG-100 crosses the epithelial barrier from the lumen to block lymphocyte trafficking in gut lymphoid tissues and blood

Marsal and Agace, J Intern Med, 2012, 272; 411–429

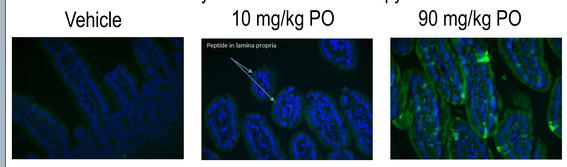
RESULTS IN VITRO ASSAYS

Table 1. PTG-100 is selective for human circulating α4β7⁺ memory T cells.

Integrin	α4β7	α4β1	αLβ2
Ligand	MAdCAM-1	VCAM-1	ICAM-1
IC50 (nM)	1.3	>100,000	>100,000
PBMC cell adhesion assay for indicated ligand			

LOCALIZATION OF PTG-100 IN MOUSE SMALL INTESTINE

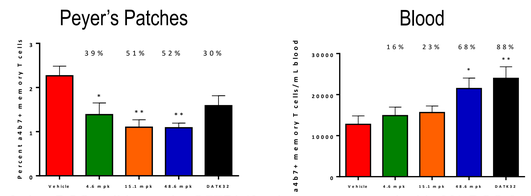
Figure 2. Localization of PTG-100 Alexa Fluor® 488 conjugate in the mouse small intestine by fluorescence microscopy.



PTG-100 was chemically conjugated to Alexa Fluor® 488 dye. C57BL/6 mice were dosed with vehicle or PTG-100 Alexa Fluor® 488 conjugate at the indicated doses. Takedown was 3 h post dose and formalin-fixed paraffin-embedded (FFPE) tissue sections of the small intestine were prepared. Shown is an overlay of images for nuclei (DAPI, blue) and PTG-100 (Alexa Fluor® 488, green) at 40X magnification.

PHARMACODYNAMICS/EFFICACY ACUTE DSS COLITIS MICE

Figure 3. PTG-100 reduces α4β7+ T cells in gut lymphoid tissues and redirects them to blood.



9 day DSS colitis study. C57BL/6 mice were treated with 3% DSS from Day 1 to Day 6, and switched to normal water until Day 10. Daily dosing was PO BID plus drinking water for PTG-100, and 25 mg/kg IP every 3 days for the anti-α4β7 Ab DATK32. PP and blood were collected and levels of α4β7+ memory T cells analyzed by FACS. Data is presented as means and SD. n=10 mice per group. Statistical significance assessed by one-way ANOVA:
*: p≤0.05; **: p≤0.01. Percentage values and statistical significance are relative to vehicle control.

CHRONIC DSS COLITIS MICE

Figure 4. PTG-100 reduces DAI AUC score in a 16 day chronic DSS study.

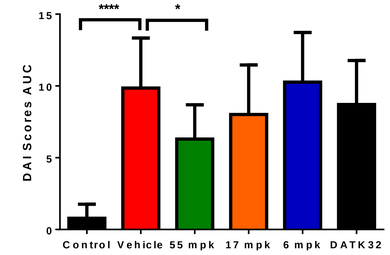


Figure 5. PTG-100 reduces number of β7⁺ cells in the lamina propria of the distal colon.

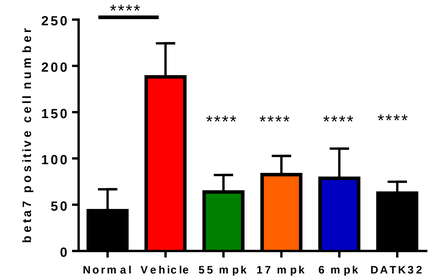
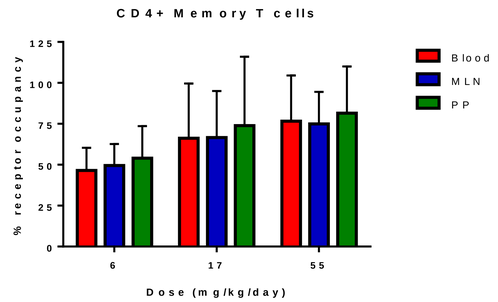


Figure 6. PTG-100 receptor occupancy of CD4+ memory T cells in whole blood, mesenteric lymph node (MLN) and Peyer's Patches (PP).



16 day chronic DSS colitis study. BALB/c mice were treated continuously with 2.5% DSS. PTG-100 total daily dose was a combination of oral gavage BID plus drug in the drinking water. The anti-α4β7 Ab DATK32 was dosed 25 mg/kg IP every 3 days. Disease activity index (DAI) score was recorded each day and is a summation of individual scores for body weight loss, stool consistency and hemoccult score. After takedown, whole blood, MLN and PP were collected for α4β7 receptor occupancy of memory CD4+ memory T cells as measured by FACS. Distal colon sections were fixed and processed for β7+ cell IHC staining using the anti-β7 antibody M293. Data is presented as means and SD. n=10 mice per group. Statistical significance relative to vehicle control assessed by one-way ANOVA: *, p≤0.05; **, p≤0.01; ***, p≤0.005; ****, p≤0.0001; ns, not significant.

CYNOMOLGUS MONKEY STUDY

Figure 7. PTG-100 increases blood receptor occupancy of α4β7+ memory CD4 T cells in cynomolgus monkey.

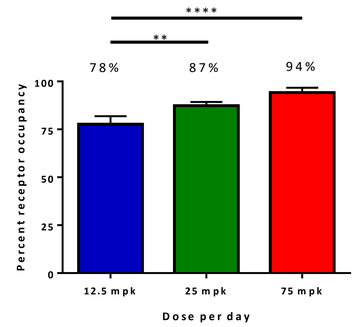


Figure 8. PTG-100 increases the percentage of circulating α4β7+ memory CD4 T cells at Day 7 normalized to pre-dose.

Cynomolgus monkeys were dosed 7 days PO QD at the indicated doses. Each group contained 4 animals (2 male, 2 female). One hour post last dose, blood was collected for PK to measure PTG-100 levels, or analyzed by FACS for α4β7⁺ memory CD4 T cells. To measure receptor occupancy, blood samples were incubated with a sub-saturating concentration of a PTG-100 Alexa Fluor® 647 conjugate. Blood was also collected from each animal prior to dosing. Receptor occupancy of each animal is defined as the level of occupied receptor one hour post dose normalized to the pre dose level.

CONCLUSIONS

- PTG-100 is the first oral antagonist selective for α4β7 integrin, a clinically validated target for IBD.
- In murine colitis models, PTG-100 alters T cell trafficking and reduces disease pathology.
- Target engagement, as measured by receptor occupancy and increase in circulating T cell populations, was demonstrated in mice and cynomolgus monkeys.
- PTG-100's low blood exposure and high GI exposure suggests it may be acting locally within the gut lymphoid compartment to block memory T cell pathology.

CONTACT INFORMATION

Thamil Annamalai, M.S.
Protagonist Therapeutics
521 Cottonwood Dr., Milpitas, CA 95035
Email: t.annamalai@protagonist-inc.com
www.protagonist-inc.com