**BACKGROUND**

PTG-100 is a gut restricted oral peptide antagonist of the T cell homing integrin α4β7, and it alters trafficking of gut homing T cells in preclinical animal models. Integrin α4β7 is the target for vedolizumab (Entyvio®), a monoclonal antibody approved for moderate to severe ulcerative colitis (UC) and Crohn’s disease. In preclinical models using healthy or dextran sulfate sodium (DSS)-induced colitis mice, we previously showed that PTG-100 causes a dose dependent increase in integrin receptor occupancy (RO) and downregulation of integrin receptor expression (RE) on peripheral blood memory T cells. Similar pharmacodynamic (PD) responses were observed in the peripheral blood of normal healthy volunteers (HV) after PTG-100 oral dosing in a randomized, double blind, Phase 1 trial. The aim of these analyses was to use modeling approaches for predicting the PD responses in UC patients.

**METHODS**

Healthy mice (C57BL/6) or colitis mice (BALB/c) treated with 3% DSS daily were dosed BID with PTG-100 or placebo control. Peripheral blood was collected and analyzed by FACS for PTG-100 receptor occupancy or α4β7 receptor expression. Semi-mechanistic, nonlinear, mixed effects models were based on the PD responses observed in healthy and colitis mice studies, and on the Phase 1 HV study. Standard model validation techniques were used including posterior predictive checks that compare model based predictions with observed data. In vitro integrin activation studies were done using memory T cells isolated from PBMCs of healthy human donors.

**RESULTS**

**PD responses in normal versus colitis mice**

We compared the PD responses in normal and colitis mice, and found that the magnitude of the PD response (both RO and RE) was greater in colitis mice compared to healthy mice at equivalent doses. Simulated models were built from the responses in healthy and colitis mice and compared to the observed data using posterior predictive checks (Fig. 3).

**Figure 3.** The relationship between PTG-100 dose versus PD is shifted in colitis mice compared to normal mice.

**Simulated models built on the PD responses in healthy mice predict the PD responses in healthy volunteers**

Human doses/kg were derived from mouse doses/kg by use of multipliers. The colitis multipliers were found that results in high affinity binding to MAdCAM-1. We show that α4β7 binding to PTG-100, but not vedolizumab, is increased in the presence of MnCl₂ (Fig. 5).

**Figure 5.** In vitro studies suggest the colitis PD dose shift is caused by PTG-100’s preference to block the activated state of α4β7.

Integrins are often expressed on the cell surface in an inactive state. Addition of MnCl₂ shifts α4β7 into an activated state that results in high affinity binding to MAdCAM-1. We show that α4β7 binding to PTG-100, but not vedolizumab, is increased in the presence of MnCl₂ (Fig. 5).

**Figure 5.** Incubation of human CD4+ memory α4β7 T cells with fluorescent conjugates of PTG-100 (A) or vedolizumab (B) in the presence or absence of 1 mM MnCl₂. Shown is the median fluorescence intensity (MFI) as measured by FACS.

**CONCLUSIONS**

- Simulated models built on the PD responses in healthy mice predicted responses that were in agreement with the PD responses in healthy human volunteers.
- Similar to the observed PD dose shifts in colitis mice, the model predicts PTG-100 will have greater PD responses in colitis patients compared to healthy volunteers at equivalent doses.
- The PD dose shifts may be explained by the greater proportion of activated α4β7 expressed under colitis versus healthy conditions. We provide supportive data by showing that PTG-100 preferentially binds to the activated state of α4β7.
- Therefore, unlike vedolizumab, PTG-100 binds selectively to the activated state of α4β7 to block homing of pathogenic T cells.

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