Establishing the human equivalent dose for PTG-100, an oral peptide antagonist of integrin α4β7

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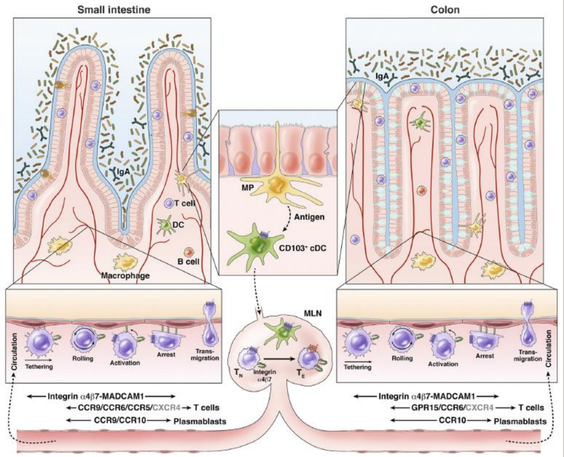
ABSTRACT

PTG-100, a selective novel oral peptide antagonist of α4β7 integrin, is being developed for the treatment of patients with moderate to severe ulcerative colitis. PTG-100 alters trafficking of gut homing T cells in preclinical animal models, and its potency and selectivity are similar to that of the approved anti-α4β7 antibody vedolizumab. Pharmacokinetic studies in rodent or cynomolgus (cyno) monkeys show that PTG-100 exposure in the blood is <0.1% of dose, but >10% of dose in the small intestine and colon and up to 40% in feces, which indicate PTG-100 is orally stable and largely gut restricted. To help establish the potential efficacious dose range in humans, we developed a receptor occupancy assay to measure occupancy of CD4+ memory α4β7+ T cells in mouse blood and gastrointestinal (GI) tissues and in cyno blood. Daily dosing of PTG-100 and other similar antagonists in DSS (dextran sodium sulfate) treated mice showed a significant reduction in disease activity index (DAI), mucosal histopathology, and number of β7+ positive cells in the distal colon lesions. At these efficacious oral doses, α4β7 receptor occupancy in the blood, mesenteric lymph nodes, and Peyer's Patches ranged from 46-81% at 4 h post dose. Single and multiple oral gavage administration of PTG-100 in healthy cynos showed that despite low systemic exposure, occupancy of blood α4β7 by PTG-100 is dose proportional, time-dependent, and influenced by the type of vehicle and fasted state of the animal. Allometric scaling from the mouse to human based on whole body surface area suggests that a similar level of blood receptor occupancy is associated with the cyno equivalent dose. The data suggests that 100% receptor occupancy over 24 h in the blood or GI in the mouse DSS model is not required for efficacy by an oral gut-restricted α4β7 antagonist. Together, these studies point to blood receptor occupancy and possibly receptor expression as useful clinical surrogates for the local effects of PTG-100 in the intestine.

BACKGROUND

Figure 1. Homing of lymphocytes to the small intestine and colon.



Habtezion et al., Gastroenterology 2016;150:340–354

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
Human PBMC memory T cell adhesion assay for indicated ligand			

PHARMACOKINETIC STUDIES

Table 2. PTG-100 exposure is largely gut-restricted (%Fp < 0.5%). 30mg/kg PO administration in healthy C57BL/6 mouse.

	C_{max} (nM)	AUC (μg*h/mL)
Small Intestine	16629	43
Colon	1156	20
Peyer's Patches	21964	63
MLN	108	0.73
Plasma	35	0.13
30 mg/kg PO QD, 24 h study, Vehicle 50 mM phosphate buffer, pH 7.4.		

EFFICACY/PHARMACODYNAMICS IN DSS COLITIS MICE

Figure 2. PTG-100 reduces DAI AUC score comparable to α4β7 mAb.

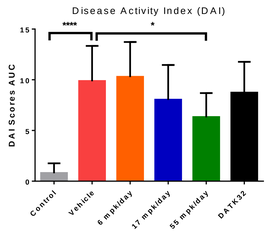


Figure 3. PTG-100 reduces number of β7+ cells in the lamina propria of the distal colon comparable to α4β7 mAb.

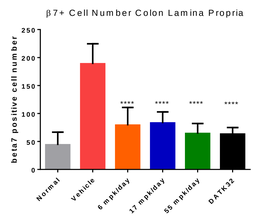
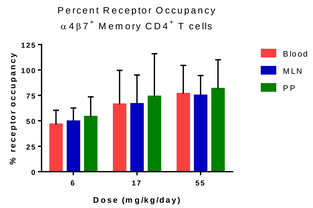


Figure 4. PTG-100 receptor occupancy of CD4⁺ α4β7⁺ memory T cells in whole blood correlates to mesenteric lymph node (MLN) and Peyer's Patches (PP).



15 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. At 4 h post last dose, 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.

