PHARMACOKINETICS AND PHARMACODYNAMICS FOLLOWING ORAL ADMINISTRATION OF PTG-100, A PEPTIDE ANTAGONIST OF INTEGRIN α4β7

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INTRODUCTION

The α4β7 integrin is a clinically validated target in inflammatory bowel disease (IBD) as reflected by the FDA-approval of the humanized monoclonal antibody vedolizumab (Entyvio®) for the treatment of moderate-to-severe ulcerative colitis (UC) and Crohn's disease. PTG-100 and vedolizumab both bind to α4β7 on circulating memory/effector T cells in the blood and block their homing to intestinal tissues that express the ligand MAdCAM-1. PTG-100 is a novel oral α4β7 antagonist peptide that has minimal systemic absorption and is therefore largely restricted to the gut tissues. A Phase 1 study in normal healthy volunteers has now been completed (see Abstract #3358). The aim of these studies was to characterize the pharmacokinetic (PK) properties and pharmacodynamic (PD) activities of PTG-100 in mice and cynomolgus monkeys and to establish a potentially efficacious dose range in UC patients.

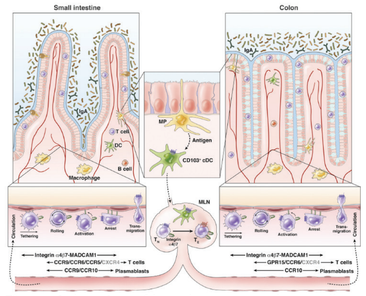


Figure 1. homing of lymphocytes to the small intestine and colon.
Habtezion et al., Gastroenterology 2016;150:340–354

METHODOLOGY

We conducted PK and PD studies in mice and cynomolgus monkeys. Peptide concentrations were measured by mass spectrometry. PD responses in whole blood were measured by α4β7+ memory T cell receptor occupancy, surface expression, and cell numbers using Fluorescence Activated Cell Sorting (FACS). Cell trafficking in blood and gut lymphoid tissues were measured by FACS or immunohistochemistry (IHC).

PHARMACOKINETICS – Mice

Oral dosing of PTG-100 in normal mice results in high exposure in the small intestine, colon, Peyer's Patches (PP), and mesenteric lymph node (MLN) with minimal exposure in the blood.

Table 1. PTG-100 exposure is largely gut-restricted (%Fp < 0.5%). 30mg/kg PO administration in healthy C57BL/6 mouse.

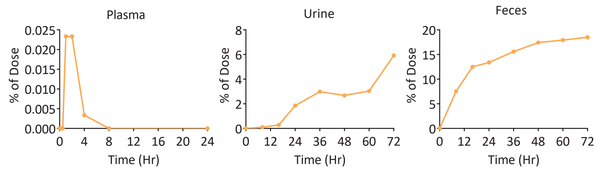
Compartment	C_{max} (nM)	AUC (μg*h/mL)	AUC ratio relative to plasma
Small Intestine	16,629	43	331
Colon	1,156	20	154
Peyer's Patches	21,964	63	485
MLN	108	0.73	6
Plasma	35	0.13	1
Feces	23% of total dose excreted up to 24 hours		

30 mg/kg PO QD, 24 h study, Vehicle 50 mM phosphate buffer, pH 7.4.

PHARMACOKINETICS – Cynomolgus Monkeys

Male cynomolgus monkeys (n=3) were dosed with PTG-100 (10 mg/kg, PO) formulated in 20% hydroxypropyl beta cyclodextrin and samples were collected at subsequent time points. To assess systemic exposure and recovery of intact peptide, blood was collected for up to 24 hours and urine/feces for up to 72 hours. The amount of peptide recovered in excreta was expressed as a percent of dose. All three animals had low but detectable plasma PTG-100 levels. About 5.91% was detected in the urine as parent molecule and about 18.48% was detected in the feces as unchanged parent molecule over a period of 72 hours. These results indicate that PTG-100 has low systemic exposure, and the peptide is stable in the GI tract of non-human primates.

Figure 2. Time-course of percent dose exposure in male cynomolgus monkeys (10 mg/kg, PO) in plasma, urine, and feces. Each point represents the mean of three animals.



PHARMACODYNAMICS – T Cell Trafficking

Daily dosing with PTG-100 in murine DSS colitis models showed a dose-dependent reduction in $CD4^+$ $CD44^{high}$ $CD45RB^{low}$ $\beta7^+$ T cells in the Peyer's Patches, and concomitant increase in the blood as measured by FACS. There was also a strong reduction of $\beta7^+$ cell infiltration into lamina propria lesions of the distal colon as measured by IHC.

Protocol: 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 mouse anti-α4β7 Ab DATK32. PP and blood were collected and levels of α4β7+ memory T cells analyzed 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≤0.001; ****, p≤0.0001.

Figures 3a & 3b. PTG-100 reduces α4β7+ T cells in gut lymphoid tissues and redirects them to blood.

