**Integrin α4β7: discovery of gut-restrictive oral peptide antagonists that are active in murine models of inflammatory bowel disease**


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**ABSTRACT**

**Background**

The α4β7 integrin is a clinically validated target for inflammatory bowel disease (IBD). The anti-α4β7 antibody vedolizumab is approved by the FDA for treating moderate-to-severe ulcerative colitis and Crohn’s disease. Vedolizumab binds to α4β7 on circulating mucosal 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 develop orally stable α4β7 antagonist peptides that act locally in the intestinal tissue. These peptides have minimal systemic exposure, yet are effective in blocking T cell homing and are efficacious in murine models of IBD.

**Methods**

Potent, selective and orally stable peptide antagonists of α4β7 integrin were identified through Protagonist’s peptide and peptidomimetic technology platform. To evaluate oral stability, the peptides were incubated in a variety of ex vivo intestinal/colonic washes or simulated gastric/intestinal fluids, and half-lives determined by mass spectrometry. Potency and selectivity assays used transformed cell lines or primary cells from PBMC donors. Pharmacokinetic (PK), pharmacodynamic (PD) and chronic studies were conducted in mice.

**Results**

The peptides are potent against α4β7, but not α4β1 and α4β2 as measured in biochemical and cell adhesion assays. In α4β7 specific cell adhesion assays, the peptides block adhesion of the human RPMI 8466 cell line (B cell lymphoblastoid) or mouse TK-1 (T cell lymphoblastoid) to immobilized MAdCAM-1 (IC50 < 20 nM). In the α4β1 or α4β2 specific cell adhesion assay using human Jurkat cells, they are inactive up to the highest tested concentration, 100 μM. To facilitate oral delivery, we chemically engineered the peptides to be resistant to chemical and proteolytic degradation in a variety of gastric and intestinal fluids, while maintaining their potency and selectivity. PK studies in normal or dextran sodium sulfate (DSS) treated mice and rats showed that oral dosing results in exposure in the small intestine, colon and mesenteric lymph nodes (MLN), but no significant measurable levels in the blood and urine. A PD assay was used to assess the effect of oral dosing on trafficking of endogenous memory T cells in the mouse. Mice treated with DSS were orally dosed daily with peptides for 9 or 13 days, and harvested tissues were analyzed by FACS. FACs analysis of tissues from animals dosed with peptides showed that there is a reduction of CD4+ CD45RA-CD45RB+ α4β7+ T cells in the MLN and Peyer’s Patches, but not in the spleen or blood. There was also marked reduction of clinical disease symptoms. We also evaluated these peptides in a T cell adoptive transfer chronic colitis model. Daily oral dosing with peptides reduced the severity of disease as measured by colon weight length ratio and histology.

**Conclusions**

Potent, selective and orally stable peptide antagonists of α4β7 integrin were shown in oral PK studies to have significant exposure in intestinal tissues, but not blood and urine. Despite low blood exposure, these peptides block T cell homing to gut associated lymphoid tissue and attenuate disease in murine models of IBD. These results support the therapeutic potential of locally blocking T cell homing while minimizing immunogenicity and the risk of opportunistic infections associated with systemically delivered immunosuppressants and biologics.

**RESULTS**

**Table 1. PTG-100 is potent and selective for α4β7 integrin.**

<table>
<thead>
<tr>
<th>Integrin</th>
<th>α4β7</th>
<th>α4β1</th>
<th>α4β2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
<td>MAdCAM-1</td>
<td>VCAM-1</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>Cell Line</td>
<td>RPMI8466</td>
<td>TK1 (Mu)</td>
<td>Jurkat (Hu)</td>
</tr>
<tr>
<td>IC50 (nM)</td>
<td>0.72</td>
<td>0.50</td>
<td>&gt;100,000</td>
</tr>
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</table>

Cell adhesion assay for indicated ligand.

**Figure 1. Lymphocyte trafficking.**

**Figure 2. Gut-homing T cell binding to MAdCAM-1 and extravasation.**

**Table 2. PTG-100 is selective for human circulating α4β7+ memory T cells.**

<table>
<thead>
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<tr>
<td>Ligand</td>
<td>MAdCAM-1</td>
<td>VCAM-1</td>
<td>ICAM-1</td>
</tr>
<tr>
<td>IC50 (nM)</td>
<td>1.3</td>
<td>&gt;100,000</td>
<td>&gt;100,000</td>
</tr>
</tbody>
</table>

PBMC cell adhesion assay for indicated ligand.

**Figure 3. PD study design.**

**Figure 4. PTG-100 significantly reduces endoscopy score.**

**Table 3. Binding kinetics to α4β7 by SPR. Tool peptide PN-10742 has a slower dissociation rate compared to vedolizumab**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Kd (M1 sec^-1)</th>
<th>Kd (sec^-1)</th>
<th>Kd (nM)</th>
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</thead>
<tbody>
<tr>
<td>PN-10742</td>
<td>793</td>
<td>5.3 X 10^5</td>
<td>67.0</td>
</tr>
<tr>
<td>Vedolizumab</td>
<td>6460</td>
<td>4.1 X 10^-4</td>
<td>64.0</td>
</tr>
</tbody>
</table>

Biotinylated peptide or Ab was immobilized to chip for binding of integrin in solution.

**Figure 5. PTG-100 reduces colonic friability and mucosal injury.**

**Figure 6. PTG-100 reduces α4β7+ T cells in gut lymphoid tissues and redirects them to blood and spleen.**

**CONCLUSIONS**

- The α4β7 integrin is a specific IBD target that is clinically validated by the intravenous antibody vedolizumab.
- PTG-100 is an oral α4β7 antagonist peptide that has low systemic exposure, and it is effective in blocking T cell homing and preventing mucosal injury in a murine model of IBD.
- PTG-100 has the potential to be an oral therapeutic for the treatment of IBD while minimizing the risk of immunogenicity associated with systemically delivered biologics.

**CONTACT INFORMATION**

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**DISCLOSURE**

Dr. Aida Habtezion’s contribution to this publication was as a paid consultant, and was not part of her Stanford University duties or responsibilities