PHARMACODYNAMICS IN DSS COLITIS MICE

PTG-100 TARGET ENGAGEMENT RESULTS IN DOWNREGULATION OF α4β7 INTEGRIN EXPRESSION AND INCREASES IN CIRCULATING EFFECTOR MEMORY T CELLS IN BLOOD

Figure 5. Dose dependent increase of PTG-100 receptor occupancy on CD4⁺ α4β7⁺ effector memory T cells in whole blood.

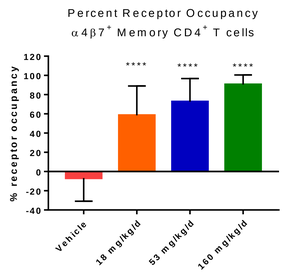


Figure 6. Specific downregulation of α4β7 expression on CD4⁺ effector memory T cells in blood.

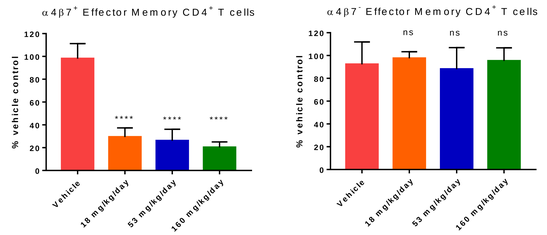
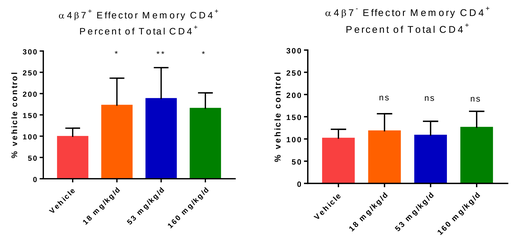


Figure 7. PTG-100 increases circulating numbers of α4β7⁺, but not α4β7⁻, CD4⁺ effector memory T cells in blood.



15 day chronic DSS colitis study. BALB/c mice were treated continuously with 3% DSS. PTG-100 total daily dose was a combination of oral gavage BID plus drug in the drinking water. At 4 h post last dose, whole blood was collected for α4β7 receptor occupancy, α4β7 expression, and circulating numbers of effector memory T cells using FACS. 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.

ALLOMETRIC SCALING (MOUSE TO CYNO TO HUMAN)

Mouse effective dose	Cyno equiv.	Human equiv.
18 mg/kg/day	4.5 mg/kg/day	102 mg
53 mg/kg/day	13.4 mg/kg/day	302 mg
160 mg/kg/day	40.3 mg/kg/day	911 mg
Whole body surface area. Equivalent dose based on 4 kg cyno and 70 kg human.		

CYNOMOLGUS MONKEY STUDIES

Figure 8. Blood receptor occupancy of CD4+ α4β7+ memory T cells in cyno is similar under fasted and non-fasted dosing conditions.

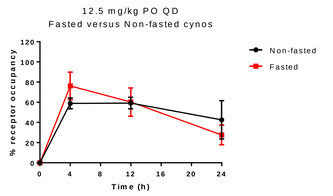
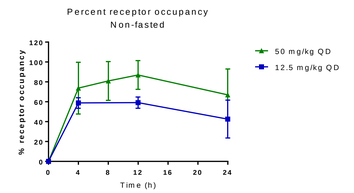


Figure 9. Dose dependent increase in blood receptor occupancy of CD4+ α4β7+ memory T cells in cynos after a single dose.



Male cynomolgus monkeys were dosed by nasogastric intubation at the indicated doses using 50 mM sodium phosphate buffer pH 7.4 as vehicle. Each group contained 3 animals. At the indicated time points, whole blood was collected for FACS analysis to measure receptor occupancy of α4β7⁺ memory CD4 T cells. Receptor occupancy of each animal is defined as the level of occupied receptor at the indicated time point normalized to the pre dose level.

CONCLUSIONS

- PTG-100 is the first oral antagonist selective for α4β7 integrin, an IBD target clinically validated by the approval of vedolizumab.
- PK studies show that PTG-100 exposure is gut restricted. Exposure in the small intestine, colon and Peyer's Patches is 150 to 480-fold higher compared to plasma based on AUC.
- PTG-100 reduces Disease Activity Index and β7⁺ cell number in the colon lamina propria in the mouse DSS colitis model comparable to α4β7 mAb.
- Target engagement by PTG-100 is accompanied by specific downregulation of α4β7 expression and increase in circulating CD4⁺ effector memory T cells in the blood of colitis mice which indicate pharmacological activity of PTG-100.
- High exposure in gut tissues and loss of α4β7 expression may explain PTG-100's significant pharmacological activity at less than 100% blood receptor occupancy.
- PTG-100 exhibits dose dependent target engagement in cynomolgus monkeys.
- Human equivalent doses established by allometric scaling based on blood target engagement and pharmacological activity observed in colitis mice and healthy monkeys.
- PTG-100 is currently being investigated in a Phase 1 clinical trial in normal healthy volunteers.

CONTACT INFORMATION

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