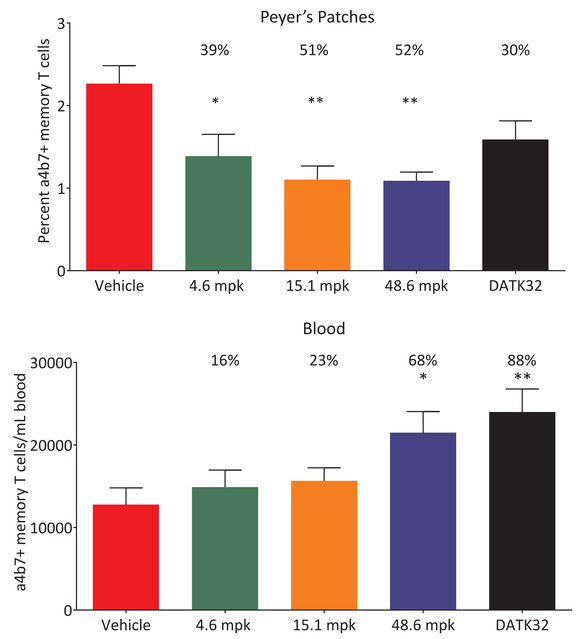
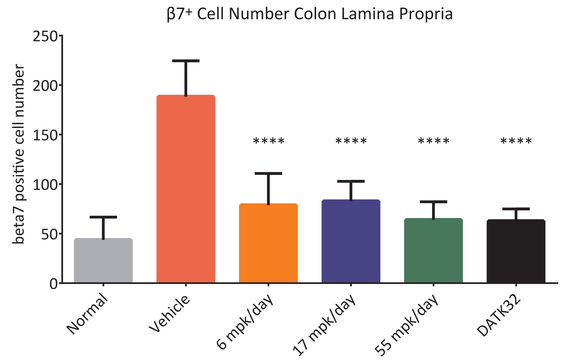


Figure 4. PTG-100 reduces number of β7+ cells in the lamina propria of the distal colon comparable to the mouse anti-α4β7 mAb.



PHARMACODYNAMICS – Target Engagement

PTG-100 receptor occupancy results in downregulation of α4β7 integrin expression and increases in circulating effector memory T cells in blood.

Protocol: 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≤0.001; ****, p≤0.0001.

Figure 5. Dose dependent increase of PTG-100 receptor occupancy on $CD4^+$ $\alpha4\beta7^+$ effector memory T cells in whole blood.

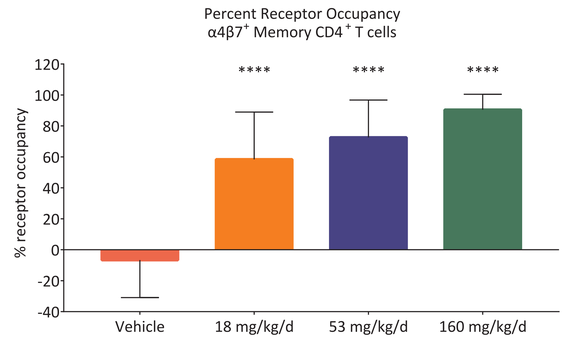


Figure 6. Specific downregulation of α4β7 expression on $CD4^+$ effector memory T cells in blood.

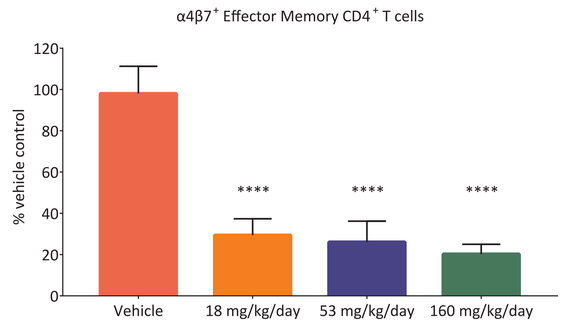
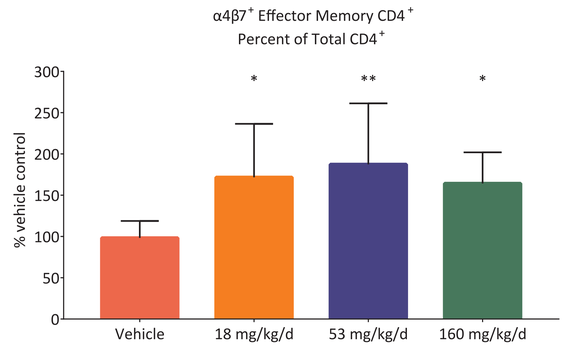


Figure 7. PTG-100 increases circulating numbers of $\alpha4\beta7^+$ $CD4^+$ effector memory T cells in blood.



ESTIMATED HUMAN DOSE

Table 2. Estimation of Human Equivalent Dose

Mouse effective dose	Cyno equivalent	Human equivalent
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

Allometric scaling by whole body surface area. Equivalent dose based on 4 kg cyno and 70 kg human.

SUMMARY AND CONCLUSIONS

- PTG-100 is a novel oral α4β7-selective antagonist being developed for the treatment of patients with ulcerative colitis.
- It is largely gut restricted in mice and cynomolgus monkeys.
- Mouse blood PD responses indicate that PTG-100 binds to α4β7 integrin and causes a reduction in integrin expression resulting in the inhibition of memory T cell trafficking to gut lymphoid tissues.
- Allometric scaling was used to estimate the human effective dose range based on PD responses in the mouse and cynomolgus monkey (data not shown).
- A Phase 1 study in normal healthy volunteers (Abstract #3358) confirmed that PTG-100 blood receptor occupancy and receptor expression in the preclinical models is consistent with the responses observed in human

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